Synergist coactivation and substitution pattern of the human masseter and temporalis muscles during sustained static contractions

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Abstract

OBJECTIVE: Previous reports indicated that between-muscle substitution of active motor unit pools can be found in a variety of synergist muscles, including shoulder and leg muscles, but little information is available for the masticatory muscles. We hypothesized that, during a prolonged clenching effort performed at low- to moderate-bite force levels, a substitution pattern of activity can be found also in the masseter and anterior temporal muscles. METHODS: Ten healthy volunteers were recruited and were asked to clench unilaterally on a force transducer for 10min at 10%, 15%, and 20% of the maximum bite force. During each session, bite force, perceived muscle pain and electromyographic activity were continuously assessed. Data analyses were performed by means of cross-correlation and periodogram analyses. RESULTS: During sustained static contractions, different contraction patterns of jaw elevator muscles could be identified. These included a coactivation pattern, a substitution pattern, and several intermediate situations between coactivation and substitution. CONCLUSIONS: The findings support the concept that the masticatory muscles are functionally heterogeneous and provide evidence that the neuromuscular strategies used by the masticatory system to perform sustained static contractions differ between individuals. SIGNIFICANCE: Individual neuromuscular strategies might play a role in the development of masticatory muscle pain conditions.
Title: Synergist coactivation and substitution pattern of the human masseter and temporalis muscles during sustained static contractions.

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Running head: EMG activity of jaw muscles during prolonged clenching
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Abstract

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Keywords: clenching, masticatory muscles, bite force, electromyography, pain.
Introduction

Muscle hyperactivity has long been considered an initiating or perpetuating factor of masticatory muscle pain (Glaros et al., 1998; Laskin, 1969; Moller et al., 1984; Sheikholeslam et al., 1982). The hyperactivity hypothesis has been tested by attempting to overload the masticatory muscles under experimental controlled conditions (Bakke et al., 1996; Clark et al., 1984; Clark and Carter, 1985; Clark et al., 1989; Farella et al., 2001; Glaros and Burton, 2004; Jow and Clark, 1989; Svensson and Arendt-Nielsen, 1996; Svensson et al., 2001; Christensen, 1989). The findings of these studies showed that moderate to high (i.e. ≥ 25% of maximum voluntary contraction; MVC) sustained static contractions of the masticatory muscles leads to unbearable pain and fatigue that impede the subjects from continuing the contraction task (Christensen, 1989; Clark and Carter, 1985; Clark et al., 1989). Both pain and fatigue, however, disappear very quickly after task cessation and the masticatory muscles do not develop post-exercise tenderness, even when the endurance tests are repeated several consecutive days (Svensson and Arendt-Nielsen, 1996). On the other hand, there is evidence that prolonged static contractions at lower contraction levels (i.e. < 25% MVC) may significantly reduce bite force and jaw opening capacity (Svensson et al., 2001), and lead to pain and tenderness in the masticatory muscles (Glaros et al., 2000; Glaros and Burton, 2004), which are the most common signs and symptoms of temporomandibular disorders (TMD).

Tooth clenching is frequently reported by patients experiencing persistent masticatory muscle pain (Huang et al., 2002; Velly et al., 2003). It should be noted, however, that so-called bruxers (i.e. people who clench or grind the teeth at wake and/or sleep time) do not always feel masticatory muscle pain (Dao et al., 1994), and may react to bruxism with an enlargement and/or hypertrophy of the masticatory muscles (Balatsouras et al., 2004; Da Silva and Mandel, 2006; Kato et al., 2001; Mandel and Kaynar, 1994). Thus, it’s still unclear why hyperactivity may result in persistent masticatory muscle pain only in some individuals. One possibility is that the development of muscle injury and pain might depend upon the individual neuromuscular control strategies used for maintenance of the sustained contraction. These include, for instance, a substitution of the motor unit drive either between- or within-muscles in order to optimize the workload and to prevent
damage to muscle fibers (Westgaard and de Luca, 1999; Zennaro et al., 2003b; Zennaro et al., 2003a). Indeed, a between-muscle substitution of active motor unit pools has been found in a variety of synergist shoulder and leg muscles (Holtermann et al., 2005; Kouzaki and Shinohara, 2006; McLean and Goudy, 2004).

A rotation of synergistic activity between the masseter and anterior temporalis muscles during sustained static contractions has been reported only in one study (Hellsing and Lindstrom, 1983). During a clenching test performed at high force level, five subjects experienced a sudden relief of pain, which corresponded to a marked reduction of EMG amplitude in the masseter muscle and to an increase of EMG amplitude in the anterior temporal muscle. An alternate switch of activity between the two synergistic muscles was found in all the subjects after repeated tests (i.e. a facilitation effect occurred over consecutive trials). The authors concluded that a trade-off mechanism turns part of the load over to the more rested synergist, when a jaw elevator muscle is fatigued and painful. Unfortunately, the findings arising from this study were mostly based about a qualitative description of the phenomenon under study and subjective reports of the subjects investigated.

The aim of the present investigation was to quantitatively study the co-contraction pattern of the masseter and anterior temporal muscles during sustained static contractions performed at low- to moderate bite force levels. It was hypothesized that, during these tasks, the main load can alternately switch between the two most important synergistic jaw elevator muscles.

Material and methods

Subjects
Ten subjects (five males and five females) were recruited among post-graduate students and staff members attending the Dental Clinic of the University of Naples Federico II. The group mean age ± SD was 28.2 ± 7.6 years. Eligible subjects were screened for temporomandibular disorders (TMD) according to Axis I of the Research and Diagnostic Criteria (Dworkin and LeResche, 1992). To be included in the study, the subject had to fulfil following criteria: a) adult age (> 18 yrs); b) pain-free active mouth opening > 40 mm (including overbite), pain-free active protrusion and laterotrusion > 7 mm; c) difference
between active and passive opening ≤ 2 mm; d) positive overjet and overbite between 0 and 4 mm; e) willingness to participate to the experiment and to sign a written informed consent. Exclusion criteria were: a) any TMD diagnosis; b) chronic pain conditions (> 3 month duration) in other parts of the body; c) current orofacial inflammatory conditions; periodontal diseases; d) removable dental prostheses; e) absence of any teeth (except third molars); f) neurological and movement disorders; g) habitual intake of drugs influencing the activity of the central nervous system. The subjects were carefully informed about the experimental protocol, but were left naïve to tested hypothesis.

Bite force equipment
Subjects were assisted in the clenching tests by visual feedback from bite force. This was recorded by a steel housing strain gauge force transducer (15 × 11 × 4 mm, length × width × height) with a working range between 0-1000 N, connected to an amplifier with an analog output (Floystrand et al., 1982). The force transducer was covered with a plastic foil to prevent moisture and then fitted into a protective polymethylene bite block (Eggen, 1969).

Pain rating
The pain perceived in the masseter and anterior temporal muscles during clenching efforts was assessed by means of electronic visual analog scales (VAS; (Scott and Huskisson, 1976)) with the anchors points “no pain” and “worst pain imaginable”. The equipment consisted of 100-mm linear cursors (variable resistors). Four cursors, each corresponding to one of the four masticatory muscles, were placed in a custom-made case (Stebi srl, Napoli, Italy). The location of each pain site was indicated to the subject by means of a schematic drawing of the face and masticatory muscles attached to the front of the case.

Electromyographic equipment
Muscle activity was picked up bilaterally from the masseter and anterior temporal muscles by means of self-adhesive pre-gelled disposable bipolar surface electrodes (Duotrode, Myotronics Inc. Seattle, Washington, US). Electromyographic (EMG) signal
was low-pass filtered (1000 Hz) and amplified (3000×) by means of BM623 (Biomeccanica Mangoni, Pisa, Italy) with a noise level of less than one μV and a common mode rejection ratio higher than 100 dB.

**Software and data acquisition**

The bite force, raw EMG, and VAS signals were A/D-converted at 2 kHz by means of a multifunction data acquisition board (PCI 6024; National Instrument, Austin, Texas, USA) connected to a computer motherboard (Intel Pentium IV). Digital signals were then processed using custom-made software developed in the LabView environment (LabView 8.0; National Instrument, Austin, Texas, USA). This software provided the subjects with visual feedback for keeping constant the bite force level. This consisted of a vertical bar whose height was proportional to the force produced. A green lamp was switched on when the subjects kept the force level within ±20% of the value. Conversely, excessive or insufficient bite force level was indicated by the appearance of a red arrow pointing up or down, respectively. Raw EMG signals were digitally filtered using a narrow band-stop centred at 50 Hz and were converted to the root mean square amplitude values ($EMG_{RMS}$) using a 3 sec window. The output of the software consisted of nine signals: one bite force signal, four $EMG_{RMS}$ signals, and four VAS signals. Recorded signal were all stored as ASCII files for subsequent off-line analyses.

**Procedure**

The study included a clinical session and three experimental sessions, separated by at least one-week. The study protocol was fully respectful of the principles of the Helsinki Declaration and received approval from the ethical committee.

During the clinical session, subjects were asked to report their preferred chewing side (later this was the ipsilateral side of the experiment). Lacking a preferred side, the right side was chosen for the application of the force transducer. For all subject, dental impressions (Extrude XP and Wash, Kerr, Romulus, MI, USA) of the lower arch were taken (SA) and poured with stone (Vel-Mix Stone type IV, Kerr, Scafati, Italy). A 1-2 mm thick layer of self-curing acrylic resin (Duralay, Reliance Dental, IL, US) was applied to the lower surface of the bite block in order to ensure reproducible and stable seating of
the transducer. Thereafter, the bite block was placed upon the dental cast so that the transducer centre coincided with the mesio-buccal cusp of the lower first molar. After removal of resin excesses and undercuts, a second layer of acrylic resin was applied to the upper surface of the bite block; this was then fitted intra-orally to the lower teeth, and the subject was asked to close the mouth slowly until the upper teeth contacted the resin layer. The force transducer in place caused a jaw opening (i.e. the interincisal distance corrected for overbite) ranging from 13.4 mm to 20.1 mm (mean = 17.6 mm) and was used for all clenching tests. After bite block construction, the subjects got acquainted with the procedures used in the study including the assessment of maximal bite force, the use of the force visual feedback, and the use of VAS equipment.

The three experimental sessions included three clenching tasks performed at 10%, 15%, and 20% of the maximum voluntary bite force (MVBF), respectively, with the order of tasks being randomized. At the beginning of each experiment, the bite block was placed into the mouth. Since a temperature-dependent offset shift of force signal had been observed in a pilot study, the subject was asked to keep the bite block-transducer in the mouth without clenching for at least 5 minutes. Then, the skin overlying the masseter and anterior temporal muscles was rubbed vigorously by cotton pads soaked into 90% ethanol. The surface EMG electrodes were placed bilaterally along the main direction of the muscle fibres as determined by palpation with a center to center distance of 21 mm (Farella et al., 1999). The reference electrode was attached to the ipsilateral mastoid process. In order to allow reproducible repositioning of the electrodes across each experimental session, a transparent pliable plastic template was aligned to the ear, to the labial margin and to the eye, and the location of the electrode sites was marked. The subjects were then asked three times to clench as hard as possible. The duration of each maximum clench was 1-2 sec with an inter-clench rest interval of 30 sec. The maximum peak value of bite force and of EMG_{RMS} obtained during clenching efforts were stored and used as estimates of the maximum voluntary bite force level (MVBF) and of the maximum voluntary contraction activity (MVC), respectively. After maximal clenching efforts, the subjects were allowed to rest for three minutes. Thereafter, subjects were asked to perform the clenching task at the required force level (i.e. 10%, 15%, or 20% MVBF) by the aid of visual feedback. Subjects had to rate
continuously the intensity of the pain perceived in each of the four muscle throughout the whole experiment. During the experiment the subjects were not allowed to look at the amount of EMG amplitude produced during contraction in each muscle. Each clenching task had to be performed for 10 minutes and the subjects were verbally encouraged to endure even if they experienced pain and fatigue. However, the subjects had also been allowed to stop clenching if they felt too exhausted. After the clenching task, the subject rested for 30 seconds and then the recording equipment was disconnected.

Data analysis and statistics

Each experimental session included an initial rest period, three maximum voluntary efforts, the clenching test, and a final rest period. The bite force signal typically showed an initial jump from zero to the target force, a sustained clenching effort, and a sudden decrease back to zero at task cessation, the sustained clenching effort being the segment of interest in the present investigation. This segment was selected by using leading and lagging zero values as initial and final markers of the task. The first and the last two seconds of the clenching segment were always discarded in order to avoid boxcar artefacts during subsequent harmonic analyses. $\text{EMG}_{\text{RMS}}$ and VAS signals were also cut along the bite force segment selected. $\text{EMG}_{\text{RMS}}$ amplitude was expressed as proportion of the $\text{EMG}_{\text{RMS}}$ peak values obtained during maximum clenching (i.e. relative EMG amplitude = $\text{EMG}_{\text{%MVC}}$) within each muscle.

Preliminary analyses consisted of descriptive statistics, normality tests, and tests for homogeneity of variances. Means and standard deviations were computed for each outcome variable (i.e. bite force, $\text{EMG}_{\text{%MVC}}$, and VAS) across subjects and across the different experimental factors (i.e. clenching force level, time).

Bite force, $\text{EMG}_{\text{%MVC}}$, and VAS were modelled as a function of the factors involved in the experiment. The following experimental factors and their first-order interactions were entered into a repeated measurements model: subject (block: random factor), clenching force level (fixed factor: three levels) and time (fixed factor: ten levels consisting of one-minute intervals). Least square means and their standard errors were used to plot the temporal profile of the response variables. Orthogonal contrasts up the fourth degree
were also calculated to estimate the time-related linear and non linear effects of each response variable. The time series of EMG\textsuperscript{%MVC} signal was then detrended only with respect to the linear component, since the quadratic and cubic components might be representative of a substitution pattern. Henceforth in this report, detrended EMG relative amplitude was indicated as EMG\textsubscript{detr\%}.

The occurrence of a substitution pattern between synergist muscles during the clenching test was analysed using three approaches. The first approach consisted of computation of unlagged cross-correlation coefficients between EMG\textsubscript{detr\%} of each pair of masseter and anterior temporalis muscles obtained across the entire recording. The second approach consisted of calculation of correlation coefficients by segmenting the recording in ten one-minute segments. Significant positive correlation coefficients indicated a coactivation pattern between synergist muscles occurring during the clenching test whereas significant negative correlation coefficients were indicative of a substitution pattern. The third approach consisted of a periodogram analysis of the difference of EMG\textsubscript{detr\%} between the two synergistic muscles (i.e. diffEMG\textsubscript{(t)} = EMG\textsubscript{detr\% (t)} masseter – EMG\textsubscript{detr\% (t)} anterior temporalis) using a Fast Fourier Transform (FFT) algorithm (Monro and Branch J.L., 1976). In the case of a substitution pattern, it can be expected that the diffEMG will fluctuate with a cyclic pattern showing significant periodogram peaks. Periodogram intensities of diffEMG were calculated for each subject, for each force level, and for each pair of synergistic muscles. Each periodogram had a temporal resolution of 6 sec and was normalized by dividing each intensity estimate by the sum of all periodogram ordinates (i.e. the overall variance), thus allowing the estimation of the proportion of variance provided by each rhythmic component (or frequency band) represented in the periodogram. Periodogram intensities were plotted as a function of period length (from 0 to 10 minutes). The $g$ statistic was used to test the significance of periodogram peaks (Russel, 1985). The sum of normalized periodogram intensities for the band extending from 2 min to 10 min was used as rhythmicity index to test the effect of bite force level, and of the order of the clenching sessions (i.e. facilitation factor) on the periodic components of diffEMG signals. The relationship between pain and EMG activity was analyzed using repeated measurement ANOVAs, where EMG\textsuperscript{%MVC} obtained
from each muscle investigated represented the dependent variables and VAS scores
and time were entered into the model as response variables.
All data processing and statistical analyses were performed using SAS package (SAS
8.01, SAS Institute, Cary, NC USA). Type I risk error for all statistical tests was set at
0.05 with the only exception of $g$ statistics ($\alpha = 0.001$).

Results

Bite force, endurance and pain

Mean $\pm$ SD maximal unilateral bite force was 420.7 $\pm$ 55.7 N. No polynomial component
of the time-related profile of bite force level was significant ($F \leq 3.6; p \geq 0.06; \text{Figure 1A}$),
thus indicating that the mean bite force was not affected by time.

All subjects could sustain the 10% MVBF trial for 10 minutes, but endurance time
became shorter at higher clenching level being 9.9 $\pm$ 0.15 min at 15% MVBF, and 7.5 $\pm$
2.1 min at 20% MVBF. One out of the ten subjects investigated (i.e. subject 8) was not
able to sustain the 15% MVBC for 10 minutes, whereas only three subjects (i.e. subjects
2, 4, 5) could sustain the 20% MVBC trial for 10 minutes. With the only exception of
subject 4, all the other subjects experienced moderate to severe pain 4-5 minutes after
starting of the clenching tests; when endurance time was shorter than 10 min, the
subjects reported that unbearable pain was the reason for stopping the task. Endurance
time and peak pain values experienced during the 20% MVBC trial were negatively
correlated ($R^2 = 0.68; p = 0.003$). Mean values of VAS pain scores for pain gradually
increased throughout the clenching task for all muscle sites. Orthogonal polynomial
decomposition of time-related profiles of VAS scores indicated a significant linear
positive trend in the amount of perceived pain ($4.15 \leq F \leq 137.0; 0.0001 < p \leq 0.045$).

Higher order components, however, were not statistically significant ($F \leq 1.4; p \geq 0.23;
\text{Figure 1B}$). Perceived pain during the clenching tasks was more intense at the masseter
than at the anterior temporal muscles sites ($p<0.001$), but it did not differ between
ipsilateral and contralateral facial sides. Perceived pain was significantly influenced from
the clenching intensity level ($F \geq 3.1; p \leq 0.01$), being higher during the 15% and 20%
MVBF tasks. After adjusting for the effect of time, there was not significant relationship
between VAS scores and EMG$\%\text{MVC}$ ($F \leq 1.1; p \geq 0.44$).
Coactivation and substitution pattern

Mean EMG relative amplitude (i.e. EMG%\text{MVC}) gradually increased throughout the clenching tasks for all muscles investigated (Figure 1C), with the anterior temporalis ipsilateral to the bite force transducer showing the highest EMG%\text{MVC} values.

EMG%\text{MVC} of both masseter and anterior temporalis significantly increased with time ($51.8 \leq F \leq 137.0; \ p \leq 0.0001$), the mean square (MS) due to the linear component being by far higher than that due to the quadratic and cubic components ($1478.8 \leq MS \leq 3755.2$, $28.8 \leq MS \leq 88.9$, and $37.0 \leq MS \leq 323.1$, respectively).

Visual examination of all individual recordings showed that the EMG\text{detr\%} amplitude fluctuations of synergist muscles exhibited a variable degree of synchronization among different subjects and among different trials (Figure 2).

The magnitude and significance level of unlagged cross correlation coefficients between the detrended EMG relative amplitudes of synergist muscles during the whole clenching tasks for each subject, force level, and facial side are summarized in Table 1. In most sessions (48 out of 60) the EMG\text{detr\%} values of synergist muscles were positively interrelated (Table 2). Only in 4 out of 60 sessions, significant negative correlation coefficients were obtained (Table 2). The examination of the magnitude and of the signs (+, -, =) of correlations coefficients indicated that in three subjects (i.e. subject 1, 4, 7), all the unlagged cross-correlation coefficients obtained across the entire recording were positive, significant, and greater than 0.5 (Table 1). In these subjects, more than 70% of coefficients obtained across the one-minute segments were positive, while the remaining coefficients were not significant (Table 2). No negative correlation coefficient was ever found across all one-minute segments (Table 2).

Negative cross-correlation coefficients across the entire recording were found only in two subjects (i.e. subject 2, 5), and more frequently in subject 5 (Table 1). In these subjects, significant negative correlation coefficients across the one-minute segments occurred much more frequently than in all the other subjects (Table 2).
The remaining five subjects (*i.e.* subject 3, 6, 8, 9, 10) showed less straightforward results, as a number of positive as well as of non-significant correlation coefficients across the entire recording were found in each subject (Table 1). The average strength of positive correlation coefficients was low (< 0.5), and in four (*e.g.* subject 3, 6, 8, 0) out of these five subjects, at least one negative correlation coefficient was found across the one-minute segments (Table 2).

Periodogram analysis of *diff*EMGs showed the occurrence of significant intensity peaks in four out of ten subjects (*i.e.* subject 2, 5, 8, 10) investigated and most frequently in subject 5 (Table 3). The mean period (± SD) of these rhythmic components was 5.7 min (± 2.4 min) and ranged from 4.1 to 10 min. The rhythmicity index of *diff*EMGs was not affected by the bite force level (*F* = 0.69; *p*=0.42) and by the order of the clenching sessions (*F* = 0.22; *p* = 0.64).

**Discussion**

Maximum unilateral bite forces obtained from the sample investigated were in agreement with previous data obtained in healthy subjects (Bakke et al., 1989; Bakke et al., 1990). The observation that bite force levels did not vary significantly throughout the clenching tasks indicated that the visual feedback helped the subjects in maintaining a steady level of biting force throughout the whole experimental session.

Endurance time decreased with increasing clenching force levels and pain was the reason for stopping the higher level clenching tasks. This pain can be ascribed to the impairment of blood flow due to an increased intramuscular pressure and subsequent ischemia (Clark et al., 1989; Rasmussen et al., 1977), and was more pronounced in the masseter muscles than in the temporalis muscles. Mean values of the EMG relative amplitude continuously increased throughout the submaximal clenching tasks. This agrees with previous findings obtained from the same muscles (Svensson et al., 2001) and has been explained through facilitated motor unit recruitment (Moritani et al., 1986), increase in the degree of synchronization between motor units (Krogh-Lund and Jorgensen, 1991), and decrease of action potential conduction velocity along the muscle fibers (Lindstrom et al., 1977).
In order to assess the synchronicity between masseter and temporalis in each subject during the clenching tests we used analytic approaches of progressive refinement. The first approach consisted in determining the unlagged cross-correlation coefficients between the detrended activities of each pair of jaw elevator muscles (i.e. ipsilateral and contralateral masseter and temporalis muscles) obtained during the whole clenching task. The second approach consisted in calculating the cross-correlation coefficients by segmenting the entire EMG recording in one-minute intervals. As a last approach, we used the periodogram analysis, looking for the occurrence of cyclic components in the difference of detrended EMG amplitude between the two synergist muscles investigated. Even though the findings resulting from these different analytic approaches were not totally in agreement, they all indicated that individuals differ in the co-contraction patterns of the synergist masseter and anterior temporalis during sustained clenching efforts. Based upon critical evaluation of the findings, we could describe several patterns of activity.

Indeed, three out of ten subjects (subject 1, 4, and 7) demonstrated a marked synchronicity with continuous coactivation of the two elevator muscles, which was reflected in positive sign, magnitude, and significance of the cross-correlation coefficients, and in the absence of any significant peak emerging from the periodogram analysis. Conversely, significant negative cross-correlation coefficients and significant periodogram peaks were found more frequently in two out of the ten subjects investigated (subject 2, 5). These findings indicated that an activity substitution during the clenching test occurred in these subjects with a cycle period of about 5-6 min. This phenomenon was similar to the so-called “rotation” of synergist activity (Hellsing and Lindstrom, 1983), and could be interpreted as a protective mechanism, as a trade-off of activity between synergist muscles during prolonged fatiguing contractions would allow time for the contractile elements to recover, while the overall force being produced by the masticatory system remains constant. The observation that the subjects showing this phenomenon could hold the whole 20%MVBF clenching task may further corroborate this hypothesis. Unfortunately, in the present study, duration of clenching sessions was limited to 10 minutes at maximum. It would be interesting in future studies, to investigate
substitution activity patterns in relation to endurance time resulting from different clenching efforts performed up to exhaustion.

Contrary to previous findings (Hellsing and Lindstrom, 1983), which reported a rotation of activity in the whole sample investigated, the substitution phenomenon occurred in only two out of the ten subjects participating to the present study. Furthermore, we did not find a facilitation effect over consecutive trials, as the factor “order of clenching sessions” did not affect significantly the periodogram peaks, and the substitution phenomenon occurred since the first clenching session. Also, we could not demonstrate any significant relationship between EMG relative amplitude values and the amount of current pain experienced at each muscle investigated.

Due to a high content of type I muscle fibers, the jaw muscles are less susceptible to pain and fatigue than limb muscles (Eriksson and Thornell, 1983). Indeed, the pain perceived during the clenching tests, became moderate or severe only 4-5 minutes after starting the 15% and 20% MVFB tasks. We cannot exclude that the substitution phenomenon might occur more frequently with stronger pain. This hypothesis may be tested in future studies using higher clenching forces and/or injecting algesic substances in the masticatory muscles during the clenching tasks. It needs to be emphasized, however, that we did not find any relationship between the substitution pattern and the clenching intensity, at least in the range of bite force investigated in the present study (i.e., 10 to 20% of MVBF), which could be considered representative of low- to moderate clenching efforts.

The EMG co-contraction pattern of the masseter and anterior temporalis in five out of ten subjects was difficult to interpret, as neither a marked synchronous coactivation nor an evident substitution could be identified. These patterns were considered as “intermediate” situations between coactivation and substitution, and may reflect spatial inhomogeneous activation of jaw elevator muscles during sustained static contractions of the masticatory muscles. In other words, it is possible that different part of the masseter and anterior temporalis muscles are predominantly active during the clenching tests and that only the most active parts are picked up by the bipolar surface electrodes as they were placed in the present study.
Since the masticatory system is mechanically redundant (van Eijden et al., 1990) an infinite number of muscle activity patterns is theoretically capable of producing a specific task. The observation that the motor strategy employed for the performance of the clenching tasks varied among different individuals is therefore not surprising and is consistent with previous EMG studies showing heterogeneous activation in masticatory muscles during other tasks (Blanksma and van Eijden, 1995; Farella et al., 2002; Turp et al., 2002), and with histochemical and immunohistochemical studies showing that these muscles exhibit an uneven fiber type distribution and an architectural and nervous compartmentalization (Eriksson and Thornell, 1983; Korfage and van Eijden, 2003). It is also possible that substitution or rotation phenomena occurred with other synergist not investigated in the present study (e.g. medial pterygoid muscle) or within the motor unit pool of each masticatory muscle. This last hypothesis would be consistent with previous findings obtained in the trapezius (Westgaard and de Luca, 1999), in the limb muscles (Bawa et al., 2006) and in the masseter muscle (Nordstrom and Miles, 1991).

It is also possible that during prolonged clenching, a number of agonist and antagonistic jaw muscles undertook a progressive co-contraction. This type of co-contraction, for instance, has been previously shown for several limb muscles during fatiguing contractions (Levenez et al., 2005; Psek and Cafarelli, 1993). It needs to be emphasized, however, that our analytical approach aimed to investigate the synchronicity between synergist muscles after linear detrending of all EMG signals. Therefore, a possible co-contraction between antagonistic jaw muscles should not affect our results.

A limitation of the present study is the potential confounding factor of cross-talk between the jaw elevators muscles investigated. This cross-talk might have some impact on the magnitude of unlagged cross-correlation coefficients, particularly for the subjects exhibiting a marked synchronicity of EMG signals. To the best of our knowledge, no investigation has ever tried to estimate the cross-talk between the masseter and anterior temporalis muscles during sustained contractions, and therefore we cannot infer from previous literature on this argument. It may be worth mentioning, however, that a recent study (Jaberzadeh et al., 2007) has investigated the EMG cross-talk between right and left digastric muscles, using focal transcranial magnetic stimulation. The cross-talk
between surface EMG recordings obtained from these two muscles was rather low (i.e. estimated at approximately 10%). Since masseter and anterior temporalis are anatomically well distinct muscles, and since unilaterally they are at greater distance than left and right digastic muscles, we consider that their cross-talk should be negligible. On the other hand, the alternative hypothesis that the significant cross-correlation between the two EMG signals would reflect a synergist co-activation pattern due to common drive is consistent with the “sharing principle” proposed by Stephens and co-workers (Stephens et al., 1999). According to this model, muscles acting around a common joint, such as the temporomandibular joint, receive common synaptic inputs to their motoneuron pools. The hypothesis of a central common drive to several masticatory muscles is also supported by other findings (Carr et al., 1994; Jaberzadeh et al., 2006).

Another limitation of the present study is the small number of subjects investigated, which, for instance, did not allow us to investigate for potential gender differences in the contraction pattern of the masticatory muscle. It’s interesting to note, however, that out of the two subjects showing a substitution activity pattern, one was male and the other was female. Despite the small sample size, our findings support the idea that neuromuscular strategies used by the masticatory system to perform sustained static contractions differ between subjects and that several distinct contraction patterns can be identified. This finding may account to explain why prolonged masticatory load of long duration such as that of bruxers may be at risk of developing muscle pain only in some individuals. Differences in the individual motor control strategies occurring during prolonged clenching contractions can be implicated in protective mechanisms aiming to reduce the potential detrimental effects of prolonged fatigue and overload of the jaw muscles.

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Reference List


Figure legends

Figure 1: Mean values (± standard error of the means) of bite forces, VAS scores and relative EMG amplitude (EMG\%MVC) averaged across subjects, and time interval (each time interval = 1 min) obtained during the clenching tasks at 10% (A), 15% (B), and 20% (C) of maximum voluntary bite force. Used abbreviations: EMG = electromyographic VAS = visual analog scales; IpsMass = ipsilateral masseter; IpsTemp = ipsilateral anterior temporalis; CntMass = contralateral masseter; CntTemp = contralateral anterior temporalis.

Figure 2: Examples of different individual patterns of detrended EMG relative amplitude (EMG\%detr) obtained from the masseter and anterior temporal muscles ipsilaterally to the bite force transducer in three subjects (subject 4, 5, and 10, respectively) during a clenching test performed at 10% of maximal voluntary bite force: A) coactivation pattern, B) substitution pattern, C) intermediate pattern in between coactivation and substitution. Note that negative values of EMG values are the consequence of linear trend removal.
Figure 2
TABLE 1. Unlagged cross-correlation coefficients between detrended EMG relative amplitude of the masseter and of the anterior temporalis muscle ipsilaterally and contralaterally to the bite force transducer, obtained over the whole recording

<table>
<thead>
<tr>
<th>Bite force level</th>
<th>Facial side</th>
<th>10% MVBF</th>
<th>15% MVBF</th>
<th>20% MVBF</th>
</tr>
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<tbody>
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<td></td>
<td>Ipsilateral</td>
<td>Contralateral</td>
<td>Ipsilateral</td>
<td>Contralateral</td>
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<td>Subject 1</td>
<td>0.82**</td>
<td>0.59**</td>
<td>0.89**</td>
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<tr>
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<td>0.45**</td>
<td>-0.19*</td>
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<td>0.81**</td>
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MVBF = maximum voluntary bite force; Level of significance: * = p< 0.05; ** = p<0.01
TABLE 2. Signs counts of correlation coefficients between detrended EMG relative amplitude of the masseter and of the anterior temporalis muscle ipsilaterally and contralaterally to the bite force transducer obtained sequentially over one-minute intervals

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<thead>
<tr>
<th>Facial side</th>
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<th>15% MVBF</th>
<th>20% MVBF</th>
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<tr>
<td>Sign of coefficients</td>
<td>+ -</td>
<td>+ -</td>
<td>+ -</td>
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| Subject 1   | 10 0 0 9 0 1 | 10 0 0 10 0 0 | 6 0 4 8 0 2 |
| Subject 2   | 5 0 5 3 4 3 | 5 0 5 0 3 7 | 7 0 3 1 0 9 |
| Subject 3   | 1 0 9 10 0 0 | 4 0 6 3 1 6 | 2 0 4 3 1 2 |
| Subject 4   | 10 0 0 8 0 2 | 10 0 0 8 0 2 | 10 0 0 10 0 0 |
| Subject 5   | 3 3 4 6 2 2 | 1 2 7 7 0 3 | 2 1 5 3 2 3 |
| Subject 6   | 8 0 2 3 0 7 | 9 0 1 6 0 4 | 5 0 0 0 0 5 |
| Subject 7   | 5 0 5 7 0 3 | 9 0 1 7 0 3 | 7 0 1 7 0 1 |
| Subject 8   | 4 0 6 3 0 7 | 6 1 3 4 0 6 | 5 0 1 3 0 3 |
| Subject 9   | 6 0 4 4 0 6 | 8 0 2 4 0 6 | 3 0 4 2 0 5 |
| Subject 10  | 7 0 3 8 1 1 | 5 0 5 2 0 8 | 7 0 3 4 1 5 |

MVBF = maximum voluntary bite force; '+' = number of positive correlation coefficients; '-' = number of negative correlation coefficients; '=' = number of non-significant correlation coefficients.
<table>
<thead>
<tr>
<th>Bite force level</th>
<th>10% MVBF</th>
<th>15% MVBF</th>
<th>20% MVBF</th>
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<td>Contralateral</td>
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