Spatio-temporal Effects of Standardized Palpation on Referred Sensations and Pain from the Masseter Muscle in Healthy Individuals

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Abstract

The aims of this study were to investigate the influence of different spatio-temporal conditions of palpation stimulus on occurrence of referred pain/sensations in healthy participants.

Participants comprised 32 pain-free individuals (mean age, 25.7 ± 5.3 years) according to the Diagnostic Criteria for Temporomandibular Disorders. The right masseter muscle was equally divided into 15 test sites. Mechanical sensitivity was assessed with three mechanical stimuli (0.5-kg, 1.0-kg, or 2.0-kg) applied to the 15 test sites with three different durations (2 s, 5 s, or 10 s) using palpmeters. Participants scored the intensity of perceived pain and unpleasantness for the three mechanical stimuli on a 0-50-100 numerical rating scale (NRS) after each stimulus. If the participant reported referred pain/sensations after a stimulus, they indicated areas of referred pain/sensations on a digital drawing.

The occurrence of referred pain/sensations was significantly higher with 10 s palpation stimulus than with 2 s palpation stimulus for 1.0-kg and 2.0-kg stimuli (P < 0.05 each). There was no significant relationship between test site and occurrence of referred pain/sensations. Stimulus intensity, duration and test site significantly affected NRS scores for pain and unpleasantness (P < 0.05 each).

These findings showed that referred pain/sensations from the masseter muscle were time- and intensity-dependent, but not site-dependent processes originating from standardized palpation of the masseter muscle. In addition, our results indicate that duration, intensity and test site of palpation stimuli for masseter muscle is associated with muscle pain diagnosis related to referred pain in the orofacial area.

Keywords:
referred pain, palpation, mechanical sensitivity, masseter muscle.

Introduction

Muscle 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. Despite this the extensive convergence of afferent input from various tissues onto wide-dynamic range neurons and central sensitization is believed to be crucially involved (6).

Our previous study investigated referred pain/sensations evoked by three different mechanical stimuli applied to the masseter muscle in healthy participants and demonstrated

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that referred pain/sensations in the orofacial region is a frequent phenomenon among healthy individuals during standardized palpation of the masseter muscle (7). However, our previous study did not clarify the relationship between occurrence of referred pain and duration of the palpation stimulus. Furthermore, there are remaining questions about potential mechanical sensitivity site-to-site differences within the masseter muscle. To clarify the relationship between mechanical sensitivity and referred pain in the orofacial area, identifying possible duration-, site- and/or intensity-dependent relationships with local pain in the masticatory muscles appears to be essential for clinical examination and diagnostic procedures.

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 hypothesis of the present study was that referred pain/sensations and mechanical sensitivity of the masseter muscle were not only intensity-dependent but also duration-dependent from standardized palpation of the masseter muscle. The aims of this study were to investigate the influence of different spatio-temporal conditions of palpation stimulus on occurrence of referred pain and/or sensation in healthy participants.

Materials and Methods

Participants

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 (8). The Danish translation of the DC/TMD was used to rule out presence of a TMD in healthy participants. 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).

Study design

This study was performed as a single-blinded, randomized study. The participants involved in the study were blinded to the experimental conditions. The anterior-posterior and superior-inferior borders of the right masseter muscle were identified by palpation during repetitive clenching and the area was equally divided into 15 test sites (3 horizontal rows × 5 vertical columns; Fig. 1A). Mechanical sensitivity was assessed on each of the 15 test sites at different stimulus intensities (0.5 kg, 1.0 kg, 2.0 kg) using a palpometer (Palpeter; Sunstar Swiss SA, Etoy, Switzerland) (9, 10). 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 kg, 1.0 kg, or 2.0 kg), duration of palpation stimulus (2 s, 5 s, or 10 s) and test site (15 sites) was randomized. A randomization program determined the combination of 135 ways (3 stimulus intensities x 3 duration of palpation stimulus x 15 test sites) in random order. 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 (11). 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. Participants were also instructed to use a different 0–100 NRS for unpleasantness where 0 denoted “no unpleasantness at all” and 100 “the most unpleasant sensation imaginable”. To avoid the effect of peripheral sensitization, the interval between each palpation stimulus was 10 s. This was based on results regarding after sensations evoked by palpation of the masseter muscle in our previous study (7).

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
Fig. 1. Design of the 15 test sites on masseter muscle and digital anatomical drawing of referred pain/sensations. Anterior-posterior and inferior-superior borders of the masseter muscle were identified and the muscle area was divided into 15 test sites (5 vertical columns × 3 horizontal rows) (A). The participant was asked to indicate the area of any referred pain/sensations on a digital anatomical drawing (B).
drawing (Navigate Pain; Aglance Solutions, Aalborg, Denmark) if the participant reported referred pain/sensations (Fig. 1B) (12).

Statistical analysis
Mean pain and unpleasantness NRS scores for the three mechanical stimulus intensities were analyzed by 3-way repeated measures analysis of variance (ANOVA) with the following factors: stimulus intensity, duration of palpation stimulus, and test site. 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.

Results

NRS scores
Mean pain NRS scores were 13.4±10.9 using a duration of 2 s, 15.4 ± 12.4 with 5 s duration, and 19.9 ± 14.3 palpating with 10 s when using 0.5 kg stimulus intensity. Mean pain NRS scores were 24.2 ± 15.1 using a duration of 2 s, 31.6 ± 16.6 with 5 s duration, and 38.3 ± 17.1 palpating with 10 s when using 1.0 kg stimulus intensity. Mean pain NRS scores were 49.9 ± 17.4 using a duration of 2 s, 54.6 ± 18.0 with 5 s duration, whereas it was 62.5 ± 19.2 using 10 s when using 2.0 kg stimulus intensity.

Mean unpleasantness NRS scores were 6.0±8.9 using a duration of 2 s, 8.2 ± 12.1 with 5 s duration, and 11.0 ± 12.9 palpating with 10 s when using 0.5 kg stimulus intensity. Mean unpleasantness NRS scores were 11.3 ± 13.2 using a duration of 2 s, 16.8 ± 16.9 with 5 s duration, and 23.3 ± 17.4 palpating with 10 s when using 1.0 kg stimulus intensity. Mean unpleasantness NRS scores were 31.2 ± 19.7 using a duration of 2 s, 38.1 ± 22.5 with 5 s duration, whereas it was 48.0 ± 23.4 using 10 s when using 2.0 kg stimulus intensity (Fig. 2A and B).

Table 1 shows the statistical outcome and interactions between factors for NRS scores. Overall, there were significant differences in pain and unpleasantness NRS scores were seen between duration of the palpation stimulus, stimulus intensity, and test site (P < 0.05, 3-way ANOVA). Figure 2 shows a comparison of pain NRS scores and unpleasantness NRS scores between duration of palpatation 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. 2A) (P < 0.05, Tukey post hoc). Unpleasantness NRS scores for a 10 s palpation stimulus were significantly higher than for 2 s palpation stimulus when using 1.0 kg and 2.0 kg stimulus intensities (Fig. 2B) (P < 0.05, Tukey post hoc). The interactions between duration x intensity and intensity x test site (P < 0.05, 3-way ANOVA) with regard to NRS pain and unpleasantness were also significant. However, there were no other significant interactions (P > 0.05, 3-way ANOVA) with regard to NRS pain and unpleasantness. The location of the highest pain NRS scores for 2 s palpation stimulus were site 3, site 9, and site 9 when using 0.5 kg, 1.0 kg and 2.0 kg stimulus intensity, respectively. The location of the highest pain NRS scores for 5 s palpation stimulus were site 9, site 9, and site 3 when using 0.5 kg, 1.0 kg and 2.0 kg stimulus intensity, respectively. The location of the highest pain NRS scores for 10 s palpation stimulus were site 3, site 9, and site 6 when using 0.5 kg, 1.0 kg and 2.0 kg stimulus intensity, respectively.

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 kg 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 kg 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 kg stimulus intensity (Fig. 3). In an individual analysis of the participants that had referred pain/sensations, referred pain/sensations were evoked in 10 of the healthy participants when palpating for 10 s using a 2.0 kg stimulus intensity. Of those 10 participants, 60% (n = 6) also experienced referred pain/sensations with 5 s palpation using 2.0 kg. Referred pain/sensations were evoked in 5 of the 10 using 10 s/1.0 kg, in 2 of the 10 participants using 2 s/2.0 kg or 5 s/1.0 kg or 10 s/0.5 kg. Finally, 1/10 experienced referred pain/sensation using 5
The areas of referred pain/sensations elicited by the 0.5 kg 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 kg 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 kg stimulus intensity were the temporal region (9.4%; n = 3), 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 kg stimulus intensity were the temporal region (6.3%; n = 2) 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 2).

McNemar’s test assessing the number of participants with referred pain/sensations evoked at each test site showed no significant differences between test sites (P > 0.05, McNemar’s). 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 kg stimulus intensities (P < 0.05, McNemar’s; Fig. 3).

**Discussion**

The main findings in this study were that: 1) a positive correlation existed between the duration of the palpation stimulus and occurrence of referred pain/sensations at each stimulus intensity; and 2) a positive correlation existed between the duration of the palpation stimulus and pain and unpleasantness NRS scores at each stimulus intensity. Moreover, a prominent finding was that stimulus site did not show any specific relation to the occurrence of referred pain/sensation.

Some studies have suggested that referred pain represents a combination of central sensitization, convergence of sensory nerve inputs from multiple sites, changes in second-order neuron connectivity, and descending facilitation in the central nervous system (4–6, 13). Castrillon et al. (14) reported that a 2.0 kg stimulus intensity applied to the masseter muscle would be sufficient to elicit a painful sensation. The present results showed that mean pain NRS scores were in the non-painful range for 0.5 and 1.0 kg, whereas mean pain NRS scores were in the painful range for 2.0 kg, supporting previous findings (14). The results also

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**Table 1** Statistical relationship of factors for NRS scores

<table>
<thead>
<tr>
<th></th>
<th>Duration</th>
<th>Intensity</th>
<th>Test site</th>
<th>Duration x Test site</th>
<th>Duration x Intensity</th>
<th>Intensity x Test site</th>
<th>Duration x Intensity x Test site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain NRS</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>0.345</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>0.306</td>
</tr>
<tr>
<td>Unpleasantness NRS</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>0.572</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>0.229</td>
</tr>
</tbody>
</table>

Fig. 3. Comparison of the number of participants with referred pain/sensations between durations of palpatation 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 kg and 2.0 kg stimulus intensities (P < 0.05, McNemar’s test).
Table 2  Area of referred pain/sensations at each stimulus intensity
The most common areas for referred pain/sensations were the mandibular region at 5 s, and the temporal and mandibular region at 10 s when using 0.5 kg. The most common areas for referred pain/sensations were the orbital region and lower teeth at 5 s, and the temporal, lower teeth, orbital, and mandibular region at 10 s when using 1.0 kg. The most common areas for referred pain/sensations were the temporal region at 2 s, the temporal region at 5 s, and the temporal region at 10 s when using 2.0 kg.

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Duration</th>
<th>Referred Area</th>
<th>Number of participants with referred pain</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 kg</td>
<td>5 s</td>
<td>Angle of mandible</td>
<td>3.1% (n = 1 / 32)</td>
</tr>
<tr>
<td></td>
<td>10 s</td>
<td>Temporal</td>
<td>3.1% (n = 1 / 32)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Angle of mandible</td>
<td>3.1% (n = 1 / 32)</td>
</tr>
<tr>
<td>1.0 kg</td>
<td>5 s</td>
<td>Orbital</td>
<td>3.1% (n = 1 / 32)</td>
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<tr>
<td></td>
<td></td>
<td>Lower posterior teeth</td>
<td>3.1% (n = 1 / 32)</td>
</tr>
<tr>
<td></td>
<td>10 s</td>
<td>Temporal</td>
<td>9.4% (n = 3 / 32)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lower posterior teeth</td>
<td>3.1% (n = 1 / 32)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Orbital</td>
<td>3.1% (n = 1 / 32)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Angle of mandible</td>
<td>3.1% (n = 1 / 32)</td>
</tr>
<tr>
<td>2.0 kg</td>
<td>2 s</td>
<td>Temporal</td>
<td>6.3% (n = 2 / 32)</td>
</tr>
<tr>
<td></td>
<td>5 s</td>
<td>Temporal</td>
<td>9.4% (n = 3 / 32)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Occipital</td>
<td>3.1% (n = 1 / 32)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Orbital</td>
<td>3.1% (n = 1 / 32)</td>
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<tr>
<td></td>
<td></td>
<td>Upper posterior teeth</td>
<td>3.1% (n = 1 / 32)</td>
</tr>
<tr>
<td></td>
<td>10 s</td>
<td>Temporal</td>
<td>15.6% (n = 5 / 32)</td>
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<tr>
<td></td>
<td></td>
<td>Orbital</td>
<td>3.1% (n = 1 / 32)</td>
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<tr>
<td></td>
<td></td>
<td>Lower posterior teeth</td>
<td>3.1% (n = 1 / 32)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Angle of mandible</td>
<td>3.1% (n = 1 / 32)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Occipital</td>
<td>3.1% (n = 1 / 32)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Labrum</td>
<td>3.1% (n = 1 / 32)</td>
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suggested that the 2.0 kg stimulus intensity is not suitable for clinical palpation of masseter muscles and the results indicated that 1.0 kg stimulus intensity is appropriate to use in DC/TMD for masseter muscle.

Sessle et al. (6) suggested that stimulation from deep muscle (e.g. masseter and temporal muscle) not only excites wide-dynamic-range (WDR) neurons, but also may activate low-threshold mechanoreceptive (LTM) and nociceptive-specific (NS) neurons, and that WDR and NS neurons may be involved in referred pain. Additionally, they suggested that convergence of afferent inputs onto spinal dorsal horn neurons may contribute to the neural basis of referred pain (6). In the present study, it was interesting to find that some healthy participants reported referred pain/sensations with non-noxious palpation stimuli (0.5 kg and 1.0 kg stimulus intensities). Our results suggest that non-noxious palpation of the masseter muscle may excite WDR neurons and convergence of afferent input may cause referred pain/sensations in healthy participants. In addition, a positive correlation existed between the duration of the palpation...
stimulus and occurrence of referred pain/sensations at each stimulus intensity. Of the three stimulus intensities, 2.0 kg was the only stimulus intensity to produce pain NRS scores around the pain threshold. According to Mense (15), 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 kg evokes pain in healthy participants and may cause an increase in the neural changes required to elicit referred pain. Since the present study only applied palpation to the masseter muscle, further studies are needed to investigate mechanical sensitivity and referred pain/sensations when mechanical stimuli are applied to other masticatory muscles (e.g., temporalis muscle).

Arendt-Nielsen et al. (16) reported that the occurrence of evoked referred pain depends on both the perceived intensity of the stimulus and the duration of that stimulus. The present results likewise showed positive correlations between occurrence 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 standardized palpation of the masseter muscle. Individual analysis among participants who have referred pain/sensations also demonstrated that referred pain/sensations were found by 5 s and 10 s palpation stimulus when using each stimulus intensity. To consider the diagnosis criteria about referred pain in orofacial area, our results suggests that it is necessary to apply 5 s palpation stimulus as lowest duration for masseter muscle.

Castrillon et al. (17) 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. (18) 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. Since the present results showed that NRS scores of perceived pain and unpleasantness were time-, intensity- and site-dependent, this observation could indicate that the duration, intensity and test site of the palpation stimulus to the masseter muscle could influence the diagnosis of myofascial pain with referral. Although the target of pain and unpleasantness NRS score in this study was an indicator of mechanical sensitivity in the masseter muscle using palpation, pain and unpleasantness NRS score about referred pain/sensations was not the focus of this study. Further studies are needed to investigate the pain and unpleasantness of referred pain/sensations using a similar experimental design to further clarify the mechanisms of referred pain/sensations related to the palpation of the masseter muscle.

In conclusion, the present study showed that referred pain/sensations were time- and intensity-dependent, but not site-dependent processes originating from standardized palpation of the masseter muscle. Furthermore, since NRS scores of perceived pain and unpleasantness were time-, intensity- and site-dependent, this observation could indicate that duration, intensity and test site of palpation stimuli for masseter muscle is associated with muscle pain diagnosis related to referred pain in the orofacial area.

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Conflict of interest

All authors declare that there are no conflict of interests.

References


