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### Masticatory muscle pain: Causes, consequences, and diagnosis

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**Normalization reduces the spatial dependency  
of the jaw-stretch reflex activity in the  
human masseter muscle**

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### **ABSTRACT**

The jaw-stretch reflex is the short-latency response in the jaw-closing muscles after a sudden stretch. The hypothesis whether normalization of the jaw-stretch reflex amplitude with respect to prestimulus electromyographic (EMG) activity will make the amplitude more independent of the location of the electrodes over the masseter muscle was tested. A 5 × 6 electrode grid was used to record the jaw-stretch reflex from 25 sites over the right masseter muscle of 15 healthy men. The results showed that there was a significant site dependency of the prestimulus EMG activity and the reflex amplitude. High cross-correlation coefficients were found between the spatial distribution of mean prestimulus EMG activities and reflex amplitude. When the reflex amplitude was normalized with respect to the prestimulus EMG activity, no site dependency was found. In conclusion, normalization of the jaw-stretch reflex amplitude by the pre-stimulus EMG activity strongly reduces its spatial dependency.

**Key words:** Jaw-stretch reflex, multichannel EMG, normalization, human masseter muscle

### **ACKNOWLEDGMENTS**

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## **INTRODUCTION**

The jaw-stretch reflex is the short-latency excitatory response in the jaw-closing muscles after the application of a sudden stretch. It is considered the trigeminal equivalent of the monosynaptic spinal stretch reflex in limb muscles (Lund et al., 1983). The reflex is important in the maintenance of the posture of the mandible against the gravitational load during running (Miles, 2007), for compensating sudden changes in load during chewing, and for development of powerful bite forces in aggressive or defensive situations (Scutter and Türker, 2001). The simplest way to provoke the jaw-stretch reflex is by tapping the chin with a reflex hammer (Murray and Klineberg, 1984). However, this method does not allow the control of important parameters such as the amount of displacement of the mandible or the applied force. More complex systems that control the displacement of the mandible allow the reflex provocation under standardized conditions (Lobbezoo et al., 1993a; Miles et al., 1993; Svensson et al., 2000).

The jaw-stretch reflex can be recorded from the jaw-closing muscles with the use of bipolar surface electrodes (Wang and Svensson, 2001). It appears as a biphasic excitatory potential in the raw electromyographic (EMG) signal (Wang et al., 2000). The voluntary EMG activity has been reported to vary between different recording sites over the masseter muscle (Schumann et al., 1994; Castroflorio et al., 2005) but little is known about the spatial dependency of the amplitude of the jaw-stretch reflex. If the reflex amplitude follows the same spatial distribution as the voluntary EMG activity, it may be expected that normalizing the amplitude with respect to the voluntary activity preceding the reflex stimulus, could reduce the dependency on the recording site.

The aim of the study was to test the hypothesis that normalization of the jaw-stretch reflex amplitude with respect to the voluntary EMG activity preceding the reflex stimulus makes the amplitude more independent of the electrode location over the masseter muscle.

## **MATERIALS AND METHODS**

### **Participants**

Fifteen men (mean age  $\pm$  SD = 24.6  $\pm$  2.1 years) who were free of temporomandibular disorders (Dworkin and LeResche, 1992) participated in the study. All had full, healthy dentition ( $\geq$ 28 teeth), without periodontal or endodontic problems, or extensive restorative works. Before the start of the experiment, the participants were given detailed information about the experimental procedure, and when they agreed to proceed, they signed a written informed consent for their voluntary participation. The experiment was conducted in accordance to the Helsinki Declaration, and the protocol was approved by the local Ethics Committee of the University of Aalborg (VN20040074).

### **Elicitation of the Jaw-Stretch Reflex**

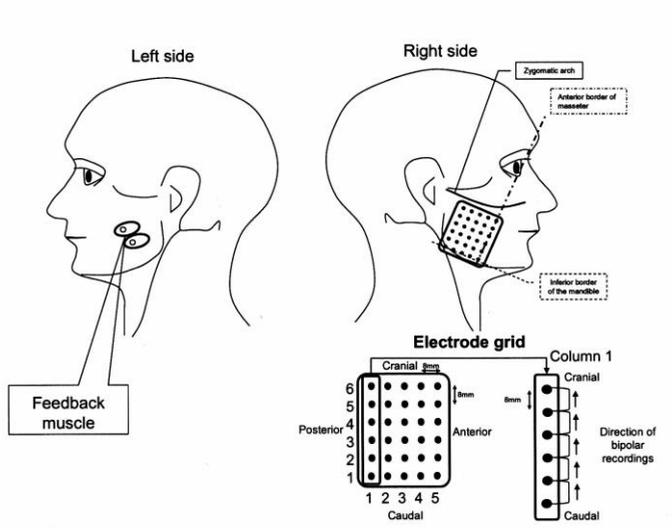
The jaw-stretch reflexes were evoked with a custom-built muscle stretcher based on the original design of Miles (Miles et al., 1993). Briefly, it consisted of two stainless-steel jaw bite-bars which were 1.5 cm wide and 2 cm long each. The upper jaw bite-bar was mounted on a frame firmly attached to the floor. A powerful electromagnetic vibrator (Ling Dynamic Systems, model 406, UK) imposed servo-controlled displacements of the lower jaw bite-bar. A 200 N load cell (Kistler 5039 A312, Switzerland), placed in series with the moveable probe of the vibrator, measured forces on the lower jaw bar. The displacement of the vibrator probe was measured with a linear potentiometer (Sakae Type 20FLP 30A-5K, Japan) mounted parallel to the vibrator probe. Acceleration in the vertical plane was measured with an accelerometer (Delta Tron Accelerometer Type 4399, Brüel and Kjær, Denmark) mounted on the lower jaw bar. The jaw-stretch reflex was evoked by a displacement of the lower bar of 1 mm with a ramp time of 10 ms. The initial jaw gape, determined by the distance between the outer surfaces of the upper and lower jaw bars, was 4 mm (Poliakov and Miles, 1994).

### **Surface EMG recordings**

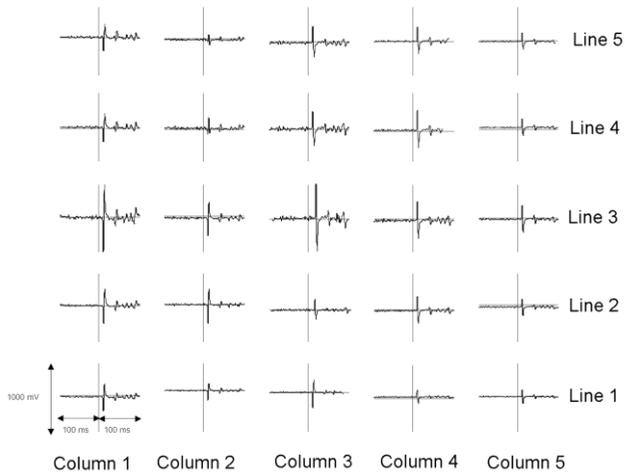
A bipolar surface EMG signal was recorded from the left masseter muscle (electrodes 720-01-k, 4 x 7 mm recording area, Neuroline, Medicotest, Ølstykke, Denmark). The electrodes were placed 10 mm apart over the muscle belly (Fig. 1) after skin preparation with alcohol. The bipolar EMG signal was amplified (200 - 5000 times; Counterpoint MK2, Skovlunde, Denmark), filtered (bandwidth 20 Hz – 1 kHz), and sampled at 4 kHz.

Multi-channel bipolar surface EMG recordings were obtained from the right masseter muscle using a 5 × 6 electrode grid (LISiN-OT Bioelettronica, Torino, Italy). The electrode diameter was 1 mm, and there was a distance of 8 mm between electrodes in both the posterior-anterior and caudal-cranial directions. For each column of electrodes, multi-channel bipolar recordings were obtained from adjacent electrodes in the caudal-cranial direction (Fig. 1). This analysis provided a total of 25 bipolar recordings. To locate the grid, the inferior borders of the mandible and the zygomatic arch, together with the posterior border of the mandibular ramus were marked on the skin using a skin-marking pen. The anterior border of the masseter was identified by palpation during subsequent clenching and relaxing tasks. After the skin was prepared using abrasive paste, the electrode grid was mounted with the first anterior column of electrodes corresponding to the anterior border of the masseter and the last row corresponding to the inferior border of the mandible (Fig. 1). The multi-channel bipolar EMG signals were amplified (64-channel surface EMG amplifier, SEA 64, OT Bioelettronica, Torino, Italy; -3dB bandwidth 10 - 500 Hz) with a gain of 2000 - 5000 and sampled at 2048 Hz with 12-bit resolution. A ground electrode was placed around the right wrist to serve as common reference for the EMG signals recorded from the left and right masseters.

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**Figure 1.** Schematic representation of the placement of the  $5 \times 6$  electrode grid. For each column of electrodes, multi-channel bipolar recordings were obtained from adjacent electrodes in the caudal-cranial direction, providing 25 multi-channel bipolar recordings.



**Figure 2.** Example of the reflex response as recorded at the 25 recording sites over the right masseter of one subject. Each signal is the average of 20 sweeps.

### **Procedures**

During the recording of the reflexes the subjects were seated upright in front of the muscle stretcher while they were biting with the incisors on the two jaw bars. The jaw bars were covered with a thin piece of rubber to protect the teeth and with a disposable small plastic coverage as a hygiene measure. The thickness of these two plastic layers was negligible and did not increase the initial vertical bite.

Each participant performed three, 5-s maximal clenches with the incisor teeth on the jaw bars of the muscle-stretcher. The mean value of the root mean square of the three maximum EMG recordings of the left masseter muscle was regarded as the maximum voluntary contraction (MVC) and was used for visual feedback for the remainder of the experiment. The participants were then instructed to contract their muscles at a level corresponding to 15% of the MVC. The muscle stretcher was automatically triggered each time the EMG activity of the left masseter was stable between 13.5% and 16.5% of the MVC value for 400 ms. For each stimulus and for each of the multichannel bipolar recordings, 300 ms of peristimulus EMG activity was recorded, with 100 ms prestimulus and 200 ms poststimulus. In total, 20 reflexes were evoked per participant.

### **Signal analysis**

For each of the multichannel bipolar recordings, the EMG signal in the 100 ms preceding the stimulus was first full-wave rectified and then averaged. The mean of the 20 reflex sweeps was considered the prestimulus EMG activity of that bipolar recording.

For analysis of the reflex amplitude of each multi-channel bipolar recording, the EMG signals of the 200 ms following a stimulus were averaged over the 20 individual sweeps. The jaw-stretch reflex appeared as a biphasic potential in this averaged sweep (Scutter and Türker, 2001). The amplitude of this reflex was the peak-to-peak value of this biphasic potential. The reflex amplitude was also normalized (%) with respect to the mean pre-stimulus EMG activity of that multi-channel bipolar recording.

### **Statistical analysis**

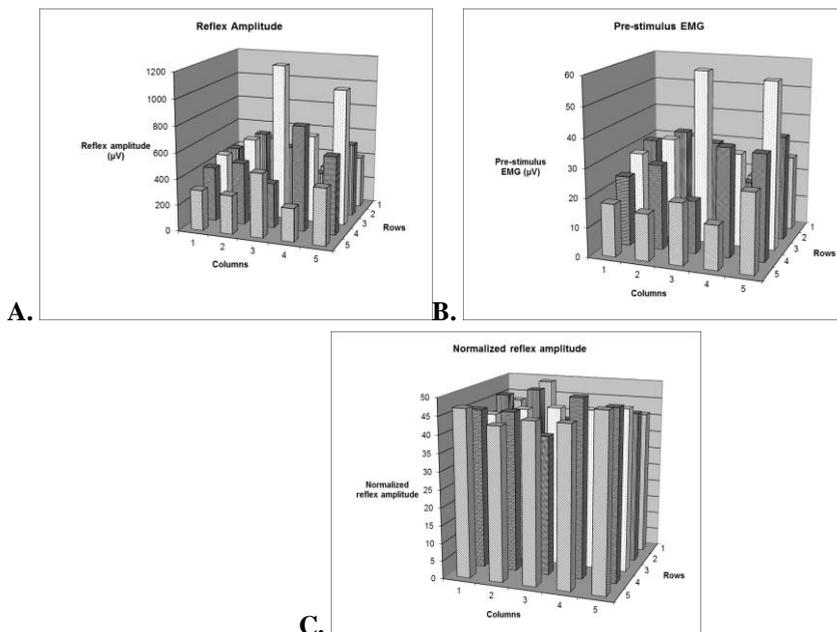
The mean pre-stimulus EMG activity, the mean reflex amplitude, and the mean normalized reflex amplitude were analyzed separately with two-way analysis of variance (ANOVA) with as factors the posterior-anterior and caudal-cranial direction. When sphericity was violated (i.e., a non-equal level of dependence between the axes was present), conservative Greenhouse-Geisser corrected values of the degree of freedom were used (Mauchly, 1940; Greenhouse, 1959). For each participant, a cross-correlation analysis was also performed between the 25 mean pre-stimulus EMG activities and the corresponding reflex amplitudes. Significance was accepted for  $P < 0.05$  and results are presented as mean and standard deviation (mean  $\pm$  SD).

## RESULTS

The jaw-stretch reflex amplitude depended on the recording site (Fig. 2, Fig. 3A, and Table IA). The two-way ANOVA showed that this site dependency was statistically significant, both for the posterior-anterior ( $P = 0.006$ ,  $F = 5.6$ ) and caudal-cranial direction ( $P = 0.001$ ,  $F = 11.2$ ). An interaction was also found between the two directions ( $P < 0.001$ ,  $F = 5.2$ ). Also, the mean pre-stimulus EMG activity depended on the recording site, both in the posterior-anterior ( $P < 0.001$ ,  $F = 15.7$ ) and in the caudal-cranial directions ( $P < 0.001$ ,  $F = 9.1$ ) (Fig. 3B, Table IB). Again, an interaction was found between the two directions ( $P = 0.005$ ,  $F = 5.1$ ).

High cross-correlation coefficients were found between the 25 mean prestimulus EMG activities and reflex amplitudes (mean correlation coefficient over the 15 volunteers,  $0.88 \pm 0.17$ ), indicating that the reflex and voluntary EMG activities had a similar spatial distribution over the muscle.

When the reflex amplitude was normalized with respect to the prestimulus EMG activity, no dependency was found for the posterior-anterior direction ( $P = 0.26$ ,  $F = 1.4$ ) or the caudal-cranial direction ( $P = 0.68$ ,  $F = 0.4$ ) (Fig. 3C and Table IC, II). Some site dependency was still present as indicated by the interaction between the two factors of the ANOVA which just reached statistical significance ( $P = 0.05$ ,  $F = 2.3$ ).



**Figure 3.** Schematic representation of the mean values ( $\pm$  SD,  $n = 15$  subjects) of the reflex amplitude ( $\mu V$ ) (A), the full-wave rectified and averaged (over 100 ms) pre-stimulus EMG activity ( $\mu V$ ) (B), and the normalized reflex amplitude (C) (data presented in Table I).

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**Table 1.** Mean values ( $\pm$  SD,  $n = 15$  subjects) of the reflex amplitude ( $\mu$ V) (A), the full-wave rectified and averaged (over 100 ms) pre-stimulus EMG activity ( $\mu$ V) (B), and the normalized reflex amplitude (C).

<b>A.</b>	Column 1	Column 2	Column 3	Column 4	Column 5
Row 5	311 $\pm$ 265	296 $\pm$ 304	491 $\pm$ 569	254 $\pm$ 193	433 $\pm$ 292
Row 4	418 $\pm$ 375	477 $\pm$ 439	344 $\pm$ 263	802 $\pm$ 893	598 $\pm$ 419
Row 3	459 $\pm$ 418	602 $\pm$ 466	1193 $\pm$ 1284	669 $\pm$ 663	1039 $\pm$ 778
Row 2	452 $\pm$ 387	591 $\pm$ 502	504 $\pm$ 306	313 $\pm$ 205	566 $\pm$ 369
Row 1	255 $\pm$ 187	500 $\pm$ 419	468 $\pm$ 336	299 $\pm$ 249	401 $\pm$ 257

<b>B.</b>	Column 1	Column 2	Column 3	Column 4	Column 5
Row 5	18 $\pm$ 16	16 $\pm$ 14	21 $\pm$ 21	15 $\pm$ 11	27 $\pm$ 19
Row 4	24 $\pm$ 24	29 $\pm$ 29	18 $\pm$ 12	37 $\pm$ 36	36 $\pm$ 26
Row 3	29 $\pm$ 29	35 $\pm$ 27	59 $\pm$ 56	32 $\pm$ 27	57 $\pm$ 43
Row 2	31 $\pm$ 36	35 $\pm$ 31	32 $\pm$ 21	19 $\pm$ 13	36 $\pm$ 24
Row 1	19 $\pm$ 13	31 $\pm$ 27	28 $\pm$ 20	18 $\pm$ 14	26 $\pm$ 19

<b>C.</b>	Column 1	Column 2	Column 3	Column 4	Column 5
Row 5	47 $\pm$ 12	43 $\pm$ 13	45 $\pm$ 16	45 $\pm$ 9	49 $\pm$ 7
Row 4	45 $\pm$ 14	45 $\pm$ 10	39 $\pm$ 10	50 $\pm$ 11	48 $\pm$ 11
Row 3	42 $\pm$ 13	44 $\pm$ 14	45 $\pm$ 14	45 $\pm$ 11	46 $\pm$ 11
Row 2	46 $\pm$ 12	48 $\pm$ 9	40 $\pm$ 12	38 $\pm$ 9	43 $\pm$ 12
Row 1	43 $\pm$ 14	49 $\pm$ 8	42 $\pm$ 15	39 $\pm$ 14	41 $\pm$ 12

**Table 2.** Results from the two-way ANOVA on the data presented in Table I. (\* statistically significant results).

	Caudal-cranial direction		Posterior-Anterior direction		Interaction	
	F	p-value	F	p-value	F	p-value
Reflex amplitude	11.2	0.001*	5.6	0.006*	5.2	< 0.001*
Pre-stimulus EMG activity	9.1	< 0.001*	15.7	< 0.001*	5.1	0.005*
Normalized reflex amplitude	0.4	0.68	1.4	0.26	2.3	0.05

## **DISCUSSION**

The results of this study showed that, when the jaw-stretch reflex amplitude of the masseter muscle is normalized with respect to the pre-stimulus EMG amplitude, it becomes independent of the location of the electrodes over the muscle.

Human jaw-stretch reflexes are extensively studied because they give insight into the neurophysiologic trigeminal mechanisms responsible for the motor control of jaw-closing muscles (Poliakov and Miles, 1994). The monosynaptic jaw-stretch reflex is part of the response to a sudden perturbation that activates the muscle stretch-sensitive receptors. Two methods have been proposed for quantifying the excitatory part of the reflex. The first method uses the peak-to-peak amplitude of the biphasic potential appearing in the EMG signal (Biasiotta et al., 2007; Kimura et al., 1994; Lobbezoo et al., 2003; Murray and Klineberg, 1984; Peddireddy et al., 2005; 2006; Svensson et al., 2000; Svensson et al., 2001; Svensson et al., 2003; Türker, 2002; van Selms et al., 2005; Wang et al., 2000; Wang and Svensson, 2001), whereas the second one uses only the first depolarizing peak (Widmer et al., 1989; Lobbezoo et al., 1993a; b; Lobbezoo et al., 1993c; 1996). In the present study, the first method was used for analysis of the data. Separate analysis using the second method led to the same results (data not shown).

The development of highly controlled jaw-muscle stretchers (Miles et al., 1993; Svensson et al., 2000) has enabled the provocation of the jaw-stretch reflex under standardized conditions. With the use of such equipment, it has been shown that the reflex amplitude is proportionally dependent on the level of the pre-stimulus EMG amplitude (Wang and Svensson, 2001), making it necessary to control the excitability of the motoneuronal pool during reflex provocation (Türker, 2007). In the present experiment, in order to take this into account, the level of background EMG amplitude was controlled through visual feedback from the left masseter muscle.

The EMG activity in the masseter muscle has been reported to vary between different recording sites (Schumann et al., 1994; Castroflorio et al., 2005). The results of the present study show that a similar degree of variability exists in the amplitude of the jaw-stretch reflex. This may substantially increase the variability of results among studies which use different criteria for electrode placement. The high cross-correlation coefficients between the pre-stimulus EMG amplitude and the reflex amplitude imply that muscle areas with high voluntary EMG amplitude also show high amplitudes of the stretch-reflex. Anatomical and physiological factors, such as the dispersed innervation zones of the masseter muscle (Castroflorio et al., 2005) and the relatively small motor unit territories in relation to limb muscles (van Eijden and Turkawski, 2001), may account for these similar spatial distributions. Normalization of the reflex by the pre-stimulus EMG activity reduced its spatial dependency and made the recording of the reflex independent of the location of the electrodes. Thus, it would be expected that the reproducibility of the recording of the normalized reflex amplitude would be increased.

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In conclusion, in young, healthy participants, normalization of the jaw-stretch reflex amplitude by the pre-stimulus EMG amplitude strongly reduces the spatial variability typical of non-normalized reflex responses.

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