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博 士 論 文

Fatigue resistance to experimental contraction in human craniofacial muscle groups

（顎顔面筋群の実験的収縮運動に対する抗疲労性）

平成２８年３月申請

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Abstract

Aim: Hyperactivity or abnormal contractions of craniofacial muscles, e.g. bruxism, are traditionally linked to pain and unpleasantness in the active muscles. The aim of this study was to investigate the effects of different standardized craniofacial muscle contractions on perceived muscle symptoms. **Materials and Methods:** Sixteen healthy volunteers (seven men, mean ± SEM, 25.7 ± 1.4 years and nine women, 24.9 ± 2.3 years) performed six 5-minutes bouts of 20% maximal voluntary experimental contraction task of jaw-closing (Jaw), orbicularis-oris (O-oris), and orbicularis-oculi (O-oculi) muscles. Participants rated their perceived pain, unpleasantness, fatigue, and stress levels before, during, and after the contraction tasks on 0-10 Numeric Rating Scales (NRS). Each muscle contraction task (= one session) was separated by at least one week and the order of performed sessions was randomised in each participant. Data were analysed using repeated measurements ANOVAs. **Results:** All muscle contraction tasks evoked significant increases in NRS scores of pain, unpleasantness, fatigue, and stress ($P < .034$). Jaw contractions were associated with higher NRS scores compared to O-oris and O-oculi contractions ($P < .005$) without differences between O-oris and O-oculi ($P > .063$). All craniofacial muscle symptoms had disappeared within a day (P

> .469). **Conclusion:** The results showed that submaximal static contractions of different craniofacial muscle groups could evoke mild to moderate levels of transient symptoms. Further studies are warranted to better understand the contribution of specific craniofacial muscle groups for the characteristic presentation of musculoskeletal pain conditions in the head.

Introduction

Temporomandibular disorders (TMD) and tension-type headaches (TTH) are frequently encountered musculoskeletal pain conditions in the craniofacial region [1)] with overlapping clinical presentations and significant impact on quality of life. The craniofacial muscles are suspected to be involved in the pathophysiology and both peripheral and central sensitization as well as impaired endogenous inhibitory controls have implicated in the development and maintenance of the pain. Nevertheless, the triggering causes or event leading to such neuroplastic changes in the trigeminal and cervical parts of the nociceptive system have not yet been convincingly demonstrated.

Continued contraction of craniofacial muscles, e.g. the frontalis or temporalis muscles, have however been linked to TTH [2)]. The suggested aetiology of myofascial TMD pain in the jaw muscles has been that intense jaw-muscle activities leads to muscle fatigue and pain, which normally are seen in limb muscle during 24-48 hours after eccentric muscle contractions [3)], and has been well known as delayed onset muscle soreness (DOMS) [4, 5)]. Armstrong et al. (1991) suggested that eccentric muscle exercise damage and break down muscle fibres [6)]. Local muscle inflammation may occur,

causing sensitization of primary afferent nerve fibres [7, 8]. DOMS has also been considered as secondary to fatigue and pain, such as allodynia, hyperalgesia, and edema in limb muscles [9-11]. The DOMS theory has been applied to the jaw-closing muscles by the use of different types of experimental contractions performed in humans in order to manipulate myofascial pain in the jaw-muscles [4-6]. For tooth grinding, Christensen (1971) first investigated the effect of experimental tooth grinding on healthy individuals and reported long-lasting jaw-muscle pain [12]. Arima et al. (1999) made the first attempt to induce jaw-muscle pain by a standardized tooth-grinding exercise [13]. For tooth clenching, some studies have used short-time clenching at submaximal level of maximal-voluntary occlusal-bite force (MVOBF) [13-20]. Svensson et al. (1996) made the first attempt to evoke orofacial muscle pain by standardized experimental tooth clenching and reported that the tooth-clenching procedure failed to induce a progressive increase in masticatory muscle pain [14]. Some studies reported that low-level tooth-clenching tasks (≤ 10% MVOBF) do not produce long-lasting pain and fatigue [15, 19]. However, perceived levels of fatigue were significantly higher after low-level tooth-clenching tasks than after high-level tooth-clenching tasks (≥ 15% MVOBF) [14].

Takeuchi et al. recently reported that prolonged (two hours) and low-level (10% MVOBF) tooth-clenching evoked short-lived TMD-like pain, but the tasks could not induce longer lasting jaw-muscle or temporomandibular joint pain [21].

Furthermore, the International Classification of Headache Disorders (ICHD-III) and the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) have recently identified headache and facial pain attributed to TMD [1, 22]. It has also been reported that oral habits, such as teeth clenching, may be related to migraine and TTH [23].

Thus, a number of studies are published about the relationship that exists between orofacial pain and oral habits, however, to our knowledge, such information does not exist on activities of other craniofacial muscles like orbicularis-oris and orbicularis-oculi.

The aim of this study was, therefore, to investigate and compare, in a randomized and controlled cross-over clinical trial in healthy participants, the effects of continuous low-level contractions of various craniofacial muscles groups on subjective ratings of muscle symptoms. We hypothesized that (1) perceived levels of pain,

unpleasantness, fatigue, and stress increases after experimental craniofacial muscle contractions, and (2) the subjective symptoms from contractions of the jaw-closing, the orbicularis-oris, and the orbicularis-oculi muscles are different from each other.

Materials and Methods

Participants

Seven men (25.7 ± 1.4 years) and nine women (24.9 ± 2.3 years) participated in this study. All participants were healthy students or staff recruited from Aarhus University. Exclusion criteria were: (1) younger than 18 years of age; (2) less than 24 teeth in complete dental arches; (3) craniofacial pain complaints or other pain conditions, based in self-report; (4) systemic inflammatory connective tissue diseases e.g. rheumatoid arthritis; (5) consumption of analgesics e.g. paracetamol, non-steroidal anti-inflammatory drugs, salicylate drugs, or opioids, or other medication that would influence pain perception e.g. antidepressants and antiepileptic drugs; (6) pregnancy; and (7) severe skeletal malocclusions. All participants received both written and oral information about the experiment before they signed an informed consent document and they were monetary compensated for their participation.

Study Design

The participants performed 30 minutes (consisted of six 5-minutes bouts) of

standardized experimental muscle contractions of three different craniofacial muscle groups, i.e. jaw-closing (Jaw), orbicularis oris (O-oris), and orbicularis oculi (O-oculi) muscles. This study was single-blinded, and the order of the contraction sessions was randomized in each participant and the sessions were separated by at least one week (= wash out period). Before, during, and after the standardized experimental muscle contractions, the participants were asked to report their perceived levels of pain, unpleasantness, fatigue, and mental stress (Fig. 1). Eight out of the 16 participants were randomly selected and joined an additional fourth session as a control in which no contractions were performed (Control). All sessions were conducted at the laboratory of the Section of Orofacial Pain and Jaw Function, Department of Dentistry, Aarhus University, Denmark. All data were collected and analysed by the same female investigator (TI). The experimental protocol was approved by the Central Denmark Region Committees on Biomedical Research Ethics (No. 20110101) and was conducted in accordance with the World Medical Association Declaration of Helsinki.

Standardized experimental muscle contractions

First, maximal voluntary contraction force (MVCF) was measured with a force meter (7 mm high, 1.1 x 1.1 cm area, Aalborg University, Denmark [13, 24]). The same force meter was used for all three craniofacial muscle group contractions (Jaw, O-oris, and O-oculi). The force transducer had a 2 mm thick layer of self-curing acrylic resin. For the Jaw contraction task, participants held the centre of the force transducer between the first molars on the right side (Fig. 2: a1, a2). For the O-oris contraction task, participants held the force transducer coated with a customized silicone impression template (President putty, Coltene, Switzerland) that was shaped to fit the upper and lower lips and thereby minimized the forces from other craniofacial muscles (Fig. 2: b1, b2). All 16 participants used the same silicone template for this O-oris contraction task. The silicone template was disinfected with an alcohol prep pad and covered by cellophane wrap prior to use. For the O-oculi contraction task, the participants held the silicone coated force transducer that was customized individually to fit the right side eye orbit prior to the experimental session (Fig. 2: c1, c2). MVCF was performed for 2-3 seconds and the peak force level was assessed. The examiner verbally encouraged the participants to perform the maximum contraction. The recording was repeated for three

times and the values were averaged and set as the MVCF before the contraction task as a baseline [25]. The submaximal MVCF was then calculated and used for the standardized experimental muscle contraction tasks using visual feedback from the force transducer display to obtain the correct level of force. The contraction level was set at 20% [19, 20] of MVCF in accordance with several studies on e.g. jaw-muscle pain and symptoms. The operator continually encouraged the subjects to maintain the contraction force. The MVCF recordings were performed again immediately after the completion of the contraction task.

Perceived levels of pain, unpleasantness, fatigue and mental stress

Numeric Rating Scales (NRS), McGill Pain Questionnaire (MPQ) with Pain Rating Index (PRI), and Drawing of Perceived Pain Area were used for assessment of pain, unpleasantness, fatigue, and mental stress levels.

Numeric Rating Scales

Four 11-point NRS were administered to the participants at baseline, immediately after

each 5-minutes contraction task (at rest: period 1-6) and one (1 h), 24 (24 h), and 48 hours (48 h) after the last contraction task (Fig. 1). Levels of pain, unpleasantness, fatigue, and perceived mental stress intensity with 0 corresponding to "no pain / unpleasantness / fatigue / stress at all" and 10 to "the worst pain / unpleasantness / fatigue / stress imaginable" were obtained [26-28].

McGill Pain Questionnaire and Pain Rating Index

The participants were asked to fill out the MPQ to describe their pain sensation from a list of words, which fall into four groups: sensory, effective, evaluative, and miscellaneous [29]. A total PRI was subsequently calculated. Participants completed MPQ at baseline, period 6, 1 h, 24 h, and 48 h (Fig. 1).

Drawing of Perceived Pain Area

Participants marked the spatial extent of their perceived pain on an anatomical drawing of the head from the lateral and frontal view, the oral cavity (intraoral view) and a whole body perspective. Subsequently, all drawings were digitized (Sigma Scan Pro

4.01.003) to obtain a quantitative measure of the perceived pain area (arbitrary units) that included potential referred pain area [30, 31]. The drawings of the experimentally evoked pain areas were assessed at baseline, period 6, 1 h, 24 h, and 48 h (Fig. 1).

Statistical Analyses

NRS scores (absolute values of pain, unpleasantness, fatigue, and stress) were tested with the use of analyses of variance (ANOVAs) with session (Jaw, O-oris, O-oculi, and Control) and time (baseline, period 1-6, 1 h, 24 h, and 48 h) as repeated measurement factors. MPQ, total PRI, and Drawing of Perceived Pain Area were analysed with ANOVAs with session (Jaw, O-oris, O-oculi, and Control) and time (baseline, period 6, 1 h, 24 h, and 48 h) as repeated measurement factors. When appropriate, the Tukey HSD test was used for post-hoc analyses. Results are presented as mean ± standard error of the mean (SEM). Values of $P < .050$ were considered statistically significant.

Results

All participants completed the study and no side effects except the expected muscle symptoms were observed.

Maximal Voluntary Contraction Force

Table 1 shows the mean MVCF of Jaw, O-oris, and O-oculi muscles at baseline and period 6. The mean Jaw MVCF at baseline, 306.0 ± 33.3 N, was by far the highest force of the three muscles groups (baseline MVCF: O-oris: 6.9 ± 1.0 N and O-oculi: 1.1 ± 0.1 N). There were no major changes of the MVCF in any of the muscles groups by the experimental contractions ($P > .056$).

Numeric Rating Scales

The overall analysis showed that all pain, unpleasantness, fatigue, and stress NRS scores demonstrated significant main effects of both session (ANOVA: $P < .001$) and time (ANOVA: $P < .001$, Fig. 3).

In the Jaw session, NRS scores of pain, unpleasantness, fatigue, and stress

were increased at all time points between period 1 to 6 compared with baseline (Tukey: $P < .001$). In the O-oris session, NRS scores of pain at period 6 (Tukey: $P = .014$), NRS scores of unpleasantness and fatigue at period 4 to 6 (Tukey: $P < .014$), and stress at period 3 to 6 (Tukey: $P < .034$) were significantly increased at these time points compared with the control. In the O-oculi session, NRS scores of pain at period 4 to 6 (Tukey: $P < .001$), NRS scores of unpleasantness, fatigue, and stress at period 1 to 6 (Tukey: $P < .001$) were significantly increased at these time points compared with baseline. All symptoms disappeared within one hour after the experiment (Fig. 3).

Jaw contractions evoked significantly higher NRS pain, unpleasantness, fatigue, and stress scores at all time points between period 1 to 6 compared to O-oris (Tukey: $P < .001$). Jaw contractions also resulted in significantly higher NRS scores of pain (Tukey: $P < .001$), unpleasantness (Tukey: $P < .005$), and fatigue (Tukey: $P < .001$) at Period 1 to 6, and stress at period 3 compared to O-oculi (Tukey: $P = .005$). O-oculi contractions evoked higher NRS scores of stress at period 1 compared with O-oris (Tukey: $P = .027$), otherwise there were no differences in any of the other NRS scores at any time point from contractions of these two muscles groups (Tukey: P

> .063, Fig. 3).

McGill Pain Questionnaire and Pain Rating Index

The participants used several words from the McGill Pain Questionnaire (MPQ) to describe their perceived pain and muscle symptoms. The most frequently used words by at least 30% of the participants are presented in Table 2. The mean sensory, affective, evaluative, miscellaneous and total Pain Rating Index (PRI) scores are presented in Table 3. At baseline in all sessions and at 24 h and 48 h follow up in the O-oris and the O-oculi clenching sessions, none of the participants reported any pain. The mean total PRI score showed significant effects of both session (ANOVA: $P = .029$) and time (ANOVA: $P < .001$). A post-hoc analysis revealed that the mean total PRI score in the Jaw and the O-oculi sessions were significantly higher than in the control session (Tukey: $P < .001$).

Drawing of Perceived Pain Area

When the participants were asked to draw the spatial extent of the perceived pain, the

pain occurred on both sides of the face from a lateral, intraoral, and frontal perspective (Fig. 4 and Table 4). In the Jaw session, 14 out of 16 participants reported pain on the lateral aspect of the face at period 6. There were significant main effect of session and time (ANOVA: $P < .001$). In the O-oris and the O-oculi sessions, 11 of 16 participants reported pain on the lateral aspect of the face at period 6. The post-hoc analysis revealed that period 6 on the lateral aspect of the face in the Jaw session, the average pain drawing area was significantly larger than the control session (Tukey: $P < .001$). There was no main effect of session (ANOVA: $P > .099$), but a significant main effect of time (ANOVA: $P < .004$) on the frontal pain drawings. The post-hoc analysis revealed that period 6 on the frontal of the face in the O-oris session, the average pain drawing area was significantly larger than the control session (Tukey: $P < .001$). The pain distribution assessed on the drawings differed markedly in terms of location without any significant overlaps (Fig. 4). At baseline in all sessions and at 24 h and 48 h follow up in the O-oris and the O-oculi clenching sessions, none of the participants reported any pain.

Headache occurred in 6 out of 16 participants in the Jaw session, in 1 out of

16 participants in the O-oris session, and in 5 out of 16 participants in the O-oculi session (Fig. 4), however, it disappeared within one hour after a session end except for one participant (in a Jaw session).

Discussions

The primary findings of this study were that (1) low-level and continuous contractions of jaw-closing, orbicularis-oris, and orbicularis-oculi muscle groups caused pain, unpleasantness, fatigue, and stress symptoms and (2) jaw-closing muscle contractions caused more intense symptoms than that of orbicularis-oris or orbicularis-oculi contractions.

The mean maximum voluntary contraction force of jaw-closing muscles at baseline 306.0 ± 33.3 N was by far the highest force in these muscle groups (for orbicularis-oris: 6.9 ± 1.0 N and for orbicularis-oculi: 1.1 ± 0.1 N). This is the first report of the maximum force of voluntary contraction from both orbicularis-oris and -oculi muscle groups since these facial expression muscles do not act on a lever or tendon and there might be individual variation in the ability to produce a strong contraction. Thus, it might be better to pronounce that this study is firstly reporting the "approximate" maximum voluntary contraction force of orbicularis oris and oculi muscle groups.

However, this study needed to estimate the maximum voluntary contraction

forces of orofacial expression muscles in order to set the certain standardized clenching levels for the experimental muscle pain / fatigue. The orbicularis oculi and oris muscles consist mostly of fast-twitch type II fibres [32-34], while the major of masseter muscle is slow-twitch type I [35]. The fast-twitch type II fibres normally make speedy and fine contractions as compared to the slow twitches. The low-level and continuous contractions were adopted in this study to produce "low-frequency fatigue", which can typically be seen in limb muscles by the activities of weight lifting and long-distance running. The results showed that pain, unpleasantness, fatigue, and stress symptoms appeared after the experimental contractions but the symptoms disappeared within 24 hours after the exercises in all muscles without decreased force output (Table 1). This means that the current experimental muscle exercises did not cause either "metabolic fatigue" or "low-frequency fatigue". It is suggestive that, as mentioned, jaw-closing muscles are originally fatigue resistant (mostly slow twitch type I), and it is an only probability that orbicularis oris and oculi muscles (fast twitch type IIB) could be converted to fatigue resistant (fast twitch type IIA) by the normal and daily facial appearance muscle contractions for many lifetime years. It could also be suggested that

the other factor may play an important role in aetiology of masticatory-muscle pain and headache evoking “central fatigue”. An interesting finding in the present study was that some subjects reported pain not localized around the contraction region. One possibility is that the experimental muscle contractions in the present study were “jaw-closing”, “orbicularis oris”, and “orbicularis oculi”, and these muscles work in conjunction with other muscles, i.e. craniofacial muscle “groups”. Thus, the experimental craniofacial muscle group contractions evoked pain in both the area of contraction and the other locations.

In conclusion, this study suggested that submaximal static contractions of different craniofacial muscle groups could evoke mild to moderate levels of symptoms but the symptoms short lived. Further studies are warranted to better understand the contribution of specific craniofacial muscle groups for the characteristic presentation of various musculoskeletal pain conditions such as in myofascial TMD patients and TTH.

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Table 1 Maximal Voluntary Contraction Force (MVCF) of three muscles at baseline and after the contraction tasks.

MVCF (Mean ± SEM) (N)		
	Baseline	Period 6
Jaw	305.6 ± 33.6	313.4 ± 39.3
O-oris	6.7 ± 0.5	6.5 ± 0.6
O-oculi	1.1 ± 0.1	1.3 ± 0.2

Table 2 The most frequently used words on the McGill Pain Questionnaire (MPQ)

The Most frequently Used Words on the MPQ (%)								
	Jaw				O-oris		O-oculi	
Words	Period 6	1 h	24 h	48 h	Period 6	1 h	Period 6	1 h
QUIVERING	31.3 *	6.3	0.0	0.0	12.5	6.3	18.8	6.3
PRESSING	25.0	31.3*	0.0	0.0	18.8	0.0	37.5*	0.0
DULL	31.3*	12.5	6.3	6.3	6.3	6.3	18.8	0.0
ANNOYING	8.0	0.0	0.0	0.0	6.3	0.0	31.3*	0.0

Table 3 Pain Rating Score (PRI) after the experimental contraction sessions.

PRI after the experimental contraction (Mean ± SEM)								
	Jaw				O-oris		O-oculi	
	Period 6	1 h	24 h	48 h	Period 6	1 h	Period 6	1 h
Sensory	6.6 ± 1.7	2.4 ± 1.0	0.3 ± 0.2	0.1 ± 0.1	4.2 ± 1.3	0.4 ± 0.2	3.5 ± 1.2	0.3 ± 0.2
Affective	1.2 ± 0.5	0.4 ± 0.3	0.1 ± 0.1	0.0 ± 0.0	0.4 ± 0.4	0.1 ± 0.1	0.4 ± 0.3	0.0 ± 0.0
Evaluative	1.3 ± 0.4	0.3 ± 0.2	0.1 ± 0.1	0.0 ± 0.0	0.4 ± 0.2	0.0 ± 0.0	0.6 ± 0.3	0.0 ± 0.0
Miscellaneous	2.1 ± 0.8	0.3 ± 0.2	0.0 ± 0.0	0.1 ± 0.1	0.7 ± 0.4	0.0 ± 0.0	1.0 ± 0.4	0.3 ± 0.2
Total Score	11.1 ± 3.1*	3.4 ± 1.6	0.6 ± 0.4	0.2 ± 0.2	5.8 ± 2.1	0.4 ± 0.2	5.6 ± 1.8*	0.6 ± 0.4

Table 4 Drawing of Perceived Pain Area

Area of Pain Drawing (Mean ± SEM) (mm^2)									
	Jaw			O-oris			O-oculi		
	Lateral	Intraoral	Frontal	Lateral	Intraoral	Frontal	Lateral	Intraoral	Frontal
Period 6	91.9 ± 21.1*	26.8 ± 13.6	4.2 ± 4.0	22.4 ± 7.9	22.4 ± 11.3	9.5 ± 4.5*	34.3 ± 10.0	6.0 ± 4.2	13.0 ± 4.8
1 h	13.4 ± 5.6	5.1 ± 4.9	5.8 ± 5.6	5.9 ± 3.2	0.7 ± 0.7	1.1 ± 0.8	4.9 ± 4.4	0.0 ± 0.0	0.6 ± 0.5
24 h	12.2 ± 10.3	0.3 ± 0.2	0.0 ± 0.0	5.0 ± 4.9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
48 h	0.8 ± 0.8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Fig. 1 Experimental design.

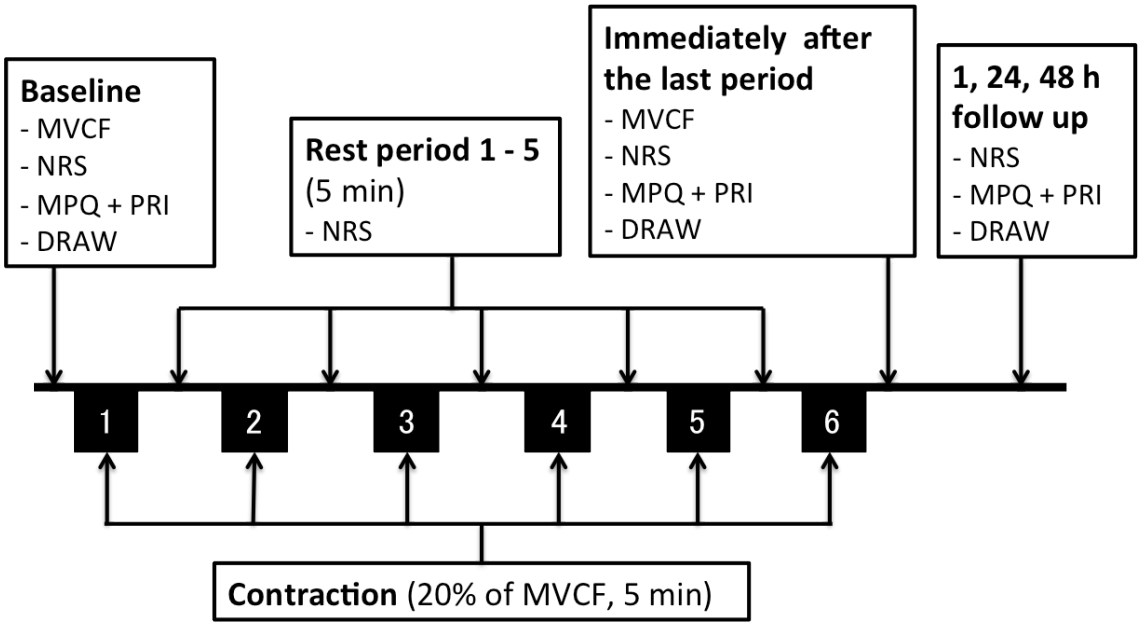

Fig. 2 The force transducer

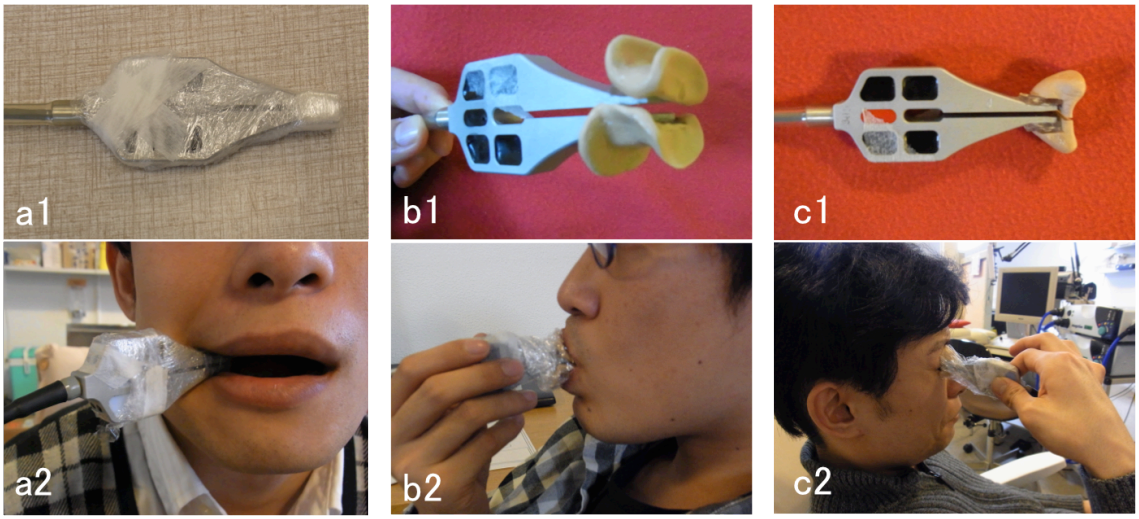

Fig. 3 Mean values of the subject-based scores on Numeric Rating Scales (NRS) (pain, unpleasantness, fatigue, and stress) from the three experimental contraction sessions.

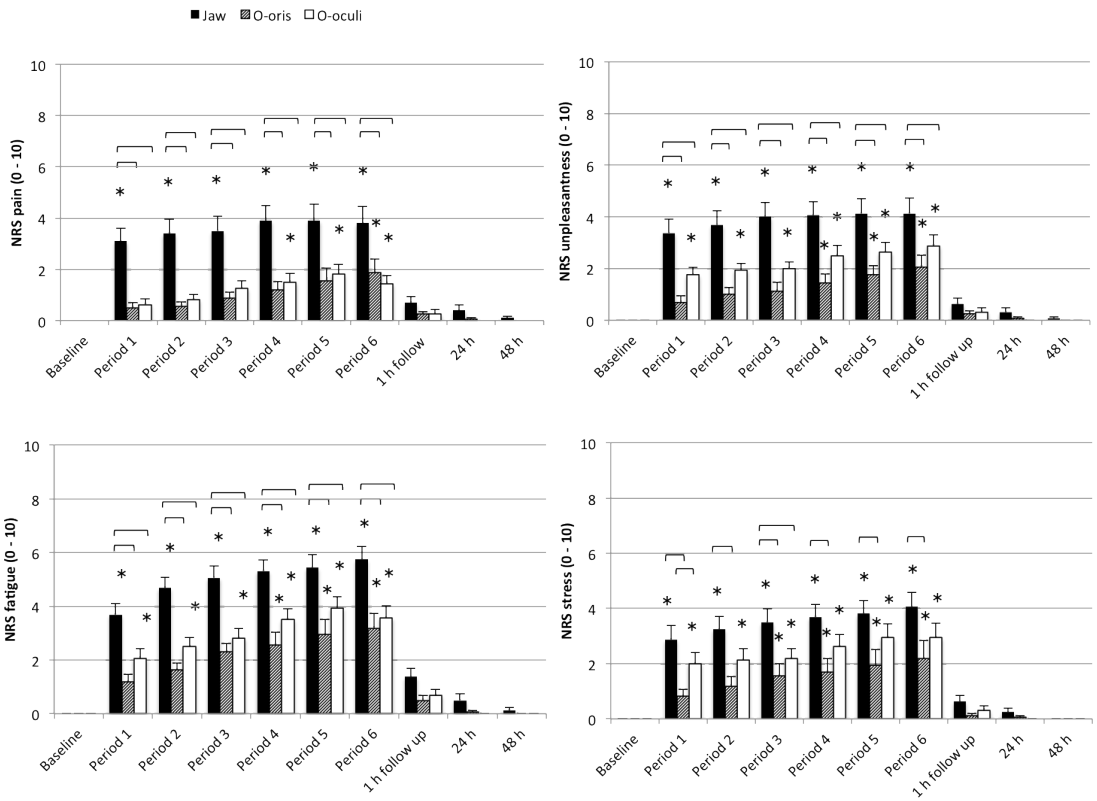

Fig. 4 Illustrations of the patient-based drawings of the spatial extent of the perceived pain from the three contraction sessions.

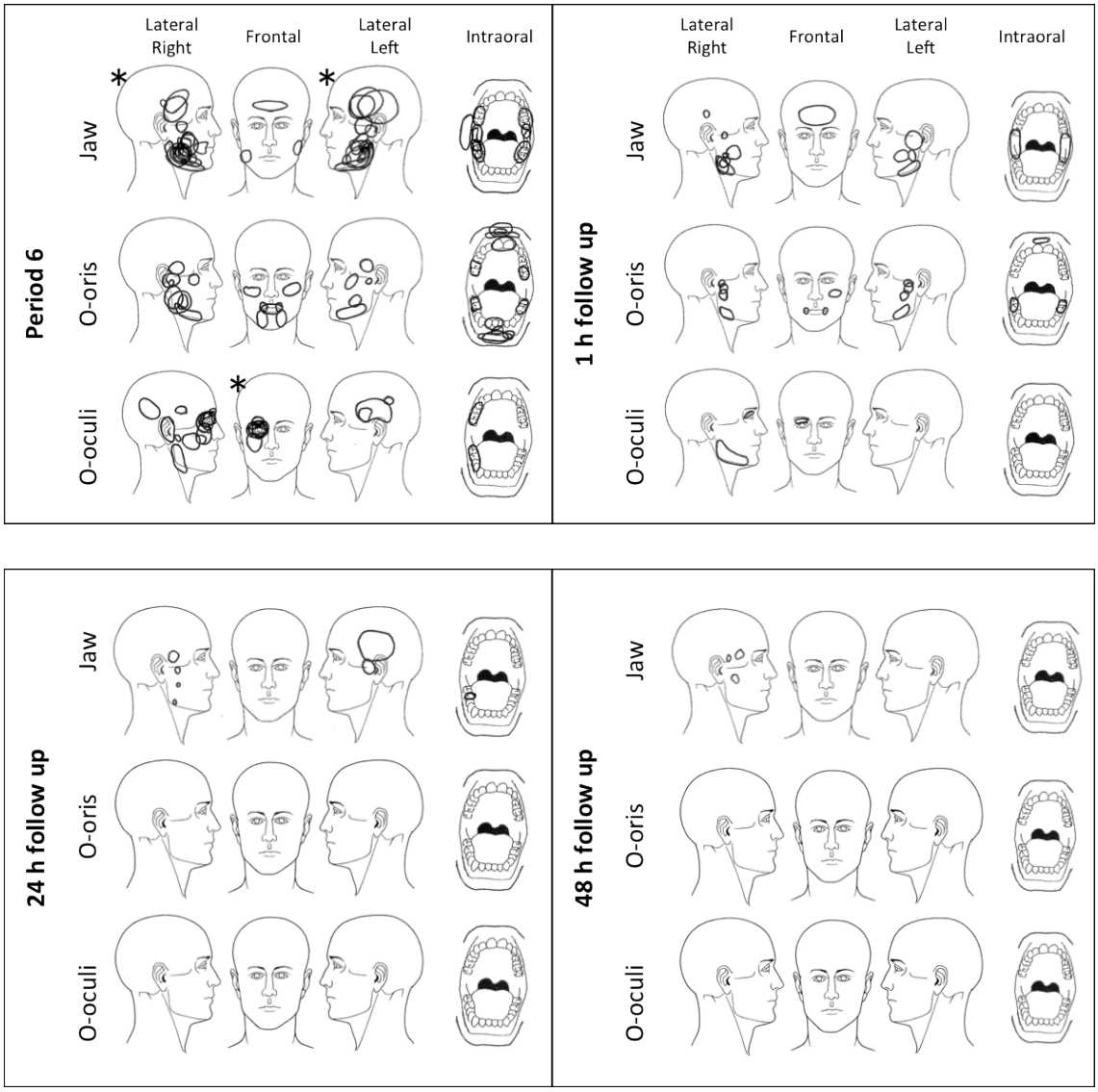

Table 1 The mean of maximal voluntary contraction force (MVCF) in the jaw-closing (Jaw), the orbicularis oris (O-oris), and the orbicularis oculi (O-oculi) sessions. MVCF were measured at baseline and immediately after the experimental contractions (Period 6).

Table 2 The numbers with * are words used by at least 30% of the 16 participants to describe their pain. Jaw = jaw-closing muscles, O-oris = orbicularis oris, O-oculi = orbicularis oculi. At baseline in all sessions and at 24 h and 48 h follow up in the O-oris and the O-oculi clenching sessions, none of the participants reported any pain.

Table 3 At baseline in all sessions and at 24 h and 48 h follow up of the orbicularis oris (O-oris) and the orbicularis oculi (O-oculi) clenching sessions, none of the participants reported any pain. * Significantly different from control.

Table 4 Subjective descriptions of the pain extent on an anatomical drawing from a lateral, intraoral, and frontal perspective. There were no pain reports at baseline in any of the sessions and at 24 h and 48 h follow up of the orbicularis oris (O-oris) and the orbicularis oculi (O-oculi) clenching sessions. (n = 16). * Significantly different from

control.

Fig. 1 Experimental design. Black boxes = Six bouts of five minutes contraction task at 20% maximal voluntary contraction force (MVCF); NRS = Numeric Rating Scales; MPQ = McGill Pain Questionnaire; PRI = Pain Rating Index; and DRAW = Drawing of perceived pain area.

Fig. 2 (a1) A force transducer for the Jaw contraction task. (a2) The participants held the force transducer between the first molars on the right side. (b1) A force transducer for the O-oris task. (b2) The participants held the force transducer coated with a customized silicone impression template between the upper and lower lips. (c1) A force transducer for the O-oculi task. (c2) the participants held the silicone coated force transducer that was customized individually to fit the right side eye orbit prior to the experimental session.

Fig. 3 Mean (± SEM) values of the subject-based scores on Numeric Rating Scales (NRS) (pain, unpleasantness, fatigue, and stress) from the three experimental

contraction sessions (jaw-closing (Jaw), orbicularis oris (O-oris), and orbicularis oculi (O-oculi)). * = $P < .050$ significant different from control; ¬ = $P < .050$ significant different between sessions; Period 6 = immediately after the last contraction task1 h = one hour, 24 h = 24 hours, and 48 h = 24 hours after the last experimental contraction in a session.

Fig. 4 Illustrations of the patient-based drawings of the spatial extent of the perceived pain from the three contraction sessions (jaw-closing (Jaw), orbicularis oris (O-oris), and orbicularis oculi (O-oculi)). * at the upper left of the drawing = $P < .050$ significant different from control; Period 6 = immediately after the last contraction task1 h = one hour, 24 h = 24 hours, and 48 h = 48 hours after the last experimental contraction in a session.