Effects of eccentric jaw exercise on temporal summation in jaw-closing muscles of healthy subjects

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Abstract

Eccentric jaw exercises has been known to cause muscle soreness but no studies have so far examined to what extent temporal summation mechanisms within the exercised muscles are changed. The purpose of the present study was to investigate the effects of an eccentric biting exercise on the temporal summation, mechanical pressure sensitivity and jaw muscle activity.

A total of 15 healthy men participated in a two-session-experiment: In one session, they performed 30 min controlled eccentric jaw exercise and the other session served as a no-exercise control. Soreness sensations at rest and during maximal biting, pressure pain thresholds (PPTs) and electromyographic (EMG) activity during maximal jaw biting were recorded before (baseline), immediately after (Post-task), and 1 day after the exercise (1-day-after). The temporal summation ratio using intramuscular electrical stimulation of the masseter was investigated at baseline and at 1-day-after. The eccentric jaw exercise was associated with significant increases in soreness sensation and decreased PPTs at Post-task and at 1-day-after. The EMG activity and biting force did not change. The summation ratio was significantly decreased at 1-day-after in both sessions. The present findings demonstrate that eccentric jaw exercise does not induce detectable changes in temporal summation. However, the summation ratio may have clinical utility to differentiate the location of sensitization.
Introduction

Orofacial pain is one of the common chronic pain conditions (Dworkin and LeResche, 1992; LeResche et al., 1997). Musculoskeletal conditions are the major cause of non-odontogenic pain in the orofacial region (McNeill and Dubner, 2001), and it is suggested that some types of chronic pain in the orofacial region are associated with delayed-onset muscle soreness (DOMS) (Lund et al., 1991). Eccentric or uncustomed exercise induces DOMS (Gleeson et al., 1995). DOMS is associated with muscle injury, inflammation and/or tissue necrosis (Allen, 2004; Lieber and Fridén, 2002; Gleeson et al., 1995). A DOMS-like phenomenon has been reported also in jaw muscles (Christensen, 1971; Arima et al., 1999; Svensson and Arendt-Nielsen, 1995; Svensson et al., 1997). One of the main symptoms of DOMS is tenderness, manifested by a reduction in pain threshold to normally innocuous mechanical stimulation, i.e., allodynia/hyperalgesia (Barlas et al., 2000; Arendt-Nielsen and Graven-Nielsen, 2003). Central and/or peripheral sensitizations are suggested to contribute to these sensitized conditions (Arendt-Nielsen and Graven-Nielsen, 2003). Various measures, e.g., visual analogue scale (VAS) of pain (or soreness), pressure pain thresholds (PPTs), pain threshold to electrical stimulation, have been investigated to evaluate muscle sensitization (Gleeson et al., 1995; Arima et al., 1999; Fenger-Grøn et al., 1998; Graven-Nielsen et al., 2000; Arendt-Nielsen and Graven-Nielsen, 2003). Tools to differentiate the localization, i.e, peripheral or central sensitization by using these measures would be important to provide appropriate pain treatment for each patient. It has been suggested that there is a relationship between central hyperexcitability and temporal summation using single and repeated electrical stimulation to leg muscles in human study (Graven-Nielsen et al., 2000). Moreover, temporal summation induced by repeated stimulation is related with wind-up (Price et al., 1994), which is considered an
initiator of central sensitization (Woolf, 1996). Meanwhile, the eccentric exercise can induce peripheral sensitization (Smith, 1991; Proske and Morgan, 2001). Measuring the difference between the pain threshold (PT) to single and repeated stimuli after the eccentric exercise may thus provide information to differentiate peripheral sensitization from central sensitization. So far, little has been known about the effect of eccentric exercise on various sensory measures in the masticatory system, especially on temporal summation in the jaw muscles. The aim of the present study, therefore, was to investigate the effects of eccentric exercise on mechanical pressure sensitivity and jaw muscle activity, and to investigate the utility of the difference between the PTs to single and repeated stimuli in an attempt to differentiate peripheral sensitization from central sensitization.

2. Materials and methods

2.1. Subjects

Fifteen healthy men (25.6 ± 0.5 years) (mean ± SEM) participated in this study. The subjects did not have signs or symptoms of TMD (Dworkin and LeResche, 1992), no daily headache (less than 12 days per year), nor were they aware of excessive parafunctional activities such as jaw-clenching. The study was approved by the local ethics committee (County of Aarhus: 20040074). All individuals gave their informed consent in accordance with the Helsinki Declaration, and understood that they were free to withdraw from the experiment at any time.

2.2. Experimental protocol and recordings

The experimental protocol is illustrated in Fig. 1. All subjects participated in two randomized sessions with a task of “eccentric open-close jaw exercise” or “no-exercise”
(control) with at least one-week interval. Each session had two successive experimental days.

The intensity of soreness sensation, pressure pain thresholds (PPTs) of the jaw muscles, and electromyographic (EMG) activity of jaw muscles during maximal voluntary biting on a bite-force transducer were recorded before the task (baseline), immediately after the task (Post-task) and 1 day after the task (1-day-after) (Fig. 1). Soreness sensations were recorded using a 0-10 cm visual analogue scale (VAS) in two conditions, i.e. at rest and during maximal biting (Fig. 1). Biting force was also measured during maximal voluntary biting on a force transducer. Pain threshold (PT) to intramuscular (i.m.) electrical single and repeated stimuli to the left masseter muscle was also measured at baseline and 1-day-after (Fig. 1).

2.3. Eccentric jaw open-close movement

Eccentric jaw open-close movement was carried out against resistance of spring-coils based on that described in previous reports (Svensson and Arendt-Nielsen, 1995; Svensson et al., 1997). Briefly, eight constant torque spring-coils (250 g each, Tensator ltd, UK) were attached from below to the lower canine via a string to pull the lower jaw. The other side of the coils was attached to the frame of the cephalostat. The subjects were instructed to open maximally and close the lower jaw steadily in a rhythm dictated by a metronome (0.3 Hz) for 5 min on the first day in the exercise session. Since the subjects were asked to open slowly they had to contract their jaw-closing muscles during opening which is negative work for the jaw-closing muscles. DOMS can be evoked when the eccentric exercise is carried out on the plateau or the descending limb of the muscle’s length-tension relation (Proske and Morgan, 2001; Talbot and Morgan, 1998). Therefore, the subject was also asked to open maximally
beyond the mandibular postural position to include the descending limb of the muscle’s length-tension relation in their open-close pathway. These jaw movements were repeated in 6 blocks with 1 min interval, i.e., a total of 30 min eccentric jaw exercise.

2.4. Pressure Pain Threshold (PPT)

The PPTs of the jaw muscles were measured by a pressure algometer (Somedic AB, Farsta, Sweden) to assess the general sensitivity to painful stimulation. The PPT was defined as the amount of pressure (kPa) that the subject first perceived as painful (Svensson et al., 1995). The probe with area of 1 cm² was applied perpendicular to the central part of the jaw muscles. The subject pushed a button when the threshold was reached. The PPTs were determined three times with a constant application rate of 30 kPa/s. The time between repeated measurements was 1 min. The mean value was used for further statistical analysis.

2.5. Pain threshold to intramuscular electrical stimulation (i.m. PT) and summation ratio

The i.m. PT was investigated based on a previously described method (Fenger-Grøn et al., 1998; Graven-Nielsen et al., 2000). Two insulated needle electrodes (9013R0271, Medtronic, Denmark) with 2 mm uninsulated tip were inserted into the left masseter, 20 mm apart, and to a depth of 15 mm. A computer-controlled constant current stimulator (Counterpoint MK2, Dantec, Denmark) was used. Each stimulus consisted of a train of two 1-ms rectangular pulses with 100 ms interval. The i.m. PTs to single and five repeated (2Hz) stimuli were determined.

For single stimulation the initial stimulation intensity (0.25 mA) was increased in steps of 0.25 mA until the subjects detected the pain sensation. Then the stimulation
intensity was decreased in steps of 0.05 mA until the pain sensation has vanished. Thereafter, the stimulation was increased again in steps of 0.05 mA until the subjects detected the pain sensation. The inter-stimulus interval between steps was about 5 sec. The i.m. PT to single stimulation was defined as the mean of the last two stimulations around PT. A similar sequence was repeated three times with 20 sec. interval. Thereafter, determination of the i.m. PT to repeated stimuli was also carried out and repeated three times using the initial intensity of 0.05 mA and steps of 0.05 mA. The repeated i.m. PT was defined as the stimulus intensity to induce pain at least at the 5th stimulus (Graven-Nielsen et al., 2000). The mean values of the three trials were calculated for the i.m. PTs to single and repeated stimuli respectively for further statistical analysis. To measure the difference in changes between the PT to single and repeated stimuli, the summation ratio (Arendt-Nielsen et al., 1997) was calculated as: (the i.m. PT to single stimulus – the i.m. PT to repeated stimuli) / the i.m. PT to single stimulus. Thus, if the degree of temporal summation increases the i.m. PT to repeated stimuli shows a lower value, and then the summation ratio increases.

2.6. EMG activity and maximal biting force

The subjects held a 6-mm thick strain-gauge bite-force transducer (SN002, JNI Biomedical, Denmark) between the upper and lower incisors and were instructed to bite twice with maximal force for 3 sec with 60 sec interval. For the EMG recordings, bipolar disposable surface electrodes (720-01-k, Neuroline, Medicotest, Denmark) were placed 10 mm apart along the central part of the left and right masseter and anterior temporalis muscles (MAL, MAR, TAL, TAR). The common electrode (879100, PALS, Axelgaard, USA) was attached to the left wrist. The EMG signals were amplified, filtered with bandpass 20 Hz-1 kHz (Counterpoint MK2, Dantec, Denmark), sampled at
4 kHz, and stored together with the biting force for off-line analysis.

The RMS amplitude during maximal voluntary biting (3 s) in each experimental condition was calculated. The mean value calculated from two trials was used for further statistical analysis. Also the mean value of the peak maximal biting forces was calculated.

2.7. Statistics

To test the effects of task type and time effect, a two-way repeated measurements ANOVA was performed and followed by post-hoc comparisons with the use of Tukey tests. The factors in the ANOVA were session (two levels: eccentric exercise and no exercise) and time effect (3 levels: baseline, Post-task, and 1-day-after). For i.m. PT and summation ratio, 2 levels of time effect (baseline and 1 day after) were applied. The level of significance was set at $P < 0.05$. Mean values ± SEM are given in the text and figures.

3. Results

3.1. Soreness sensation

In the exercise session, soreness sensation at rest condition significantly increased at Post–task ($P < 0.001$) and at 1-day-after ($P = 0.014$) as compared to baseline (Post–task: $P < 0.001$; 1-day-after: $P = 0.014$) but not in the control session. The soreness sensation at rest condition at Post-task and at 1-day-after in the exercise session showed a higher value than that in the control session (Post-task: $P < 0.001$; 1-day-after: $P = 0.045$) (Fig. 2a).

Soreness sensation during maximal biting in the exercise session also significantly increased at Post-task and 1-day-after compared to baseline (Post-task: $P < 0.001$;
1-day-after: \( P < 0.001 \), and in the exercise session showed a higher value than that in the control session (Post-task: \( P = 0.021 \); 1-day-after: \( P = 0.013 \)) (Fig. 2b).

3.2. PPTs

There were no main effects of sessions on PPTs in any muscle \( (P > 0.417) \), and the interaction between sessions and time \( (P > 0.09) \) was not significant in any muscle \( (P > 0.09) \). However, the PPT of the jaw muscles significantly decreased at Post-task and 1-day-after in the exercise session as compared to baseline in the left and right masseter muscles (MAL and MAR) \( (P < 0.01) \). In the control session there were no significant changes from baseline \( (P > 0.053) \) (Fig. 3).

3.3. Intramuscular PT and summation ratio

The i.m. PTs to the single electrical stimulation in the exercise session were 1.6 ± 0.3 mA and 1.2 ± 0.3 mA, at baseline and 1-day-after, respectively. In the control session the i.m. PT were 1.4 ± 0.3 mA and 1.2 ± 0.3 mA, respectively. There was no significant difference in the i.m. PTs to single electrical stimulation between baseline and 1-day after the task in neither session \( (P > 0.310) \) (Fig. 4a). The i.m. PTs to repeated stimuli in the exercise session were 0.6 ± 0.1 mA at both the baseline and 1-day-after. In the control session the i.m. PT to repeated stimuli were 0.7 ± 0.1 mA at both the baseline and 1-day-after. There was no significant difference in the i.m. PTs to repeated stimuli between baseline and 1-day after the task in neither session \( (P > 0.777) \) (Fig. 4b).

The summation ratio decreased at 1 day after as compared to baseline in both sessions (Exercise session: \( P = 0.003 \); Control session: \( P = 0.041 \)). In the exercise session the summation ratios were 0.51 ± 0.05 and 0.29 ± 0.05, at baseline and
1-day-after, respectively. In the control session the summation ratios were 0.42 ± 0.05 and 0.27 ± 0.05, respectively. There was no significant difference between the two sessions in the summation ratio \((P > 0.205)\) (Fig. 4c).

3.4. EMG activity and maximal biting force

In general, the EMG activity during maximal biting showed no significant sessions or time effects. The EMG activity significantly decreased at 1-day-after as compared to baseline only in the left anterior temporalis muscle (TAL) \((P = 0.034)\). The biting forces in the exercise session were 219.4 ± 5.2 N, 206.9 ± 5.2 N and 209.2 ± 5.2 N for baseline, Post-task and 1-day-after, respectively. In the control session, the force values were 210.4 ± 5.2 N, 219.1 ± 5.2 N and 208.8 ± 5.2 N, respectively. There was no significant difference in any experimental condition in maximal biting force \((P = 0.145)\).

4. Discussion

The soreness sensations both at rest and during biting increased immediately after the eccentric exercise and 1 day after the exercise. The PPTs were reduced at 1 day after the exercise. The summation ratio of the PT to the i.m. electrical stimulation was decreased 1 day after the exercise in the both sessions. The present findings demonstrate that eccentric exercises of jaw muscles can induce short-lasting sensitization in the orofacial area, but the summation ratio by using the i.m. electrical stimulation was not increased, suggesting central sensitization is not a prominent feature of this type of symptoms evoked by eccentric exercise. Rather, we suggest that peripheral sensitization is the dominant mechanism underlying the jaw pain associated with DOMS of the jaw closing muscles.
4.1. Methodological consideration

In the present study, the PPTs of jaw muscles decreased after the eccentric exercise and remained lower 1 day after the exercise. Short lasting sensitization might therefore be induced by the eccentric exercise. Increase in the soreness sensation and decrease in the PPT immediately after the eccentric exercise may be influenced by fatigue-related changes in the intramuscular circumstance, e.g., acidity (Ugawa et al., 2002). The soreness sensations both at rest and during biting remained, however, increased even at 1 day after the exercise, although no significant decreases in the EMG activities or the biting force were observed in the present study. The latter markers usually decrease under DOMS conditions (Clarkson and Hubal, 2002). We consider a DOMS-like phenomenon to have been evoked in the present study since the subjects were instructed to open maximally beyond the mandibular postural position and consequently, the open-close pathway of the eccentric exercise included the descending limb of the muscle’s length-tension relation (Proske and Morgan, 2001; Talbot and Morgan, 1998). However, the subjects appeared to recover from fatigue-related muscle soreness 1 day after the exercise probably because jaw muscles are very fatigue-resistant (Lund, 2001). Therefore, it could be argued that the present results at 1 day after the exercise were unrelated to fatigue but rather linked to a DOMS-like phenomenon following the eccentric exercises.

In the present results, the eccentric exercise resulted in different modulation patterns among the indexes, i.e., the PPT and soreness sensation showed different patterns between the sessions, while the summation ratio of i.m. PT did not, even if the decline of the summation ratio tended to be steeper after the eccentric exercise in comparison to the no-exercise task. These differences might occur from the difference
of the tissues involved in the sensitization. The PPT and soreness are influenced by both superficial and deep structures, while i.m. PT is mainly thought to express pain from a muscle (Fenger-Grøn et al., 1998). Inflammation and the breakdown products evoked by the eccentric exercise sensitize nociceptors (Proske and Morgan, 2001; Lieber and Fridén, 2002; Smith, 1991; MacIntyre et al., 1995) not only in muscles but also in skin and subcutaneous tissues. Thus, the effect of the eccentric exercise might be enhanced more, and the differences between the sessions might become more evident in the PPT and soreness sensation. On the other hand, the determination of the i.m. PT is an invasive method, which causes microinjury of the inserted muscle. Microinjury will cause hypersensitivity around it, and this might have an influence on the i.m. PTs in both sessions. Consequently, the microinjury might mask the specific effects of the eccentric exercise. Comparison of PT and summation ratio between surface electrodes and intra-muscular electrode might solve these questions in future studies. Indeed, the surface electrode has the advantage that it is applicable without tissue damage. In addition, also the specificity of the eccentric exercise needs to be examined.

Identical levels of electrical stimulation is also important for impartial evaluation of the changes in the pain threshold, and constant twitch force and exact location of the stimulus electrode may be needed to solve this problem. To avoid the effects of microinjury during the i.m. PT methods, a similar but not exact same position was used in the two sessions. The use of ratio between single and repeated stimuli applied in the present study would diminish these concerns. Though we should recognize the limitation of this method, we believe that the current method is adequate to evaluate the pain threshold in the muscle structures and that the changes in the i.m. PT reflect the changes in nociceptive processing and not just efficacy of stimulation. Measurements of twitch and subtetanic forces, and again comparison of PT between surface electrodes and
intra-muscular electrodes might be helpful in future experiments for a more refined characterization of the electrical stimulation.

4.2. Eccentric exercise evoked sensitization

Eccentric exercise is known to cause micro-damage in the muscles (Proske and Morgan, 2001; Lieber and Fridén, 2002), and then the breakdown products from injured tissues may sensitize nociceptors (Smith, 1991; MacIntyre et al., 1995), i.e., peripheral sensitization processes are likely to be involved. On the other hand, it has been suggested that central sensitization is likely to increase temporal summation (Graven-Nielsen et al., 2000). These authors reported that the degree of temporal summation was reflected in the summation ratio: a low summation ratio indicated minor efficacy of temporal summation compared with a high value of the summation ratio. Thus, central hyperexcitability might increase the summation ratio. Moreover, if central hyperexcitability progresses, the i.m. PT to repeated stimuli will decrease because of temporal summation (Graven-Nielsen et al., 2000). Thus, if the observed sensitization in the present study had mainly been caused by central sensitization, the i.m. PT to repeated stimuli would have been reduced, and then the summation ratio would have shown higher values after the eccentric exercise than in the control session. However, in the present results the i.m. PT to repeated stimuli did not show any changes in either sessions, but the i.m. PT to single stimuli tended to decrease. Moreover, the summation ratio did not show significant difference between sessions. Thus, these findings in the i.m. PT 1 day after the exercise might indicate that peripheral sensitization mechanisms contribute more to the present results than central sensitization phenomena, i.e., nociceptive sensitization in DOMS condition might be mainly due to the peripheral sensitization in the exercised muscles. These findings are consistent with that obtained
by Nie et al., (2009), where they reported that facilitation of temporal summation was not induced only by the eccentric exercise.

However, we can not ignore the contribution of central sensitization to the sensitization after other types of eccentric exercise (Barlas et al., 2000; Gibson et al., 2006). In the present study, reduction of the summation ratio seemed to be induced not by modification (i.e., increase) of the i.m. PT to repeated stimuli but by reduction of the i.m. PT to single stimuli (Fig. 4). C-fiber input following deep tissue stimulation, e.g., muscle, produces robust and long-lasting neural hyperexcitability and leads to central sensitization (Wall and Woolf, 1984; Ren and Dubner, 1999). Thus, after the eccentric exercise, chemical mediators of inflammation and breakdown products may stimulate nociceptive fibers (Smith, 1991) including C-fibers, and then not only peripheral sensitization but also central sensitization might be induced. The i.m. PT to a single stimulus will, therefore, reduce under this situation as shown in the present results. The summation ratio finally will reduce without any change in the i.m. PT to repeated stimuli because the difference of the PTs between single and repeated stimuli diminishes. Thus, the reduction of summation ratio might not always mean a reduction of central hyperactivity. From the above consideration it is suggested that summation ratio, i.e. the difference between the PT to single and repeated stimuli, is useful to differentiate peripheral sensitization from central sensitization, though we ought to take into account changes in the i.m. PTs both to single and repeated stimuli for a proper understanding of modification of the summation ratio.

5. Conclusion

The present study has shown that 30 min eccentric jaw exercise causes increased soreness in the jaw muscles and increased sensitivity to mechanical stimuli, but no
significant difference from control in terms of temporal summation. This might indicate that a DOMS-like phenomenon in jaw muscles is mainly related to peripheral sensitization. Summation ratio may have clinical utility to differentiate the location of the sensitization but further studies will be needed.
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References


Gleeson M, Almey J, Brooks S, Cave R, Lewis A, Griffiths H. Haematological and


Price DD, Mao J, Frenk H, Mayer D. The N-methyl-D-aspartate receptor antagonist


Figure legends

Fig. 1. Experimental protocol. “Exercise session” and “No-exercise session” (control) were carried out with at least one week interval. A gray-colored block means one bout of eccentric jaw open-close movement for 5 min. Baseline: baseline recordings. VAS, visual analogue scale; EMG-RMS, root-mean square value of electromyographic activity.

Fig. 2. Mean (+SEM) VAS scores of soreness sensation at rest (a) and during maximal biting (b). Eccentric: the exercise session; No-exercise: the no-exercise session. \*\( P < 0.05 \): Significantly different from baseline (Tukey test). \#\( P < 0.05 \): Significantly different between sessions (Tukey test). \( n = 15 \).

Fig. 3. Mean (+SEM) values of the pressure pain thresholds (PPTs) of the jaw muscles. MAL and MAR: left and right masseter muscles; TAL and TAR: left and right anterior temporalis muscles. Note that there were no significant differences between sessions at any period in any muscle. \*\( P < 0.05 \): Significantly different from baseline (Tukey test). \( n = 15 \).

Fig. 4. Mean (+SEM) values of intra-muscular pain thresholds (i.m. PT) to single- (a), repeated stimulation (b) and summation ratio (c) to the left masseter muscle (see text). Note that there were no significant differences between sessions at any period in any measure. \*\( P < 0.05 \): Significantly different from baseline (Tukey test). \( n = 15 \).
**Fig. 1**

**No-exercise session**
- Baseline
- No exercise (Control)
- Task: Eccentric open-close: for 5 min × 6 blocks
- Post-task
- 1-day-after

**Exercise session**
- Baseline
- Task
- Post-task
- 1-day-after

- Soreness (VAS) at rest condition
- Pressure Pain Threshold
- Maximal biting (with force-transducer)
  - *EMG-RMS*
  - *Force*
  - *Soreness (VAS) during biting*

- Pain Threshold to intramuscular electrical stimulation
Fig. 2

During maximal biting

At rest

10
2
4
6
8

Eccentric

Control

Soreness (VAS 0-10 cm)

Baseline
Post-
task
1-day-
after

Baseline
Post-
task
1-day-
after

During maximal biting

0
2
4
6
8
10

Baseline
Post-
task
1-day-
after
Fig. 3