Analysis of mechanism of referred pain in orofacial region

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Ⅰ. Abstract

[Objective]

Referred pain is defined as pain felt in a different region or structure away from the source of pain. Although several studies have proposed mechanisms for referred pain, the actual processes underlying referred pain in the orofacial area have yet to be clarified. This study was consisted of two researches. The aim of Study 1 was to investigate somatosensory function of the skin over the masseter muscle in healthy participants that were divided into a masseter pain prone group (MPP) (n = 22) and non-MPP group (n = 22), according to the response to a 1.0-kg palpation. Study 2 aimed to determine if standardized palpations of the temporalis muscle evoke referred pain and/or sensations in individuals without temporomandibular disorder (TMD).

[Materials and methods]

Study 1: Quantitative sensory testing (QST) was performed at the skin above the right masseter muscle (homotopic). In an additional experiment, 13 individuals each from MPP and non-MPP received application of 60% topical lidocaine tape to the skin over the masseter muscle for 30 min. Immediately after, mechanical pain sensitivity (MPS), dynamic mechanical allodynia, and pressure pain threshold were tested.

Study 2: The mechanical sensitivity of the right temporalis muscle was assessed in 32 participants without TMD with nine different stimulations to 15 test sites using palpometers (different stimulus intensities (0.5, 1.0, and 2.0 kg) and durations (2, 5, and 10 s). After each stimulus, participants were asked to score perceived pain intensity and

intensity of unpleasantness on a 0–100 numeric rating scale as an indicator of mechanical sensitivity in the temporalis muscle and to indicate any areas of referred pain/sensations on a body chart.

[Result]

Study 1: Homotopic MPS was significantly higher and PPTs significantly lower in MPP than in N-MPP ($P < 0.05$). Strikingly, no other differences in QST outcomes were observed between the groups ($P > 0.05$). After lidocaine application, no significant differences in homotopic MPS were observed between groups.

Study 2: Pain intensity significantly differed between palpation durations, intensities, and test sites ($P < 0.001$). In contrast, unpleasantness significantly differed between palpation duration and intensities ($P < 0.001$), but not test sites. Participants more frequently reported referred pain/sensations evoked by the 10-s (34.4%) as opposed to the 2-s (6.3%) and 5-s (15.6%) palpation duration at the 2.0-kg stimulus intensity ($P < 0.05$).

[Conclusion]

The presence or absence of acute provoked pain in masseter muscle is exclusively associated with differences in homotopic MPS which is decreased following topical anesthesia. And referred pain/sensations in the orofacial region can be evoked by standardized palpation of the temporalis muscle and influenced by the palpation duration in individuals without TMD. Clinical relevance Referred pain/sensations from the temporalis muscle were duration- and intensity-dependent processes originating from local stimuli.

Ⅱ. Introduction

Myofascial orofacial pain is difficult to localize and often referred to regions remote from the muscle regions [1]. Local pain is defined as pain located to the source of pain, referred pain is defined as pain felt in a different region or structure away from the source of pain [2]. Although several studies have proposed mechanisms for referred pain [3-5], the actual processes underlying referred pain in the orofacial area have yet to be clarified. The extensive convergence of afferent input from various tissues onto wide-dynamic range neurons and central sensitization is believed to be crucially involved [6].

Current international classifications of myofascial orofacial pain rely heavily on the response to palpation of the jaw muscles [7–9]. To distinguish between “pain” and “healthy” an palpation pressure of 1.0 kg for 2s has been recommended, however, however, it is the common clinical observation that even healthy and pain-free individuals may report pain on such standardized palpation with 1.0 kg [10]. It is not known if such a difference in responsiveness which could be conceptualized as being “masseter pain prone” (MPP) has any bearing on other somatosensory stimuli and effect of topical anesthesia within the same region (homotopic site). The study 1 aimed to explore the normal somatosensory physiology in order better to comprehend the pathophysiology involved in chronic orofacial pain.

A previous study has demonstrated that experimental masseter muscle pain induced by glutamate injections influence either pain intensity or pressure pain sensitivity in the masseter muscle [11,12], moreover other studies have indicated a significant sensitization of the homotopic muscle following noxious stimulation with glutamate injections [13–15]. Costa et al. reported that short-lasting experimental muscle pain was capable of causing loss of tactile sensitivity and perceptual distortions of the face [16]. It

has also been shown that longer-lasting pain in the masseter muscle caused by continuous infusion of hypertonic saline is associated with significantly higher sensitivity over the skin of the masseter muscle [12].

The German Research Network on Neuropathic Pain has recommended a protocol of 13 quantitative sensory testing (QST) measures for detecting somatosensory abnormalities [17]. Pigg et al. evaluated the inter- and intra-examiner reliabilities of QST measures for assessing somatosensory function and concluded that the reliability of QST in the orofacial area is adequate for future application of the method, such as for the establishment of normative values [18]. Moreover, Costa et al. investigated short-lasting experimental muscle pain by applying QST measures to the skin over the masseter muscle and suggested a capacity for causing loss of tactile sensitivity as well as perceptual distortion of the face regardless of preconditioning with a topical lidocaine patch. In addition, short-term application of a lidocaine patch did not significantly affect the mechanical somatosensory profile [3]. However, to date, no studies have investigated changes in somatosensory sensitivity following topical lidocaine patches in MPP and non-MPP individuals.

Our previous study investigated referred pain/sensations evoked by three different mechanical stimuli and by three different durations of a palpation stimulus applied to the masseter muscle in participants without TMD and demonstrated that referred pain/sensations from the masseter muscle are duration- and intensity-dependent, but not site dependent, processes that originate from a local stimulus with prolonged aftersensations [19]. However, questions remain about potential site-to-site differences in mechanical sensitivity within the temporalis muscle. To increase the accuracy of clinical examination and diagnostic procedures for myofascial TMD,

investigation of the relationship between mechanical sensitivity and referred pain in the orofacial area is needed.

The aim of the study 1 was to investigate the normal physiological mechanisms associated with acute masseter muscle pain in order better to understand somatosensory abnormalities in patients with chronic masseter muscle pain. And the study 2 aimed to determine if standardized palpation of the temporalis muscle can evoke referred pain and/or sensations in individuals without TMD and compare the mechanical sensitivities in response to three different stimulus levels of palpation force and three different stimulus duration of palpation time.

Ⅲ. Materials and methods

Study 1: Drop homotopic effects of masseter-muscle pain on somatosensory sensitivity in healthy participants

Forty-four participants (22 men, mean ± standard deviation [SD] age, 27.3 ± 3.2 years; 22 women, mean age 27.6 ± 2.6 years) were recruited from the community of students and staff members at Nihon University, Chiba, Japan. Inclusion criteria were as follows: age > 18 years; unassisted pain-free jaw opening, > 40 mm; and no pain during maximum unassisted or assisted jaw-opening movements. The number of participants were calculated by power analysis [20]. Exclusion criteria comprised: pregnancy; any mental disorder; allergy to lidocaine; scheduled dental treatment as of the time of the study; or intake of medications (analgesics, antidepressants, or hypnotics) within 48 h of the investigation [21]. The Patient Health Questionnaire (PHQ-9, PHQ-15), Generalized Anxiety Disorder (GAD-7) were used to screen for depression, somatic symptoms, and anxiety disorder severity. The Score of PHQ-9, PHQ-15, GAD-7 in all participants were

within normal range. Prior to enrollment in the study, all participants received written and oral explanations about the experiment and provided their informed written consent to participate. The study 1 was conducted in accordance with the guidelines established by the Declaration of Helsinki, and all protocols were approved by the ethics committee of Nihon University School of Dentistry at Matsudo (EC 18-024).

This study comprised two experiments, as a main experiment and an additional experiment. During the experiment, participants were seated on a comfortable chair in a relaxed state. First, 44 participants were divided into a masseter muscle pain prone (MPP) group (n = 22) and a non-masseter muscle pain prone (non-MPP) group (n = 22), according to the response to a 1.0-kg mechanical pressure stimulation for 2 s to the center of the right masseter muscle, using a mechanical device (PALPETER; Sunstar Swiss SA, Swiss, 1.0 kg) to standardize the site and force of the palpation (Fig. 1) [22]. The center of the right masseter muscle was identified by palpation during repetitive clenching. After application of the pressure stimulus, participants were asked to answer the presence/absence of pain during palpation.

In the main experiment, all 22 individuals of the MPP group (9 men, 13 women), and 22 individuals of the non-MPP group (13 men, 9 women) participated. QST was performed on the right masseter, the skin over the center of the right masseter, and the right first dorsal interosseous (FDI) muscle as a control. QST was conducted according to the methods proposed by the DFNS (Fig. 1).

In the additional experiment, 13 individuals from the MPP group (5 men, 8 women) and 13 individuals from the non-MPP group (8 men, 5 women) participated. In this experiment, 1 cm^2 of 60% topical lidocaine tape (PENLES TAPE; Maruho, Osaka, Japan) was applied to the skin over the center of the right masseter and right FDI for 30

min [23]. After application, mechanical pain sensitivity (MPS), dynamic mechanical allodynia (DMA), and pressure pain threshold (PPT) were performed at the right masseter, the skin over the center of the right masseter, and the right FDI (Fig. 1).
In the MPP group, QST was assessed at center of masseter muscle. In the non-MPP group, the center of the masseter muscle was used as test site. Moreover, for the heterotopic site, the central part of the FDI was tested.

Quantitative sensory testing (QST)

The standardized battery for QST applied to the right masseter muscle and right FDI involved 13 thermal and mechanical tests [18,24]. In this study, QST was performed using the following method for both the main and additional experiments. These tests included cold detection threshold (CDT), warm detection threshold (WDT), thermal sensory limen (TSL), paradoxical heat sensation (PHS), cold pain threshold (CPT), heat pain threshold (HPT), mechanical detection threshold (MDT), mechanical pain threshold (MPT), MPS, DMA, wind-up ratio (WUR), vibration detection threshold (VDT), and PPT.
A thermal sensory testing device (THERMOCEPTION ANALYZER INTERCROSS-210; Intercross Inc., Tokyo, Japan) was used to perform thermal tests. A probe with a 25-mm^2 surface area was used for all tests [18,25,26]. CDT and WDT were first measured using cold and warm stimuli, followed by the TSL. In the TSL, when the ramped stimulus reached a point where the participant first perceived the temperature as warm, the participant pressed a button. Subsequently, the direction of the temperature ramp was reversed and the thermode cooled down until the participant perceived a temperature change and again pressed the button. During this procedure, the number of occurrences of PHS was recorded, after which the CPT and HPT were determined [24]. Ramped

stimuli of 1 °C/s were used with the procedure ending when the participant pressed the button [24,25], and the participant was unable to see the computer screen during these measurements. The starting temperature on the right masseter muscle and right FDI was 32 °C, and cut-off temperatures were set at 0 °C for CPT and 50 °C for HPT [24,25]. Interstimulus interval between each thermal measurement was 4–6 s. CDT, WDT, CPT, and HPT were calculated as the mean of three measurements. Each measurement was repeated if the thermode slipped and provoked a mechanically induced pain sensation [24,25]. MDT was measured using a standardized set of modified von Frey filaments (20 PIECE MONOFILAMENT KIT PRODUCT # 10-2000; Texas Medical Design, Texas, USA) [12,19,20]. The set of von Frey filaments contains monofilaments that exert different forces on bending. Each monofilament doubled the force exerted by the previous monofilament, ranging from 0.25 to 512 mN. All monofilaments were applied perpendicular to the examination site, with contact times ranging from 1 to 2 s. The five threshold measurements were made by applying a series of ascending and descending stimulus intensities, and the threshold value was calculated using the geometric mean of these five measurements [24,25]. Geometric mean was calculated as the average can be influenced by a few unrepresentative high judgements [26]. For MPT measurements on the right masseter muscle and right FDI, a custom-made set of seven weighted pinprick stimulators was used [18,25,26]. The pinprick stimulators had a flat contact surface 0.2 mm in diameter. The range of forces of pinprick stimulators was from 8 to 512 mN, and contact time for the measurement areas was approximately 2 s. All pin-prick tests were made with the stimulator in a vertical position and perpendicular to the measurement area. The method-of-limits technique, similar to the one used to determine the MDT, was also used to determine the MPT. Similar to the MPT evaluation, seven

weighted pinprick stimulators were used for MPS determinations. DMA was estimated using three tactile stimulators including a cotton wisp, a cotton wool tip (Q-tip) attached to a flexible handle, and a disposable toothbrush (G.U.M #211 M; Sunstar Inc, Osaka, Japan). For the measurement of DMA, the three tactile stimulators were applied in a single stroke over a distance of 1–2 cm of the right masseter and FDI. MPS and DMA measurements comprised five stimulations with each of the 10 stimulators (7 weighted pinprick stimulators, 3 tactile stimulators) in randomized order according to the DFNS protocol [18,24]. In each of the total of 50 stimuli, the participant rated pain on a 0–100 numeric rating scale (NRS) with endpoints of 0 indicating no pain and 100 indicating most intense pain imaginable. MPS was calculated as the geometric mean of all numeric ratings using the seven weighted pinprick stimulators [24,25]. DMA value was calculated as the geometric mean of all numeric ratings using the three tactile stimulators [24,25].

To measure WUR, 10 pinprick stimuli were repeated at a rate of 1 Hz according to a metronome and the perceived magnitude on the 0–100 NRS for pain was determined [24]. The WUR assessment used the same custom-made pinprick stimulators as used in MPT determinations. A pinprick stimulator that delivered a force that the participant perceived as slightly painful was selected, trying the 128-mN stimulator first. If the response from the participant to the 128-mN pinprick stimulus was 0 (not painful), WUR assessment was performed using a greater force. If the participant perceived the stimulus as intolerable, less force was used [18,24]. If a participant did not perceive the 512-mN stimulator as painful, the WUR assessment was abandoned. The participant was asked to give a pain rating representing the single stimulus, and the estimated mean over the whole series of 10 stimuli using a ‘0–100’ numerical rating scale. The whole procedure was repeated three times [17]. VDT was assessed using a Rydel-Seiffer

graded tuning fork (64 Hz, 8/8 scale) [3,24–26]. In the VDT assessment, the participant was asked to raise a hand to indicate when the vibration could no longer be sensed. A 9-point scale (0–8) was used to measure the intensity of vibration, with all values recorded to an accuracy of 0.5 units. The VDT assessment consisted of three trials, and the mean VDT from three trials was calculated for each participant. PPT was measured using a digital pressure algometer (SOMEDIC ALGOMETER; Somedic Sales, Sösdala, Sweden) with a pinch handle and a probe surface area of 0.18 cm^2. PPT assessment used a rate of increase in pressure of 50 kPa/s. The participant pressed a button to interrupt the stimulation when the first painful sensation was perceived. The PPT assessment consisted of three trials, using the mean value from three trials for analysis.

Statistical analyses

Some QST parameters (with the exception of PHS and DMA) were not normally distributed, but normal distribution was achieved by logarithmic transformation (secondary normal distribution). Rolke et al. recommend executing log-transformation in the following QST parameters: CDT, WDT, TSL, MDT, MPT, MPS, DML, WUR, and PPT [17,24]. All data are presented as the mean ± the standard deviations of the mean (SD). The normal distribution of variables was analyzed using the Shapiro–Wilk test ($P < 0.05$). In the first experiment, a t-test was applied for comparisons of QST data between the two groups. Values of $P < 0.05$ were considered statistically significant. A z-score > 1.96 was regarded as a gain in somatosensory function, while a z-score < − 1.96 was regarded as indicating a loss of somatosensory function [24,25,27]. Z-scores were calculated (subtracting the non-MPP group value mean from the MPP group value mean and dividing by the sample baseline SD) for all QST parameters. Z-score values > 0

indicate higher somatosensory sensitivity than the sample mean and values < 0 indicates lower sensitivity.

In the additional experiment, QST data were analyzed using two-way analysis of variance (ANOVA) with groups (MPP and non-MPP group) and time (pre- and post-application) as factors. When appropriate, ANOVA was followed by post-hoc Tukey testing to compensate for multiple comparisons. Data were analyzed using the SPSS statistical package (version 23.0; IBM Japan, Tokyo, Japan).

Study 2: Standardized palpation of the temporalis muscle evoke referred pain and sensations in individuals without TMD

Thirty-two volunteers without TMD (16 men, mean (± standard deviation (SD)) age 26.9 ± 3.0 years; 16 women, mean age 28.4 ± 3.5 years) were recruited. Inclusion criteria were as follows: (a) age > 18 years and (b) good systemic health with (c) no orofacial pain complaints in the last 6 months or chronic pain disorders. The Diagnostic Criteria for Temporomandibular Disorders (DC/ TMD) Axis I and II were applied to all participants to assess orofacial pain and TMD symptoms by a certified examiner [2]. The DC/TMD Axis I consisted of a Pain Drawing, Graded Chronic Pain Scale (GCPS), Jaw Functional Limitation Scale -8 (JFLS -8), Patient Health Questionnaire -4 (PHQ -4), and Oral Behaviour Checklist (OBC) [2]. The PHQ -4 was used as a screening tool for anxiety and depression [28]. The OBC was used to identify and quantify the frequency of jaw overuse behaviours, e.g., bruxism [2]. Exclusion criteria were as follows: (a) the presence of medical illness or regular intake of medications such as antidepressants, anticonvulsants, muscle relaxants, hypnotics, or nonsteroidal anti-inflammatory

medications, (b) muscle-skeletal problems, (c) diagnosis of psychiatric or personality disorders, and (d) current pregnancy (as reported by the participant).

The study was conducted in accordance with the Helsinki Declaration II and after receiving approval from the Ethics Committee of Nihon University School of Dentistry at Matsudo (EC18-024). All participants gave their voluntary consent after a full explanation of all procedures.

This was a randomized, single-blinded study. Figure 2a illustrates the 15 test sites (three horizontal rows and five vertical columns) of the temporalis muscle, which we palpated. The borders of the temporalis muscle were identified by palpation during repetitive clenching. Mechanical sensitivity was assessed using three different stimulus intensities (0.5 kg, 1.0 kg, 2.0 kg) at each of the 15 test sites. To standardize the palpation, the examiner used a palpometer (Palpeter; Sunstar Swiss SA, Swiss) [2, 29]. The duration of a single palpation at each test site was 2 s, 5 s, or 10 s. The order of stimulus intensity (0.5 kg, 1.0 kg, or 2.0 kg), duration of palpation stimulus (2 s, 5 s, or 10 s), and test sites (15 sites) was randomized using a randomization program (www.randomization.com). After each stimulus, participants were asked to score perceived pain intensity and intensity of unpleasantness on a numerical rating scale (NRS) as an indicator of mechanical sensitivity in the temporalis muscle. Participants were carefully instructed in the use of the NRS for pain and unpleasantness. Figure 2b shows the NRS for pain. 0 denotes “no sensation at all,” 50 as “just barely painful,” and 100 as “the worst pain imaginable” for pain intensity [30]. Mean pain NRS scores were assessed for each of the 15 test sites on the right temporalis muscle as an overall assessment of mechanical sensitivity. On a different 0–100 NRS, the participants scored the intensity of unpleasantness, with 0 denoting “no unpleasantness at all” and 100 as

“the most unpleasantness imaginable” (Fig. 2c). In addition, participants were asked to raise their hand when they felt the absence of any sensations in their temporalis muscles after removal of the stimulus, and the examiner counted the time it took until they raised their hand. Aftersensations were recorded in seconds using a stopwatch as the duration of the sensation perceived after removal of the stimulus [31]. Pain/sensations were considered as referred pain/sensations if the participant reported pain or any sensation beyond the boundary of the temporalis muscle being palpated (i.e., perceived in another structure). Pain/sensations were not considered referred if the participant reported pain or sensation within the boundary of the temporalis muscle. After each stimulus, if the participant reported referred pain/sensations, they were asked to indicate the area of referred pain/ sensations on a digital body chart with detailed anatomical landmarks of the face, head, and neck (Navigate Pain; Aglance Solutions) (Fig. 2d) [32].

Since entropy measures complexity and the degree of diversity of information, it could be used to assess localized muscle mechanical sensitivity in response to standardized palpation with a palpometer and may be useful for establishing optimal stimulus intensity of muscle palpation to cause referred pain for diagnosing myofascial pain in the muscle [19]. Also, it may be helpful for better comprehension of the mechanical sensitivity and referred pain mechanisms in the temporalis muscle. In the context of the diversity of mechanical sensitivity scores for the right temporalis muscle, entropy indicates the degree of such diversity of 0–100 NRS sensitivity scores, with higher entropy values corresponding to more diverse intensity registers of NRS scores over the grid. Entropy was calculated for both pain and unpleasantness intensity NRS scores of the 15 test sites for each assessment within the right temporalis muscle according to a previously described method [33].

Statistical analyses

Assumption of normality was tested using the Shapiro–Wilk test, and homogeneity of variance was tested using Levene's test. The differences in mean pain, unpleasantness NRS scores, and the aftersensation time were analysed using analysis of variance (ANOVA). The different test factors were stimulus intensity (three levels), duration of palpation stimulus (three levels), and test site (15 levels). Post hoc tests were performed by using Tukey's honestly significant difference test with correction for multiple comparisons. Entropy scores for palpation were analysed with two-way ANOVA with the factor of stimulus intensity (three levels) and duration of palpation stimulus (three levels). Furthermore, McNemar's test was used to test differences in frequency of referred pain/sensations (percentage of participants with referred pain/sensation) evoked by each test site for the three mechanical stimulus intensities and durations of palpation. For all tests, the significance level was set at $P < 0.05$. All data are presented as mean values and SDs. The data were analyzed using Sigma Plot (version 14.0; HULINKS Inc., Tokyo, Japan).

Ⅳ. Results

Study 1: Drop homotopic effects of masseter-muscle pain on somatosensory sensitivity in healthy participants

1. Main experiment.

There were no participants who reported any referred pain with 1.0-kg mechanical pressure stimulation for 2 s to the center of the right masseter muscle.

Table 1 shows the comparison of QST results between the MPP and non-MPP groups for the masseter muscle. MPS on the masseter muscle was significantly higher

in the MPP group than in the non-MPP group ($P < 0.05$), and PPT was significantly lower in the MPP group than in the non-MPP group ($P < 0.05$) (Table 1). Figure 3 shows z-scores on the masseter muscle for the MPP group based on the non-MPP data as reference values. Only the PPT values were outside the range between − 1.96 and 1.96. Table 2 shows the comparison of QST results between the MPP and non-MPP groups for the FDI muscle with no significant differences for any QST parameter.

2. Additional experiment.

Table 3 shows the comparison of MPS, DMA, and PPT on the masseter muscle between before and after lidocaine application in the MPP and non-MPP groups. In both the MPP and non-MPP groups, the MPS on the masseter muscle was significantly higher before lidocaine application than after lidocaine application ($P < 0.05$) (Fig. 4a). After lidocaine application, no significant differences in MPS on the masseter muscle were evident in the MPP and non-MPP groups (Fig. 4a). In both the MPP and non-MPP groups, no significant differences in PPT on the masseter muscle were evident between before lidocaine application and after lidocaine application (Fig. 4b). Furthermore, both before lidocaine application and after lidocaine application, PPT on the masseter muscle was significantly lower in the MPP group than in the non-MPP group ($P < 0.05$) (Fig. 4b).

Study 2: Standardized palpation of the temporalis muscle evoke referred pain and sensations in individuals without TMD

1. NRS scores

Table 4 shows the statistical relationship of factors for NRS scores and aftersensation times. Significant differences were seen between pain and duration of the palpation stimulus ($F\ 2 = 121.52$, $P < 0.001$), stimulus intensity ($F\ 2 = 2723.26$, $P < 0.001$),

and the test site ($F_{14} = 3.55$, $P < 0.001$) (Table 4). Significant differences were also seen between unpleasantness and duration of the palpation stimulus ($F_{2} = 73.8$, $P < 0.001$) and stimulus intensity ($F_{2} = 638.6$, $P < 0.001$), but not the test site ($F_{14} = 0.98$, $P = 0.477$) (Table 4). Figure 5 shows a comparison of pain NRS scores. Scores for 10 s of duration of palpation stimulus were significantly higher than for 2 s of duration of palpation stimulus when using each stimulus intensity ($P < 0.05$) (Fig. 5a). Pain NRS scores for 5 s of duration of palpation stimulus were significantly higher than for 2 s of duration of palpation stimulus when using 2.0-kg stimulus intensity ($P < 0.05$) (Fig. 5a). Moreover, 78.1% (25/32) of participants reported an NRS score over 50 (pain report) with 2.0-kg stimulus intensity ($P < 0.05$) (Fig. 5a). Unpleasantness NRS scores for 10 s of duration of palpation stimulus were significantly higher than for 2 s of duration of palpation stimulus when using 1.0-kg and 2.0-kg stimulus intensities ($P < 0.05$) (Fig. 5b). Unpleasantness NRS scores for 5 s of duration of palpation stimulus were significantly higher than for 2 s of duration of palpation stimulus when using 2.0-kg stimulus intensity ($P < 0.05$) (Fig. 5b). Aftersensation times for 10 s of duration of palpation stimulus were significantly longer than for 2 s and 5 s of duration of palpation stimulus when using each stimulus intensity ($P < 0.05$) (Fig. 5c). Aftersensation for the 5-s palpation stimulus was significantly longer than the 2-s palpation stimulus when using 0.5-kg stimulus intensity ($P < 0.05$) (Fig. 5c).

Furthermore, there were significant interactions for stimulus intensity × duration of palpation stimulus and for stimulus intensity × test site with regard to pain and unpleasantness ($P < 0.001$).

2. Referred pain/sensation

Referred pain/sensations were evoked in 3.1% of participants (n = 1/32) for 5 s

and 10 s of duration of palpation with the 0.5-kg stimulus intensity. Referred pain/sensations were evoked in 3.1% (n = 1/32) for 2 s, 3.1% (n = 1/32) for 5 s, and 9.4% (n = 3/32) for 10 s of duration of palpation in participants with the 1.0-kg stimulus intensity. Referred pain/sensations were evoked in 6.3% (n = 2/32) for 2 s, 15.6% (n = 5/32) for 5 s, and 34.4% (n = 11/32) for 10 s of duration of palpation in participants with the 2.0-kg stimulus intensity (Fig. 6).

The number of participants with referred pain/sensations elicited by 10-s palpation stimulus was significantly higher than the 2-s and 5-s palpation stimulus when using the 2.0-kg stimulus intensity ($P < 0.05$; Fig. 6). Table 5 shows the area of referred pain/sensations elicited by each stimulus intensity and each duration of palpation. The most frequent areas of referred pain/sensations were the posterior teeth (15.6%; n = 5) for 10-s palpation stimulus at 2.0-kg stimulus intensity. The anterior teeth, ear, cervical, occipital, and temporalis muscle were also frequently reported as areas of referred pain/sensations. Seven participants reported more than one area of referred pain/sensation.

Six of 11 (54.5%) participants had referred pain/sensations elicited by 2.0-kg stimulus intensity for 10 s of palpation in areas with known prior medical history. For example, the posterior teeth with referred pain/sensations had a history of caries and root canal treatment. In addition, a participant reporting referred pain/sensations in the masseter muscle had a history of masseter muscle pain, and a participant reporting referred pain/sensations in the ear had a history of otitis media.

3. Aftersensations

Significant differences were seen in the duration of aftersensation between duration of palpation stimulus ($F 2 = 95.0$, $P < 0.001$) and stimulus intensity ($F 2 = 435.5$,

P < 0.001) (Table 4, Fig. 5c).

4. Entropy analysis of mechanical sensitivity

Figure 7 a and b show entropy values for pain NRS scores and unpleasantness NRS scores. ANOVA analyses of entropy values for pain NRS scores and unpleasantness NRS scores showed overall significant differences between intensity and duration and between stimulus intensities, respectively (P < 0.05 each). Post hoc tests showed that entropy values of pain NRS scores elicited with 10 s of palpation stimulus with the 0.5-kg stimulus intensity were significantly higher than those with 2 s of palpation stimulus (P < 0.05) (Fig. 7a). However, there was no significant difference in entropy values for unpleasantness NRS scores (Fig. 7b).

Ⅴ. Discussion

Study 1: Drop homotopic effects of masseter-muscle pain on somatosensory sensitivity in healthy participants

The study 1 investigated whether short-lasting pressure-evoked masseter muscle pain is associated with alterations in somatosensory sensitivity of the overlying skin in healthy individuals. The main findings in this study were: (1) MPS on the masseter muscle (homotopic) was significantly higher in the MPP group than in the non-MPP group; and (2) no significant differences in MPS before and after lidocaine patch application were evident between MPP and non-MPP groups. As expected, the PPTs were lower in the MPP group compared to non-MPP group. There was no impact on thermal or tactile sensitivity.

According to the main experiment, MPS on the masseter muscle was significantly higher and PPT was significantly lower in the MPP group than in the non-

MPP group. The study 1 found no significant differences in CDT, WDT, TSL, PHS, CPT, HPT, MDT, MPT, DMA, WUR or VDT on masseter muscle between the MPP and non-MPP groups in the main experiment. MPS was assessed using the same set of seven weighted pin-prick stimuli to obtain a stimulus–response function for pinprick-evoked pain, designed to detect pin-prick hyperalgesia [27]. Meints et al. found that patients with chronic low back pain demonstrated greater deep-tissue hyperalgesia as well as increased sensitivity for mechanical punctate pain compared to pain-free controls [34]. In addition, it is well known that widespread hyperalgesia on quadriceps femoris muscle in deep tissue is a common finding in patients with muscle pain and could be related to a dysfunction of the descending inhibitory system [35]. Puta et al. have reported that widespread changes of somatosensory sensitivity were found in chronic low back pain patients. Furthermore, significantly enhanced pain thresholds were found not only at the back, but also at a non-painful hand [36]. While the innervating nerves and anatomical location of chronic pain area of previous studies differ from those involved in masseter muscle pain, increased pain sensitivity may occur in the skin overlying the masseter muscle. The present findings suggest that masseter muscle pain is at least partially related to subjective changes of the mechanical pain sensitivity of the skin overlying the masseter muscle.

Costa et al. investigated the effect of experimental short-lasting muscle pain on the tactile sensitivity of the skin overlying the masseter muscle [16]. Glutamate-evoked jaw muscle pain is well known to simulate aspects of myogenous temporomandibular disorders [37]. Costa et al. found that the MDT on the masseter muscle was significantly lower before glutamate injection than after glutamate injection and concluded that experimental short-lasting muscle pain impair touch perception [18]. That result appears

to conflict with the results from the study 1, potentially due to several factors. However, Svensson et al. previously demonstrated mechanical hyperesthesia to pin prick stimuli following prolonged nociceptive stimulation of the masseter muscle [30]. To further clarify the mechanism of normal physiological masseter muscle pain, studies will need to investigate the effect of different types of masseter pain, e.g., post-exercise muscle soreness or nerve-growth factor-induced sensitization on somatosensory sensitivity.

According to the additional experiment, the MPS on the masseter muscle was significantly lower after lidocaine application than before lidocaine application in both the MPP and non-MPP groups. On the other hand, no difference in MPS on the masseter muscle was seen between MPP and non-MPP group after lidocaine patch application. Wehrfritz et al. reported that lidocaine tape applied to healthy skin on the volar forearm can alter the mechanical pain threshold, mechanical wind-up, and tactile threshold [38]. Okayasu et al. also reported NRS pain intensity of the cheek skin decreased after application of 8% lidocaine spray [39]. In addition, Pillai et al. found that 5% local anesthetic agent containing 2.5% lidocaine and prilocaine application caused significant somatosensory loss in thermal and mechanical parameters CDT, WDT, TSL, CPT, MDT, MPT, MPS, and VDT when compared to baseline in the right infraorbital (V2) region [40]. Inada et al. investigated the efficacy of lidocaine tape for alleviating the pain associated with a stellate ganglion block [23]. They also found that the lidocaine tape reduced visual analog scale evaluations of pain after application for as little as 7 min [23]. The results for MPS from the main and additional experiments suggest subjective change of mechanical pain within the range of effect of the topical lidocaine. Further studies are needed to investigate the subjective change of mechanical pain sensitivity for the skin over the masseter muscle, to elucidate the mechanisms of related pain among patients

with masseter muscle/fascial pain. For PPTs, no differences on the masseter muscle were seen between before and after lidocaine patch application in the MPP and non-MPP groups. However, a significant difference was evident between the MPP and non-MPP groups. Past studies have demonstrated no difference in PPT sensitivity after lidocaine patch application [16,41]. Such results agree well with past results [16,41]. The lack of difference in PPT on the masseter muscle between before and after lidocaine patch application in the MPP and non-MPP groups may indicate that pressure pain sensation in the human masseter muscle was not derived predominantly from cutaneous tissues, but rather from the muscle itself. No difference in DMA on the masseter muscle was evident between before and after lidocaine patch application in the MPP and non-MPP groups. This finding was not unexpected, given that only healthy participants were recruited to this study.

It must be acknowledged that even though the study 1 applied mechanical devices to standardize palpation for participants and allow division into two groups, then the evoked pain was in any case short-lasting (seconds). It may therefore not be an effect of ongoing nociceptive input which alters the MPS in MPP individuals but rather a trait. Not surprisingly, the PPTs were also lower in the MPP but no other of the standardized QST measures indicated any significant difference. It could be of interest to test if participants who report referred pain sensations in response to longer and more intense palpation pressure would display any difference in homotopic and heterotopic (referred pain area) somatosensory sensitivity compared to participant who only report local pain on palpation. Therefore, further study into levels of pressure-evoked pain, including the duration of pain, need to be conducted. In addition, psychological factors were not investigated in the study 1, as only healthy participants were recruited. However, pain

perception is well known to occur with a high frequency of psychological comorbidities and sleep deprivation [41,42]. Future studies will thus be required to standardize other participant conditions.

Study 2: Standardized palpation of the temporalis muscle evoke referred pain and sensations in individuals without TMD

Overall, the study 2 supported the hypothesis that the duration and intensity of palpation of the temporalis muscle influence the frequency of referred pain/sensations.

The study 2 demonstrated that referred pain/ sensations can, indeed, be evoked by standardized palpations in the painful range (2.0 kg of palpation) and in the pain-free range (0.5 kg and 1.0 kg of palpation). These findings are consistent with our previous findings from standardized palpation of the masseter muscle and referred pain/sensations [19]. More specifically, our previous study also showed that the number of participants with referred pain/sensations evoked by 2.0 kg of standardized palpation pressure was higher than by 1.0 kg and 0.5 kg of palpation on the masseter muscle [19]. Exposto et al. reported that referred pain/sensations can be evoked by both painful and nonpainful stimuli, and this was true for stimuli applied to the orofacial region [43]. Moreover, Torebjörk et al. reported a positive correlation between pain intensity and the frequency of reported referred pain [44]. In line with these results, our study showed a positive correlation between the duration of the palpation stimulus and the number of participants with referred pain/sensations and stimulus intensity. The study 2 also suggest that referred pain from the temporalis muscle is an intensity dependent process originating from a local stimulus.

Wang et al. found that after prolonged nociceptive input, these silent synapses

appeared to mature [45]. Furthermore, they suggested silent synapses as potential cellular substrates that are recruited by pain experience to remodel key neural circuits that modulate pain perception and sensitivity [45]. Some studies propose that referred pain/sensation is caused by activation of silent synapses converging in the CNS by persistent intense nociceptive input [6, 46]. The study 2 may indicate that prior diseases activated silent synapses as persistent intense nociceptive input, and palpation stimulus could cause referred pain/sensations. Further studies are needed to clarify the impact of prior diseases and activation of silent synapses.

The study 2 showed that the mean pain NRS scores were in the nonpainful range for 0.5 and 1.0 kg of all duration stimuli and in the painful range for the 2.0 kg of 5 s and 10 s stimulus. These results are in line with our previous study investigating the mechanical sensitivity of the masseter muscle [19]. Previous study also showed a positive correlation between mechanical stimulation forces (5 N, 10 N, and 20 N) and NRS scores in participants for durations of 2 s in the masseter muscle [19]. Our results also showed positive correlations between mean pain/ unpleasantness NRS scores and three different stimulus intensities for each duration of palpation in the temporalis muscle. Our results suggested that when palpating the temporalis muscle, stimulus intensity is tightly linked to the intensity of pain and unpleasantness.

Some studies reported that the measure of entropy may represent the diversity of mechanical sensitivity scores within the spatial distribution [33]. The study 2 showed significant differences in entropy values of pain NRS between 10 s of palpation stimulus and 2 s of palpation stimulus duration compared to palpation stimulus with the 0.5-kg stimulus intensity. Moreover, entropy values of pain NRS scores tended to increase according to the duration of palpation stimulus for the 0.5-kg stimulus intensity, but not

for the 1.0-kg or 2.0kg intensities. Furthermore, a similar pattern was shown for entropy values of unpleasantness NRS scores, with increases found according to the duration of the palpation stimulus at 0.5-kg and 1.0-kg stimulus intensities, but not at 2.0 kg. The present results are in agreement with previous studies [19] and suggest that an extended duration of palpation stimulus is associated with higher entropy values. However, this may not have occurred for the 2.0-kg stimulus intensity because pain NRS scores were already quite high and diverse, and thus, extending the duration of palpation stimulus did not cause further increases in entropy values (diversity).

Ⅵ. Conclusion

In study 1, our present results suggested that brief, acute pain in the masseter muscle is linked to increased MPS which can be reversed by transient deafferentation of the superficial nociceptive input. Chronic pain in the masseter muscle could therefore influence homotopic sensitivity. In study 2, our present results suggested that referred pain/sensations in the orofacial region can be evoked by both painful and non-painful standardized palpation of the temporalis muscle, and the frequency of these responses is influenced by the palpation duration in individuals without TMD. Furthermore, these findings show that referred pain/ sensations from the temporalis muscle are duration- and intensity-dependent processes originating from local stimuli. Clinicians should be aware of the epiphenomenon of referred pain/sensations triggered by standardized palpation of the cranial muscles.

Further studies are needed to reveal actual processes of the mechanisms of related pain among patients with masseter muscle/fascial pain such as increased MPS. However, these findings may have implications for proposing the mechanisms of referred

pain in the orofacial area and, the relation between related pain among patients with masseter muscle/fascial pain and referred pain.

Ⅶ. References

1. Mense S (1993) Nociception from skeletal muscle in relation to clinical muscle pain. Pain 54:241–289. https://doi.org/10.1016/0304-3959(93)9002

2. Svensson P (2000) Clinical and experimental aspects of temporomandibular disorders. Curr Rev Pain 4: 158-165.

3. Craig AD (2003) Pain mechanisms: Labeled lines versus convergence in central processing. Annu Rev Neurosci 26:1–30.

4. Perl ER (2011) Pain mechanisms: A commentary on concepts and issues. Prog Neurobiol 94:20–38. https://doi.org/10.1016/j.pneurobio.2011.03.001

5. Hombrebueno JR, Chen M, Penalva RG, Xu H (2014) Loss of synaptic connectivity, particularly in second order neurons is a key feature of diabetic retinal neuropathy in the Ins2Akita mouse. PLoS One 9. https://doi.org/10.1371/journal.pone.0097970

6. Sessle BJ, Hu JW, Amano N, Zhong G (1986) Convergence of cutaneous, tooth pulp, visceral, neck and muscle afferents onto nociceptive and non-nociceptive neurones in trigeminal subnucleus caudalis (medullary dorsal horn) and its implications for referred pain. Pain 27:219–235. https://doi.org/10.1016/0304-3959(86)90213-7

7. Dworking SF, LeResche L (1992) Research diagnostic criteria for temporomandibular disorders : review , criteria , examinations and specifications , critique. J Craniomandib Disord Facial Oral Pain 6:301–55.

8. Schiffman E, Ohrbach R, Truelove E, et al (2014) Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) for Clinical and Research Applications: Recommendations of the International RDC/TMD Consortium Network* and Orofacial Pain Special Interest Group†. Polym Bull 28:6–27. https://doi.org/10.1007/BF00338494

9. Orofacial T, Classification P (2020) International Classification of Orofacial Pain, 1st

edition (ICOP). Cephalalgia 40:129–221. https://doi.org/10.1177/0333102419893823

10. Andersen S, Petersen MW, Svendsen AS, Gazerani P (2015) Pressure pain thresholds assessed over temporalis, masseter, and frontalis muscles in healthy individuals, patients with tension-type headache, and those with migraine-a systematic review. Pain 156:1409–1423. https://doi.org/10.1097/j.pain.0000000000000219

11. Svensson P, Wang K, Arendt-Nielsen L, et al (2005) Pain effects of glutamate injections into human jaw or neck muscles. J Orofac Pain 19:109–118.

12. Arendt-Nielsen L, Svensson P, Sessle BJ, et al (2008) Interactions between glutamate and capsaicin in inducing muscle pain and sensitization in humans. Eur J Pain 12:661–670. https://doi.org/10.1016/j.ejpain.2007.10.013

13. Cairns BE, Gambarota G, Svensson P, et al (2002) Glutamate-induced sensitization of rat masseter muscle fibers. Neuroscience 109:389–399.

14. Cairns BE, Hu JW, Arendt-Nielsen L, et al (2001) Sex-related differences in human pain and rat afferent discharge evoked by injection of glutamate into the masseter muscle. J Neurophysiol 86:782–791. https://doi.org/10.1152/jn.2001.86.2.782

15. Svensson P, Cairns BE, Wang K, et al (2003) Glutamate-evoked pain and mechanical allodynia in the human masseter muscle. Pain 101:221–227. https://doi.org/10.1016/S0304-3959(02)00079-9

16. Costa Y, Castrillon E, Bonjardim L, et al (2017) Effects of Experimental Pain and Lidocaine on Mechanical Somatosensory Profile and Face Perception. J Oral Facial Pain Headache 31:115–123. https://doi.org/10.11607/ofph.1758

17. Rolke R, Baron R, Maier C, et al (2006) Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): Standardized protocol and reference values. Pain 123:231–243. https://doi.org/10.1016/j.pain.2006.01.041

18. Pigg M, Baad-Hansen L, Svensson P, et al (2010) Reliability of intraoral quantitative sensory testing (QST). Pain 148:220–226. https://doi.org/10.1016/j.pain.2009.10.024

19. Masuda M, Iida T, Exposto F et al (2018) Referred pain and sensations evoked by standardized palpation of the masseter muscle in healthy participants. J Oral Facial Pain Headache 32:159–166. https://doi.org/10.11607/ofph

20. Faul F, Erdfelder E, Lang A-G, Buchner A (2007) G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 39:175–191.

21. Dagsdóttir LK, Skyt I, Vase L, et al (2015) Experimental orofacial pain and sensory deprivation lead to perceptual distortion of the face in healthy volunteers. Exp Brain Res 233:2597–2606. https://doi.org/10.1007/s00221-015-4330-9

22. Futarmal S, Kothari M, Ayesh E, et al (2011) New palpometer with implications for assessment of deep pain sensitivity. J Dent Res 90:918–922. https://doi.org/10.1177/0022034511402997

23. Inada T, Uesugi F, Kawachi S, Inada K (1997) Lidocaine tape relieves pain due to needle insertion during stellate ganglion block. Can J Anaesth 44:259–262.

24. Rolke R, Baron R, Maier C, et al (2006) Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): Standardized protocol and reference values. Pain 123:231–243. https://doi.org/10.1016/j.pain.2006.01.041

25. Baad-Hansen L, Pigg M, El'Masry Ivanovic S, et al (2013) Intraoral somatosensory abnormalities in patients with atypical odontalgia – a controlled multicenter quantitative sensory testing study. Pain 154:1287–1294.

26. Svensson P, Baad-Hansen L, Pigg M, et al (2011) Guidelines and recommendations for assessment of somatosensory function in oro-facial pain conditions - a taskforce

report. J Oral Rehabil 38:366–394. https://doi.org/10.1111/j.1365-2842.2010.02196

27. Lu S, Baad-Hansen L, List T, et al (2013) Somatosensory profiling of intra-oral capsaicin and menthol in healthy subjects. Eur J Oral Sci 121:29–35. https://doi.org/10.1111/eos.12014

28. Kroenke K, Spitzer RL, Williams JBW, Löwe B (2009) An ultra-brief screening scale for anxiety and depression: The PHQ–4. Psychosomatics 50:613–621. https:// doi. org/ 10. 1016/ s00333182(09)70864-3

29. Kothari SF, Kothari M, Zambra RF et al (2014) Standardization of muscle palpation - methodological considerations. Clin J Pain 30:174–182.

30. Svensson P, Graven-Nielsen T, Arendt-Nielsen L (1998) Mechanical hyperesthesia of human facial skin induced by tonic painful stimulation of jaw muscles. Pain 74:93–100. https://doi.org/10. 1016/S0304-3959(97)00156-5

31. Zagury JG, Eliav E, Heir GM, et al (2011) Prolonged gingival cold allodynia: A novel finding in patients with atypical odontalgia. Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology 111:312–319. https://doi.org/10.1016/j.tripleo.2010.10.008

32. Boudreau SA, Badsberg S, Christensen SW, Egsgaard LL (2016) Digital pain drawings: assessing touch-screen technology and 3D body schemas. Clin J Pain 32:139–145. https://doi.org/10.1097/ AJP.0000000000000230

33. Castrillon E, Exposto F, Sato H et al (2017) Entropy of masseter muscle pain sensitivity: a new technique for pain assessment. J Oral Facial Pain Headache 31:87–94. https://doi.org/10.11607/ ofph.1756

34. Meints SM, Mawla I, Napadow V, et al (2019) The relationship between catastrophizing and altered pain sensitivity in patients with chronic low back pain. Pain 160:833–843. https://doi.org/10.1016/j.physbeh.2017.03.040

35. Kosek E, Ekholm J, Hansson P (1996) Modulation of pressure pain thresholds during and following isometric contraction in patients with fibromyalgia and in healthy controls. Pain 64:415–423. https://doi.org/10.1016/0304-3959(95)00112-3

36. Puta C, Schulz B, Schoeler S, et al (2013) Somatosensory Abnormalities for Painful and Innocuous Stimuli at the Back and at a Site Distinct from the Region of Pain in Chronic Back Pain Patients. PLoS One 8. https://doi.org/10.1371/journal.pone.0058885

37. Castrillon EE, Cairns BE, Ernberg M, et al (2008) Glutamate-evoked jaw muscle pain as a model of persistent myofascial TMD pain? Arch Oral Biol 53:666–676. https://doi.org/10.1016/j.archoralbio.2008.01.008

38. Krumova EK, Zeller M, Westermann A, Maier C (2012) Lidocaine patch (5%) produces a selective, but incomplete block of Aδ and C fibers. Pain 153:273–280. https://doi.org/10.1016/j.pain.2011.08.020

39. Okayasu I, Komiyama O, Ayuse T, et al (2018) Effect of 8% lidocaine spray on the sensory and pain thresholds of the skin of the face and hands evaluated by quantitative sensory testing. J Dent Anesth Pain Med 2018;18(6):361-365

40. Pillai R, Pigg M, List T, et al (2020) Assessment of Somatosensory Function, Pain, and Unpleasantness in Two Surrogate Models of Trigeminal Nerve Damage: A Randomized, Double-Blind, Controlled Crossover Study. J Oral Facial Pain Headache 34:92–107. https://doi.org/10.11607/ofph.2423

41. Fujisawa M, Shoji S, Ishibashi K, Clark GT (1999) Pressure pain threshold with and without iontophoretic anesthesia of the masseter muscle in asymptomatic males. J Oral Facial Pain Headache 13:97–103

42. Lautenbacher S, Kundermann B, Krieg JC (2006) Sleep deprivation and pain perception. Sleep Med Rev 10:357–369. https://doi.org/10.1016/j.smrv.2005.08.001

43. Exposto FG, Udagawa G, Naganawa T, Svensson P (2018) Comparison of masseter muscle referred sensations after mechanical and glutamate stimulation: a randomized, double-blind, controlled, cross-over study. Pain 159:2649–2657. https://doi.org/10.1097/j.pain.0000000000001379

44. Torebjörk HE, Ochoa JL, Schady W (1984) Referred pain from intraneural stimulation of muscle fascicles in the median nerve. Pain 18:145–156. https://doi.org/10.1016/0304-3959(84)90882-0

45. Wang YQ, Wang J, Xia S et al (2020) Neuropathic pain generates silent synapses in thalamic projection to anterior cingulate cortex. Pain Publish Ah. https://doi.org/10.1097/j.pain.0000000000 002149

46. Hoheisel U, Mense S, Simons DG, Yu XM (1993) Appearance of new receptive fields in rat dorsal horn neurons following noxious stimulation of skeletal muscle: a model for referral of muscle pain? Neurosci Lett 153:9–12.

Ⅷ. Tables and Figures

Table 1. Comparison of Quantitative Sensory Testing (QST) results between Masseter Muscle Pain Prone (MPP) group and Non-Masseter Muscle Pain Prone (non-MPP) group for masseter muscle.

Applications	CDT (℃)	WDT (℃)	TSL (℃)	PHS (/3)	CPT (℃)	HPT (℃)	MDT (mN)	MPT (mN)	MPS (NRS)	DMA (NRS)	WUR (ratio)	VDT (/8)	PPT (kPa)
<MPP>	27.3	38.5	11.1	0.0	11.8	44.0	0.1	76.1	1.0	0.0	3.9	7.6	130.6
SD	(2.2)	(2.4)	(4.2)	(0.0)	(7.2)	(1.8)	(0.0)	(29.9)	(0.4)	(0.0)	(3.1)	(0.2)	(27.6)
<non-MPP>	25.5	39.5	13.0	0.0	12.3	44.1	0.1	75.5	0.7	0.0	3.7	7.7	186.3
SD	(3.3)	(4.8)	(4.4)	(0.0)	(7.4)	(2.2)	(0.1)	(27.7)	(0.3)	(0.0)	(3.2)	(0.1)	(23.6)
P value	0.06	0.67	0.12	-	0.81	0.93	0.12	0.96	0.04*	-	0.51	0.24	0.01*

All data are presented as mean and standard deviations of the mean. CDT= cold detection threshold (°C); WDT= warm detection threshold (°C); TSL= thermal sensory limen (°C); PHS= paradoxical heat sensation (score/3); CPT= cold pain threshold (°C); HPT= heat pain threshold (°C); MPT= mechanical pain threshold (mN); MPS= mechanical pain sensitivity (mean pain rating, 0–100); DMA= dynamic mechanical allodynia (NRS); WUR= wind-up ratio; MDT= mechanical detection threshold (mN); VDT= vibration detection threshold (score/8); PPT= pressure pain threshold (kPa). (*: $P < 0.05$, T-test).

Table 2. Comparison of Quantitative Sensory Testing (QST) Results Between Masseter Muscle Pain Prone (MPP) group and Non-Masseter Muscle Pain Prone (non-MPP) group for First Dorsal Interosseous (FDI) muscle.

Applications	CDT (℃)	WDT (℃)	TSL (℃)	PHS (/3)	CPT (℃)	HPT (℃)	MDT (mN)	MPT (mN)	MPS (NRS)	DMA (NRS)	WUR (ratio)	VDT (/8)	PPT (kPa)
<MPP>	26.0	36.6	7.4	0.0	14.1	43.1	0.2	128.1	0.6	0.0	4.1	7.6	240.4
SD	(4.2)	(1.9)	(3.0)	(0.0)	(5.9)	(1.9)	(0.6)	(76.8)	(0.2)	(0.0)	(3.2)	(0.1)	(55.0)
<non-MPP>	25.4	34.6	8.2	0.0	13.6	42.6	0.1	117.9	0.5	0.0	3.0	7.7	256.3
SD	(4.3)	(4.7)	(3.5)	(0.0)	(6.9)	(1.9)	(0.1)	(38.3)	(0.2)	(0.0)	(1.9)	(0.1)	(42.5)
P value	0.67	0.09	0.44	-	0.39	0.88	0.89	0.14	0.18	-	0.24	0.21	0.24

All data are presented as mean and standard deviations of the mean. CDT= cold detection threshold (°C); WDT= warm detection threshold (°C); TSL= thermal sensory limen (°C); PHS= paradoxical heat sensation (score/3); CPT= cold pain threshold (°C); HPT= heat pain threshold (°C); MPT= mechanical pain threshold (mN); MPS= mechanical pain sensitivity (mean pain rating, 0–100); DMA= dynamic mechanical allodynia (NRS); WUR= wind-up ratio; MDT= mechanical detection threshold (mN); VDT= vibration detection threshold (score/8); PPT= pressure pain threshold (kPa).

Table 3. Comparison of Mechanical Pain Sensitivity (MPS), Dynamic Mechanical Allodynia (DMA), and Pressure Pain Threshold (PPT) on masseter muscle between before and after Lidocaine Application in the Masseter Muscle Pain Prone (MPP) and Non-Masseter Muscle Pain Prone (non-MPP) groups.

			BEFORE APPLICATION		AFTER APPLICATION	
		Applications	Mean	SD	Mean	SD
MPP	MPS	(NRS)	1.2	(0.4)	0.3	(0.4)
	DMA	(NRS)	0.0	(0.0)	0.0	(0.0)
	PPT	(kPa)	131.7	(29.6)	116.4	(25.8)
NON-MPP	MPS	(NRS)	0.7	(0.2)	0.3	(0.9)
	DMA	(NRS)	0.0	(0.0)	0.0	(0.0)
	PPT	(kPa)	182.6	(29.0)	184.7	(42.4)

All data are presented as mean and standard deviations of the mean. MPS= mechanical pain sensitivity (mean pain rating, 0–100); DMA= dynamic mechanical allodynia (NRS); PPT= pressure pain threshold (kPa).

Table 4. Statistical relationship for factors related to NRS scores and aftersensation times.

	Duration	Intensity	Test site	Duration x Test site	Duration x Intensity	Intensity x Test site
Pain NRS	P <0.001	P <0.001	P <0.001	0.999	P <0.001	0.958
Unpleasantness NRS	P <0.001	P <0.001	0.477	1	P <0.001	1
Aftersensation time	P <0.001	P <0.001	0.649	1	0.053	0.994

The p-values from ANOVAs testning differences in means of pain NRS scores and unpleasantness NRS scores and aftersensation times for three mechanical stimulus intensities with the following factors: duration of palpation stimulus (three levels), stimulus intensity (three levels), and test site (15 levels).

Table 5. The area of referred pain/sensations in each stimulus intensity.

0.5 kg	2 s	-	-	-
	5 s	masseter	1	3.1 % (n =1/32)
	10 s	masseter	1	3.1 % (n =1/32)
1.0 kg	2 s	masseter	1	`3.1 % (n =1/32)
	5 s	masseter	1	3.1 % (n =1/32)
	10 s	masseter	2	6.3 % (n =2/32)
		posterior teeth	1	3.1 % (n =1/32)
2.0 kg	2 s	masseter	2	6.3 % (n = 2/32)
		temple	1	3.1 % (n =1/32)
		posterior teeth	1	3.1 % (n =1/32)
	5 s	masseter	2	6.3 % (n = 2/32)
		anterior teeth	2	6.3 % (n = 2/32)
	10 s	posterior teeth	5	15.6 % (n = 5/32)
		masseter	2	6.3 % (n = 2/32)
		ear	2	6.3 % (n = 2/32)
		cervical	1	3.1 % (n =1/32)
		occipital	1	3.1 % (n =1/32)

The most common area of referred pain/sensations was the masseter region (3.1%; n = 1/32) for 5 s and 10 s when using 0.5 kg. The most common area of referred pain/sensations were the masseter region (3.1%; n = 1/32) for 2 s and 5 s, and the masseter region (6.3%; n = 2/32) for 10 s when using 1.0 kg. The most common areas of referred pain/sensations were the masseter region (6.3%; n = 2/32) for 2 s, the masseter region and anterior teeth (6.3%; n = 2/32) for 5 s, and the posterior teeth (15.6%; n = 5/32) for 10 s when using 2.0 kg.

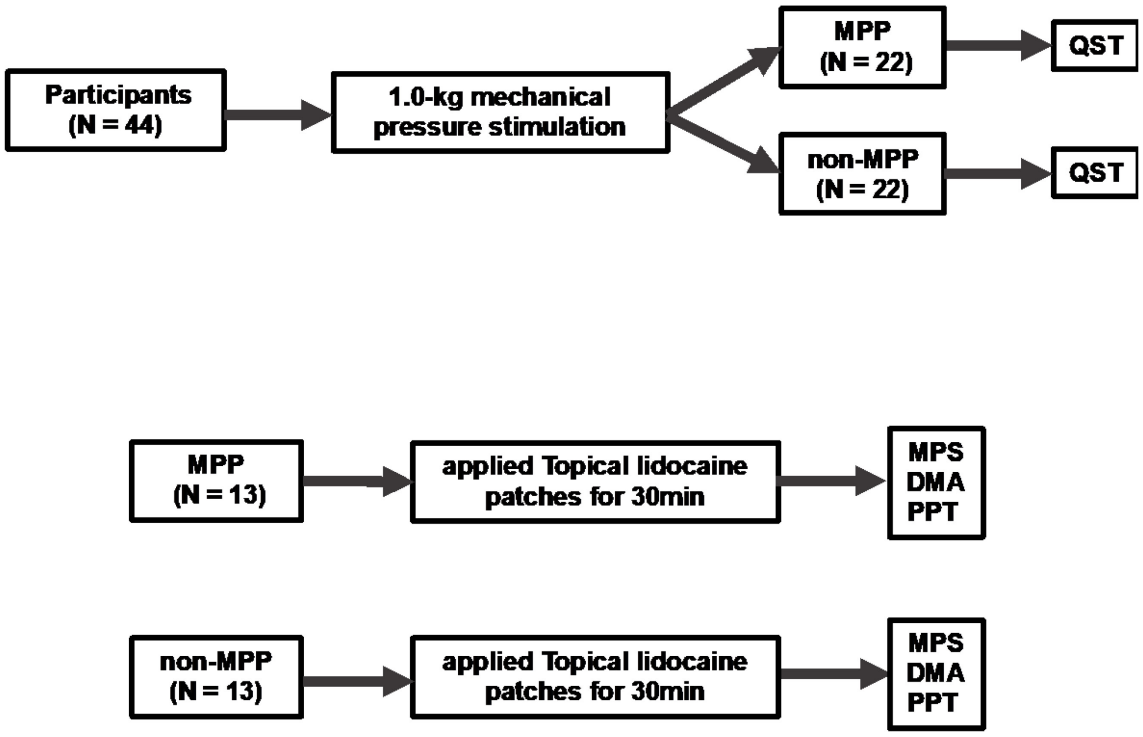
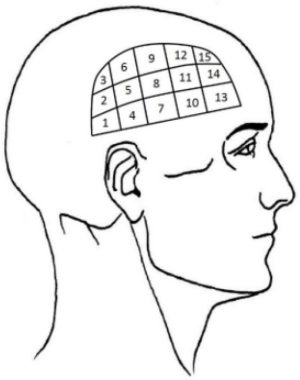
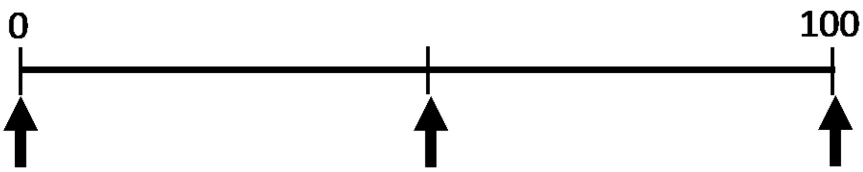


Figure 1. Flowchart of the main experiment procedure and the additional experiment procedure.

a



b

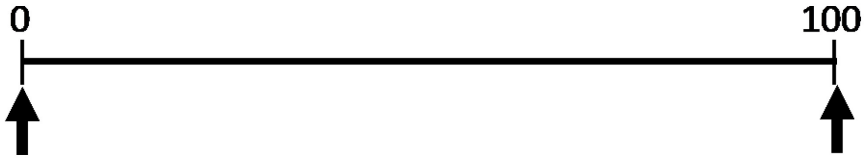


"0" no sensation at all

"50"just barely pain sensation

"100" the most imaginable pain sensation

c



"0" no unpleasantness at all

"100" the most imaginable unpleasantness sensation

d

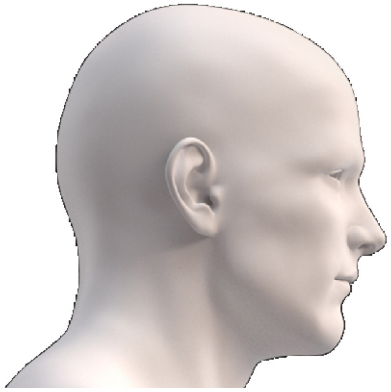


Figure 2. The design of 15 test sites on the temporalis muscles, the numerical rating scale (NRS), and a digital anatomical drawing of referred pain/sensations. The anterior–posterior and inferior-superior borders of the temporalis muscles were identified, and the areas were divided into 15 test sites (five vertical and three horizontal) (a). Pain intensity was scored on a 0–50–100 NRS with 0 denoting “no sensation at all,” 50 as “just barely painful,” and 100 as “the worst pain imaginable” (b). Unpleasantness intensity was scored on a 0–100 NRS with 0 denoting “no unpleasantness at all” and 100 as “the most unpleasantness imaginable” (c). The participants were asked to indicate the area of referred pain/sensation on a digital anatomical drawing (d).

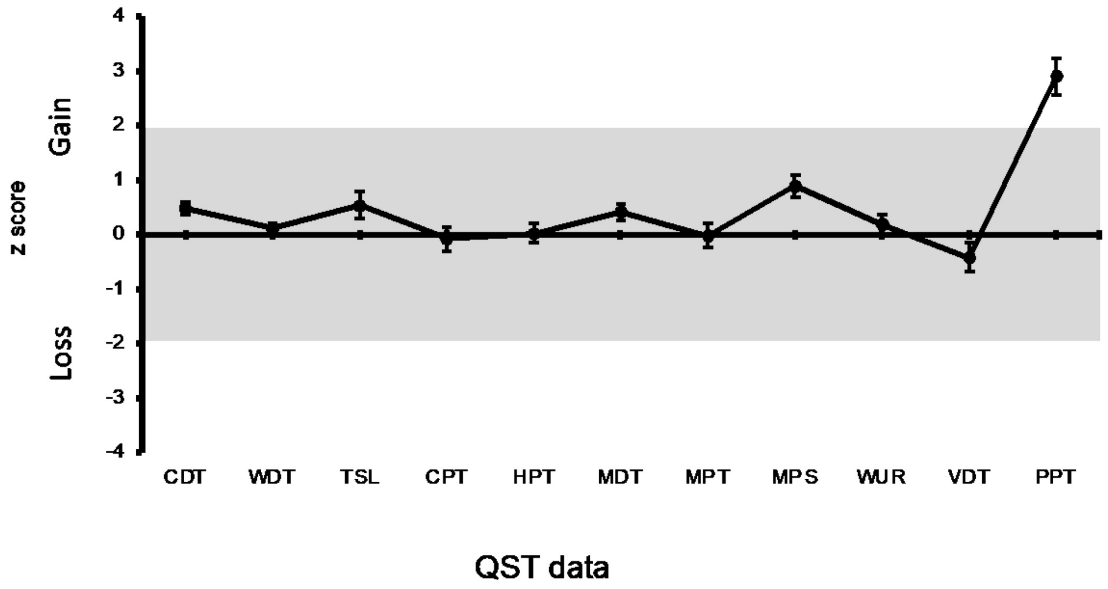
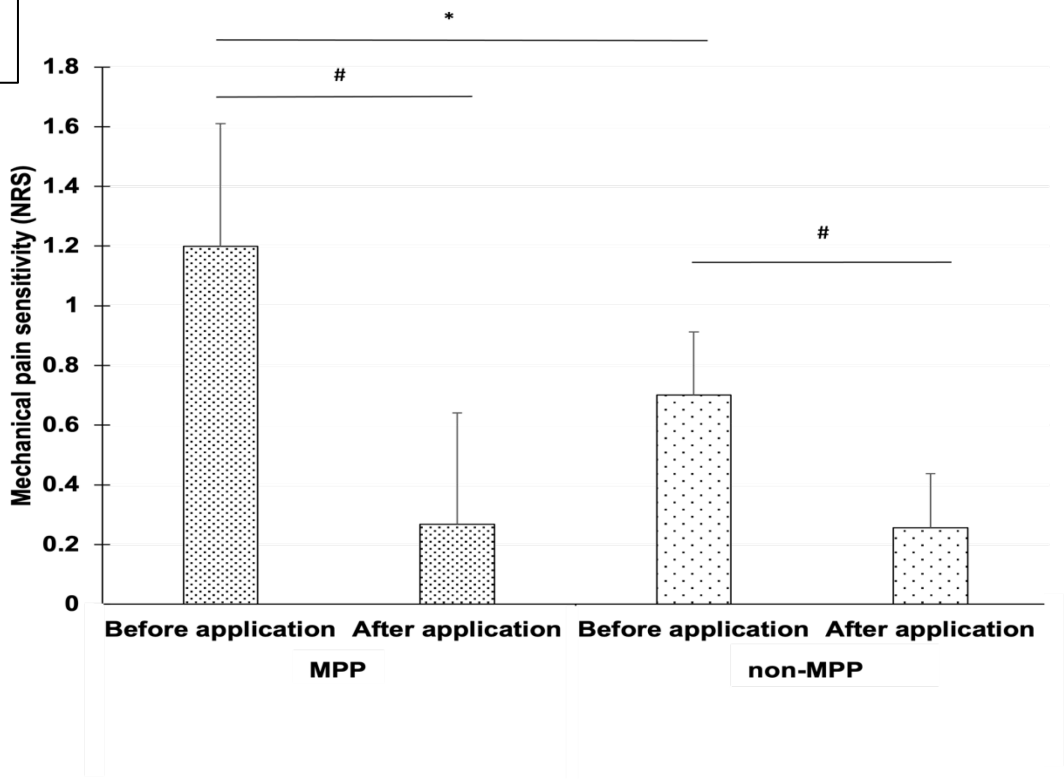


Figure 3. Z-scores on the Masseter for the MPP group. Error bars indicate the standard deviation of the mean.

CDT = cold detection threshold; WDT = warm detection threshold; TSL = thermal sensory limen; CPT = cold pain threshold; HPT = heat pain threshold; MPT = mechanical pain threshold; MPS = mechanical pain sensitivity; DMA = dynamic mechanical allodynia; WUR = wind-up ratio; MDT = mechanical detection threshold; PPT = pressure pain threshold. The gray zone (z score between − 1.96 and 1.96) represents the 95% confidence interval of baseline values.

a


*
#
#
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Mechanical pain sensitivity (NRS)
Before application
After application
Before application
After application
MPP
non-MPP

b

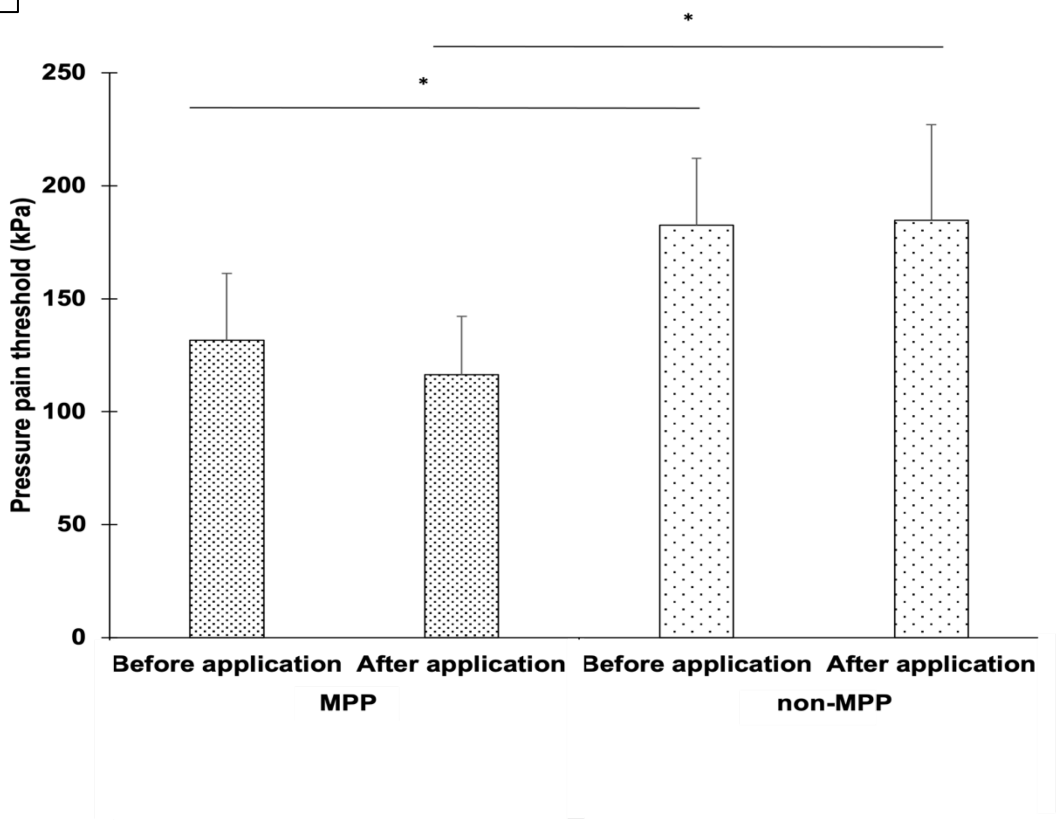
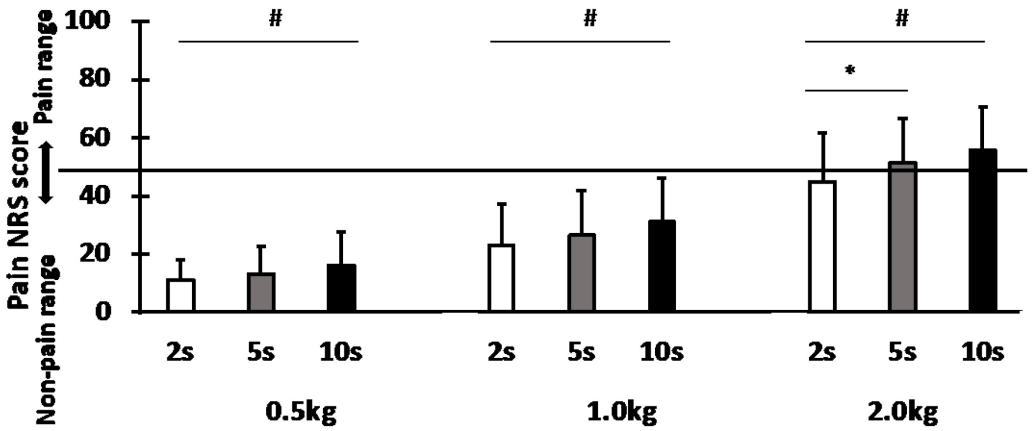

*
*
250
200
150
100
50
0
Pressure pain threshold (kPa)
Before application
After application
Before application
After application
MPP
non-MPP

Figure 4. Comparison of MPS on Masseter muscle between before lidocaine application than after lidocaine application in the MPP and non-MPP groups (a), comparison of PPT on Masseter muscle between before lidocaine application than after lidocaine application in the MPP and non-MPP groups (b).

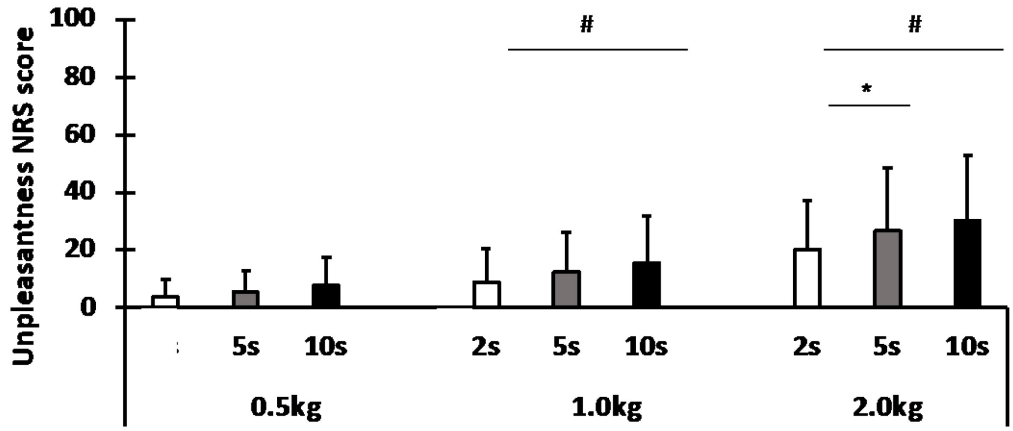
MPS on the masseter muscle was significantly higher in the MPP group than in the non-MPP group (* $P < 0.05$, Tukey post hoc test). In both the MPP and non-MPP groups, the MPS on the masseter muscle was significantly higher before lidocaine application than after lidocaine application (# $P < 0.05$, Tukey post hoc test) (a).

PPT was significantly lower in the MPP group than in the non-MPP group (* $P < 0.05$, Tukey post hoc test) (b).

a


Pain NRS score
Pain range
Non-pain range
100
80
60
40
20
0
#
#
#
*
2s
5s
10s
2s
5s
10s
2s
5s
10s
0.5kg
1.0kg
2.0kg

b


Unpleasantness NRS score
100
80
60
40
20
0
#
#
*
5s
10s
2s
5s
10s
2s
5s
10s
0.5kg
1.0kg
2.0kg

c

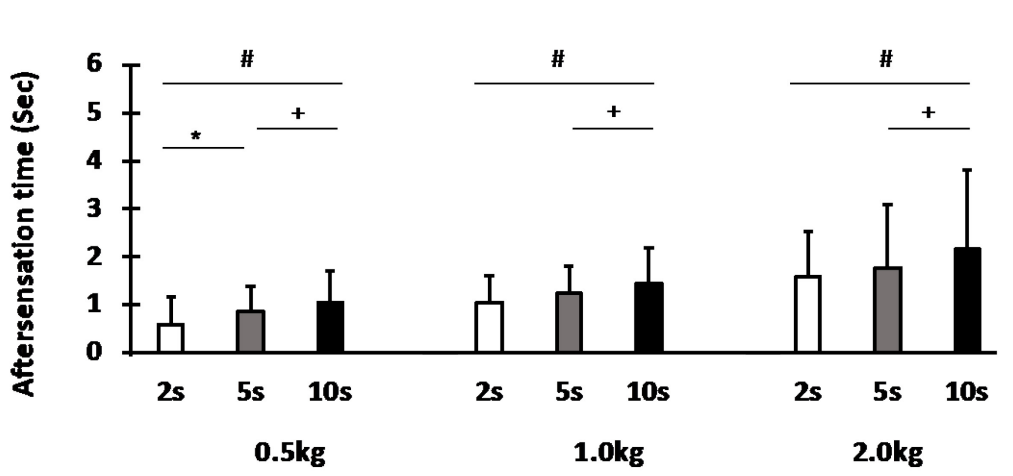

Aftersensation time (Sec)
6
5
4
3
2
1
0
#
#
#
*
+
+
+
2s
5s
10s
2s
5s
10s
2s
5s
10s
0.5kg
1.0kg
2.0kg

Figure 5. Comparison of pain NRS score (a), comparison of unpleasantness NRS score (b), and comparison of aftersensation time (c) for the duration of palpation stimulus at each stimulus intensity.

Pain NRS scores for 10 s of duration of palpation stimulus were significantly higher than for 5 s of duration of palpation stimulus when using each stimulus intensity, and scores for 5 s of duration of palpation stimulus were significantly higher than for 2 s of duration of palpation stimulus when using the 2.0 kg stimulus intensity (# * P < .005, Tukey post hoc test) (a). Unpleasantness NRS scores for 10 s of duration of palpation stimulus were significantly higher than for 2 s of duration of palpation stimulus when using the 1.0-kg and 2.0-kg stimulus intensities, and NRS scores for 5 s of duration of palpation stimulus were significantly higher than for 2 s of duration of palpation stimulus when using the 2.0-kg stimulus intensity (# * P < .005, Tukey post hoc test) (b). Aftersensation times for 10 s of duration of palpation stimulus were significantly higher than for 2 and 5 s of duration of palpation stimulus when using each stimulus intensity, and scores for 5 s of duration of palpation stimulus were significantly higher than for 2 s of duration of palpation stimulus when using the 0.5-kg stimulus intensity (# + * P < .005, Tukey post hoc test) (c).

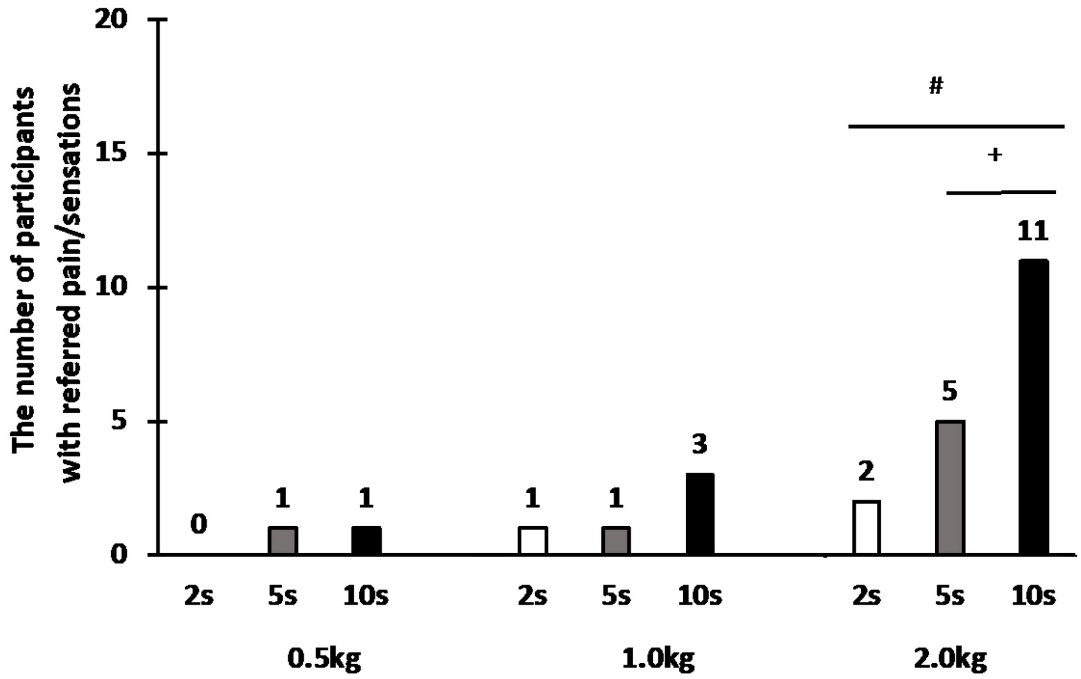


Figure 6. Comparison of the number of participants with referred pain/sensations for duration of palpation stimulus of each stimulus intensity.

The number of participants with referred pain/sensations elicited by 10 s of duration of palpation was significantly higher than by 2 s and 5 s of duration of palpation when using the 2.0-kg stimulus intensity (# + $P < .05$, McNemar's test)

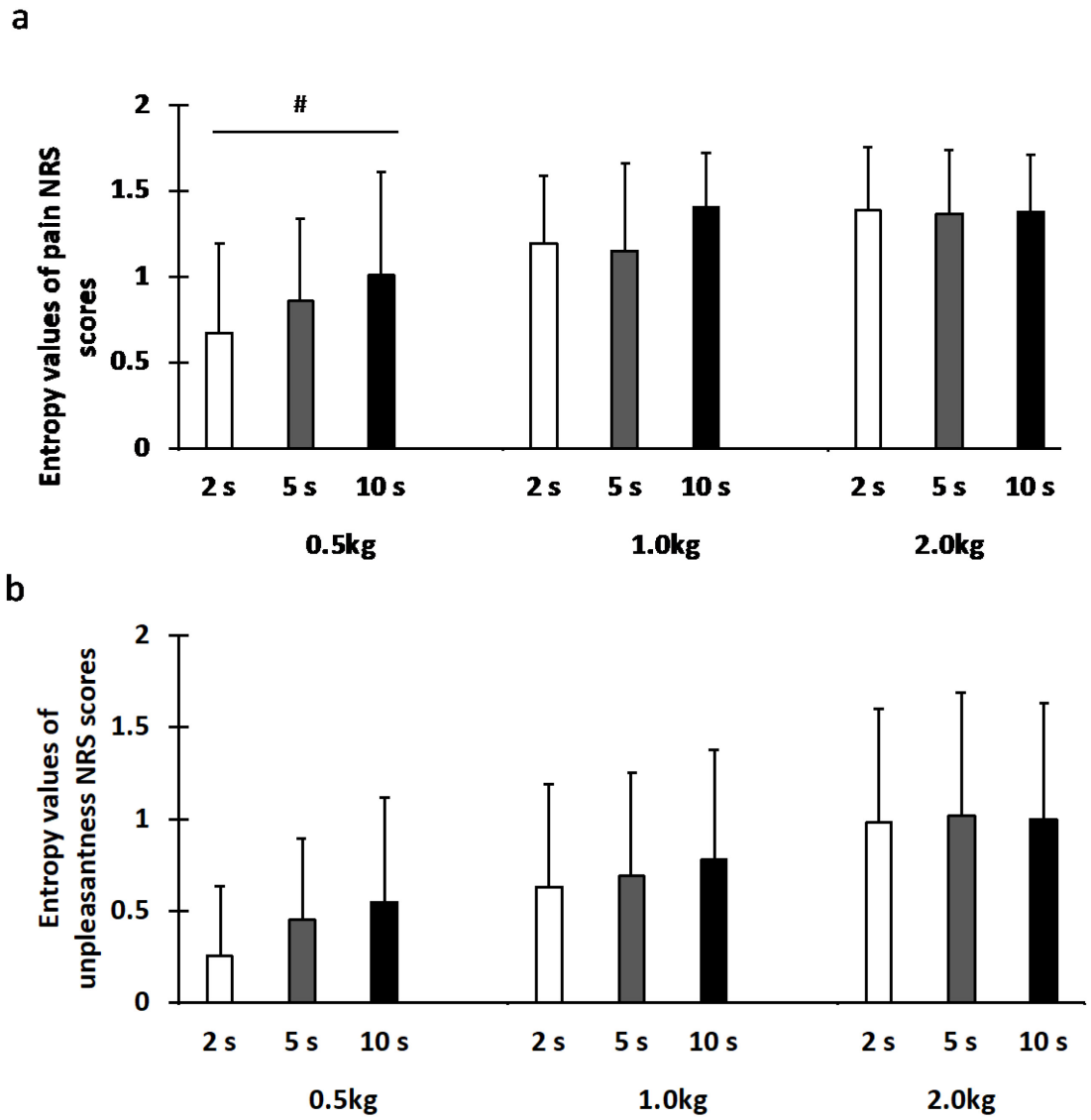


Figure 7. Comparison of entropy values of pain NRS scores (a) and comparison of entropy values of unpleasantness NRS scores (b) for duration of palpation stimulus of each stimulus intensity.

Entropy values of pain NRS scores elicited with 10 s of duration of palpation stimulus were significantly higher than those with 2 s of duration of palpation stimulus when using 0.5-kg stimulus intensities (# $P < .05$, Tukey post hoc test) (a)