Time-frequency analysis of rhythmic masticatory muscle activity

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Running head: Time-frequency analysis of rhythmic EMG activity

Keywords: Electromyography, masticatory muscles, chewing, central pattern generator, Fast Fourier Transform.

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Time-frequency analysis of rhythmic masticatory muscle activity

Abstract

The aim of this study was to develop and to validate under laboratory conditions an algorithm for a time-frequency analysis of rhythmic masticatory muscle activity (RMMA). The algorithm base-band demodulated the electromyographic (EMG) signal providing a frequency vs. time representation. Using appropriate thresholds for frequency and power parameters, it was possible to automatically assess the features of RMMA without examiner interaction. The algorithm was first tested using synthetic EMG signals and then using real EMG signals obtained from the masticatory muscles of eleven human subjects, who underwent well-defined rhythmic, static, and possible confounding oral tasks. The accuracy of detections was quantified by receiver operating characteristics (ROC) curves. Sensitivity and specificity values were ≥ 90% and ≥ 96%, respectively. The areas under the ROC curves was ≥ 95% (standard error = ± 0.1%). The proposed approach represents a promising tool to effectively investigate rhythmical contractions of the masticatory muscles.

Introduction
Nipple sucking during breastfeeding is probably the first oral behavior associated with rhythmic contractions of the masticatory muscles presenting in human newborn infants\textsuperscript{8,19,23}. With brain maturation and craniofacial development, the masticatory system evolves, becoming able to perform more complex rhythmic movements such as those of mastication.\textsuperscript{15} The intrinsic rhythm of this oral behavior is regulated by a central pattern generator (CPG) that is located in the pons and medulla, but is also influenced by descending neural pathways as well as by peripheral periodontal and muscle afferents.\textsuperscript{15,16} The mean chewing frequency shows large inter-individual variability\textsuperscript{18,20} and is influenced by age and gender.

Rhythmical contractions of the masticatory muscles can also occur during sleep (\textit{i.e.} jaw movements accompanied with tooth grinding) in normal subjects and more frequently in sleep bruxers.\textsuperscript{12} These contraction episodes last on average 8-10 seconds with a burst frequency of approximately 1 Hz, which is very close to the frequency of the basic rhythm of CPG.\textsuperscript{14} In sleep bruxers, this rhythmical activity occurs during an arousal reaction and is often accompanied by tooth-to-tooth grinding sound.\textsuperscript{13}

The occurrence of rhythmic as well as of sustained static masticatory activity (\textit{e.g.} tooth clenching and abnormal jaw posturing) can be identified during awake and sleep stages by using multiple physiological measurements supplemented with audio and video recordings.\textsuperscript{10,13} A major disadvantage of this equipment is the high cost and amount of time needed for manual/visual scoring. Furthermore, this equipment can be used only in the laboratory setting, thus providing values that are not representative of oral behaviors as they occur in the natural environment. Last but not least, the scoring of masticatory muscle activity under these conditions is mainly based upon subjective evaluation and skill of the examiner.

The use of portable electromyographic (EMG) equipment has opened the possibility to explore masticatory function in the natural environment, interfering minimally with spontaneous behavior.\textsuperscript{3,7} Conventional analyses of long-term surface EMG signals, however, are generally limited to the assessment of number, amplitude, and duration of bursts in the time domain.\textsuperscript{7} Using this approach, it may be rather difficult to selectively extract rhythmic muscle contractions, as they may be
confounded with other activities (e.g. talking, drinking, head movements, etc.) and/or with possible artifacts.

A surface raw EMG signal can be regarded as a carrier signal containing frequencies in the range 20-500 Hz and modulated by a baseband signal with frequency content much lower (i.e. < 4 Hz) than the carrier frequency. This low-frequency content reflects the dynamics used by central nervous system (CNS) for muscle activation patterns. This model resembles the traditional technique of amplitude modulation (AM) used in radio transmission. Using the reverse operation (i.e. demodulation), it is possible to recover the signal corresponding to muscle activation and to assess rhythmic masticatory activity in terms of frequency and power (i.e. energy per time), over short-term time windows. Based upon available data from masticatory muscle physiology, it can be expected that rhythmic masticatory muscle activity will mostly occur in the frequency band lying around 1 Hz. Higher frequency content is most likely due to other activities or movement artifacts.

When the frequency of the modulating signal is close to zero, this will indicate sustained masticatory muscle activity.

The aim of this study was to develop and to validate under laboratory conditions an algorithm for the automated assessment of rhythmic masticatory muscle activity using single-channel surface EMG signals.

**Material and methods**

*Synthetic EMG signals*

EMG low-level postural activity was recorded at 4096 Hz in a human test subject (male, age 40 years) from the masseter muscle using surface EMG electrodes. The subject was asked to keep the mandible as relaxed as possible for three minutes. An amplitude modulated signal, which included sustained and rhythmic activity segments, was then generated using square waves and half-wave rectified sinusoidal waves. The modulating signal (signal-to-noise ratio: 30 dB) included three sustained activity segments lasting 5 seconds, and five rhythmic activity segments with burst frequencies ranging from 0.50 to 1.50 Hz (step: 0.25 Hz), lasting 15 seconds. Activity segments were separated by ten-second rest pauses. A second modulating signal was generated using two
rhythmic activity segments (signal-to-noise ratio: 30) lasting 25 seconds separated by fifteen-second rest pauses. The first segment had a burst frequency linearly increasing from 0.75 Hz to 1.25 Hz, whereas the second segment had a burst frequency linearly decreasing from 1.25 Hz to 0.75 Hz. Synthetic EMG signals were then obtained multiplying point-wise the EMG low-level postural activity by the modulating signals. Duration of the first synthetic EMG signal was 180 seconds, whereas the second synthetic EMG lasted 60 seconds.

Subjects
Eleven healthy subjects (5 men, 6 women; age = 34.6 ± 10.8 years), were recruited among the staff of the University of Zurich by means of local advertisements. A detailed description of sample characteristics can be found elsewhere. The study protocol complied fully with the principles of the Helsinki Declaration, and was approved from the Local Ethics Committee (reference number: StV 08/02). The volunteers were carefully informed about the procedures and signed an informed consent form.

EMG recordings
Muscle activity was recorded unilaterally from masseter and anterior temporalis by means of self-adhesive pre-gelled disposable Ag-AgCl rectangular (20 x 15 mm) surface electrodes (Type 9013S0212, Alpine Biomed ApS, Skovlunde, Denmark). Electromyographic (EMG) signal was band-pass filtered (20-1000 Hz; -3dB) and amplified (5000×) by means of DISA 15C 01 (Disa Elektronik, Skovlunde, Denmark) with an input impedance of 250 MΩ, noise level of 0.7 μV, and a common mode rejection ratio of 100 dB. The amplifier output was connected to a notebook computer (Asus A6J, Intel Centrino Duo T2300, RAM 1024 MB) by means of a 4-channel USB analog data acquisition system (NI cDAQ-9172 and NI 9233, National Instruments Co, Austin, TX, USA). The skin overlying masseter, anterior temporalis, and the mastoid process of the ipsilateral side was rubbed vigorously by cotton pads covered with abrasive paste (Lubex peeling, Permamed AG, Therwil, Switzerland) for impedance reduction. Surface EMG electrodes were placed unilaterally along the main fiber direction of the masseter and anterior temporalis as determined by
palpation with a center to center distance of 20 mm. The reference electrode was attached on the skin overlying the mastoid process.

Clinical procedure
Masticatory muscle activity was recorded under computer guidance during performance of deliberate oral tasks using the Oral Task Collector software (OTC). The software and the procedure have been extensively described elsewhere. Briefly, the OTC displays detailed instructions about the oral task to be performed by means of explanatory text, still and animated images, audio and video files. Timing was given by countdown and progress bars, which facilitated the subject to be sharp with onset, maintenance, and cessation of each task. Couples of dual-tone multi-frequency sounds (DTMF) lasting 300 ms each were generated by the OTC at the beginning, and the end of each oral task acting as task definer and time markers for off-line analyses. During oral task collection, a video was synchronously recorded by a commercially available digital camera (QuickCam® Pro 9000, Logitech). The subjects were trained to perform the requested oral tasks by OTC software during home training.

At the beginning of the experiment, the subjects were asked to perform the following eight oral tasks (i.e. first task paradigm): three sustained clenchings using light, moderate, and strong effort, and five rhythmic clenchings performed at 0.50, 0.75, 1.00, 1.25, 1.50 Hz, using moderate effort. Ten-second rest pauses were allowed in-between consecutive clenching efforts. This paradigm was similar to that used to generate the first synthetic EMG signal. During the following 20 minutes, subjects were asked to perform thirty-six additional tasks (i.e. second task paradigm). These included rhythmic and sustained oral tasks, which are generally accompanied by light to vigorous contractions of the masticatory muscles, and tasks that can be considered potential confounders, e.g. tasks in which the EMG signals can be altered by activity of the mimic muscles and by movement artifacts. The investigated tasks are summarized in Table 1. Unilateral oral tasks (i.e. hard and gum chewing, grinding, head-turned, cupping of the jaw on the hand, and holding the mandible in laterotrusion) were performed both on the right and on the left side. The unilateral gum chewing task was performed using one piece of a commercially available gum (Spearmint, Migros, Zurich, Switzerland). The hard chewing task was performed using one piece (1 x 1 x 1 cm) of dried
meat (“Bündnerfleisch”, Migros, Zurich, Switzerland). The natural chewing task was performed by asking the subject to chew a piece (3 g) of bread (“Bürli”, Migros, Zurich, Switzerland) on the most convenient side. The biting tasks were performed on a hollow rubber tube (diameter = 6.3 mm; thickness = 1.5 mm). With the exception of the natural chewing task, during which the subject was asked to chew at his habitual pace, all the other rhythmic tasks were visually cued, using animations showing one cycle per second (i.e. 1 Hz). This pacing was also used for several confounding activities such as coughing, deep breathing, jumping on a chair, touches of the electrodes, and traction of electrodes.

Duration of rhythmic and sustained static oral tasks was set to last 15 and 5 seconds, respectively. Head movements, grimacing, smiling, and swallowing were set to last 5 seconds, whereas all the other confounding tasks were set to last at 15 seconds. All requested tasks were separated by rest pauses ranging from 3 to 30 seconds, the length of each pause depending on the amount of effort produced during each previous task. With the only exception of the initial task paradigm, task sequences were randomized in order to avoid the occurrence of learning bias throughout the recordings. The correct performance of the whole task paradigm was verified by checking off-line all the video clips synchronized with EMG signals. Further details on the performed tasks can be found elsewhere.4

Data processing, algorithm, and analyses

The two raw EMG signals and one audio signal with DTMF sounds were A/D-converted (24 bit) at 4096 Hz using custom made software (Labview Signal Express, National Instruments Co, Austin, TX, USA). Raw EMG signals were base-band demodulated using root mean square amplitude values (EMGRMS) calculated over 125 msec contiguous rectangular windows by Matlab™ (Matlab 8.0, The Mathworks, Natick, MA, USA).

EMGRMS was then analysed using the windowed Short-Time Fast Fourier Transform (ST-FFT) applied to 64 points with a one-point sliding Hamming window. Before FFT computation, each data point was adjusted for the mean value of the whole data points included in the sliding window. The resulting spectrum (i.e. spectrogram) had a frequency band ranging from 0 to 4 Hz, a frequency
resolution of 0.125 Hz, and a time resolution of 125 msec. Time shifts due to EMG demodulation and FFT windowing were compensated by software. For each spectrum, peak frequency (Freq\text{\textsubscript{peak}}; Hz) and peak power (Pow\text{\textsubscript{peak}}; dB) were calculated. Pow\text{\textsubscript{peak}} was normalized to the maximum power found during the ipsilateral hard chewing task (Pow\text{\textsubscript{%}}). In the subsequent step of the algorithm, the spectral estimates were included in the Time-Frequency matrix, provided that both Freq\text{\textsubscript{peak}} and Pow\text{\textsubscript{%}} were equal to or greater than predefined thresholds. If this condition was not verified, the corresponding time-point spectral array was set to 0. The resulting “cleaned” spectrogram was then used for automated detection of a rhythmic episode. This was defined as a portion of the cleaned spectrogram lasting more than 1.5 seconds and containing at least three bursts. This condition was satisfied for the minimum duration by frequencies of at least 2 Hz, whereas for lower frequencies, the episode duration had to be greater than 1.5 second. A schematic representation of the proposed approach applied to the first synthetic EMG signal is given in Figure 1.

As the main steps of the algorithm were the Demodulation of the EMG signal and the Frequency vs. Time representation, henceforth in this report, it was indicated using the abbreviation DEFT. Robust linear regression analysis\textsuperscript{28} was then used to evaluate the time-related profile of Freq\text{\textsubscript{peak}} across each rhythmic episode. The resulting slope was expressed in mHz/sec and could be preceded by a positive or negative sign indicating either an increase (\textit{i.e.} “up-chirp frequencies”) or a decrease (\textit{i.e.} “down-chirp frequencies”) of Freq\text{\textsubscript{peak}} across each episode. Statistically non-significant positive or negative slopes indicated a steady frequency of the rhythmic episode. An example of rhythmic EMG activity with up-chirp and down-chirp frequencies is given in Figure 2. Freq\text{\textsubscript{peak}} and Pow\text{\textsubscript{%}} were also averaged across each episode (Mean\_Freq\text{\textsubscript{peak}} and Mean\_Pow\text{\textsubscript{%}}).

The audio signal recorded during both task paradigms was used for the labelling of each masticatory task. This was obtained by further analysing the audio trace using ST-FFT, but this was applied to 1024 points with a 512-point sliding Hamming window. The resulting spectrum had a frequency band ranging from 0 to 2048 Hz, a frequency resolution of 4 Hz, and a time resolution of 125 msec. DTMF codes were thus detected by custom-made software (Matlab 8.0, The Mathworks, Natick, Massachusetts, US) so that each EMG segment corresponding to a specific task could be automatically extracted for subsequent analyses.
The performance of the rhythmic detection algorithm was assessed by receiver operating characteristics curves (ROC) using the requested type of oral tasks (i.e. rhythmic, static, confounders) as true classification variable. Rhythmic oral tasks (n = 10) represented the reference standard, which was compared with non-rhythmic oral tasks that included sustained tasks and confounders (n=26). \( F_{\text{peak}} \) and \( \text{Pow}_{\%} \) were used as predictor outcomes in a two-step procedure. ROC curves were assessed time-point-wise for both masseter and anterior temporalis EMG signals. For each ROC curve, the area under the curve (AUC) was determined along with its 95% confidence interval (CI). Sensitivity (Se) and specificity (Sp) values were assessed both time-point-wise and episode-wise, and were maximized using Youden’s index (i.e. \( \text{Se} + \text{Sp} -1 \)). Robust regressions, as well as all the other statistical analyses were performed using SAS package (SAS 9.1, SAS Institute, Cary, NC, USA).

Results

Synthetic EMG signals
The DEFT algorithm was initially tested on synthetic EMG signals. At this stage, thresholds were determined on an empirical basis (\( F_{\text{peak}} \) threshold =0.37 Hz; \( \text{Pow}_{\%} \) threshold = 10%). In this way, all sustained clenching episodes were systematically discarded, whereas all rhythmic clenching episodes were correctly identified (Figure 1). The point estimates of \( F_{\text{peak}} \) throughout the whole detected rhythmic episodes always corresponded exactly to the modulation frequencies used for burst generation (i.e. 0.50, 0.75, 1.00, 1.25, 1.50 Hz). From the second synthetic EMG signal, the time-varying features of the modulation frequency originally used for burst generation could be accurately estimated by robust linear regression analysis (Figure 2).

First task paradigm
The DEFT algorithm was then applied to real EMG signals obtained from both masseter and anterior temporalis during the first task paradigm, and settings the algorithm as for synthetic EMG signals. Also in this case, all the sustained clenching episodes were systematically discarded,
whereas all the rhythmic clenching episodes were correctly identified. Mean burst frequencies of voluntary rhythmic episodes resulted very close to the target values (i.e. 0.50, 0.75, 1.00, 1.25, 1.50 Hz) and the coefficient of variations were relatively small (< 4.2%). There was no significant linear trend of $\text{Freq}_{\text{peak}}$ (i.e. slopes) across all the rhythmic episodes, suggesting that the subjects were able to keep a constant frequency during task performance. Complete descriptive statistics for the first task paradigm is given in Table 2.

**Second task paradigm**

The EMG signals obtained from jaw elevator muscles during thirty-six sustained static, rhythmic, and confounding tasks were used for threshold optimization. Therefore, the DEFT algorithm was initially run without using any frequency or power threshold. ROC curves were then calculated using $\text{Freq}_{\text{peak}}$ as the outcome measure. The resulting ROC curves are shown in Figure 3A. The AUC was 87.3 % (CI = 86.9-87.8 %) and 86.3 % (CI = 85.9-86.8 %) for the masseter and the anterior temporalis, respectively. The resulting optimal threshold for $\text{Freq}_{\text{peak}}$ was 0.62 Hz for both jaw elevator muscles. The use of $\text{Pow}_\%$ as an additional outcome measure yielded a further improvement of the accuracy of the algorithm, as the corresponding AUC was 95.4 % (CI = 95.2-95.7%) for the masseter muscle and 96.5 % (CI = 96.3-96.7 %) for the anterior temporalis muscle (Figure 3B). The resulting optimal threshold for $\text{Pow}_\%$ was 2% for both muscle investigated. The combined use of two thresholds yielded 90.0% sensitivity and 97.4% specificity for the masseter muscle, and 90.2% sensitivity and 96.3% specificity for the anterior temporalis muscles.

After calibration of the algorithm, the accuracy of DEFT was also assessed episode-wise. The resulting overall sensitivity/specificity were 93.6 % / 97.2 % for the masseter, and 95.5 % / 96.1 % for the temporalis. The most frequent false positive detected episode was the coughing task, as this was erroneously classified as a rhythmic task in three out of the eleven subjects investigated. The most frequent false negative episode detected was the jaw play task, as this was not identified in two out of the eleven subjects investigated. None of the chewing tasks was ever misclassified either as false positive or as false negative episode. Two examples of time-frequency representation obtained from demodulated EMG signals recorded during chewing are given in
Figure 4. The mean duration (± standard deviation) of all detected rhythmic episodes was 15.6 ± 1.9 sec for the masseter, and 15.5 ± 2.2 sec for the anterior temporalis, which was very close to the target task duration (i.e. 15 sec).

Discussion

Using time-frequency analysis by short-time Fourier or wavelet transforms, it is possible to collect information from a signal in both time and frequency domains, simultaneously. The resulting time-frequency representation is generally defined as a spectrogram. These are widely used in medicine and biology to investigate human voice\(^2\),\(^5\) and a variety of animal sounds such as those of bats, birds, and whales.\(^1\) More recently, time-frequency analysis has also been used to investigate surface raw EMG activity recorded from different muscles during fatiguing contractions, giving new insight into motor unit recruitment strategies.\(^{17,21,25}\) Indeed, raw surface EMG activity can be regarded as a bipolar signal with zero mean, in which the action potentials of all active motor units detectable under the electrode site are electrically superimposed. The frequency content of raw EMG signals is influenced by the duration and shape of the motor unit action potentials,\(^{9,22}\) as well as by the firing frequencies of each motor unit action potential train.\(^6\)

Demodulation of raw EMG signals is equivalent to the extraction of its linear envelope. This envelope is generally obtained by signal rectification followed by low-pass filtering (e.g. < 8 Hz) or by calculation of root-mean-square amplitude over specific time windows, as in the case of the present study. This transformation causes a shift of the signal spectrum to baseband, whose content reflects the rhythmicity pattern that is characteristic of the muscle under investigation. Power spectra obtained from demodulated EMG activity assessed during deliberate mastication have been previously used to assess the individual ability to maintain a prescribed chewing frequency.\(^{11}\) In this study, however, the spectra were obtained by applying the Fourier transform to the whole EMG recording. This approach did not allow determination of the occurrence time of spectral peaks and their possible variation over time. This might be a critical issue when the chewing frequency is nonstationary, as often happens during masticatory rhythmogenesis.\(^{15}\)
To the best of our knowledge, only one study has assessed the time-frequency features of demodulated EMG activity, which was recorded from the human trapezius muscle during sleep, and no study has ever described the time-frequency features of rhythmic masticatory muscle activity.

The analysis of synthetic EMG signals used in the present study indicated that the spectrogram of demodulated EMG masticatory activity may provide valuable information on muscle contraction patterns. Indeed, looking at the simulated contractions with known timing, amplitude, and frequency modulation, it was observed that the static contraction episodes showed spectral peaks in a frequency band that is much lower than that of rhythmic contraction episodes. Using the DEFT algorithm, it was therefore possible to selectively extract rhythmic contraction episodes and to assess their frequency variation time with high accuracy.

Application of this algorithm to real EMG masticatory activity recorded during sustained and rhythmic clenching tasks performed under computer guidance showed that the subjects investigated in the present study were able to match the requested target frequency and to maintain this frequency constant across the whole episode.

It needs to be emphasized that in the natural environment, the masticatory system is involved in many functional as well as non-functional activities. These activities can be either sustained or rhythmic. The masticatory system, however, is also involved in quasi-rhythmic or pseudo-rhythmic activities such as speaking, coughing, deep breathing, facial mimics, and a variety of activities, which can represent a possible source of movement artifacts. The findings obtained from numerous additional sustained, rhythmic, and confounding tasks indicated that the time frequency-representation of masticatory EMG activity can be used to distinguish true rhythmic tasks performed at vigorous as well as light contractions, from both sustained static episodes and possible confounders. Indeed, it was possible to make an automated detection of most rhythmic contraction episodes with high accuracy for both the masseter and anterior temporalis EMG signals. A few false positive detections were found during coughing. It should be emphasized, however, that coughing episodes occur rather infrequently in healthy human subjects. In a few cases, the rhythmic jaw play was not correctly identified. This can be explained by the fact that this
oral task did not involve tooth contact, yielding a very low-level of jaw-closing muscle activity. The jaw play behavior, however, is also not frequent in the general population.27 We found that the detection accuracies obtained from EMGs of the masseter and anterior temporalis muscles were slightly different. This can be ascribed to the fact that the activation balance of these muscles varies across different tasks. We have previously shown, for instance, that the masseter is more active than the anterior temporalis during rhythmic biting tasks performed in a protruded jaw position whereas the anterior temporalis is more active than the masseter during simulated grinding, and rhythmic tasks performed in intercuspal position. Noteworthy is that none of the chewing episodes was ever misclassified from both masseter and anterior temporalis EMG signals. This indicates that the proposed algorithm is particularly suitable to investigate the chewing function in long-term EMG recordings obtained in the natural environment. Furthermore, it can also be used to assess the time frequency feature of nocturnal rhythmic masticatory contractions (i.e. tooth grinding) occurring in sleep bruxers. These issues are currently under investigation in our laboratory.

In the present study, only few spectral indices (i.e. frequency and power peaks) obtained from the complete time-frequency representation of demodulated EMG masticatory activity have been analyzed. It is possible, however, to assess additional spectral indices, such as secondary frequency peaks, mean or median power frequency, and power integral, to name just a few. More complex algorithms have the potential of more refined pattern recognition of specific masticatory tasks. In this respect, it is worth mentioning that a number of speech recognition techniques are based upon the time-frequency representation of human voice.24 Finally, although the proposed approach was specially developed to investigate rhythmic masticatory activity, it can be also used to investigate rhythmic contractions involving other muscles or muscle groups in physiological (e.g. gait analysis) as well as pathological conditions (e.g., tics, dystonia and other movement disorders).

In conclusion, time frequency analysis applied to demodulated EMG activity represents a promising tool to effectively investigate rhythmical contractions of the masticatory muscles and can be potentially extended to other muscle groups. The use of a few spectral indices and of a simple
algorithm allowed us to provide an automated assessment of masticatory rhythmic contraction episodes. A good validity of the proposed approach was demonstrated under laboratory conditions. The assessment of the time-varying features of EMG masticatory muscle activity opens the possibility of gaining new insights on chewing rhythmogenesis, and into the functioning of the masticatory central pattern generator as well as the functioning of other rhythmic pattern generators.
Reference List


List of abbreviations:

**RMMA:** rhythmic masticatory muscle activity

**EMG:** electromyography

**DEFT:** demodulated electromyography frequency vs. time representation

**ROC:** receiver operating characteristics

**CPG:** central pattern generator

**CNS:** central nervous system

**AM:** amplitude modulation

**OTC:** oral task collector software

**DTMF:** dual-tone multi-frequency

**ST-FFT:** short-time fast Fourier transform

**AUC:** area under the curve

**Se:** sensitivity

**Sp:** specificity

**CI:** confidence intervals
Figure legends

Figure 1: Schematic representation of the various steps of the algorithm used for detection of rhythmic masticatory muscle activity and applied to a synthetic raw EMG signal: (a) EMG preprocessing; (b) demodulation of EMG signal by calculation of root mean square amplitude; (c) computation of spectrogram using Short-Time Fast Fourier Transform; (d) application of thresholds for frequency (Th1) and power (Th2); (e) automated assessment of rhythmic masticatory muscle activity.

Figure 2: Synthetic raw EMG signal and time-frequency representation of its demodulated EMG activity. The signal included a first rhythmic episode with a frequency linearly increasing from 0.75 Hz ($F_{\text{start1}}$) to 1.25 Hz ($F_{\text{stop1}}$), and a second episode with a frequency linearly decreasing from 1.25 Hz ($F_{\text{start2}}$) to 0.75 Hz ($F_{\text{stop2}}$). Variations of frequency over time were $+20$ mHz/sec ($\Delta F_1$) and $-20$ mHz/sec ($\Delta F_2$) for the first and second episodes, respectively. These variations (i.e. slopes) could be correctly estimated using robust linear regression analysis ($p<0.001$).

Figure 3: Receiver operating characteristics curves showing the accuracy of the proposed algorithm using only $\text{Freq}_{\text{peak}}$ as predictor (A), and using both $\text{Freq}_{\text{peak}}$ and $\text{Pow}_{\%}$ as predictors (B). Separate curve were calculated using EMG signals recorded from the masseter (unbroken line) and anterior temporalis (broken line).

Figure 4: Examples of real raw EMG signals recorded during the gum chewing task (A) and during the natural chewing task (B), along with the time-frequency representation of their demodulated EMG activity. The gum chewing task was performed at a constant frequency of 0.92 Hz. The natural chewing task started at a frequency of 1.12 Hz and gradually decreased to 0.87 Hz. Variation of frequency over time showed a significant ($p<0.01$) negative linear trend (- 14.0 mHz/sec).
<table>
<thead>
<tr>
<th>Rhythmic (n = 10)</th>
<th>Sustained (n = 10)</th>
<th>Confounders (n = 16)</th>
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<tbody>
<tr>
<td>Canine grinding IPSI</td>
<td>Jaw cupping CONTRA</td>
<td>Coughing</td>
</tr>
<tr>
<td>Canine grinding CONTRA</td>
<td>Jaw cupping IPSI</td>
<td>Deep breathing</td>
</tr>
<tr>
<td>Gum chewing IPSI</td>
<td>Jaw laterotruded CONTRA</td>
<td>Drinking water</td>
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<tr>
<td>Gum chewing CONTRA</td>
<td>Jaw laterotruded IPSI</td>
<td>Grimacing</td>
</tr>
<tr>
<td>Hard chewing IPSI</td>
<td>Jaw protrusion</td>
<td>Head extension</td>
</tr>
<tr>
<td>Hard chewing CONTRA</td>
<td>Light clenching</td>
<td>Head flexion</td>
</tr>
<tr>
<td>Natural chewing</td>
<td>Lip biting</td>
<td>Head-turned IPSI</td>
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<tr>
<td>Jaw play</td>
<td>Maximum clenching</td>
<td>Head-turned CONTRA</td>
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<tr>
<td>Molar tapping</td>
<td>Sustained incisal biting</td>
<td>Jumping on the chair</td>
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<tr>
<td>Rhythmic incisal biting</td>
<td>Yawning</td>
<td>Reading aloud</td>
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<td>Whistling</td>
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Table 2: Descriptive statistics (means ± standard deviations) for average frequency (M_Freqpeak), power (M_Pow%), and duration (Dur) of rhythmic episodes performed at different target frequencies, as assessed from EMG activity recorded from the masseter and anterior temporalis muscles.

<table>
<thead>
<tr>
<th>Target frequency</th>
<th>Masseter</th>
<th>Anterior Temporalis</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>M_Freqpeak (Hz)</td>
<td>M_Pow%</td>
</tr>
<tr>
<td>0.50 Hz</td>
<td>0.48 ± 0.02</td>
<td>37.6 ± 16.3</td>
</tr>
<tr>
<td>0.75 Hz</td>
<td>0.76 ± 0.02</td>
<td>38.5 ± 17.8</td>
</tr>
<tr>
<td>1.00 Hz</td>
<td>0.90 ± 0.01</td>
<td>43.2 ± 22.0</td>
</tr>
<tr>
<td>1.25 Hz</td>
<td>1.13 ± 0.02</td>
<td>41.9 ± 15.8</td>
</tr>
<tr>
<td>1.50 Hz</td>
<td>1.46 ± 0.09</td>
<td>39.7 ± 13.4</td>
</tr>
</tbody>
</table>