# Delayed-onset muscle soreness does not influence occlusal sensitivity and position sense of the mandible

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SUMMARY Masticatory muscle-pain patients often complain about sensorimotor changes, but the effects of pain on the psychophysical properties remain unclear. This study aimed to investigate the effects of delayed-onset muscle soreness (DOMS) on the jaw's position sense (PS) and occlusal sensitivity (OS). In all, 12 participants underwent intense concentric–eccentric jaw exercises. Self-reported muscle fatigue and pain, pain-free maximum mouth opening (MMO), pain pressure thresholds (PPTs) at right and left masseter and right and left anterior temporalis, maximum voluntary bite force (MVBF), PS and OS were recorded before, immediately after, 24 h, 48 h and 1 week after the exercises. Data were analysed

with repeated measures ANOVA. Pain and fatigue increased significantly after the exercises, while fatigue also increased 24 h afterwards. Time and site had a significant effect for PPTs, not for MVBF. MMO decreased significantly 24 h after the exercises. OS and PS did not change significantly. Experimentally induced DOMS does not influence the psychophysical properties of the masticatory system.

KEYWORDS: exercise, masticatory muscles, facial pain, muscle contraction, myofascial pain syndromes, proprioception

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# Introduction

The motor system has the function to organise and coordinate the activities of individual muscles to generate fine movements. The activity of this system involves a complex hierarchical structure, including the cerebral motor cortex, the cerebellum and the basal ganglia as well as the brain stem and the spinal cord. The motor neurons, which synapse on and activate muscle fibres, are at the lowest level of the hierarchy of signal integration (1). This system of feedforward and feedback information processing has a crucial role in the execution of behaviours, which are essential for the adaptation and the survival of humans. In particular, oral motor behaviours are a fundamental component of food-seeking, mastication and vocalisation (2). During the execution of a movement, peripheral proprioceptive mechanical, chemical and thermal feedback originating from sense organs are continuously used to monitor the accuracy and adjust the precision of a movement (3). For instance, during mastication, jaw movements might require large, rapid and unpredictable changes, due to the sudden change of food consistency and the narrow margin of safety from damage (4).

Human periodontal membrane receptors provide sensory feedback regarding tooth contact during function and the presence of fine objects between antagonist teeth (3, 5). This task, which goes under the name of occlusal tactile perception, plays a major role in the regulation of the occlusal bite forces and in the control of jaw movements, especially in the jaw-opening reflex (6, 7). The occlusal tactile perception ability can be tested by assessing the minimum interdental threshold ability [or occlusal sensitivity (OS)], that is the minimal thickness that can be detected between the teeth during maximum intercuspation (7). In natural dentate humans, the OS has been measured to be between 8 and 60  $\mu$ m (8), while after the application of local anaesthesia a substantial increase in the threshold has been observed (8). However, in patients lacking periodontal receptors, such as those with dental implants or full dentures, the OS was altered but not totally lost (3, 8, 9), suggesting that the occlusal tactile perception is based on a complex transmission process in which periodontal receptors are not the only contributors. Also temporomandibular joint capsule receptors (10), muscle receptors (muscle spindles) and pulpal receptors seem to be involved in the determination of the minimum interdental threshold, but the contribution of the single signals is still unclear (3).

On the other hand, non-periodontal receptors, and in particular muscle spindles, seem to have a dominant role in the macro-thickness discrimination, that is interdental thickness discrimination ability of the mouth at larger inter-incisal distances (3, 4). The interdental dimension discrimination is a measure of mandibular proprioception, and in turn it deals both with the position sense (PS), that is the sense of a stationary position, and the kinaesthetic perception, that is the sense of a movement (11). Both senses share inputs from the same mechanoreceptors. More specifically, the PS of the mandible is defined as the ability to perceive or to produce a predetermined mandibular posture repetitively (9) and it involves an individual's ability to perceive the position of a part of the body without the aid of vision.

If muscle receptors, especially muscle spindles, are mainly responsible for the PS, muscle damage resulting from intense muscle activity is likely to affect this ability. To gain insight into the mechanisms of muscle activity and its relation to the PS, several experimental studies have been conducted performing highintensity eccentric contraction in limb muscles. It has been proved that strenuous bouts of eccentric exercises can determine a sensation of discomfort and stiffness in the muscles, commonly described as a sense of tautness or a dull pain, that usually lasts for several days after the muscular activity, and is described as Delayed-Onset Muscle Soreness (DOMS) (12). It is generally agreed that DOMS is the result of small microscopic damage in the muscle fibres although the precise mechanisms responsible for this phenomenon remains unclear. In fact, two possible conditions have been proposed as being responsible for muscle fibre damage: damage to the excitation–contraction coupling system and disruption of the sar-comeres (12–14). Changes in the perception of muscle force and in the perceived position and movement of the limbs during DOMS experience have been reported at the elbow flexors, elbow extensors and quadriceps muscles (15–18).

Since patients suffering from pain in the orofacial region frequently complain about unpleasant sensations associated with disturbance in the somatosensory function (19-21), a relation between this disturbance and the effect of the pain on their proprioceptive system can be hypothesised. Previous papers reported decreased jaw PS in subjects presenting unilateral temporomandibular joint dysfunction (22), and in subjects prone to develop muscle fatigue (9). However, since several confounding factors can affect studies in myofascial pain patients, experimental pain models have been proposed to study the functional consequences of masticatory muscle pain (19). Recently, an experimental model involving intense eccentric-concentric contractions, able to induce DOMS in the jaw-closing muscles, has been developed (23). In this experimental setting, the transient diagnosis of myofascial pain according to the RDC/ TMD in healthy individuals was set (24). Therefore, this model offers the possibility to study the behaviour of the masticatory system under experimentally induced muscle pain.

The aim of the present study was, therefore, to investigate the effects of experimental DOMS on the psychophysical properties of the masticatory system, focusing on the PS of the jaw and the OS.

The hypothesis was that experimentally induced DOMS would negatively influence the PS, resulting into increased position-matching errors, while the OS would remain unchanged.

# Materials and methods

#### Participants

In all, 12 healthy participants (five males and seven females, mean age  $\pm$  SD = 26.8  $\pm$  5.5 years) agreed

to participate in the study after receiving detailed information about the procedure. The participants were all free of orofacial pain and temporomandibular pain complaints. Participants with reduced anterior or posterior overbite, on-going orthodontic treatment, use of pain-killers or medications active on the nervous system, and presence of prosthetic crowns and/ or endodontic treatments of the first permanent molars were excluded from the study sample. The protocol was approved by the review board of the Netherlands Institute for Dental Sciences, and written informed consent was obtained from all participants.

#### Provocation part

The provocation part (PP) used in the current study was described in detail in previous publications (23, 24). Briefly, first the maximum electromyographic (EMG) activity of the right masseter (RM) muscle was recorded with the use of bipolar surface electrodes. The PP included six exercise sets of 5-min-long bouts of concentric-eccentric contractions, with 1 min of rest in between (total time for the PP: 35 min). During each bout, the participants were biting with their anterior teeth on one edge of a custom-made jaw muscle stretcher (Fig. 1), with their teeth protected by an acrylic upper and lower mouth guard\*. The participants continuously received visual feedback of the root mean squared (RMS) values of 15% of their maximum EMG activity, and were instructed to keep their muscle activity as constant as possible during the open-close cycles.

When the apparatus was opened, the participant's mouth was gently forced to open, allowing the jawclosing muscles to contract while being elongated, thus contracting eccentrically. When the experimenter closed the apparatus, the masticatory muscles contracted concentrically.

#### Protocol overview and data collection

All data were collected by two operators (R.B. and M.K.), following exactly the same protocol. Data regarding self-reported level of pain and fatigue, Pain Pressure Thresholds (PPTs), Maximum Voluntary Bite



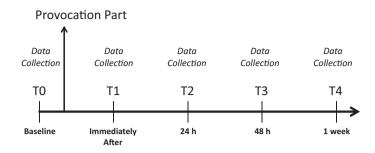
**Fig. 1.** Custom-made muscle stretcher apparatus. When the experimenter opens the apparatus, participant's mouth is gently forced open, while keeping a constant contraction of the jaw-closing muscles (*eccentric contraction*) (Appendix S1).

Force (MVBF), pain-free Maximum Mouth Opening (MMO), Occlusal Sensitivity (OS) and Position Sense of the jaw (PS) were collected at the baseline (TO), immediately after the PP (T1), 24 h (T2), 48 h (T3) and 1 week afterwards (T4). The protocol overview is described in detail in Fig. 2.

*Pain and fatigue.* The self-reported levels of pain and fatigue were recorded using 100 mm Visual Analogue Scales (VAS), with left anchor words 'No fatigue/pain at all' and right anchor words 'Fatigue/pain as bad as it could be'.

Pain pressure threshold. Pain pressure threshold is defined as the amount of pressure (kPa) necessary for a participant to experience pain (25). Two different algometers were used based on availability: a custommade electronic algometer and a hand algometer. The two operators have been previously trained for the use of both devices. Both devices presented a tip of 1 cm diameter, and the hand algometer was used at the beginning of the study to calibrate the electronic device. For the PPT measurements, the participants sat on a dental chair and were asked to relax with their mandible in rest without performing any jaw contractions. A pressure increase of 30 N  $s^{-1}$  was set, with a visual feedback on the computer screen during the electronic registration. The pressure was applied with the tip of the algometer perpendicular to the skin and with the participant's head held by a counter-pressure from the hand of the operator. The participants indicated the threshold level by pressing a

<sup>\*</sup>Bioplast, Ref 3188.1, 4-0  $\times$  125 mm, clear, Scheu Dental Technology, Iserlohn, Germany.



**Fig. 2.** Overview of the protocol and time points for data collection. Each data collection comprises the following: self-reported levels of pain and fatigue, pain pressure thresholds (PPTs), maximum voluntary bite force (MVBF), maximum mouth opening without pain (MMO), occlusal sensitivity (OS) and position sense of the mandible (PS).

push-button (with the electronic algometer) or by raising their hand (with the manual algometer). The records were taken from four muscle sites (right masseter: RM, left masseter: LM, right anterior temporalis: RAT and left anterior temporalis: LAT). As previously described (26), for the masseter muscle, the site was located over the most bulky part of the muscle, as determined by palpation during voluntary contraction, while for the temporalis muscle it was located on the line between the upper orbital margin to the upper point of the outer ear, 2 cm behind the anterior border of the muscle. This border was determined by palpation during forceful voluntary contraction. At each site, 4 PPT recordings were randomly made. As the first PPT value measured is commonly higher than the followings (27), the mean values of the last three recordings were used for the analysis of the data.

*Maximum voluntary bite force.* The MVBF was measured unilaterally using a force-transducer<sup>†</sup>. The transducer was placed on the mesial cusp of the lower first molar of the preferred chewing side, as reported by the participant. When a preferred chewing side was not mentioned, the left side was chosen. The participants sat on a dental chair and were verbally encouraged to bite on the transducer as hard as possible for 3 s. Three recordings were made, with 1 min of interval in between. The highest value of the three records was used for the data analysis.

*Maximum mouth opening without pain.* The participants were asked to open their mouth as wide as possible without experiencing any pain. The distance between

the incisal edges of the upper and lower central incisors was measured (in mm) with a plastic ruler. The overbite measure (in mm) was added for the final measurement.

*Position sense of the mandible.* In all, 10 wooden biting plates with thicknesses ranging from 1 to 10 mm (increment 1 mm) were used during the measurements for the PS. First, the participants were asked to familiarise with a reference biting plate of 5.5 mm thickness for 5 min, while biting it with their front teeth (Fig. 3a). Afterwards, each of the testing biting plates was presented to the subjects for 10 times in a random order (hence 100 tests in total), and the participant was asked whether the biting plate was felt 'thicker' of 'thinner' than the reference. During all the experiment, the participants were asked to keep their eyes closed and the experimenter recorded the participants' answers.

Occlusal sensitivity. In all, 10 different thicknesses were tested: nine aluminium foils ranging from 12  $\mu$ m to 108  $\mu$ m and one sham test without any foil. The testing thicknesses were put at the area of the first permanent molars, preferably in correspondence of the mesio-labial cusp, and presented 10 times in random order (hence 100 tests in total). The participants were asked whether or not they felt the aluminium foil between their teeth. To avoid any additional information influencing the measurements, the cheek mucosa was retracted with a mouth mirror and headphones with white noise were used to mask any noises of the foils (Fig. 3b). Again, during the experiment, the participants were asked to keep their eyes closed and the experimenter recorded the participants' answers.

<sup>&</sup>lt;sup>†</sup>GM-10 Occlusal Force Meter; Nagano Keiki, Tokyo, Japan.



**Fig. 3.** (a) Recording of the *position sense of the jaw* (PS): participant biting a reference wooden bite plate (thickness: 5-5 mm) with her anterior teeth, while keeping her eyes closed. (b) Recording of the *occlusal sensitivity* (OS): the operator is presenting one testing aluminium foil to the participant while she is hearing white noise and keeping her eyes closed (Appendix S1).

#### Statistical analysis

For the analysis of the PS and the OS, a custom-made macro for the Rosin–Rammler's model was used to generate curve-plots, for each participant at each time point, based on the number of 'thicker than the reference' answers (an example of the curve-plot is given in Figure S1). From each curve-plot, data regarding the mean testing thickness detected as 'thicker than the reference' for at least the 50% of times (X50) and the maximum slope of the curve (MAXRR) were collected.

The Statistical Package for the Social Sciences<sup>‡</sup> was used for data analysis. Analyses of variance (ANOVA) for repeated measures with pairwise comparisons adjusted with Sidak test were used for the analysis of the VAS pain and fatigue, PPT, MVBF, MMO, PS (X50 and MAXRR) and OS (X50 and MAXRR). In the pairwise comparisons, the variables were compared with the baseline values. For all the variables, 'time' (with five levels: T0–T4) was set as withinsubject factor. For the PPTs, a further within-subject factor ('site', four levels: RM, LM, RAT and LAT) was set. Statistical significant difference was set at  $\alpha < 0.05$ .

Sample size was calculated using G-Power software analysis assuming an ANOVA repeated measure test with five time points and a significant level of 0.05. A sample size of 12 participants achieves 80% power to detect a difference in terms of effect size of 0.34.

#### Results

Descriptive statistics of all variables and the results of the ANOVA and of the Sidak *post-hoc* are shown in Table 1.

- **1** *VAS pain and fatigue:* Self-reported pain was increased immediately after the PP, but overall no statistically significant changes were found (P = 0.059) across time. Fatigue was found to be significantly changed over time (P < 0.001). In the pairwise comparisons, VAS scores for fatigue were significantly increased at both T1 and T2 (immediately after and 24 h after) as compared to T0.
- **2** *PPTs:* Due to mechanical problems related to the electronic device, the data collected from one subjects were excluded from the analysis. Statistically significant effects of time (P < 0.001) and site (P < 0.001) were found. In the *post-hoc* tests, a statistically significant increase was found at T4 (after 1 week) compared to the baseline values.
- **3** *MVBF:* No statistically significant effect of time was found regarding the MVBF (0.490).
- **4** *MMO:* Changes in MMO were found statistically significant over time (P < 0.001). In the pairwise comparison with the baseline, a significant reduction of the opening range 24 h after the PP (T2) was present (P < 0.005).
- **5** *PS* and *OS*: Both PS and OS did not show any statistical significant changes throughout the entire experiment, concerning both the average thickness and the maximum slope (PS\_X50: P = 0.947; PS\_MAXRR: P = 0.346; OS\_X50: P = 0.722; OS\_MAXRR: P = 0.284).

<sup>&</sup>lt;sup>‡</sup>version 21.0, SPSS Inc., Chicago, IL, USA.

	Baseline (T0)	Immediately after (T1)	After 24 h (T2)	After 48 h (T3)	After 1 week (T4)	Р
VAS pain (mm)	$2.58 \pm 2.24$	$11.50 \pm 6.10$	$7.83 \pm 3.58$	$6.08 \pm 3.80$	$1.50 \pm 1.33$	0.059
VAS fatigue (mm)	$4{\cdot}92\pm2{\cdot}96^{a.b}$	$36{\cdot}00\pm7{\cdot}79^a$	$20{\cdot}67\pm5{\cdot}33^b$	$13{\cdot}42\pm6{\cdot}78$	$4{\cdot}25\pm2{\cdot}19$	<0.001
PPT LM (kPa)	$205{\cdot}81\pm21{\cdot}10^a$	$196.39 \pm 17.63$	$196{\cdot}49\pm19{\cdot}73$	$210{\cdot}50\pm23{\cdot}93^a$	$268{\cdot}89\pm26{\cdot}12^a$	<0.001
PPT RM (kPa)	$204{\cdot}06\pm22{\cdot}07^a$	$200.76 \pm 15.56$	$215{\cdot}16\pm24{\cdot}76$	$225{\cdot}81\pm21{\cdot}28^a$	$256{\cdot}93\pm26{\cdot}34^{a}$	<0.001
PPT LAT (kPa)	$259.33 \pm 30.57^{a}$	$250.24 \pm 27.87$	$257{\cdot}26\pm28{\cdot}83$	$279.32 \pm 33.75$	$317{\cdot}74 \pm 29{\cdot}23^a$	<0.001
PPT RAT (kPa)	$231{\cdot}28\pm26{\cdot}83^a$	$233{\cdot}22\pm24{\cdot}42$	$224{\cdot}05\pm31{\cdot}71$	$261{\cdot}21\pm32{\cdot}40$	$301.89 \pm 34.77^{a}$	<0.005
MMO (mm)	$46{\cdot}09\pm2{\cdot}46^a$	$44{\cdot}82\pm2{\cdot}82$	$39{\cdot}83\pm3{\cdot}05^a$	$42{\cdot}42~\pm~3{\cdot}11$	$47{\cdot}25\pm2{\cdot}36$	<0.001
MVBF (kN)	$0.56 \pm 0.07$	$0.54 \pm 0.06$	$0.56 \pm 0.06$	$0.55 \pm 0.06$	$0.59 \pm 0.06$	0.490
PS_X50	$6.50 \pm 1.40$	$6.56 \pm 1.38$	$6.65 \pm 1.40$	$6.59 \pm 1.40$	$6.55 \pm 1.42$	0.947
PS_MAXRR	$0.73 \pm 0.46$	$0.72 \pm 0.42$	$0.66 \pm 0.42$	$0.90 \pm 0.46$	$0.67 \pm 0.40$	0.346
OS_X50	$1.98 \pm 0.27$	$2.02 \pm 0.28$	$2.01 \pm 0.32$	$2.04 \pm 0.32$	$1.80 \pm 0.29$	0.722
OS_MAXRR	$0.48 \pm 0.07$	$0.76 \pm 0.22$	$0.89 \pm 0.27$	$0.49 \pm 0.08$	$0.52 \pm 0.07$	0.284

Table 1. Descriptive statistics of the variables analysed and results of the ANOVA for repeated measures

Data are reported as mean  $\pm$  SE.

<sup>a,b</sup>same letter indicates statistically significant difference in the *post-hoc* comparison between time points.

## Discussion

The present study aimed to evaluate the effect of DOMS of the jaw-closing muscles, induced by a series of intense eccentric–concentric contractions on the psychophysical properties of the masticatory system, particularly the OS and the PS. The results, in contrast with the hypothesis, revealed that none of these variables showed a statistically significant change throughout the experiment.

Healthy young volunteers free from orofacial and/ or temporomandibular pain complaints underwent the experimental exercises. Participants with reduced overbite were excluded as the lack of dental interdigitation in maximal intercuspation could have affected the assessment of the OS and PS. Three participants taking part in the current study reported subjective history of sleep bruxism, and this might have potentially influenced the results, since lower level of pain and fatigue after the exercises can be expected. Nevertheless, controversial results are reported in literature, with some previous researches suggesting that the OS of bruxers was lower than that of non-bruxers due to the excessive occlusal force for prolonged periods (28, 29), and one more recent study reporting no significant difference in OS between bruxers and controls (6).

In previous studies assessing the PS of the mandible (30–32), the reference and testing biting blocks of various thicknesses were presented as a pair during the whole experimental session, while in the current study the subjects were asked to familiarise with the reference stick only at the beginning of each session. It can be argued that a gradual increase in position-matching errors could be found throughout the session, due to the fact that participants might progressively forget the initial reference position. For this reason, considering that each experimental session is composed of 100 answers, the first 50 answers were compared to the last 50 ones, revealing no differences in the interdental dimension discrimination ability and thus rejecting the hypothesis that this could have negatively influenced the results.

In the present study, several variables (VAS, MMO, MVBF and PPT) were recorded to assess the successful provocation of DOMS in the jaw-closing muscles. Immediate and delayed effects were recorded regarding the self-reported level of pain and fatigue and the pain-free maximum mouth opening. The symptoms evoked immediately after the provocation part are probably the result of an accumulation of metabolites. The delayed response resembles the set of symptoms previously attributed to the DOMS phenomenon, such as fall in force, decreased range of motion, pain at rest and the muscle being painful to palpation (24). However, a slighter pain effect was present in the examined sample, as compared to previous studies with the same or similar methodology (23, 24). The low-mild level of pain can be attributed to the high inter-individual variability in pain perception (33).

Interesting results were found concerning the PPTs, as the thresholds were not significantly reduced during the experimental protocol and the values did not show return at the baseline after 1 week, but instead resulted in significant increase. These results are in accordance with a previous study in which with low-load exercises of wrist extensors revealed reduced mechanical sensitivity of deep tissues (34).

The findings of the present study concerning the effects of a slight DOMS on the psychophysical variables of the masticatory system were in contrast with the hypothesis that during the DOMS experience the PS would be impaired. The hypothesis was derived from previous findings supporting the role of the muscle spindles in the interdental dimension discrimination. In particular, experimentally induced vibration of the mandible, which is likely to stimulate muscle spindles, significantly altered the position matching of the mandible (35). Furthermore, in participants with muscular dystrophy syndromes, presenting an altered function of muscle spindles, impaired dimension discrimination ability was found (30). On the other hand, several studies pointed out that edentulous patients performed as well as participants with natural dentition in the interdental dimension discriminating ability of the mouth, (36) and that the application of local anaesthesia to upper and lower teeth did not affect size judgements (31, 36), indicating a marginal role of periodontal receptors in this task.

The effects of DOMS on the PS have been extensively studied in the limb muscles, often measured as the difference in position between a reference and a matching limb, reporting an increased number of position-matching errors at the joint at which the muscle is exercised. In particular, the errors were observed in the same direction from exercising each of the antagonists (37), which means that exercised arms were perceived as being more extended than they really were (16, 17), while the exercises of knee muscles lead to the perception of a more flexed knee (18). At the masticatory muscles, only one previous study evaluated the PS during pain experience, focusing on myofascial pain subjects (32). In contrast with our findings, this study pointed out worse discrimination ability in the myofascial pain group, as compared to that of control group, concluding that occlusal restorative procedures should be discouraged in subjects with acute symptoms of muscle pain. However, the diagnosis of myofascial pain was based only on self-reported symptoms, without a proper clinical examination. Furthermore, in that study, pain patients were compared with healthy controls, and

the inter-individual variability could have significantly accounted for the difference, while in our experimental setting each participant acted as control for him/ herself.

One possible explanation for the absence of significant changes in the PS during the provoked slight DOMS experience can be the contribution of receptors other than the muscle spindles in the maintenance of the dimension discrimination, when the muscle fibres are weakened. As a matter of fact, also pulpal, joint and extraoral skin receptors might play a marginal role in this task, but the literature on this topic is scarce and controversial (4). Another possible explanation is the low level of pain and fatigue reached in the current experiment. In previous limb studies, a reduction of force of at least 30% of the baseline MVC torque was considered a sufficient goal to observe position-matching errors (16, 18), while in our experimental setting, although an increase in the fatigue values was observed, the MVBF remained unchanged.

Concerning the OS, one previous study pointed out a significant difference in the detection of the 0.024 foil thickness between myofascial pain patients, selected with questionnaire assessment, and healthy subjects (38). However, when comparing different subjects, the inter-individual variability in the occlusal perception could be caused by different degrees of attention (39). In the current study, the unchanged ability of OS respected the initial hypothesis, supporting the marginal role of the muscle spindles in the occlusal tactile perception. In order to limit the impairment of occlusal tactile sensation caused by an intense acute activity of the periodontal mechanoreceptors (5), during the PP the participants were invited to bite the apparatus with their anterior teeth, while the OS was tested on the first permanent molars.

## Conclusion

Taking into consideration the limitations of the present study, it can be concluded that within the level of DOMS provoked in the studied sample after intense eccentric–concentric contraction of the jaw-closing muscles, the psychophysical ability of PS and OS remained unchanged. Hence, it seems that structures other than the muscle spindles contribute to the maintenance of the position sense of the mandible whenever the masticatory muscles are fatigued.

# **Conflict of interest**

The authors have stated explicitly that there are no conflicts of interest in connection with this article. This study did not receive any funding.

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### **Supporting Information**

Additional Supporting Information may be found in the online version of this article:

**Figure S1** Example of a curve-plot. On the x axis the testing thicknesses, on the y axis the number of 'thicker than the reference' responses..

Appendix S1 Patient-Consent-Form.