Research report

Effect of experimental pain from trigeminal muscle and skin on motor cortex excitability in humans

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Abstract

The pathophysiology of many orofacial pain syndromes is still unclear. We investigated the effect of tonic muscle and skin pain on the excitability of the trigeminal motor pathways using transcranial magnetic stimulation (TMS). Motor evoked potentials (MEPs) were recorded in the masseter surface electromyogram (EMG). Magnetic pulses were delivered with a large coil at intensities 1.1 and 1.5 times the motor threshold, and for each intensity, MEPs were recorded at three different clenching levels: 15, 30 and 45% of maximum voluntary contraction (MVC). Baseline, pain and post-baseline recordings were compared in two sessions. Firstly, muscle pain was induced by infusion of hypertonic saline (5.8%) into the left masseter. Secondly, skin pain was induced by topical application of capsaicin (5%). Muscle and skin pain did not induce significant effects on the amplitude or latency of the MEPs (ANOVA: \( P > 0.50 \)). In both sessions, the amplitude of the MEPs increased with the increase of the clenching level and stimulus intensity \( (P < 0.0001; \ P < 0.005) \) whereas the latency was not significantly changed \( (P > 0.05; \ P = 0.11) \). Muscle pain was associated with an increase in the pre-stimulus EMG activity on the non-painful side compared with baseline \( (P < 0.01) \), which could be due to compensatory changes in the activation of the painful muscle. The need for voluntary contraction to evoke MEPs in the masseter muscles and compensatory mechanisms both at the brainstem and cortical level might explain the lack of detectable modulation of MEPs. Nonetheless, the present findings did not support the so-called ‘vicious cycle’ between pain — central hyperexcitability — muscle hyperactivity.

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1. Introduction

Interactions between activity in nociceptive afferent fibers and motor excitability have been described at spinal and trigeminal level \([17,20,23,35]\). In humans, there is indirect evidence that painful stimuli may modulate motor activity at the cortical level, and vice-versa \([7,36,41,42]\). Moreover, some clinical conditions involving masticatory muscle dysfunction and orofacial pain have been attributed to primary hyperactivity of the central nervous system \([11,15]\). Relatively few studies have dealt with the possible modulation of the trigeminal motor pathways related to pain \([10,32]\). Investigations in experimental models and in patients, however, have shown different effects of pain on central nervous system excitability. In particular, there is evidence of inhibitory effects in experimental pain conditions and no central modulation in patients \([10,23,32,44]\). In order to bridge the gap between different experimental conditions and to test the long-held concept of the so-called ‘vicious cycle’ between pain — central hyperexcitability — muscle hyperactivity, we investigated the effects of tonic muscle and skin pain on the trigeminal motor pathways excitability in humans with the use of transcra-
nial magnetic stimulation (TMS). This technique is now widely used to assess motor pathways both in healthy subjects and in patients [3,6,31,40]. In the trigeminal system TMS provides an indirect measure of the central excitability of the masticatory system including motor cortex and corticobulbar connections [8,25,27].

The aim of the present study was to investigate the modulation of trigeminal motor pathways during tonic muscle and skin pain. Moreover, since the recording of trigeminal MEPs requires voluntary activation of the masticatory muscles the importance of clenching levels was determined in each experimental condition. Finally, different levels of motor cortical activation were examined using two different stimulus intensities (1.1 and 1.5 times the motor threshold).

2. Materials and methods

2.1. Subjects

A total of 17 healthy subjects (12 men and 5 women) aged 21–42 years (mean age±S.E.M.: 26.2±1.7) participated. The subjects had no history of temporomandibular disorders or orofacial pain and they did not take any medications. All the subjects fulfilled the inclusion criteria for magnetic stimulation of neural tissue [28]. Informed consent was obtained prior to the study in accordance with the guidelines of the Helsinki Declaration. The Local Ethics Committee had approved the study.

2.2. Recording of MEPs

The subjects sat upright and relaxed in a dental chair with the head supported by a headrest. Electromyographic activity (EMG) was bilaterally recorded with bipolar surface electrodes (Neuroline, Medicotest, Denmark) placed on the central part of the masseter muscles along the main direction of muscle fibers, with an inter-electrode distance of 2 cm. The disposable surface electrodes had an active recording area of 4×7 mm. The EMG signals were amplified, filtered (20 Hz–1 kHz), sampled at 4 kHz (Counterpoint MK2, Dantec, Denmark) and stored on disk for off-line analysis. Initially, the EMG activity corresponding to maximum voluntary contraction (MVC) of the masseter muscles was determined while the subjects were biting in the intercuspal teeth position. The EMG level corresponding to the MVC calculated at the start of the experiment was used to set a window (±10%) around three different clenching levels: 15% (13.5–16.5%), 30% (27–33%) and 45% (40.5–49.5%) MVC. The subjects received visual feedback from markers on the computer screen indicating clearly when the level was in the predefined interval. The EMG activity recorded from the left masseter muscle served as the feedback. When the ongoing EMG activity remained within the window for more than 400 ms, the program automatically triggered the magnetic stimulator (MagLite-r25, Dantec, Denmark) [37]. The subjects were asked to maintain the clenching level for about 2–3 s after each stimulus. The inter-stimulus interval was 10–15 s. The duration of EMG activity recorded was 300 ms and included pre-stimulus (100 ms) and post-stimulus (200 ms) periods.

Transcranial magnetic stimulation was performed with the MagLite-r25 and a circular coil (140 mm diameter; peak magnetic field: 1.9 T). The coil was placed over the midline, 3–4 cm anterior to the vertex with the current flowing clockwise. After finding the optimal stimulation site on the scalp, it was marked with a dark pen and the coil was fixed in a stable position, so that the same position was kept during the whole experiment. The subject was asked to keep his head still and the position of the coil was checked after each trial. The motor threshold (Th) was measured while the subject was clenching their teeth at about 30% of maximum. The threshold was determined by descending and ascending methods and was defined as the minimum stimulus intensity that produced five discrete MEPs in both muscles, with peak-to-peak amplitude of at least 0.10 mV, discernible visually on the monitor from ten consecutive stimuli. The mean Th, measured at 30% MVC, was 48.1%±1.4 of the maximum output of the magnetic stimulator. Low-intensity stimuli (1.1×Th) had a mean intensity of 54.6±1.6%; the high intensity (1.5×Th) had a mean intensity of 70.2±1.8%.

2.3. Experimental pain

Tonic muscle pain was induced by infusion of sterile 5.8% hypertonic saline into the deep mid portion of the left masseter muscle. The saline injection was given over a 20-s period followed by a steady infusion rate of 6 ml/h for the next 440 s and finally 9 ml/h for the following 440 s with the use a computer-controlled syringe pump (IVAC, Model 770, USA) and a 10-ml plastic syringe [37]. A tube (IVAC, G30303, USA) connected the syringe to a disposable needle (27G, 20 mm) that was inserted into the left masseter muscle. Tonic skin pain was induced by capsaicin (1 ml, 500 µg/ml) applied topically in a plaster of 4-cm² area on the left cheek. This procedure has previously been shown to cause a steady, burning type of pain [30]. In both experiments subjects were instructed in the use of a 10-cm electronic visual analogue scale (VAS) with the lower extreme labeled ‘no pain’ and the upper extreme labeled ‘most pain imaginable’. The VAS score was sampled every 5 s and stored on a computer. The mean VAS score was calculated in 500-s intervals in which the subject felt constant level of pain. After the infusion or the removal of the plaster, the subjects described the quality of pain using a Danish version of the McGill Pain Questionnaire (MPQ) [13].
2.4. Protocol

The experiment was performed in two separate sessions. In the first session the effect of muscle pain was examined and in the second session skin pain was studied. Each experiment was performed in ten subjects; three subjects participated in both sessions. The recording procedure was the same on both occasions: MEPs were recorded at three clenching levels (15, 30 and 45% MVC) and for each clenching level two stimulus intensities were used: 1.1 (low intensity=L) and 1.5 (high intensity=H) times the motor threshold. With this paradigm, MEPs were measured prior to the application of experimental pain (baseline), during pain and 20 min after pain had disappeared (post-baseline). MEPs were recorded when the pain intensity was constant. In three subjects, attempts were made to obtain MEPs in the three conditions with the jaw-closing muscles at rest (0% MVC). The sequence of clenching levels and stimulus intensities was randomized. A total of 16 EMG sweeps were recorded in each trial and averaged off-line. The onset-latency and peak-to-peak amplitude were measured on the non-rectified, averaged MEPs. The root-mean-square (RMS) amplitude of the 100 ms preceding the magnetic stimulus (RMS pre) was measured from rectified averaged signals.

2.5. Statistical analysis

Mean values±S.E.M. are given in the text, tables and figures. The onset latencies, the amplitudes of the MEPs and the pre-stimulus EMG activity of both left and right masseter were compared using analysis of variance (ANOVA) with four repeated factors: muscles (two levels: painful and non-painful side); clenching level (three levels: 15, 30 and 45% of MVC); stimulus intensity (two levels: low and high intensity); conditions (three levels: baseline, pain, post-baseline). Post-hoc Tukey tests were performed to adjust for multiple pair-wise comparisons. Significance was set at $P<0.05$ for all the analyses.

3. Results

3.1. Experimental pain

The mean amount of hypertonic saline infused into the left masseter muscle was $2.2±0.3$ ml. The infusion caused a local sensation of ‘intense’ (nine subjects out of ten) pain from the masseter muscle, with a spread toward the upper or lower molar teeth and the temporomandibular joint (3/10). The mean pain intensity on the VAS was $5.4±0.3$ cm. Topical application of capsaicin produced a local painful sensation described as ‘burning’ (7/10). Spontaneous pain was located in the capsaicin-treated area only. The mean pain intensity on the VAS was $2.9±0.2$ cm.

3.2. Motor evoked responses

During clenching, MEPs were obtained bilaterally in the masseter, in all subjects. No responses were detected with the jaw muscles at rest. The MEPs appeared as biphasic and reproducible responses within individuals (Fig. 1). Furthermore, the MEPs were symmetrical and the overall four-way ANOVA showed no significant differences in latency or amplitude between the two sides. The central position of the coil prevented stimulation of the trigeminal root and no early responses were observed on either side [8].

3.3. Effect of tonic pain

Neither muscle pain nor skin pain affected the MEPs (Figs. 2 and 3). For all clenching levels and stimulus intensities, latency and amplitude did not change with muscle pain ($F (2,9)=0.19; P>0.50$) $(F (2,9)=0.19; P>0.50)$ or skin pain ($F (2,9)=0.08; P>0.50$) $(F (2,9)=0.30; P>0.50)$. Pre-stimulus EMG activity remained constant with skin pain ($F (2,9)=0.05; P=0.93$). During and after muscle pain a significant asymmetry of the pre-stimulus EMG activity was observed due to a relative increase in the pre-stimulus EMG activity in the right masseter (non-

![Fig. 1. Example of MEP recordings in one subject in the baseline condition from the left (MAL) and right masseter muscles (MAR) at different clenching levels (15, 30, 45% and rest), during low (1.1) and high intensity (1.5) stimulation. The increase in the clenching level and the stimulus intensity produced a clear increase in amplitude and tended to shorten the onset-latency. No MEPs could be recorded at rest.](image-url)

3.4. Effect of clenching level

Even at low levels of voluntary contraction (15% MVC) and low stimulus intensity reproducible MEPs were obtained in all subjects with a short latency (mean 6.2±0.6 ms) and a small amplitude (mean 0.5±0.1 mV). When the clenching level was increased the amplitude strongly increased from 0.5 mV±0.1 at 15% MVC to 1.4 mV±0.2 at 45% MVC (F (2,9)=26.4; P<0.0001) (Fig. 3), and latency slightly, but not significantly, shortened (F (2,9)=3.6; P>0.05) (Fig. 2).

3.5. Effect of stimulus intensity

Increasing the output of the magnetic stimulator from 1.1 to 1.5 times the motor threshold significantly increased the amplitude (F (2,9)=22.8; P<0.003) (Fig. 3). The latency shortened by less than 1 ms; this change, however, did not reach statistical significance (F (2,9)=3.4; P=0.05) (Fig. 2).

Our subjects perceived muscle pain as more intense and with a different quality than skin pain. This is most likely a reflection of differences in density and types of peripheral afferents, central projections and processing, and different algesic properties of hypertonic saline and capsaicin [26,36,45]. However, regardless of differences in the

4. Discussion

The present study showed that tonic muscle and skin pain did not induce detectable change in the motor evoked potentials (MEPs) in the masseter muscles. Muscle pain, however, was associated with an increase in the pre-stimulus EMG activity in the non-painful masseter. Systematic changes in pre-stimulus EMG activity of the masseter muscles and stimulus intensity influenced significantly the amplitude of the MEPs.

4.1. Effect of tonic pain on cortical excitability

Our subjects perceived muscle pain as more intense and with a different quality than skin pain. This is most likely a reflection of differences in density and types of peripheral afferents, central projections and processing, and different algesic properties of hypertonic saline and capsaicin [26,36,45]. However, regardless of differences in the
intensity and quality of two modalities of experimental pain no detectable changes in the amplitude and onset-latency of the MEPs were found.

Few studies have dealt with pain-induced changes of MEPs. Painful laser stimuli applied to the skin of the hand induced bilateral inhibitory effects on motor cortex excitability in humans [42]. However, the trigeminal central motor pathways had a normal excitability in a group of patients with painful temporomandibular disorders [10]. A direct comparison with these studies is difficult because of differences in duration and modality of the nociceptive input and because extrapolation of results from the spinal system to the trigeminal system is complicated by functional differences [2,14,24,33,35,36,44]. In the present study there was no evidence of pain-induced changes of motor cortex excitability although we can not entirely exclude this possibility. The necessity of voluntary contraction of the jaw-closing muscles to elicit MEPs could, in part, have masked an inhibitory effect induced by the nociceptive input. TMS is supposed to induce I-waves preferentially (due to indirect or transsynaptic activation of corticospinal or corticobulbar neurons); the D-wave (due to direct activation of the corticospinal or corticobulbar axons) can occur only at higher stimulus intensities [5,18,19]. The lowest intensity of the magnetic stimulus used in the present study (1.1×motor threshold) could nevertheless have activated structures downstream to the motor cortex, i.e., the corticobulbar axons directly in the white matter like electrical anodal stimulation would have done; hence an inhibitory effect could have occurred upstream to the site of excitation along the motor pathways. In this case the voluntary activation of muscles is inhibited but MEPs would not be affected. TMS is known to induce D-waves at high intensity (70–80% output) and their amplitude is smaller than that of D-waves elicited by electrical stimulation [1,5]. The D-wave induced by magnetic stimulation is also smaller than the second or third components of the I-waves [18]. There is also evidence that TMS can produce D-waves but that cranial-nerve motoneurons require the arrival of I-waves in order to fire [4,9]. Thus, we can not exclude that the magnetic stimuli in the present study induced some D-waves even at low intensity (55%), but the contribution of the I-waves to the recorded MEPs was most likely larger than the contribution of the D-waves. Hence we believe that the low-intensity TMS would have been able to detect a change in cortical excitability if it had occurred.

4.2. Effect of tonic pain on lower-motoneuron excitability

Although tonic pain had no effect on MEPs, muscle pain
Fig. 4. Effects of different conditions (■ Baseline, ▲ Pain and ● Post-Baseline) on the pre-stimulus EMG activity (pre-RMS) at different clenching levels (15, 30 and 45%) and stimulus intensity (--- Low and ---- High). Experimental muscle pain induced a significant increase in the pre-RMS on the non-painful side \( (P<0.01) \). The bars with the asterix indicate the significant increase in the pre-RMS during Pain and Post-Baseline conditions compared to the Baseline condition, at 30 and 45% clenching level (Tukey test: \( P<0.05; P<0.005 \)). No effects were observed on the pre-RMS at different stimulus intensity \( (P>0.50) \).

was associated with an asymmetry in the pre-stimulus EMG activity. The relative increase of the pre-stimulus EMG activity was only observed on the non-painful side (non-feedback muscle) and more pronounced at higher clenching levels, probably because muscle pain increases with stronger contractions. Since the painful muscle also served as the feedback muscle and necessarily had a constant EMG level, an inhibitory effect on the homonymous motoneurons could have been compensated by an increased voluntary drive. An increased activity in the descending pathways would also have an impact on the contralateral motoneuron pool [6] and cause an increase in the pre-stimulus EMG activity on the non-painful side (non-feedback muscle). Other studies have documented that experimental pain in the masseter muscle is associated with a significant decrease of the maximum voluntary occlusal force and EMG activity during agonist function [35,37,43]. It has been suggested that the inhibitory effect of muscle pain on motor function can be interpreted as an ‘adaptation’ to pain, in order to limit overall movements and protect the painful muscle [24,34]. In some studies the effect was lateralized on the painful side [38,43].

Because the asymmetry of the pre-stimulus EMG activity was also found in the post-baseline recordings, the effect of muscle pain seemed to outlast pain itself. Ro and Capra [29] recently described that injection of hypertonic saline into the cat masseter muscle caused prolonged effects. Movements and muscle contraction may increase the firing rate of muscle nociceptors primed by the chemical stimulus [44]. To explain post-infusion changes, we suggest that, although the subjects no longer reported pain, the muscle may nevertheless have been tender in the post-pain recording. Regardless of the mechanism, these long-term, pain-related changes in masseter EMG activity suggest that the nociceptive input to motoneurons was so strong that it could produce excitability changes masking any changes in corticobulbar excitability.

Tonic cutaneous pain did not induce any effect on the pre-stimulus EMG activity. This is consistent with previous studies reporting that topical application of capsaicin is unable to induce changes in the EMG activity or the electrically-evoked exteroceptive suppression responses mediated at brainstem level [21,30].

4.3. Effects of clenching level and stimulus intensity

This is the first study in the trigeminal system with automatic triggering of the TMS under highly standardized conditions. The masseter MEPs were similar to those previously described in healthy subjects [8,25,27]. An increase in background voluntary contraction induced a progressive increase of amplitude and tended to shorten the
latency of the MEPs in the present study. This phenomenon has been extensively studied in the limbs and may result from enhanced excitability at cortical level (more powerful descending volleys) or spinal level (lower threshold of motoneurons) [12,16,22,39].

In the trigeminal system voluntary pre-activation is essential to evoke masseter MEPs. The most likely reason is that masseteric motoneurons have a high activation threshold or that a relatively small proportion of the trigeminal motoneuronal pool receives monosynaptic, fast-conducting cortical projections [8]. However, once some background EMG is provided the masseter MEPs are progressively facilitated by further increases in voluntary contraction similarly to limb MEPs and likely with similar mechanisms at the segmental and cortical level.

The increase in stimulus intensity from 1.1 to 1.5 times the motor threshold also induced a strong increase in the amplitude of the MEPs in the present study. This could be due to a greater number of excited pyramidal cells and descending fibers or because the increased firing rate exerted a greater spatial-temporal summation at the lower-motoneuron synapse. The latency of the MEPs was shortened by less than 1 ms and this small latency gain confirms findings of previous studies [8,25]. Much of the latency gain, which can be observed in MEPs in the hand muscles, may be due to the absence of D-waves with low-intensity magnetic stimuli [5,16,18]. In contrast, a relatively long central delay of MEPs in cranial-nerve muscles is probably caused by the need for the arrival of the first I-waves, both with low- and high-intensity stimuli [4,9].

In conclusion, the present study shows that experimental tonic pain in the trigeminal region (whether from muscle or skin) is unable to induce detectable modulation of the MEPs. This finding does not entirely exclude pain-related changes in cortical excitability, because the magnetic stimulus may also generate some D-waves, thus bypassing the pyramidal cell and because compensatory mechanisms at the cortical or brainstem level in addition to the strong voluntary activity may raise the cortical excitability to a level that hinders the detection of small changes. Overall, the present findings argue against the so-called ‘vicious cycle’ between pain — central hyperexcitability — muscle hyperactivity.

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References


