

# **Experimental low-level jaw clenching inhibits temporal summation evoked by electrical stimulation in healthy human volunteers**

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## **Highlights**

- Low-level jaw clenching has an inhibitory action on temporal summation.
- The central nervous system may be involved to the present findings.
- Potential harmful low-level jaw clenching could continue despite painful symptoms.

## **Abstract**

**Objective:** To examine the effect of low-level jaw clenching on temporal summation in healthy volunteers.

**Design:** In 18 healthy volunteers, the pain intensities evoked at the masseter muscle and the hand palm by the first and last stimuli in a train of repeated electrical stimuli (0.3 or 2.0 Hz) were rated using 0-100 mm visual analog scales (VAS), in order to evaluate temporal summation before and after three types of jaw-muscle tasks: low-level jaw clenching, repetitive gum chewing and mandibular rest position. A set of concentric surface electrodes with different diameters (small and large) was used for the electrical stimulation.

**Results:** The temporal summation evoked by the large diameter electrode with 2.0 Hz stimulation decreased significantly both on the masseter and the hand after low-level clenching ( $P \leq 0.03$ ), but did not show any significant change after the other tasks ( $P > 0.23$ ). The VAS score of the first stimulation did not show any significant changes after low-level clenching ( $P > 0.57$ ).

**Conclusions:** Experimental low-level jaw clenching can inhibit pain sensitivity, especially temporal summation. Low-level jaw clenching can modify pain sensitivity, most likely through the central nervous system. The findings suggest that potential

harmful low-level jaw clenching or tooth contacting could continue despite painful symptoms, e.g, temporomandibular disorders.

**Key words:** temporal summation, pain sensitivity, temporomandibular disorders, masticatory muscles, concentric electrode, stimulation depth.

## 1. Introduction

Temporomandibular disorders (TMD) have been reported to have a multifactorial etiology. Parafunctions are one of the proposed risk factors for TMD<sup>(1-3)</sup> Indeed, low level jaw muscle activity has been implicated in the pathophysiology of painful TMD conditions. Several studies have reported that a limited increase of jaw muscle activity, e.g., tooth contacting habit (TCH)<sup>1-3</sup> or elevated sleep background activity,<sup>4</sup> may be a contributing factor to chronic pain in TMD patients. Meanwhile, in experimental conditions, voluntary low-level jaw clenching can cause transient jaw muscle pain symptoms in healthy subjects.<sup>5-7</sup> For example, prolonged (30 min) low-level jaw clenching at 10% maximum voluntary contraction (MVC) can induce jaw muscle fatigue and headaches after the clenching in healthy volunteers.<sup>7,8</sup> Farella et al. found that fatigue and jaw muscle pain were sustained over a long period of time after prolonged low-level clenching (30-150 min/ 7.5-10 % MVC) compared to high-level brief (1.4 min/ 40% MVC) clenching, i.e., fatigue and pain were still observed one day after prolonged low-level clenching, whereas, after the high-level brief clenching, fatigue and pain were observed only immediately after the task.<sup>9</sup> Thus, low-level jaw clenching or limited increase of jaw muscle activity has been suggested to be a contributing factor for at least some types of TMD pain.<sup>3,4,6</sup>

On the other hand, these findings cannot be simply used to support the relationship between continuous jaw muscle activity and orofacial pain. According to the pain-adaptation model,<sup>10</sup> nociceptive stimuli to, e.g., the muscle lead to inhibition of painful muscle activity. However, some types of TMD patients, especially the myofascial pain group, may have a tooth contacting habit.<sup>1, 3</sup> Tooth contact increases jaw muscle activities to about 2.0 to 3.5 times the activity during relaxed baseline.<sup>11, 12</sup> Thus the relationship between habitual limited increase of jaw muscle activity and pain of the TMD cannot simply be explained by the pain-adaptation model, and the underlying mechanism of why patients continue potentially harmful tooth contacting habits is still unclear.

Temporal summation using repeated stimulation is used as an assessment method for changes in pain sensitivity of central origin.<sup>13, 14</sup> In this way, it has been suggested that temporal summation is a useful tool to obtain valuable information with respect to central hyperexcitability.<sup>13</sup> It is also reported that wind-up is more likely to occur in the C fibers of deep tissue rather than in superficial tissue.<sup>15</sup> Therefore, changing the stimulation depth may have an effect on the magnitude of temporal summation. In previous studies, needle electrodes were used to stimulate the deep tissue.<sup>13, 14, 16</sup> The needle electrode has the advantage of selective stimulation of deep tissues with reduced

stimulation of the superficial structures.<sup>17</sup> However, micro injuries as a result of the needle electrodes are an issue with this method. In the present study, a concentric surface electrode with different diameters was used to test the effect of change in the stimulation depth, without invasion of the jaw muscle and superficial structures. The first aim of this study was to examine to what extent temporal summation evoked after jaw exercises would be influenced by differences in size of the concentric stimulating electrodes.

We speculated that low-level jaw clenching could have effects on the peripheral and/or central pain sensitivity. The second aim of this study, therefore, was to examine whether temporal summation could be influenced by low-level jaw clenching.

## **2. Materials and methods**

### *2.1. Experiment 1: Model experiment using simulation tissue*

To test the spreading pattern of the electrical stimulation evoked by the concentric stimulation electrode, a model experiment using simulation tissue was carried out. Because it has been reported that the distance between the anode and cathode can affect the spread of stimulation,<sup>18</sup> a pseudo simulation tissue made with dental silicon (Fig. 1) was stimulated by a set of concentric surface electrodes with

different diameters (KS206-010; Unique Medical, Japan). The electrode consisted of a small point-type electrode surrounded by ring-electrodes with different diameter: one with a 16 mm diameter (large-diameter electrode: large electrode), and one with a 6 mm diameter (small-diameter electrode: small electrode). The center of the concentric electrode was the cathode, and the concentric part was the anode, therefore, a set of the center electrode and a large ring-electrode (or a small ring-electrode) were used for electrical stimulation. The large electrode was intended to stimulate deep tissue (muscle), and the small electrode was intended to stimulate superficial tissue (skin). The diameter of the electrodes could be changed with a hand switch. An electrical square-wave pulse (1 ms duration, 0.3 Hz) was delivered by a constant-current stimulator (Neuropack Four mini; Nihon Kohden, Japan). The stimulation intensity was set at 10 mA for both diameters of the electrodes.

Signals evoked electrical stimulation, i.e., artifact signals, were recorded by two pairs of fine wire electrodes (KS211-018; Unique Medical, Japan) at two different depths. One pair was inserted at 2 mm depth, the other at 10 mm (Fig. 1). The recording depths were decided according to the reports, regarding skin and masseter muscle thickness.<sup>19, 20</sup> In both pairs, the electrodes were placed 16 mm apart. Electrode conductive gel was applied around the stimulation electrodes and the recording

electrodes. The artifact signals evoked by the large-diameter electrode or the small-diameter electrode were amplified, filtered with bandpass 10 Hz – 5 kHz (Neuropack Four mini; Nihon Kohden, Japan), then sampled at 40 kHz, and stored from 10 ms before to 50 ms after the electrical stimulation by use of waveform analysis system (MacLab; ADInstruments, Pty Ltd) for further analysis. Forty sweeps of the signals evoked by the stimulation were recorded six times in each condition (i.e., the large-diameter electrode or the small-diameter electrode) in random order with 5 min intervals. The forty artifact signals were averaged. The peak-to-peak amplitude of evoked signals was measured on the averaged waveform, then, the average values of six trials were calculated at each depth and in each condition.

## *2.2. Experiment 2: Test in human subjects*

### *2.2.1. Subjects*

Eighteen healthy individuals (9 women, 9 men; aged 19-29; mean  $\pm$  SEM =  $23.1 \pm 0.69$ ) participated in this study. None of the subjects had signs or symptoms of neurological disorders or abnormalities in stomatognathic, neck and shoulder functions, or had taken pain medication at least 1 month before participation. This study was approved by the local ethnics committee of Nagasaki University (approval No. 0959).

All subjects gave their informed consent in accordance with the Helsinki Declaration, and understood that they were free to withdraw from the experiment at any time.

### 2.2.2. Experimental protocol

All subjects participated in four experimental days; the first day for determination of the stimulation intensity followed by three randomized days with a task of “low level clenching”, “gum chewing” or “no exercise” (control) with at least 1-week interval, therefore, one exercise task was performed on the each experiment day. The low-level clenching task consisted of three blocks of five min voluntary jaw clenching at 10 % MCV with 1 min interval, i.e., a total of 15 min low-level clenching. In the same way, “gum chewing” and “no exercise” were carried out. Chewing rhythm was not instructed. For no exercise, subjects were instructed to spend 17 minutes in the mandibular rest position.

The VAS assessments for pain induced by repeated electrical stimuli (0.3 Hz or 2.0 Hz) to the masseter muscle or the hand palm were carried out at three points in time: before the task (baseline), immediately after the task (just after), and 30 minutes after completion of the task (30 min after). Stimulation to the masseter muscle or the hand palm was carried out in random order for each subject. A large-diameter electrode or a

small-diameter electrode (see below) was used for electrical stimulation. In each stimulation site (masseter muscle or hand palm), four combinations of conditions, i.e., two types of electrodes (large-diameter or small-diameter) x two stimulus frequencies (0.3 Hz or 2.0 Hz) were performed in random order for each subject. At each condition, the VAS assessments to stimulation were repeated three times with 1 min interval.

### 2.2.3. Recording and stimulation

For recording of the electromyographic (EMG) activity, bipolar surface disc electrodes of 10 mm in diameter were placed at a distance of 10 mm to the upper part of the habitual chewing side of the masseter muscle. The EMG signals were amplified, filtered with bandpass 10 Hz - 5 kHz, sampled at 2 kHz (MP100; Biopac Systems. Inc., USA), and stored in a computer by a waveform analysis system (AcqKnowledge; Biopac Systems. Inc., USA). The integral value of muscle EMG activity from the masseter muscle was calculated on line then displayed as a bar graph on the monitor set in front of the subject. At the start of the experiment, the EMG activities during rest and the maximum jaw clenching effort were measured. Clenching was performed three times for three seconds in the intercuspal position. The maximum voluntary contraction (MVC) using the rectified and integrated EMG was calculated as the maximum value of

the 3 efforts. During the low-level clenching task, subjects were asked to keep 10% MVC with visual feedback on the monitor. The habitual chewing side was determined by asking the subjects at the start of the experiment. In cases where this could not be determined through questioning, the chewing side was determined by having subjects chew gum for a short period of time.

Masseter and palmar electrical stimulation was performed using a set of concentric surface electrodes (KS206-010: Unique Medical Co., Ltd. Japan) tested in experiment 1. The diameter of the electrodes used for the stimulation could be changed with a hand switch, and subjects were not informed which size was being used. The stimulation electrodes were attached on the lower part of the masseter of the habitual chewing side (under the EMG electrodes) for the masseter stimulation, and on the center of the thenar eminence on the same side for the palmar stimulation. A constant-current stimulator (Neuropack Four mini: Nihon Kohden, Japan) was used for the electrical stimulation. Stimulation waveforms were rectangular with 1 ms duration. At the start of the experiment on the first day, stimulation intensity was determined by using single stimuli with 10 seconds inter-stimulus-interval. The pain evoked by the electrical stimulation was assessed using a 100 mm VAS. The left end displayed the state where there was “no pain at all”, and the right end displayed “the worst imaginable pain”.

Using two ascending and descending series of electrical stimuli, the stimulation intensity where the VAS value of pain reached 20-30 mm was determined. The stimulation intensity was increased (or decreased) in steps of 0.2 mA. The stimulus intensities were determined for the small and large electrodes separately. When the intensities for the small and the large electrode were different, the mean value was used as the stimulation intensity. Subjects were not informed about the stimulation intensity. The order of the size of the concentric electrode diameter was randomized for each subject. The stimulation intensities were determined for the masseter and the hand palm, respectively. Afterwards each of the determined stimulation intensities was used consistently throughout the experiment.

#### 2.2.4. Assessment of pain from electrical stimulation

A stimulation train consisting of four repeated electrical stimuli with the determined stimulation intensity was used for the evaluation of temporal summation. Subjects were stimulated with a train stimulation with the large-diameter or small-diameter electrode at a stimulation frequency of 0.3 Hz or 2.0 Hz before the task, immediately after the task, and 30 minutes after completion of the task. Just after the stimulation, the subjects recorded the VAS scores of the first and fourth stimuli in the

train. Afterwards, subjects recorded what they remembered of the second and third stimuli<sup>12</sup>. The train stimulation was performed three times at each point in time. The mean values of the three VAS scores of the first stimulation ( $VAS_1$ ) were calculated for each point and stimulation condition. The mean  $VAS_1$  value at 0.3 Hz and 2.0 Hz was used for further normalization. Calculation of the temporal summation ( $VAS_{4-1}$ ) was done according to Price et al.'s method:<sup>14</sup> it was calculated by subtracting the VAS score of the first stimulation ( $VAS_1$ ) from the VAS score of the fourth stimulation ( $VAS_4$ ):  $VAS_{4-1} = VAS_4 - VAS_1$ . The average of the 3 times was set as the individual score.  $VAS_1$  and  $VAS_{4-1}$  were then normalized with respect to the baseline values. Normalized  $VAS_1$  ( $norVAS_1$ ) =  $(VAS_1:each\ point - VAS_1:baseline) / VAS_1:baseline \times 100$ ; Normalized  $VAS_{4-1}$  ( $norVAS_{4-1}$ ) =  $(VAS_{4-1}:each\ point - VAS_{4-1}:baseline) / VAS_1:baseline \times 100$ . The normalized VAS scores of the first stimulation ( $VAS_1$ ) and the temporal summation ( $VAS_{4-1}$ ) were used for further statistical analysis.

#### 2.2.5. Statistics

To test the effects of task type and time effect, a two-way repeated measurements analysis of variance (ANOVA) was performed, and followed by post hoc comparisons with the use of Tukey tests. The factors in the ANOVA were task type

(three levels: low-level clenching, gum chewing, no exercise) and time (three levels: baseline, just after task, 30 min after task). In these analyses, the ANOVA were performed separately for stimulation site (masseter, palm), size of stimulation electrode (large, small) and stimulation frequency (0.3 Hz, 2.0 Hz: for norVAS<sub>4-1</sub>) (Fig. 3-5). Mean values  $\pm$  SEM are given in the text and figures. The level of significance was set at  $P < 0.05$ .

### **3. Results**

#### *3.1. Experiment 1: Effect of diameter of stimulation electrode*

The peak-to-peak amplitudes evoked by the small diameter electrode were  $1420.9 \pm 255 \mu\text{V}$  and  $737.5 \pm 241.7 \mu\text{V}$  at 2 mm depth and 10 mm depth, respectively. The peak-to-peak amplitudes evoked by the large diameter electrode were  $670.6 \pm 256.8 \mu\text{V}$  and  $1043.6 \pm 255 \mu\text{V}$  at 2 mm depth and 10 mm depth, respectively (Fig. 2). The peak-to-peak amplitude at 2 mm depth was higher than that at 10 mm depth when the small electrode was used. On the contrary, the peak-to-peak amplitude at 10 mm depth was higher than that at 2 mm depth when the large electrode was used.

### 3.2. Experiment 2

The mean stimulus intensities were  $2.63 \pm 0.79$  mA at the masseter, and  $2.34 \pm 0.56$  mA at the palm.

#### 3.2.1. VAS scores of the first stimulation (VAS<sub>1</sub>)

##### Masseter muscle

The VAS<sub>1</sub> scores at baseline did not show significant difference between task types for large or small electrode ( $P > 0.99$ ; for the large electrode,  $23.9 \pm 2.6$  for no exercise,  $26.5 \pm 2.6$  for low-level clenching,  $25.9 \pm 2.6$  for gum chewing; for the small electrode,  $24.8 \pm 2.9$  for no exercise,  $23.8 \pm 2.9$  for low-level clenching,  $25.4 \pm 2.9$  for gum chewing). In the ANOVA results of norVAS<sub>1</sub>, time was a significant factor for the large electrode ( $P < 0.05$ ), but not for the small electrode ( $P > 0.077$ ). This means that norVAS<sub>1</sub> increased just after the task ( $11.6 \pm 3.5$  %) in comparison with baseline ( $P < 0.05$ ), then returned to baseline level after 30 min ( $3.5 \pm 3.2$  %,  $P > 0.823$ ). Task type ( $P > 0.263$ ) or the interaction between time x task type ( $P > 0.223$ ) were not significant for the large electrode or the small electrode. The norVAS<sub>1</sub>, however, significantly increased ( $P < 0.014$ ) in the post hoc tests just after the task compared to baseline both using large (Fig. 3a) and small electrodes (Fig. 3b) for gum chewing only. No

significant differences were seen between baseline, just after task and 30 min after task in low-level clenching or no exercise ( $P > 0.163$ ) (Fig. 3).

#### Palm

The VAS<sub>1</sub> scores at baseline did not show significant difference between task types for large or small electrode ( $P > 0.99$ ). Time factor, task type factor, or the interaction between time x task type factor were not significant regarding norVAS<sub>1</sub> scores from the palm ( $P > 0.211$ ). No significant differences in temporal change were seen among experimental conditions or within each experimental condition in the post hoc tests ( $P > 0.440$ ).

#### 3. 2. 2. Temporal summation (VAS<sub>4-1</sub>)

##### Masseter muscle

The VAS<sub>4-1</sub> scores at baseline did not show significant difference between task types in any condition ( $P > 0.999$ , Table 1). In the ANOVA results of the norVAS<sub>4-1</sub> scores, time, task type, or the interaction between time and task type were not significant at any of the stimulation conditions (i.e., large / small electrode at 0.3 / 2.0 Hz) ( $P > 0.194$ ). However, post hoc tests showed that in the condition with the large

electrode at 2.0 Hz, norVAS<sub>4-1</sub> scores were significantly lower 30 min after the low-level clenching task in comparison to baseline ( $P < 0.03$ ) (Fig. 4b). No other significant differences were seen between baseline, just after the task and 30 min after the task for the gum chewing or no exercise conditions ( $P > 0.451$ ).

#### Palm

The VAS<sub>4-1</sub> scores at baseline did not show significant difference between task types in any condition ( $P > 0.921$ , Table 1). Similar to the results of the masseter muscle, in the ANOVA results of the norVAS<sub>4-1</sub> scores, time, task type or the interaction between time and task type were not significant for any of the stimulation conditions ( $P > 0.098$ ). However, post hoc tests showed that in the condition at 2.0 Hz, norVAS<sub>4-1</sub> scores 30 min after the low-level clenching task were significantly decreased compared to just after the task for the large electrode ( $P < 0.005$ ) (Fig. 5b), and it was also decreased compared to baseline for the small electrode ( $P < 0.043$ ) (Fig. 5d). No other significant differences were seen between baseline, just after the task and 30 min after the task for the gum chewing or no exercise conditions ( $P > 0.233$ ).

#### 4. Discussion

The main finding in this study was the demonstration of inhibitory effects of a low-level clenching task on temporal summation mechanisms; this was not observed for a chewing task or when subjects kept the lower jaw in a resting position.

#### *4. 1. Methodological consideration*

The diameter of the concentric electrode affected the spreading pattern of the electrical stimulation measured at different depths. In the present simulation study, the peak-to-peak amplitude of the stimulation artifact at 10 mm depth was higher than that at 2 mm depth when the large electrode was used. In contrast, the peak-to-peak amplitude at 2 mm depth was higher than that at 10 mm depth when the small electrode was used.

The small electrode was, indeed, intended to stimulate superficial tissues, e.g., the skin, and the large electrode was intended to stimulate deep tissues, e.g., muscles. In line with a previous report,<sup>18</sup> our findings in the pseudo-tissue suggested that distance between anode and cathode can affect the spread of stimulation, i.e., the electrical stimulation applied by a set of electrodes with large diameter can efficiently spread to deeper layers than that with a small diameter. Moreover, changes in temporal summation were unlikely to occur in cases where the small electrode was used, whereas

changes were likely to occur in cases where the large electrode was used. This is consistent with previous studies:<sup>15, 21</sup> neural hyperexcitability is more likely to occur from C fiber input originating in the muscle, than stimulation from skin C fibers. As the above-mentioned, the large electrode may be stimulating deep tissue more than surface tissue although surface tissue still should be stimulated. Pain as a result of tissue invasion is becoming a problem with needle electrodes,<sup>16</sup> but it has been demonstrated that by using concentric surface electrodes, non-invasive stimulation for different tissue depths is possible.

To test the gender difference, additionally a two-way repeated measured ANOVA was performed. The factors in the ANOVA were gender and time. In these analyses, the ANOVA were performed separately for task type. Generally, the VAS<sub>1</sub> scores and the VAS<sub>4-1</sub> scores of the masseter muscle did not show any gender differences. The gender difference is an interesting issue in the pain-related studies, however, the small sample size in the present study does not allow any firm conclusions to be made and further studies will be needed to address this issue in more detail.

#### *4. 2. Effect of task type*

The VAS<sub>4-1</sub> evoked by repeated stimuli at 2.0 Hz was used as a measure of

temporal summation which decreased after the repeated low-level clenching task. This effect, however, was influenced by the size of the stimulation electrodes and the stimulation frequency, i.e., a decrease of the norVAS<sub>4-1</sub> score of the masseter muscle could be observed when the large electrode at 2.0 Hz was used, but not by using the small electrode or the large electrode at 0.3 Hz. On the other hand, in the present condition, there were no significant changes in the norVAS<sub>4-1</sub> after control (no exercise) or gum chewing. The present findings, however, should be carefully considered because the post hoc test showed significant reduction, but the ANOVA showed no significance in the interaction between time and task type. Not only the findings from the masseter muscle, but also that from the palm showed significant decrease of the norVAS<sub>4-1</sub> scores at the post hoc test after clenching task. Thus, the present findings, we believe, suggest that low-level clenching has an inhibitory effect on pain.

To examine the characteristics of low-level clenching, gum chewing and no exercise (keeping at the mandibular rest position) were adopted as control conditions. In the present results, the normalized VAS of the first stimulation (norVAS<sub>1</sub>) at the masseter muscle was seen to increase immediately after gum chewing, then returned to the baseline level 30 minutes after gum chewing. No changes in norVAS<sub>1</sub> at masseter muscle could be observed after low-level clenching or no exercise.

Temporal summation at the masseter muscle decreased after low-level clenching under the condition of the large electrode at the stimulus frequency of 2.0 Hz, but no changes in temporal summation were seen after gum chewing or no exercise. At the stimulus frequency of 0.3 Hz, no change in temporal summation was observed even after the low-level clenching. Thus, it appears that not the peripheral region, but the central nervous system (CNS) may contribute to the decreased pain associated with temporal summation at 2.0 Hz stimulation after low-level clenching. The norVAS<sub>1</sub>, on the other hand, increased immediately after gum chewing which could be caused by the effect of changes in peripheral pain sensitivity as a result of fatigue-related chemical changes in the exercised muscle.<sup>22-24</sup>

Differences between fatigue levels might be involved in the different results between gum chewing and low-level clenching.

Prolonged gum chewing was also reported to show a pain inhibitory effect through a descending inhibitory system,<sup>25</sup> and muscle pain after prolonged gum chewing does not really last for a long period<sup>26</sup> suggesting that gum chewing is also an important exercise type for modulation of pain sensitivity. Further research by changing the duration and intensity of each exercise are required to investigate the characteristics of these exercises.

#### *4. 3. Inhibitory effect after low-level clenching*

Contrary to our expectation, in the present study, pain reports decreased after low-level clenching. Not only in the exercised masseter muscle but also in the extra-trigeminal (remote) region, i.e., the palm, reduction in temporal summation was observed 30 minutes after low-level clenching when the 2.0 Hz stimuli was used. However, the reduction was not significant immediately after the clenching task and no changes were seen in the VAS of the first stimulation. These findings suggest that peripheral mechanisms cannot explain these results, and that a mechanism via the CNS may be involved in the results after the low-level clenching in this study. It has been suggested that it takes time to develop muscle hyperalgesia since there is no change in pain threshold to the repeated stimulation of the muscles immediately after an intramuscular injection of hypertonic saline, whereas the pain threshold decreases 30 minutes after injection.<sup>27</sup> It has also been reported that long-term potentiation-like hyperalgesia increased after electrical stimulation, and reached a plateau at approximately 30 min after the stimulation.<sup>28</sup> In accordance with these findings through the present study, a significant decrease in temporal summation in both the masseter and the palm after low-level clenching was observed not immediately after exercise, but 30

minutes after the clenching. The above suggests that a certain amount of time may be required for the effect via the CNS to develop. Furthermore, for the masseter muscle stimulation, reduction of temporal summation 30 minutes after the clenching was observed when the large electrode with 2.0 Hz was used, whereas for the palm stimulation, the reduction was observed for both the large and small electrodes. This finding may also suggest the contribution of the CNS to pain modulation after the clenching. The difference between the masseter muscle and the palm may be due the difference of the pain modulation between the trigeminal region and the spinal region.<sup>29</sup>

A reduction in temporal summation, i.e., an inhibitory effect on pain was observed after the prolonged clenching in this study. Continuous tactile input originating from the periodontal ligament as a result of pressure by tooth clenching, may also be involved in the pain control system because tactile input normally controls the transmission of nociceptive input.<sup>30</sup> However, reduction of pain could not be observed after gum chewing in the present study. This may be due to the difference in duration of pressure stimulation between gum chewing and prolonged clenching.

In contrast to the present study, it has been reported that limited increase of jaw muscle activity, e.g., tooth contacting habit and elevated sleep background activity, was related to the orofacial/head pain of patients.<sup>1-4, 12</sup> Furthermore, in experimental

conditions, voluntary low-level jaw clenching can induce TMD-like experimental pain in healthy subjects,<sup>5, 6, 12</sup> and a decrease in the pain threshold of the jaw muscles was observed after prolonged low-level clenching more than after brief high-level clenching.<sup>9</sup> It is also suggested that the activation of descending inhibition is reduced after prolonged clenching.<sup>7</sup> This kind of opposite effect may have occurred due to differences in experimental conditions such as the intensity and duration of the clenching time. Moreover, from the finding of this study using only healthy subjects, it is difficult to discuss the relationship between habitual low-level clenching and TMD pain because TMD patients and healthy subjects may show differences in pain modulation. Further investigations using TMD patients require to clarify this issue.

#### *4. 4. Clinical significance*

Since only brief experimental clenching was performed in this experiment, it is difficult to transfer the present findings to TMD patients who may have more habitual low-level jaw clenching or a so-called tooth contacting activity. However, clinical pain conditions in the orofacial region (such as toothache and headache) may be decreased or inhibited through the demonstrated mechanism with decreased pain sensitivity as a result of low-level clenching. Moreover, neuroplastic changes in corticomotor control

after repeated tooth clenching has been reported.<sup>31</sup> Their result and the present results may suggest the intriguing possibility that low-level clenching could at first hand lead to neuroplasticity and temporary pain alleviation, but also to the need for more frequent activations of the jaw muscles, i.e., a potential undesirable habit. The present study has demonstrated that prolonged low-level clenching evokes pain inhibitory effects and not only pain facilitation. The present findings provide new knowledge to understand characteristics of the TMD patients who may have habitual clenching or a tooth contacting habit, and the information may be useful in educational programs.

#### *4.5. Conclusion*

In the present study, it was demonstrated that low-level clenching had an effect on the temporal summation of pain. The CNS might be involved in the modulation of pain. The effect after clenching exercises was influenced by the difference in depth of stimulation of the tissues. It is also suggested that the effect based on the difference in depth could be non-invasively examined by the use of concentric electrodes with different diameters.

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### **Competing interests**

None of the authors have potential conflicts of interest to be disclosed.

### **Ethical approval**

This study was approved by the local ethnics committee of Nagasaki University (approval No. 0959).

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## Figure legends

**Fig. 1** - Experimental condition using a pseudo-tissue made with dental silicon. A concentric electrode for electrical stimulation was set on the surface of the silicon. Two pairs of fine wire electrodes were inserted at 2 (a) and 10 (b) mm depth to record artifacts evoked by the electrical stimuli.

**Fig. 2** - Peak to peak amplitude of the artifact evoked by electrodes with different diameters at 2 and 10 mm recording depth.

**Fig. 3** - The effect of task condition and time on the masseter norVAS<sub>1</sub>. a: large electrode; b: small electrode. The norVAS<sub>1</sub> increased significantly just after the task compared to baseline and 30 min after the task in the gum chewing condition both at small and large electrodes. \* $P < 0.05$ : Significant difference from baseline in each condition. n=18.

**Fig. 4** - Effect of task condition and time on the masseter norVAS<sub>4-1</sub>. Upper columns (a, b): large electrode; Lower columns (c, d): small electrode. Left columns (a, c):

stimulation frequency of 0.3 Hz; Right columns (b, d): 2.0 Hz. In the case where the task is low-level clenching, with large electrode at 2.0Hz, the norVAS<sub>4-1</sub> 30 min after task decreased significantly compared to baseline. \* $P < 0.05$ : Significant difference from baseline in each condition. n=18.

**Fig. 5** - Effect of task condition and time on the palmar norVAS<sub>4-1</sub>. Upper columns (a, b): large electrode; Lower columns (c, d): small electrode. Left columns (a, c): stimulation frequency of 0.3 Hz; Right columns (b, d): 2.0 Hz. In the case where the task is low-level clenching with 2.0Hz stimuli, the norVAS<sub>4-1</sub> 30 min after the task decreased significantly compared to baseline or just after the task. \* $P < 0.05$ : Significant difference from baseline in each condition. # $P < 0.05$ : Significant difference between just after and 30 min after the task in each condition. n=18.

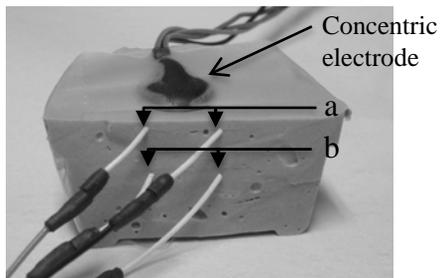


Fig. 1

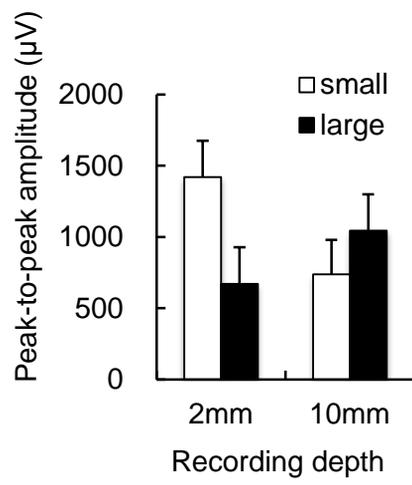
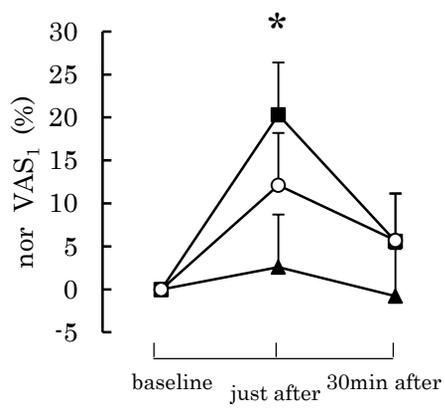


Fig. 2

a



b

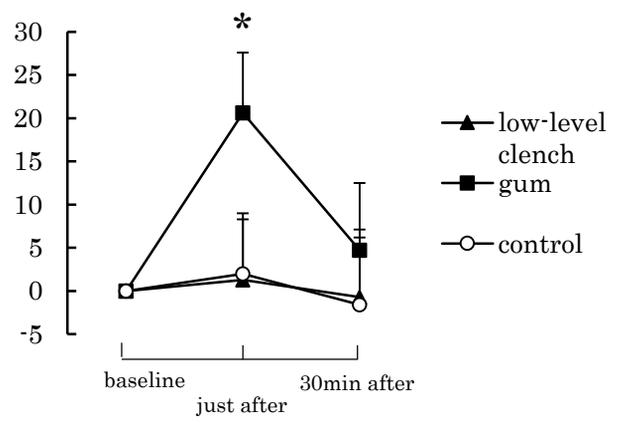


Fig.3

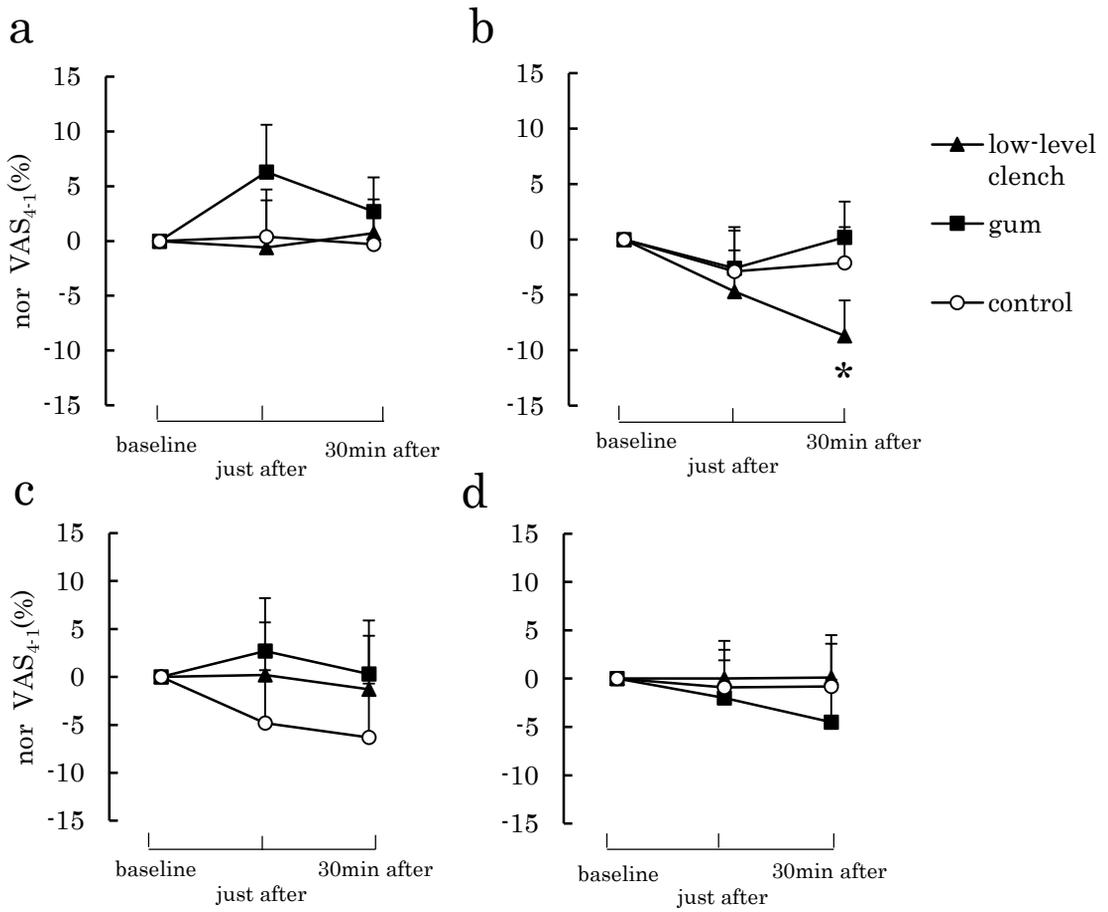


Fig.4

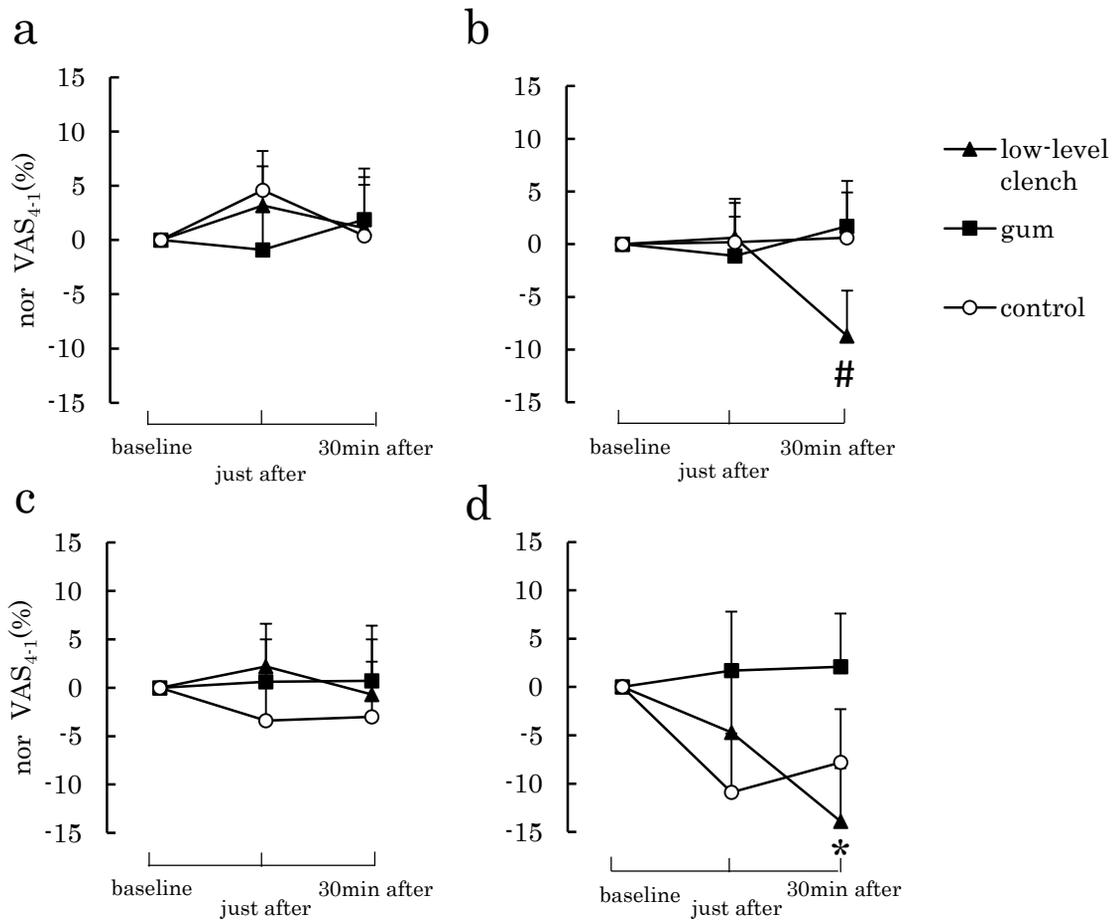


Fig.5

Table.1 Mean  $\pm$  SEM of the VAS<sub>4-1</sub> values at baseline

	Masseter				Palm			
	Large		Small		Large		Small	
	2.0Hz	0.3Hz	2.0Hz	0.3Hz	2.0Hz	0.3Hz	2.0Hz	0.3Hz
control	2.7 $\pm$ 0.9	1.8 $\pm$ 0.8	3.0 $\pm$ 1.0	2.1 $\pm$ 1.1	2.9 $\pm$ 1.0	1.2 $\pm$ 1.0	3.2 $\pm$ 0.8	0.5 $\pm$ 0.6
low level clench	3.7 $\pm$ 0.9	1.4 $\pm$ 0.8	2.6 $\pm$ 1.0	1.4 $\pm$ 1.1	4.3 $\pm$ 1.0	1.3 $\pm$ 1.0	3.3 $\pm$ 0.8	1.3 $\pm$ 0.6
gum	3.3 $\pm$ 0.9	2.0 $\pm$ 0.8	3.6 $\pm$ 1.0	2.3 $\pm$ 1.1	3.1 $\pm$ 1.0	1.9 $\pm$ 1.0	2.1 $\pm$ 0.8	0.6 $\pm$ 0.6

Table.1