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**Standardized palpation of the temporalis muscle evoke referred pain and sensations in individuals without TMD.**

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## **ABSTRACT (150 to 250 words)**

### **Objectives**

This study aimed to determine if standardized palpations of the temporalis muscle evoke referred pain and/or sensations in individuals without TMD.

### **Materials and Methods**

This was a randomized, single-blinded study. 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 seconds). 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.

### **Results**

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 ( $P < 0.05$ ).

### **Conclusions**

Our present results indicate that 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.

### **Keywords**

aftersensation, temporalis muscle, mechanical sensitivity, palpation, referred pain

## Introduction

Headache is one of the most prevalent neurological disorders. Tension-type headache (TTH) is the most common form of headache [1]. In recent years, the pathogenesis of TTH has focused on the role of abnormalities in the processing of nociceptive and non-nociceptive input from craniofacial muscles [2]. It is becoming increasingly clear that central nervous system (CNS) factors and the vascular system play a crucial role [3]. Furthermore, referred pain of muscular origin is thought to be one of the most relevant phenomena in the development of TTH [4]. Although several studies discuss various mechanisms of referred pain [5], the processes underlying referred pain have yet to be clarified not only for orofacial area but also for other body regions. There is extensive convergence of afferent input from various tissues onto wide-dynamic range neurons and central sensitization may contribute to these underlying mechanisms of referred pain in animals [6]. In human studies, referred sensations and pain can only be documented by self-reports using valid questionnaires and techniques as there are no physiological measures available.

According to the International Classification of Headache Disorders – 3<sup>rd</sup> Edition (ICHD-3), headaches are defined as pain located above the orbitomeatal line [7]. In contrast, facial pain is defined as below the orbitomeatal line, above the neck, and anterior to the pinnae [7]. Therefore, the area of the facial pain includes the frontal, temporalis, masseter, pterygoid, sternocleidomastoid, splenius, and trapezius muscles. Consequently, headaches' defined location has a distinct anatomical overlap with the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) [8]. Additionally, TMD are comorbid with some types of primary headaches, such as migraine and TTH [9].

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 [10]. 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 and headaches, investigation of the relationship between mechanical sensitivity and referred pain in the orofacial area is needed.

The present study's hypothesis was that the duration and intensity of palpation of the temporalis muscle influence the frequency of referred pain/sensations. This study 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.

## Materials and Methods

### Sample size

The sample size was calculated using G\*Power 3.1 (Heinrich-Heine-Universität Düsseldorf, Germany). The alpha value ( $\alpha$ , probability of making type I error) and power ( $1-\beta$ , probability of not making a type II error) were set as 0.05 and 0.80, respectively. The effect size in this study was set as a medium effect of 0.25 [11, 12]. Accordingly, a sample size of 28 participants was required.

### Participants

Thirty-two volunteers without TMD (16 men, mean ( $\pm$  standard deviation (SD)) age  $26.9 \pm 3.0$  years; 16 women, mean age  $28.4 \pm 3.5$  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 [13]. The DC/TMD Axis II 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 Behavior Checklist (OBC) [13]. The PHQ -4 was used as a screening tool for anxiety and depression [14]. The OBC was used to identify and quantify the frequency of jaw overuse behaviors e.g. bruxism [13]. Exclusion criteria were: (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.

## Experimental Protocol

This was a randomized, single-blinded study. Figure 1a 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) [13, 15]. 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](http://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 1b 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 [16]. 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" (Figure 1c). 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 [17].

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) (Figure 1d) [18].

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 [19].

### **Statistical analysis**

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  $P < .05$ . All data are presented as mean values and SDs. The data were analyzed using SigmaPlot (version 14.0; HULINKS Inc., Tokyo, Japan).

## Results

### NRS scores

Table 1 shows the statistical relationship of factors for NRS scores and aftersensation times. Significant differences were seen between pain and duration of the palpation stimulus ( $F_2 = 121.52$ ,  $P < .001$ ), stimulus intensity ( $F_2 = 2723.26$ ,  $P < .001$ ), and the test site ( $F_{14} = 3.55$ ,  $P < .001$ ) (Table 1). Significant differences were also seen between unpleasantness and duration of the palpation stimulus ( $F_2 = 73.8$ ,  $P < .001$ ) and stimulus intensity ( $F_2 = 638.6$ ,  $P < .001$ ), but not the test site ( $F_{14} = 0.98$ ,  $P = .477$ ) (Table 1).

Figure 2 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 ( $P < .05$ ) (Figure 2a). 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 ( $P < .05$ ) (Figure 2a). Moreover, 78.1% (25/32) of participants reported an NRS score over 50 (pain report) with 2.0 kg stimulus intensity ( $P < .05$ ) (Figure 2a).

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 ( $P < .05$ ) (Figure 2b). 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 ( $P < .05$ ) (Figure 2B).

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 ( $P < .05$ ) (Figure 2c). Aftersensation for the 5 s palpation stimulus were significantly longer than the 2 s palpation stimulus when using 0.5 kg stimulus intensity ( $P < .05$ ) (Figure 2c).

Further, there were significant interactions for stimulus intensity  $\times$  duration of palpation stimulus

and for stimulus intensity  $\times$  test site with regards to pain and unpleasantness ( $P < .001$ ).

### **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 (Figure 3).

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 ( $P < .05$ ; Figure 3). Table 2 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 masseter and posterior teeth (15.6%;  $n = 5$ ) for 10 s palpation stimulus at the 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.

### **Aftersensations**

Significant differences were seen in the duration of aftersensation between duration of palpation

stimulus ( $F_2 = 95.0, P < .001$ ) and stimulus intensity ( $F_2 = 435.5, P < .001$ ) (Table 1, Figure 2c).

### **Entropy analysis of mechanical sensitivity**

Figure 4 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 ( $P < .05$  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 ( $P < .05$ ) (Figure 4a). However, there was no significant difference in entropy values for unpleasantness NRS scores (Figure 4b).

## Discussion

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

The present results 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 [10]. 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 [10]. 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 [20]. Moreover, Torebjörk et al. reported a positive correlation between pain intensity and the frequency of reported referred pain [21]. 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 present results 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 [22]. 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 [22]. Some studies propose that referred pain/sensation is caused by activation of silent synapses converging in the CNS by persistent intense nociceptive input [6, 23]. The present result 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 present results 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 [10]. Castrillon et al. 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 [24]. 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 [19, 25, 26]. The present results 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 2.0 kg 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 [17], 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).

## **Limitations**

Several limitations need to be discussed. For example, the present study applied only the OBC

in order to screen for self-reports of bruxism. Lobbezoo et al. recommended that a 'Definite' sleep bruxism condition be based on self-report, a clinical examination, and a polysomnographic recording, preferably with audio/video recordings [27]. Since sleep or awake bruxism could influence referred pain/sensations in the masticatory muscles, further studies are needed to investigate the effect of definite or probable bruxism on the frequency of referred pain/sensations. Moreover, the effects of age and gender and prior painful conditions, including headaches, would also be interesting to test in further studies on referred pain/sensations from the temporalis muscle. However, our findings show that the most intense stimulation mechanical stimulation combination (2.0 kg and 10 seconds) most frequently evoked referred sensations suggesting that the population assessed in this study may be less sensitive, more similar and appropriately screened for influencing factors.

## **Conclusions**

In conclusion, our present results indicate 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.

## **Conflict of interest**

The authors declare that they have no competing interests.

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### **Ethical approval**

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of Nihon University School of Dentistry at Matsudo (EC18-024).

### **Informed consent**

All participants gave their informed consent prior to their inclusion in the study.



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## Figure Legends

### **Figure 1. 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 2. 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 s 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 (<sup># +</sup> \* P < .005, Tukey post-hoc test) (C).

**Figure 3. 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 (<sup>#</sup> + P < .05, McNemar's test).

**Figure 4. 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 (<sup>#</sup> P < .05, Tukey post-hoc test) (A).

**Table 1. Statistical relationship for factors related to NRS scores and aftersensation times.**

The p-values from ANOVAs testing 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).

	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

**Table 2. The area of referred pain/sensations in each stimulus intensity.**

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.

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)