Long-term antalgic effects of repetitive transcranial magnetic stimulation of motor cortex and serum beta-endorphin in patients with phantom pain

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Objectives: To assess the long-term analgesic effect of repetitive transcranial stimulation (rTMS) on chronic phantom pain using high frequency stimulation and to measure the serum beta-endorphin level pre- and post-rTMS.

Material and methods: The study included 27 patients with unilateral amputation; all patients had chronic phantom pain. The patients were classified into two groups. Seventeen patients received 10 minutes real rTMS over the hand area of motor cortex (20 Hz, 10 second trains, intensity 80% of motor threshold) every day for five consecutive days and 10 patients received sham stimulation. Pain was assessed using a visual analogue scale (VAS) and the Leeds assessment of neuropathic symptoms and signs (LANSS) scale, before and after the first, fifth sessions, one and two months after the last session. Quantitative determination of serum beta-endorphin before and after five sessions was measured.

Results: There was no significant difference between true and sham groups in the duration of illness, VAS, LANSS scores and resting motor threshold in upper and lower limb amputation at the base line. VAS and LANNS scores of the patients who received real rTMS decreased more over the course of the treatment through the different points of follow-up (after five sessions, one and two months) than those who received sham stimulation. Serum beta-endorphin was increased significantly after real stimulation with no changes in patients received shame. Serum beta-endorphin showed no significant correlation to Hamilton depression, anxiety, VAS and LANSS scores in true or sham groups before or after five sessions for rTMS.

Conclusion: These results confirm that five daily sessions of rTMS over motor cortex can produce long lasting pain relief in patients with phantom pain and it might be related to an elevation of serum beta-endorphin concentration.

Keywords: Analgesic effect, Beta-endorphin, Motor cortex, rTMs

Introduction

Peripheral, spinal, and cerebral neuronal mechanisms may generate and maintain phantom limb pain, including plastic changes occurring in the primary somatosensory cortex.¹-³ Similar plastic changes may occur in the primary motor cortex (M1), as shown after nerve transections in animals.⁴-⁶ In humans, cortical plastic changes could be shown by using an ischemic nerve block as a model for a transient deafferentation,⁷ and studying the cortical representation of proximal stump muscles after amputation by transcranial magnetic stimulation (TMS) mapping⁸,⁹ or positron emission tomography.¹⁰

Most treatments for phantom-limb pain are ineffective and do not take account of the mechanisms underlying the production of the pain.¹¹ A maximum benefit of about 30% has been reported from treatments such as local anesthesia, sympathectomy, dorsal-root entry-zone lesions, cordotomy and rhizotomy, neurostimulation methods, or pharmacological interventions such as anticonvulsants, barbiturates, antidepressants, neuroleptics, and muscle relaxants. This proportion does not exceed the placebo effect reported in other studies.¹¹,¹²

Significant analgesic effects of rTMS have been found in several studies of patients with chronic pain of various origins. M1 stimulation at high frequency was shown to reduce pain scores by 20 to 45% after active stimulation and by less than 10% after sham
stimulation. The therapeutic applications of rTMS in pain syndromes are limited by the short duration of the induced effects, but prolonged relief can be obtained by repeating rTMS sessions every day for several weeks.\textsuperscript{13}

The mechanism for the analgesic effect of rTMS, is that the noninvasive stimulation can induce plastic changes in the brain, which in turn corrects or modulates plastic changes associated with chronic pain. Initial evidence suggests that TMS affects central neurotransmitters activity in other neurological diseases.\textsuperscript{14,15} Other studies also indicate the possible role of endogenous opioid secretions triggered by long-term MCS.\textsuperscript{16,17}

Beta-endorphin is released into blood from the pituitary gland and into spinal cord and brain from hypothalamic neurons. The beta-endorphin that is released into the blood cannot enter the brain in large quantities because of the blood brain barrier.\textsuperscript{18} Khedr et al.\textsuperscript{19} measured serum dopamine after repeated sessions of rTMS as reflection of central nervous system dopamine. The present study aimed to assess the long-term analgesic effect of repetitive transcranial stimulation on chronic phantom pain using high frequency stimulation, and to measure the serum beta-endorphin level pre- and post-rTMS as an expectation of endogenous endorphins in nervous system.

\textbf{Material and Methods}

\textbf{Patients}

This study was conducted at the Department of Neurology of Assiut University Hospital, Assiut, Egypt, with participation of the Chronic Pain Unit of Anesthesiology Department, South Egypt Cancer Institute, Egypt. The study included 27 patients with unilateral amputation, 11 patients had upper limb amputation (10 of them above elbow) and 16 patients had blow knee amputation. All patients had chronic phantom pain. Clinically phantom pain was abnormally painful sensation of great intensity that they described as burning, tearing, or deep-boring either a spontaneous or commonly evoked by trivial stimuli. Neurological examination revealed an increased threshold for pinprick and thermal sensation in the painful area in all patients and a decrease in tactile sensations of varying degrees in some patients; all patients had no motor deficit.

Studied patients had been treated with various medications, including anticonvulsants, narcotic or non-narcotic analgesics and antidepressants, without satisfactory pain control. The patients were classified into two groups. Seventeen patients received true rTMS, the mean age was 52.01±12.7 years, 13 (76.5\%) were males, the mean duration of illness was 33.4±39.3 months and 10 patients received sham, the mean age was 53.3±13.3 years, 6 (60\%) were males, the mean duration of illness was 31.9±21.9 months with no significant differences between the two groups.

Patients with intracranial metallic devices or with pacemakers or any other device, as well as those with extensive myocardial ischemia and those known to have epilepsy, neurological or psychiatric diseases were excluded. All patients participated in the study after giving written informed consent and the local ethical committee of Assiut University Hospital approved the procedures.

The baseline assessment consisted of a full history and neurological examination followed by instruction in the use of a visual analogue scale (VAS). Each patient then provided two VAS ratings, and the mean was taken. After this the patients were assessed by the examiner using the Leeds assessment of neuropathic symptoms and signs (LANSS) pain scale, which is based on analysis of sensory description and bedside examination of sensory dysfunction.\textsuperscript{20} Measures of VAS and LANSS were taken at each follow-up point.

Patients were randomly assigned to one of the two groups, depending on the day of the week on which they were recruited. One group (consisting of patients recruited on Saturday to Tuesday) received real rTMS and the other group (recruited on Wednesday to Thursday) received sham-rTMS.

\textbf{Preparation}

The patient sat in a comfortable chair and was asked to relax as much as possible. Electromyography recordings from the contralateral muscle proximal to the stump (mainly deltoid in upper limb and Quadriceps in lower limb amputee) were acquired with silver–silver chloride surface electrodes, using a muscle belly-tendon set-up, with a 3 cm diameter circular ground electrode placed on the wrist. A Dantec Keypoint electromyograph was used to collect the signal (Dantec, Skovlunde, Denmark). Electromyography parameters included a bandpass of 20–1000 Hz and a recording time window of 200 ms. TMS was performed with a commercially available 90 mm figure-of-eight coil connected to Mag-Lite r25 stimulator (Dantec Medical, Skovlund, Denmark).

\textbf{Determination of resting motor threshold}

First, we determined the optimal scalp location from which TMS evoked motor potentials of greatest amplitude in the muscle proximal to the stump. We used constant suprathreshold stimulus intensity and moved the figure-of-eight coil systematically in 1 cm steps to determine the scalp position from where TMS evoked motor potentials of maximum peak to peak amplitude in the target muscle. The coil was positioned tangentially to the scalp and oriented so that the induced electrical currents would flow approximately perpendicular to the central sulcus, at a 45° angle from
the mid-sagittal line. Single pulse TMS was then delivered to the optimal location starting at suprathreshold intensity and decreasing in steps of 2% of the stimulator output. Relaxation and electromyography signals were monitored for 20 milliseconds prior to stimulation. The resting motor threshold was defined as the minimal intensity required to elicit motor evoked potentials of 50 µV peak to peak amplitude in five out of 10 consecutive trials. The optimal scalp location and coil orientation were marked using a red marker to reuse for daily rTMS.

**Repetitive transcranial magnetic stimulation**

Real rTMS involved applying a train of rTMS once per minute for 10 minutes. Each train consisted of 200 pulses at 20 Hz and 80% resting motor threshold (total duration of 10 seconds) applied through a figure-of-eight coil over the identified motor cortical area corresponding to the stimulated stump muscle of the painful side. The treatment was repeated every day for five consecutive days. Sham-rTMS was applied using the same parameters but with the coil elevated and angled away from the head to reproduce some of the subjective sensation of rTMS and yet avoid induction of current in the brain. However, none of the patients had experienced rTMS previously, they were unaware of which stimulation was real and which was sham. During the rTMS, all patients wore earplugs to protect the ears from the acoustic artifact associated with the discharge of the stimulation coil.

**Sample collection and storage**

Two blood samples were collected for each patient for the assay. The first was taken before the first session of rTMS while the second was taken 1 to 2 hours after the last session. The samples were collected 20 to 30 minutes after venipuncture to avoid physical and psychological stress on the beta-endorphin concentration. Hemolytic and especially lipemic samples were not used for the assay to avoid false values. The serum samples were stored at −20°C.

**Determination of serum beta-endorphin**

Beta-endorphin ELISA kit (Phoenix Pharmaceuticals, Inc. Burlingame, CA, USA) was used in the determination. Enzyme linked immunoassay was performed as manufacturer instructions.

The calibration curve from which the concentration of beta-endorphin in the samples was obtained by plotting the extinction values measured for the six standards (linear, y axis) against the corresponding concentrations (logarithmic, x axis). The results for unknown can be calculated using the following curve-fitting technique four-parameter logistic.

**Follow-up**

Patients were followed up after the first, fifth rTMS session, 1 and 2 months after the last session, using the VAS and LANSS scales. The second author evaluated these measures blindly, without knowing the type of rTMS.

**Data analysis**

Pain level was assessed at baseline, after the first, fifth rTMS session, and 1 and 2 months after the last session using the VAS and LANSS scales. Values for both patient groups (true and sham) and each rating scale (VAS and LANSS) were analyzed in separate two factor analysis of variance with ‘time after start of treatment’ and ‘rTMS’ as main factors. The Greenhouse–Geisser correction of degrees of freedom was used when necessary to correct non-sphericity of data. The percentage of the pain level was calculated from the VAS score measured before and after the rTMS sessions, both real and sham, by the following equation:

\[(\text{post-rTMS—pre-rTMS pain scores}) \times 100/\text{pre-rTMS pain score}\]

**Results**

There was no significant difference between true and sham groups in the duration of illness, VAS, LANSS scores and resting motor threshold in upper and lower limb amputation at the base line. Demographic and clinical results are presented in Table 1. Figure 1 shows that the VAS score of the patients who received real rTMS decreased significantly all over the course of the treatment through the different points of follow-up (after 5 sessions, 1 and 2 months) than those who received sham rTMS. This was confirmed in a two factor repeated measures analysis of variance separately in each group of patients with ‘time of assessment’ and ‘rTMS’ as main factors \((P=0.001)\) in real versus 0.69 in sham rTMs). The same results were reported in LANSS rating scale (Fig. 2). There was a significant decrease in pain ratings at time points (5 sessions, 1 and 2 months follow-up) after real rTMS compared with baseline \((P=0.001)\), but no significant change in sham patients. There were no significant
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Effect of rTMS on VAS scale - REAL GROUP vs SHAM GROUP

- BEFORE SESSION - AFTER 1 SESSION - AFTER 5 SESSIONS - AFTER 1 MONTH - AFTER 2 MONTHS

Figure 1 Effect of rTMS on VAS scale.

Effect of rTMS on LANSS scale - REAL GROUP vs SHAM GROUP

- BEFORE SESSION - AFTER 1 SESSION - AFTER 5 SESSIONS - AFTER 1 MONTH - AFTER 2 MONTHS

Figure 2 Effect of rTMS on LANSS scale.

changes in VAS and LANSS rating scales after the first session in real and sham groups (Table 2).

The percentage reduction in VAS in the real rTMS group decreased by 55% at the end of the fifth treatment session and was still reduced by 52% 1 month later and 39% after 2 months follow-up compared with baseline measures. The percentage was higher in patients with upper limb phantom pain (55, 56, and 60% respectively) compared to patients with lower limb phantom pain (55, 51, and 24% respectively). In contrast, pain ratings in the sham group declined only by 7% after 5 sessions and 2% after 1 and 2 month follow-up.

Serum beta-endorphin of normal volunteers (n=10) was 2.90 ± 1.47 ng/ml (normal value from 0 to 100 ng/ml). Serum beta-endorphin of the patients were very low (0.98 ± 0.50 in true group and 1.07 ± 0.58 in sham group) in comparison to normal volunteers. Beta-endorphin was measured 1 to 2 hours after five sessions of rTMS, it was increased significantly after real stimulation but patients received sham rTMS showed no significant change (Fig. 3, Table 3). Hamilton depression and anxiety scores showed significant decrease in patients received real rTMS but no changes were observed in those with sham sessions (Table 3). Spearman’s correlation showed that duration of illness had significant positive correlation to serum beta-endorphin before rTMS (r=0.54, P=0.02) and negative correlation to resting motor threshold (r=-0.65, P=0.004). Serum beta-endorphin showed no significant correlation to Hamilton depression, anxiety, VAS and LANSS scores in true or sham groups before or after five sessions of rTMS.

Table 2 VAS and LANSS scores before and after rTMS sessions

<table>
<thead>
<tr>
<th></th>
<th>True (Mean ± SD)</th>
<th>P value</th>
<th>Shame (Mean ± SD)</th>
<th>P value</th>
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<tbody>
<tr>
<td>VAS</td>
<td></td>
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<tr>
<td>Before sessions</td>
<td>7.4 ± 1.3</td>
<td>0.40</td>
<td>7.60 ± 0.84</td>
<td>0.59</td>
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<tr>
<td>After 1 session</td>
<td>7.1 ± 2.1</td>
<td>0.001</td>
<td>7.80 ± 0.91</td>
<td>0.59</td>
</tr>
<tr>
<td>After 5 sessions</td>
<td>3.4 ± 1.2</td>
<td>0.001</td>
<td>7.40 ± 0.84</td>
<td>0.59</td>
</tr>
<tr>
<td>After 1 month</td>
<td>3.4 ± 1.7</td>
<td>0.001</td>
<td>7.30 ± 0.62</td>
<td>0.46</td>
</tr>
<tr>
<td>After 2 months</td>
<td>4.5 ± 2.2</td>
<td>0.001</td>
<td>7.60 ± 0.96</td>
<td>1.0</td>
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<tr>
<td>LANSS</td>
<td></td>
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<tr>
<td>Before sessions</td>
<td>16.8 ± 3.4</td>
<td>0.38</td>
<td>17.30 ± 1.9</td>
<td>0.11</td>
</tr>
<tr>
<td>After 1 session</td>
<td>18.10 ± 1.9</td>
<td>0.001</td>
<td>17.8 ± 2.3</td>
<td>0.49</td>
</tr>
<tr>
<td>After 5 sessions</td>
<td>8.4 ± 3.7</td>
<td>0.001</td>
<td>17.4 ± 2.2</td>
<td>0.19</td>
</tr>
<tr>
<td>After 1 month</td>
<td>7.6 ± 2.7</td>
<td>0.001</td>
<td>16.8 ± 1.7</td>
<td>0.04</td>
</tr>
<tr>
<td>After 2 months</td>
<td>9.5 ± 3.7</td>
<td>0.001</td>
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Note: P value was between each point of follow-up and base line assessment of VAS and LANSS scores before sessions.

Discussion
The results in the present work confirmed that, 20 Hz stimulation in the motor cortex contralateral to amputated limb had long-term analgesic effect at the end of 5 sessions and 1, 2 month follow-up. The effect was reported in upper limb as well as lower limb amputee. Pain reduction in VAS in the real rTMS group decreased by 55% at the end of the fifth treatment session and was still reduced after 1 and 2 month follow-up. The percentage was high in patients with upper limb phantom pain compared to patients with lower limb pain. To our knowledge, only a few authors studied the effect of TMS on phantom pain, Rollnik and Pridmore’s protocol including only one patient with phantom pain and Irlbacher et al’s study included 14 patients with phantom pain. Both reported significant reduction in pain score and the second author reported no long-term analgesic effect of rTMS as they used low stimulation frequency (1 and 5 Hz) in comparison to the present work as we used 20 Hz on large number of patients (27 patients). Our results were parallel to others studies on different types of pain other than phantom pain. Lefaucheur et al demonstrated that rTMS was able to relieve neuropathic pain when
administered over M1 at 10 Hz but not at 0.5 Hz. André-Obadia et al.\textsuperscript{27} also showed that rTMS provided better alleviation of pain at 20 Hz than at 1 Hz and Saitoh et al.\textsuperscript{28} found that 10-Hz rTMS was more effective than 5-Hz rTMS, whereas 1-Hz rTMS did not produce significant effects. In the present study, there were no significant effects on VAS or LANS scores after the first session and the effects persist for 2 months after the end of sessions due to cumulative effects lasting for at least 2 months beyond the time of stimulation after repeated TMS. The same results were reported by Lefaucheur et al.,\textsuperscript{26} Khedr et al.,\textsuperscript{29} and Passard et al.\textsuperscript{30} Lefaucheur et al.\textsuperscript{31} also concluded that rTMS could not be considered as a therapeutic method for neuropathic pain except if the sessions of stimulation were repeated for several days or weeks. Analgesic effects in the present work occurred in all patients including eleven patient with upper limb and 16 patients with lower limbs phantom pain treated by real rTMS. The pain reduction was higher in patients with upper limb amputee in contrast to the patients with lower limb amputee. Therefore, analgesic effect of rTMS may act on neuronal networks by modulating neural activities not only in the stimulated area, but also in remote regions that are interconnected to the site of stimulation. This was proved by Garcia-Larrea and Peyron,\textsuperscript{32} as they found increase in the activity of neural structures implicated in pain processing as thalamus, anterior insula, periaqueductal gray matter, and upper brainstem after treatment by epidural MCS using positron emission tomography.

The mechanisms underlying the analgesic effects elicited by transcranial cortical stimulation of motor cortex are not fully understood yet and the exact nature of the involved pathways remains hypothetical. Raji et al.\textsuperscript{33} suggested that in chronic pain there was defective inhibition of M1 lead to pain perception so 20 Hz rTMS restored these defective mechanism and analgesia. Others reported that rTMS may increase central nervous system opioids, Maarraawi et al.\textsuperscript{16} reported that motor cortex stimulation (MCS) may induce release of endogenous opioids in brain structures involved in the processing of acute and chronic pain. Amassian et al.\textsuperscript{34} suggested that analgesic effects of rTMS in phantom pain were delivered by increase in the endogenous beta-endorphin release. Töpper et al.\textsuperscript{35} found that opiate antagonist naloxone abolished the rTMS-induced pain relief which was taken as evidence that the analgesic effect of rTMS acted via the release of endorphins. Borckardt et al.\textsuperscript{36,37} also found that a single session of high-frequency rTMS applied immediately after gastric bypass surgery at 10 Hz over the left DLPFC for a total of 4000 pulses was associated with a 40% reduction in total morphine use during the first 2 days after surgery. This reduction corresponded to the effect of active rTMS minus that of sham stimulation.

In the present work, we measured beta-endorphin in serum before and after rTMS and in volunteer group not complaining from neuropathic pain as a marker to central nervous system beta-endorphin. We found that beta-endorphin in the serum was low in patients with phantom limb pain as compared to normal volunteers which explain the persistence of pain after amputation. After true rTMS the serum level of beta-endorphin was markedly increased in comparison to the level before rTMS. In spite of the increase in the serum beta-endorphin, the level was not correlated to improvement in pain as measured by VAS and LANS score, improvement in Hamilton depression and anxiety score after five sessions. This item can be re-investigated later in a larger study than the present one.

It seems possible that some of the clinical improvement in patients following rTMS may have been due to changes in cerebral beta-endorphin, with increases in beta-endorphin causing improvement in pain score. Unfortunately, since we were only able to measure beta-endorphin concentrations at a single time point after the last rTMS treatment, we cannot say whether the

<table>
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<tr>
<th>Table 3 Serum beta-endorphin, Hamilton depression and Hamilton anxiety before and after rTMS sessions</th>
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<tr>
<td><strong>Before (Mean ± SD)</strong></td>
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<td>------------------------</td>
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<tr>
<td><strong>True</strong></td>
</tr>
<tr>
<td>Beta-endorphin (ng/ml)</td>
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<tr>
<td>Hamilton depression</td>
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<tr>
<td><strong>Shame</strong></td>
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<tr>
<td>Beta-endorphin (ng/ml)</td>
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<tr>
<td>Hamilton depression</td>
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<tr>
<td>Hamilton anxiety</td>
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</table>
increase was sustained for as long as the clinical improvement, which in the present cases persist for at least 2 month after the end of sessions. Thus, it is possible that the sustained clinical improvement could not only related to changes in beta-endorphin levels. Nevertheless, our measures of beta-endorphin were taken 1 to 2 hours after rTMS, so that we think that the effect may last for some time after the end of stimulation.

Conclusion

From this study, we can consider that rTMS for 5 consecutive days at high frequency had long-term pain relieve for at least 2 months after the last session. Serum beta-endorphin level could be considered as evidence that, rTMS can increase the central nervous system endorphins.

References


