Influence of different conditions of palpation on masseter muscle for incidence of referred pain and sensations in healthy individuals

（健常者咬筋における触診条件の相違が関連痛または関連感覚の発現に及ぼす影響）

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Ⅰ. Abstract

[Objective]

This study examined referred pain in the orofacial region during palpation of the masseter muscle. The aims of the study were twofold: to investigate the influence of differences in palpation site and stimulus intensity on incidence of referred pain and sensations in healthy individuals; and to investigate the influence of differences in duration of palpation on incidence of referred pain and sensations in healthy individuals.

[Materials and methods]

Research 1: Participants comprised 32 pain-free individuals (mean age, 28.9 ± 10.5 years). The right masseter muscle was equally divided into 15 test sites. Muscle mechanical sensitivity was assessed with three mechanical stimuli (0.5 kgf, 1.0 kgf or 2.0 kgf) applied to each of the 15 test sites for 5 s using palpometers. Participants scored the perceived pain and intensity of unpleasantness of the three mechanical stimuli on a numerical rating scale (NRS). After each stimulus, participants were asked to indicate areas within the orofacial region in which referred pain/sensations were perceived.

Research 2: Participants comprised 32 pain-free individuals (mean age, 25.7 ± 5.3 years). The right masseter muscle was equally divided into 15 test sites. Muscle mechanical sensitivity was assessed with three mechanical stimuli (0.5 kgf, 1.0 kgf, or 2.0 kgf) applied to each of the 15 test sites with three different durations (2 s, 5 s, or 10 s) using palpometers. Participants scored the intensity of perceived pain and unpleasantness for the three mechanical stimuli on a NRS after each stimulus. Furthermore, if the participant reported referred pain/sensations after a stimulus, they were asked to indicate areas of referred pain/sensations on a digital drawing.

[Results]

Research 1: Pain and unpleasantness NRS scores using the 2.0-kgf stimulus were significantly higher than using the 0.5- and 1.0-kgf stimuli ($P < 0.05$) and the 2.0-kgf stimulus intensity evoked pain on the masseter muscle in 66.7% of participants (24/32). Furthermore, pain and unpleasantness NRS scores using the 1.0-kgf stimulus were significantly higher than using the 0.5-kgf stimulus ($P < 0.05$), but the 1.0-kgf stimulus did not evoke pain on the masseter muscle in healthy participants. Referred pain/sensations were more frequently evoked with the 2.0-kgf stimulus (11/32) than with the 1.0- or 0.5-kgf stimuli (4/32 and 1/32, respectively; $P < 0.05$ each). The number of participants with referred pain/sensations evoked by each test site showed no significant differences between test sites ($P > 0.05$).

Research 2: Pain NRS scores for a 10-s palpation stimulus were significantly higher than for 2- or 5-s palpation stimuli at all stimulus intensities ($P < 0.05$ each). Unpleasantness NRS scores for a 10-s palpation stimulus were significantly higher than for 2-s palpation stimulus when using 1.0- and 2.0-kgf stimulus intensities ($P < 0.05$ each). The 2.0-kgf stimulus evoked pain on the masseter muscle with 2-s palpation (62.5%; 20/32), 5-s palpation (78.1%; 25/32) and 10-s palpation (81.3%; 26/32). Referred pain/sensations were evoked by 2.0 kgf with 2-s palpation (2/32), 5-s palpation (6/32) and 10-s palpation (10/32). Referred pain/sensations were evoked by 1.0 kgf with 5-s palpation (2/32) and 10-s palpation (6/32). Referred pain/sensations were evoked by 0.5 kgf with 5-s palpation (1/32) and 10-s palpation (2/32). The incidence of referred pain/sensations was significantly higher with 10-s palpation than with 2-s palpation for 1.0- and 2.0-kgf stimuli ($P < 0.05$ each). The number of participants with referred pain/sensations evoked by each test site showed no significant differences between test

sites ($P > 0.05$ each).

[Conclusion]

These results indicate that referred pain/sensations in the orofacial region are evoked by standardized palpation of the masseter muscle among healthy individuals. Furthermore, these findings showed that referred pain/sensations from the masseter muscle were time- and intensity-dependent processes originating from local stimuli. Because the 2.0-kgf stimulus intensity on the masseter muscle would be sufficient to elicit pain in healthy participants, the 1.0-kgf stimulus intensity appears suitable for distinguishing between patients and healthy individuals during clinical palpation of masseter muscles in The Diagnostic Criteria for Temporomandibular Disorders (DC/TMD).

Ⅱ. Introduction

The face and mouth represent sites of some of the most common pains in the body (1). The aspect of pain become very diverse and local pain due to biomedical conditions (e.g. inflammation) can be referred to regions remote from originating regions. Pain located to the source of pain is termed local pain, whereas pain felt in a different region or structure away from the source of pain is termed referred pain (2). If the source of the pain is not identified, the clinician may make a wrong diagnosis and provide inappropriate treatment. In clinical practice, temporomandibular disorders (TMD) including myofascial pain in the masticatory muscle can be referred to teeth or other orofacial region. Therefore, it is important to examine referred pain for diagnosing pain disorders in orofacial region. Although several theories of referred pain have been proposed to explain this phenomenon (3-5), the precise neural pathways of referred pain from the masticatory muscles are unknown.

The Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) provide a comprehensive assessment of the most common TMD conditions, based on the biopsychosocial model of chronic pain (6). According to the DC/TMD procedure, the examiner palpates each masseter and temporal muscle by increasing the stimulus intensity to 1.0 kgf and holding the pressure for a specified time. During palpation of the masseter and temporal muscle, durations of either 2 s (for diagnosis of myalgia) or 5 s (for diagnosis of referred pain) are recommended. However, clear evidence is lacking regarding the optimal stimulus intensity and duration of palpation for examining referred pain in the orofacial area. To clarify the relationship between mechanical sensitivity and

referred pain in the orofacial area, identifying duration- or intensity-dependent relationships with local pain in the masticatory muscles is essential.

Indeed, the establishment of optimal stimulus intensities and durations for standardized palpation of the (masseter) muscle to cause referred pain may be useful for diagnosing myofascial pain in the masseter muscle. Understanding the mechanical sensitivity of the masseter muscle may also help in a better understanding of the mechanisms of referred pain related to the masseter muscle.

The aims of the present study were to establish appropriate palpation for masseter muscle due to examine referred pain in orofacial region. The present study were consisted twofold: first, to investigate the influence of differences of palpation site and stimulus intensity for incidence of referred pain and sensations in healthy individuals, and second to investigate the influence of differences of duration of palpation for incidence of referred pain and sensations in healthy individuals.

Ⅲ. Materials and methods

Research 1: Referred pain and sensations evoked by standardized palpation of the masseter muscle in healthy participants

A total of 32 healthy volunteers (16 men, mean (±standard deviation (SD)) age 32.4 ± 12.7 years; 16 women, mean age 25.4 ± 6.1 years) were recruited from the Section of Orofacial Pain and Jaw Function, Department of Dentistry and Oral Health, Health, Aarhus University. Inclusion criteria were: no ongoing pain in the face or any other reported chronic pain in the last 6 months; no medical history of systemic disease; no current pregnancy (participant-based report); no medications (non-steroidal anti-inflammatory drugs, muscle relaxants, anxiolytics, or hypnotics); and no orofacial

pain or temporomandibular pain symptoms assessed using the DC/TMD (6). The study protocol followed the guidelines of the World Medical Association Declaration of Helsinki II. All participants signed an informed consent document agreeing to participate in the study after being provided written oral information about the experiment. This protocol was approved by the Central Denmark Region Research Ethics Committee (1-10-72-286-14).

The study was performed as a single-blinded, randomized study. The anterior-posterior and superior-inferior borders of the right masseter muscle were identified by palpation during repetitive clenching and the area was divided into 15 test sites (3 horizontal rows x 5 vertical columns) for the right masseter muscle (Fig. 1A). Mechanical sensitivity was assessed on each of the 15 test sites with different stimulus intensity (0.5 kgf, 1.0 kgf, 2.0 kgf) using a palpometer (Palpeter®; Sunstar Swiss SA, Etoy, Switzerland) (7, 8). The duration of a single palpation stimulus at each test site was 5 s in accordance with the DC/TMD (6). The order of stimulus intensity (0.5 kgf, 1.0 kgf, or 2.0 kgf) and test sites (15 sites) were randomized using a randomization program available on the internet (www.randomization.com). Each stimulus was repeated three times for all participants. 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 masseter muscle. Participants were carefully instructed in the use of the NRS for pain, with 0 denoting “no sensation at all”, 50 as “just barely pain sensation”, and 100 as “the most imaginable pain sensation” for pain intensity (Fig. 1B) (9). Mean pain NRS scores were assessed for each of the 15 test sites on the right masseter 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 imaginable unpleasantness sensation” (Fig. 1C).

Pain/sensations were considered as referred pain/sensations if the participant reported pain or any other sensation beyond the boundary of the masseter muscle being palpated (i.e., perceived in another structure). Pain/sensations were not considered referred if the participant reported pain or sensation extending beyond the area of provocation, while remaining within the boundary of the masseter muscle. After each stimulus, the participant was asked to indicate the area of referred pain/sensations on a digital anatomical drawing (Navigate Pain; Aglance Solutions, Denmark) if the participant reported referred pain/sensations (Fig. 1D).

Analysis of variance (ANOVA) was used to test differences in mean pain NRS scores and unpleasantness NRS scores for the three mechanical stimulus intensities with the following factors: stimulus intensities (3 levels), and test sites (15 levels). Before ANOVA, assumption of normality was tested using the Shapiro-Wilk test, and homogeneity of variance was tested using Levene’s test. The Tukey post-hoc test was used with correction for multiple comparisons. Furthermore, McNemar’s test was used to test differences in the number of participants with of referred pain/sensations evoked by each test site for the three mechanical stimulus intensities and for test sites. For all tests, the significance level was set at $P < 0.05$. All data are presented as mean values and SDs.

Research 2: Spatio-temporal effects of standardized palpation on referred sensations and pain from the masseter muscle in healthy individuals

This study investigated a total of 32 healthy volunteers (16 men, mean

(±standard deviation (SD)) age 24.6 ± 3.8 years; 16 women, mean age 26.8 ± 6.4 years) with no ongoing pain in the face or any other reported chronic pain in the last 3 months, no medical history of systemic disease; no pregnancy, no medications, and no orofacial pain or temporomandibular pain symptoms as assessed using the DC/TMD (6). The study protocol followed the guidelines of the World Medical Association Declaration of Helsinki II. All participants signed an informed consent document agreeing to participate in the study after being provided written and oral information about the experiment. This protocol was approved by the Central Denmark Region Research Ethics Committee (1-10-72-286-14).

This study was performed as a single-blinded, randomized study. The anterior-posterior and superior-inferior borders of the right masseter muscle were identified by palpation during repetitive clenching and the area was divided into 15 test sites (3 horizontal rows x 5 vertical columns; Fig. 1A). Mechanical sensitivity was assessed on each of the 15 test sites at different stimulus intensities (0.5 kgf, 1.0 kgf, 2.0 kgf) using a palpometer (Palpeter®; Sunstar Swiss SA, Etoy, Switzerland) (7, 8). Duration of a single palpation stimulus at each test site was 2 s, 5 s, or 10 s. The order of stimulus intensity (0.5 kgf, 1.0 kgf, or 2.0 kgf), duration of palpation stimulus (2 s, 5 s, or 10 s) and test site (15 sites) was randomized using a randomization program available on the internet (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 masseter muscle. Participants were carefully instructed in the use of the NRS for pain, with 0 denoting “no sensation at all”,1-49 means the participants feel pressure but not pain, 50 as “just barely painful”, and 100 as “the most pain imaginable” for pain intensity (Fig. 1B)

(9). Mean pain NRS scores were assessed for each of the 15 test sites on the right masseter muscle as an overall assessment of mechanical sensitivity. On a different 0-100 NRS, participants scored the intensity of unpleasantness with 0 denoting “no sensation at all” and 100 as “the most unpleasant sensation imaginable” (Fig. 1C).

Pain/sensations were considered as referred pain/sensations if the participant reported pain or sensation beyond the boundary of the masseter muscle being palpated (i.e., perceived in another structure). Pain/sensations were not considered referred if the participant reported pain or sensation extending beyond the area of provocation, but remaining within the boundary of the masseter muscle. After each stimulus, the participant was asked to indicate the area of referred pain/sensations on a digital anatomical drawing (Navigate Pain; Aglance Solutions, Aalborg, Denmark) if the participant reported referred pain/sensations (Fig. 1D).

Analysis of variance (ANOVA) was used to test differences in mean pain and unpleasantness NRS scores for the three mechanical stimulus intensities with the following factors: stimulus intensity, duration of palpation stimulus, and test site. Before ANOVA, the assumption of normality was tested using the Shapiro-Wilk test, and homogeneity of variance was tested using Levene’s test. The Tukey post hoc test was used to correct for multiple comparisons. Furthermore, McNemar’s test was used to test differences in frequency of referred pain/sensations (percentage of participants with referred pain/sensation) evoked at each test site for the three mechanical stimulus intensities and each duration time. For all tests, the significance level was set at $P < 0.05$. All data are presented as mean values and SDs.

Ⅳ. Results

Research 1: Referred pain and sensations evoked by standardized palpation of the masseter muscle in healthy participants

1. NRS scores

Mean pain NRS scores were 14.6 ± 8.8 for the 0.5 kgf stimulus intensity and 30.4 ± 14.9 for the 1.0 kg stimulus intensity, whereas the mean pain NRS score was 55.4 ± 16.5 for the 2.0 kgf stimulus intensity. The 2.0 kgf stimulus intensity evoked pain on the masseter muscle in 75% (n = 24/32) of the number of participants. However, the 0.5 kgf and 1.0 kgf stimulus intensity did not evoke pain on the masseter muscle. Mean unpleasantness NRS scores were 13.7 ± 8.5 for the 0.5 kgf stimulus intensity, 29.8 ± 14.9 for the 1.0 kgf stimulus intensity, and 52.1 ± 18.4 for the 2.0 kgf stimulus intensity. There were significantly differences in pain and unpleasantness NRS scores between stimulus intensities and test sites ($P < 0.001$) (Table 1). Pain and unpleasantness NRS scores using the 2.0 kgf stimulus intensity were significantly higher than using the 0.5 and 1.0 kgf stimulus intensities (Fig. 2A and B). Furthermore, pain and unpleasantness NRS scores using the 1.0 kgf stimulus were significantly higher than when using the 0.5 kg stimulus intensity (Fig. 2A and B). These analyses also showed significant interactions between stimulus intensity x test sites with regard to NRS pain and unpleasantness ($P < 0.001$).

2. Referred pain/sensation

Referred pain/sensations were evoked in 3.1% of healthy participants (n = 1 / 32) with the 0.5 kgf stimulus intensity, in 12.5% (n = 4 / 32) with the 1.0 kgf stimulus intensity and in 34.4% (n = 11 / 32) with the 2.0 kgf stimulus intensity. The only area of referred pain/sensations elicited by the 0.5 kgf stimulus intensity was in the temporal

region (3.1%; n = 1). The areas of referred pain/sensations elicited by 1.0 kgf stimulus intensity were the lower teeth (6.3%; n = 2), upper teeth (3.1%; n = 1), temporal region (3.1%; n = 1), and orbital region (3.1%; n = 1). The areas of referred pain/sensations elicited by the 2.0 kgf stimulus intensity were the temporal region (21.9%; n = 7), orbital region (6.3%; n = 2), frontal region (3.1%; n = 1), lip region (3.1%; n = 1), upper teeth (3.1%; n = 1), lower teeth (3.1%; n = 1), and mandibular region (3.1%; n = 1) (Table 2).

McNemar's test assessing the number of participants with referred pain/sensations evoked by each test site showed no significant differences between test sites. However, the number of participants with referred pain/sensations elicited by the 2.0 kgf stimulus intensity was significantly higher than by the 0.5 and 1.0 kgf stimulus intensities ($P < 0.05$; Fig. 3).

Research 2: Spatio-temporal effects of standardized palpation on referred sensations and pain from the masseter muscle in healthy individuals

1. NRS scores

Table 3 shows the statistical outcome and interactions between factors for NRS scores. There were significantly differences in pain and unpleasantness NRS scores between duration of the palpation stimulus, stimulus intensity, and test site ($P < 0.001$). Figure 4 shows a comparison of pain NRS scores and unpleasantness NRS scores between duration of palpation stimulus at each stimulus intensity. Pain NRS scores for a 10 s palpation stimulus were significantly higher than for 2 s or 5 s palpation stimulus at all stimulus intensities (Fig. 4A). The 2.0 kgf stimulus intensity evoked pain on the masseter muscle with 2 s palpation (62.5%: n = 20/32), with 5 s palpation (78.1%: n = 25/32) and with 10 s palpation (81.3%: n = 26/32), but the 0.5 kgf and 1.0 kgf stimulus

intensity didn't evoke pain on masseter muscle in healthy participants. Unpleasantness NRS scores for a 10 s palpation stimulus were significantly higher than for 2 s palpation stimulus when using 1.0 kgf and 2.0 kgf stimulus intensities (Fig. 4B). These analyses also showed significant interactions between stimulus intensity x duration of palpation stimulus and between stimulus intensity x test sites with regard to NRS pain and unpleasantness ($P < 0.001$).

2. Referred pain/sensation

Referred pain/sensations were evoked in 3.1% of healthy participants (n = 1 / 32) for 5 s palpation stimulus and in 6.3% of healthy participants (n = 2 / 32) for 10 s palpation stimulus when using 0.5 kgf stimulus intensity. Referred pain/sensations were evoked in 6.3% of healthy participants (n = 2 / 32) for 5 s palpation stimulus and in 18.8% of healthy participants (n = 6 / 32) for 10 s palpation stimulus when using 1.0 kgf stimulus intensity. Furthermore, referred pain/sensations were evoked in 6.3% of healthy participants (n = 2 / 32) for 2 s palpation stimulus, in 18.8% of healthy participants (n = 6 / 32) for 5 s palpation stimulus and in 31.3% of healthy participants (n = 10 / 32) for 10 s palpation stimulus when using 2.0 kgf stimulus intensity (Fig. 5). The areas of referred pain/sensations elicited by the 0.5 kgf stimulus intensity was the mandible region (3.1%; n = 1) for 5 s palpation stimulus and the temporal and mandibular region (3.1%; n = 1) for 10 s palpation stimulus. The areas of referred pain/sensations elicited by 1.0 kgf stimulus intensity were the orbital region (3.1%; n = 1) and lower teeth (3.1%; n = 1) for 5 s palpation stimulus. The areas of referred pain/sensations elicited by 1.0 kgf stimulus intensity were the temporal region (3.1%; n = 1), lower teeth (3.1%; n = 1), orbital region (3.1%; n = 1), and mandible region (3.1%;

n = 1) for 10 s palpation stimulus. The areas of referred pain/sensations elicited by the 2.0 kgf stimulus intensity were the temporal region (9.4%; n = 3) for 2 s palpation stimulus, the temporal region (9.4%; n = 3) for 5 s palpation stimulus, and the temporal region (15.6%; n = 5) for 10 s palpation stimulus (Table 4).

McNemar's test assessing the number of participants with referred pain/sensations evoked at each test site showed no significant differences between test site. However, the number of participants with referred pain/sensations elicited by 10 s palpation stimulus was significantly higher than that by 2 s palpation stimulus when using 1.0 and 2.0 kgf stimulus intensities ($P < 0.05$; Fig. 5).

Ⅴ. Discussion

Research 1: Referred pain and sensations evoked by standardized palpation of the masseter muscle in healthy participants

The main findings in this study were that: 1) referred pain/sensations occurred with 0.5, 1.0, and 2.0 kgf stimulus intensity in healthy participants; 2) a positive relationship existed between the number of participants with of referred pain/sensations and stimulus intensity; 4) applying 2.0 kgf stimulus intensity to the masseter muscle was likely to evoke pain in healthy participants.

The mechanism for referred pain is believed to represent a combination of central sensitization, convergence of sensory nerves from multiple sites, and changes in second-order neuron connectivity (3-5, 10). Some studies compared referred pain provoked by palpation between patients and healthy individuals in other regions of the body (lower part of the body or low back). Torstensson et al (11) compared referred pain provoked by palpation for 13 intra-pelvic landmarks between participants with and

without chronic pelvic pain (CPP), and participants without CPP also reported referred pain provoked by palpation. Chang-Yu et al (12) compared referred pain provoked by palpation of low back muscles between participants with and without low back pain, and 7.7% of participants without low back pain also experienced referred pain provoked by palpation. The present results also showed referred pain/sensations in the orofacial region among healthy participants upon standardized palpation of the masseter muscle. The results suggest that even participants who do not have pain or symptoms may be subject to the mechanisms of referred pain in the masseter muscles.

Some studies have reported a positive correlation between pain intensity and frequencies of referred pain (13). The present results also showed a positive correlation between the number of participants with referred pain/sensations and stimulus intensity. The present results also suggest that referred pain from the masseter muscle is an intensity-dependent process originating from a local stimulus.

Castrillon et al (9) reported that a 2.0 kgf stimulus intensity on the masseter muscle would be sufficient to elicit a mechanical pressure pain sensation. The present results showed that mean pain NRS scores were in the non-painful range for 0.5 and 1.0 kgf (14.6 ± 8.8 at 0.5 kgf stimulus intensity; 30.6 ± 14.9 at 1.0 kgf stimulus intensity), whereas mean pain NRS scores were in the painful range for 2.0 kgf (55.4 ± 16.5), supporting previous findings. The results also suggested that the 2.0 kgf stimulus intensity is not suitable for clinical palpation of masseter muscles. In addition, Rainville et al (14) demonstrated that both pain intensity and unpleasantness are tightly coupled to stimulus intensity across different stimulus types in cutaneous pain. However, information is currently lacking on comparisons between pain and unpleasantness NRS scores for the masseter muscle (deep pain). The present results suggest that when

palpating the masseter muscle, pain intensity and unpleasantness intensity are also tightly coupled to stimulus intensity.

Research 2: Spatio-temporal effects of standardized palpation on referred sensations and pain from the masseter muscle in healthy individuals

The main findings in this study were that: 1) a positive correlation existed between the duration of the palpation stimulus and the number of participants reporting referred pain/sensations at each stimulus intensity; 2) a positive correlation existed between the duration of the palpation stimulus and pain and unpleasantness NRS scores at each stimulus intensity; and 3) stimulus site did not show any specific relation to the incidence of referred pain/sensations.

The present results show that mean pain NRS scores were in the non-painful range for 0.5 and 1.0 kgf, whereas mean pain NRS scores were in the painful range for 2.0 kgf, supporting previous findings (9). In the present study, it was interesting to find that healthy participants reported referred pain/sensations with non-noxious palpation stimuli (0.5 and 1.0 kgf stimulus intensities). These results suggest that even participants who do not have pain or symptoms may have the mechanisms needed to experience referred pain in the masseter muscles through non-noxious stimuli. In addition, positive correlations were found between the duration of the palpation stimulus and the number of participants with referred pain/sensations at each stimulus intensity. Of the three stimulus intensities, 2.0 kgf was the only stimulus intensity to produce pain NRS scores around the pain threshold. According to Mense (5), recordings from dorsal horn neurons revealed that noxious stimuli to a specific receptive field in a muscle

generated new muscle receptive fields at a distance from the original one. Thus, palpation with 2.0 kgf evokes pain in healthy participants and may cause an increase in the neural changes required to elicit referred pain.

The present results likewise showed positive correlations between incidence of referred pain/sensations and both stimulus intensity and duration of stimulus. Our results thus suggest that referred pain/sensations from the masseter muscle involve time- and intensity-dependent processes originating from local stimuli. Furthermore, the present study indicated that the NRS scores were test site-dependent, but the number of participants with referred pain/sensations were not specifically test site-dependent. Further studies are needed to investigate differences in mechanical sensitivity between test sites in the masseter muscle on palpation.

Castrillon et al. (15) showed that mechanical sensitivity using NRS scores increased in parallel with three different mechanical forces (5 N, 10 N, and 20 N) applied in healthy participants for durations of 2 s. Our results also showed that mean pain NRS scores increased significantly in parallel with three different stimulus intensities for each duration of palpation. In addition, our results showed positive correlations between duration of palpation stimulus and the pain and unpleasantness NRS scores at each stimulus intensity. Rainville et al. (14) demonstrated that visual analogue scales of pain intensity and unpleasantness were tightly coupled to stimulus intensity across different stimulus types for cutaneous pain. Our results suggest that mean pain and unpleasantness NRS score are also tightly coupled to stimulus intensity when using the same duration of palpation stimulus.

Ⅵ. Conclusion

These results indicate that referred pain/sensations in the orofacial region are evoked by standardized palpation of the masseter muscle among healthy individuals. Furthermore, these findings showed that referred pain/sensations from the masseter muscle were time- and intensity-dependent processes originating from local stimulus. Because of the 2.0 kgf stimulus intensity on the masseter muscle would be sufficient to elicit pain in healthy participant, the 1.0 kgf stimulus intensity is suitable for distinguishing between patients and healthy individual on clinical palpation of masseter muscles in DC/TMD.

Ⅶ. References

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Ⅷ. Table and Figures

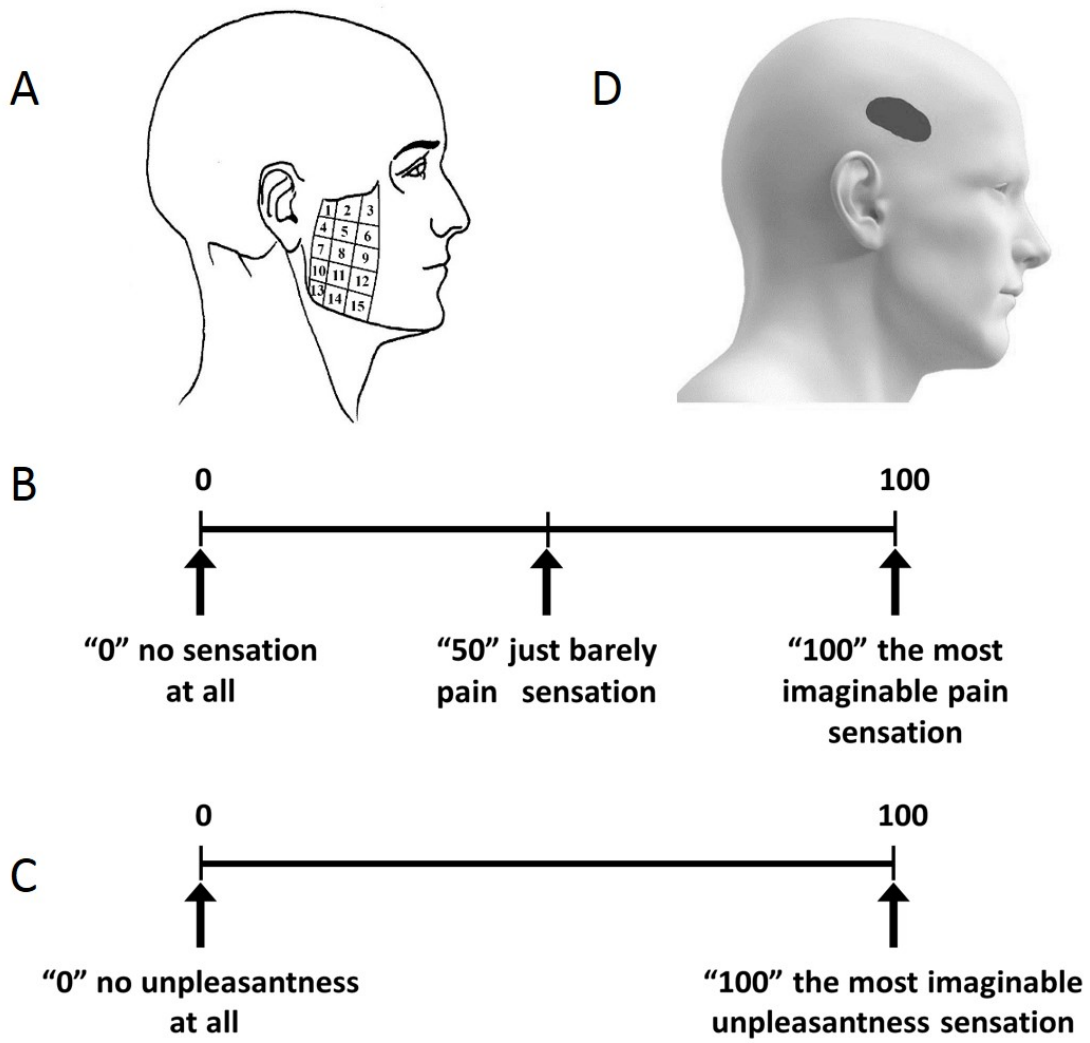

Figure 1. The design of 15 test sites on masseter muscle, numerical rating scale (NRS) and a digital anatomical drawing of referred pain/sensations. The anterior-posterior and inferior-superior borders of the masseter muscle were identified and the areas were divided into 15 test sites (5 vertical and 3 horizontal) **(A)**. Pain intensity were scored on a 0-50-100 NRS with 0 denoting “no sensation at all”, 50 as “just barely painful”, and 100 as “the most imaginable pain sensation” **(B)**. Unpleasantness intensity were scored a 0-100 NRS with 0 denoting “no unpleasantness at all” and 100 as “the most imaginable unpleasantness sensation” **(C)**. The participants were asked to indicate the area of referred pain/sensations on a digital anatomical drawing **(D)**.

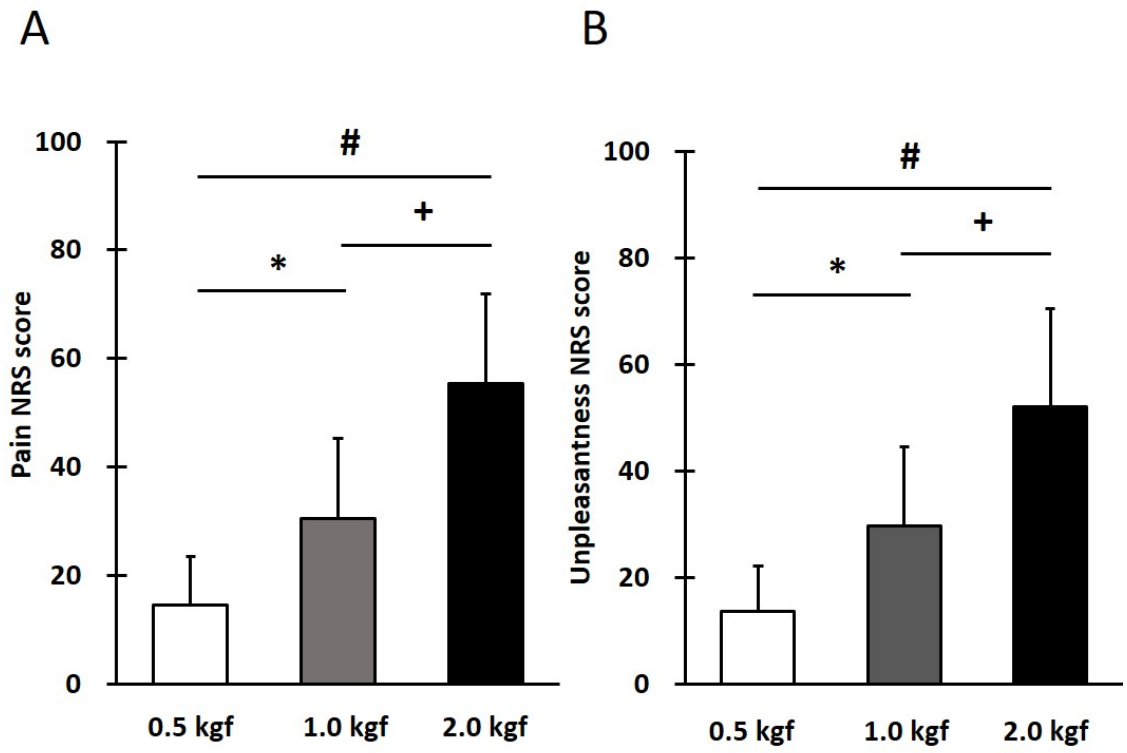

Figure 2. Comparison of pain NRS score between stimulus intensity (A) and comparison of unpleasantness NRS score between stimulus intensity (B). Pain and unpleasantness NRS score when using 2.0 kgf was significantly higher than when using 0.5 and 1.0 kgf (#, +: $P < 0.001$, Tukey post hoc test). Pain and unpleasantness NRS score when using 1.0 kgf was significantly higher than when using 0.5 kgf (∗: $P < 0.001$, Tukey post hoc test).

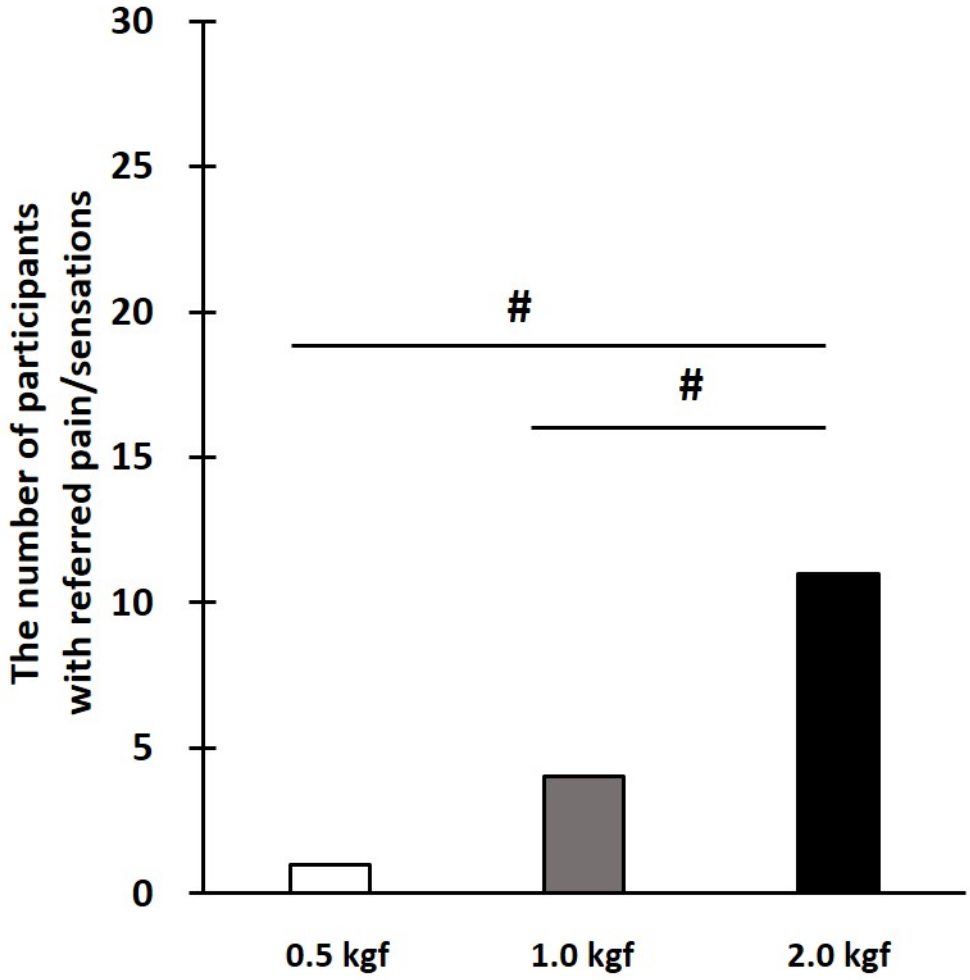

Figure 3. Comparison of the number of participants with referred pain/sensations between stimulus intensity. The number of participants with referred pain/sensations when using 2.0 kgf was significantly higher than when using 0.5 and 1.0 kgf (#: $P < 0.05$, McNemar's test).

Table 1. Statistical relationship of factors for NRS scores and duration of aftersensations. The p-values from ANOVAs testing differences in means of pain NRS scores and unpleasantness NRS scores for three mechanical stimulus intensities with following factors: stimulus intensities (3 levels) and test sites (15 levels).

	Stimulus	Test sites	Stimulus x Test sites
Pain NRS	**P < 0.001**	**P < 0.001**	**P < 0.001**
Unpleasantness NRS	**P < 0.001**	**P < 0.001**	**P < 0.001**

Table 2. The area of referred pain/sensations in each stimulus intensity. The most common area of referred pain/sensations were temporal region (3.1%; n = 1/32) when using 0.5 kgf, lower teeth (6.3%; n = 2/32) when using 1.0 kgf and temporal region (21.9%; n = 7/32) when using 2.0 kgf.

Stimuli	Referred Area	Number of participants with referred pain
0.5 kgf	Temporal	3.1 % (n = 1 / 32)
1.0 kgf	Lower teeth	6.3 % (n = 2 / 32)
	Temporal	3.1 % (n = 1 / 32)
	Upper teeth	3.1 % (n = 1 / 32)
	Orbital	3.1 % (n = 1 / 32)
2.0 kgf	Temporal	21.9 % (n = 7 / 32)
	Orbital	6.3 % (n = 2 / 32)
	Frontal	3.1 % (n = 1 / 32)
	Mandible	3.1 % (n = 1 / 32)
	lip	3.1 % (n = 1 / 32)
	Upper teeth	3.1 % (n = 1 / 32)
	Lower teeth	3.1 % (n = 1 / 32)

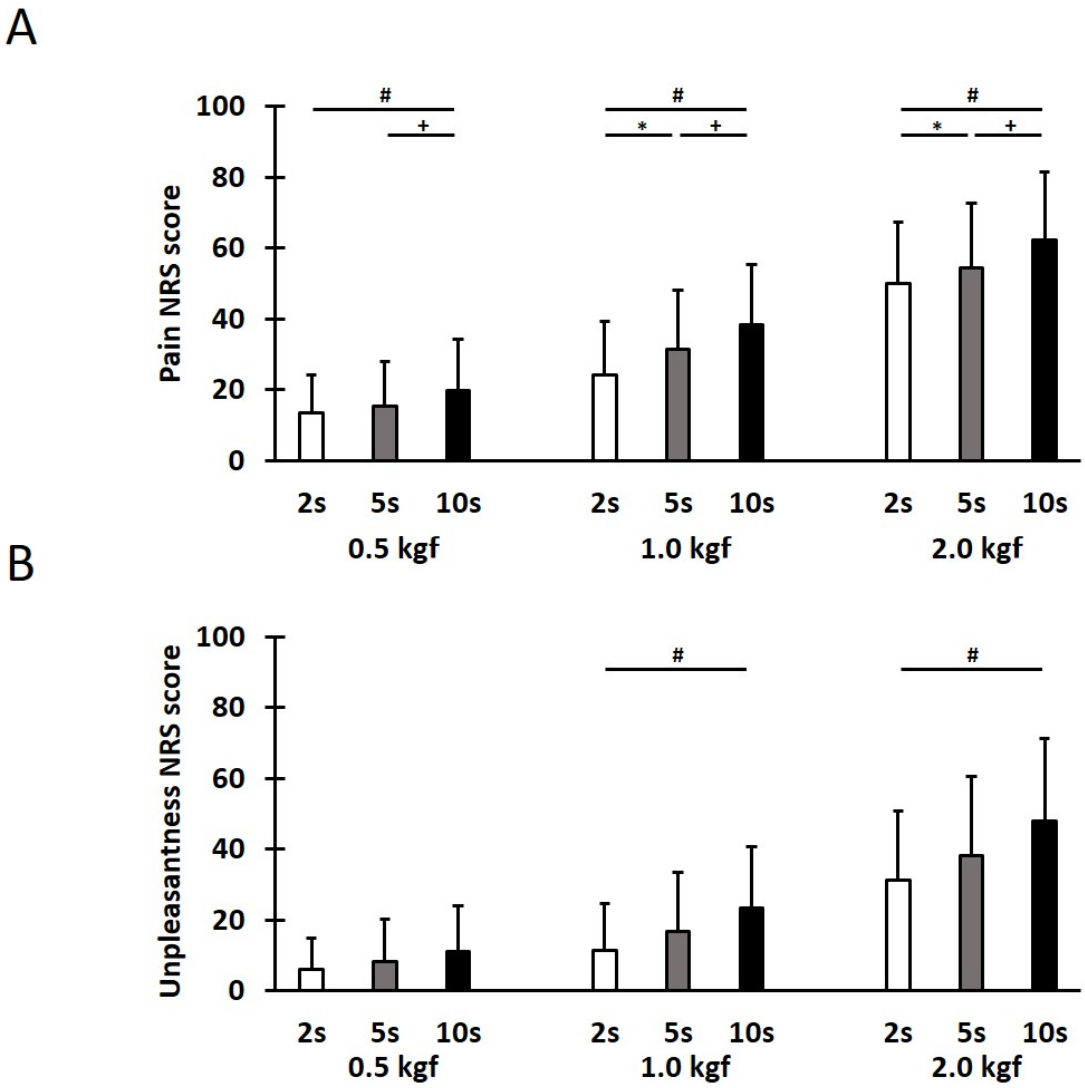

Figure. 4. Comparison of pain NRS score, (A) and comparison of unpleasantness NRS score, (B) between duration of palpation stimulus at each stimulus intensity. Pain NRS scores for a 10 s duration of palpation stimulus were significantly higher than for 2 s or 5 s duration at each stimulus intensity (#, +: $P < 0.001$, Tukey post hoc test). Unpleasantness NRS scores for a 10 s duration of palpation stimulus were significantly higher than for 2 s duration at 1.0 and 2.0 kgf stimulus intensities (#: $P < 0.001$, Tukey post hoc test).

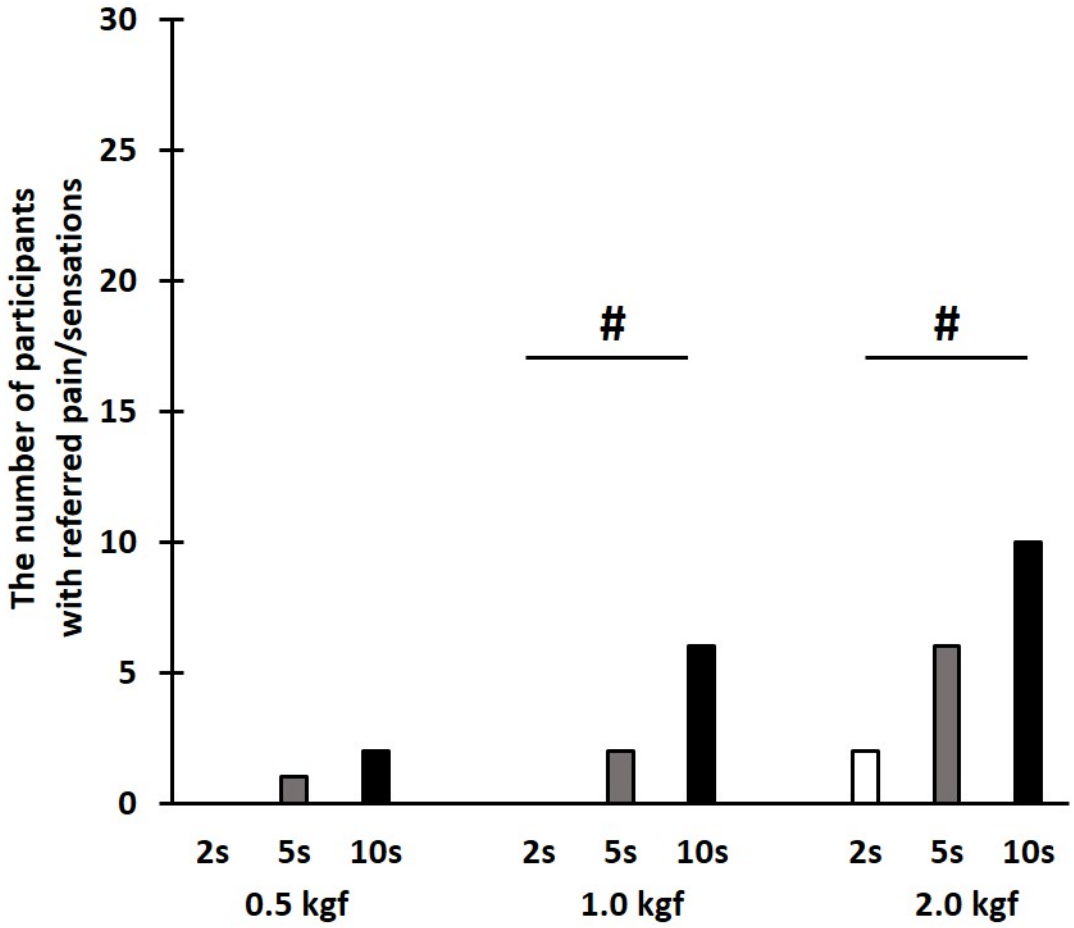

Figure. 5. Comparison of the number of participants with referred pain/sensations between durations of palpation stimulus at each stimulus intensity. The number of participants with referred pain/sensations elicited by 10 s duration of palpation was significantly higher than by 2 s duration of palpation when using 1.0 and 2.0 kgf stimulus intensities (#: $P < 0.05$, McNemar's test).

Table 3. Statistical relationship of factors for NRS scores and aftersensation times. P-values from ANOVA testing differences in mean pain NRS scores and unpleasantness NRS scores for the three mechanical stimulus intensities with the following factors: duration of palpation stimulus (3 levels), stimulus intensity (3 levels), and test site (15 levels).

	Duration	Stimulus	Test sites	Durations x Stimulus	Stimulus x Test sites
Pain NRS	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Unpleasantness NRS	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001

Table 4. Area of referred pain/sensations at each stimulus intensity. The most common areas for referred pain/sensations were the mandibular region (3.1%; n = 1/32) at 5 s, and the temporal and mandibular region (3.1%; n = 1/32) at 10 s when using 0.5 kgf. The most common areas for referred pain/sensations were the orbital region and lower teeth (3.1%; n = 1/32) at 5 s, and the temporal, lower teeth, orbital, and mandibular region (3.1%; n = 1/32) at 10 s when using 1.0 kgf. The most common areas for referred pain/sensations were the temporal region (9.4%; n = 3/32) at 2 s, the temporal region (9.4%; n = 3/32) at 5 s, and the temporal region (15.6%; n = 5/32) at 10 s when using 2.0 kgf.

Stimulus	Duration	Referred Area	Number of participants with referred pain
0.5 kg	5 s	Mandible	3.1 % (n = 1 / 32)
	10 s	Temporal	3.1 % (n = 1 / 32)
		Mandible	3.1 % (n = 1 / 32)
1.0 kg	5 s	Orbital	3.1 % (n = 1 / 32)
		Lower teeth	3.1 % (n = 1 / 32)
	10 s	Temporal	9.4 % (n = 3 / 32)
			3.1 % (n = 1 / 32)
		Orbital	3.1 % (n = 1 / 32)
		Mandible	3.1 % (n = 1 / 32)
2.0 kg	2 s	Temporal	6.3 % (n = 2 / 32)
	5 s	Temporal	9.4 % (n = 3 / 32)
		Occipital	3.1 % (n = 1 / 32)
		Orbital	3.1 % (n = 1 / 32)
		Upper teeth	3.1 % (n = 1 / 32)
	10 s	Temporal	15.6 % (n = 5 / 32)
		Orbital	3.1 % (n = 1 / 32)
		Lower teeth	3.1 % (n = 1 / 32)
		Mandible	3.1 % (n = 1 / 32)
		Occipital	3.1 % (n = 1 / 32)
		Labrum	3.1 % (n = 1 / 32)