American Pain Society
RESEARCH
EDUCATION
TREATMENT
ADVOCACY
PUBLISHED BY ELSEVIER

The Journal of Pain, Vol 17, No 8 (August), 2016: pp 911-918
Available online at www.jpain.org and www.sciencedirect.com

Repetitive Transcranial Magnetic Stimulation for Phantom Limb Pain in Land Mine Victims: A Double-Blinded, Randomized, Sham-Controlled Trial

Alejandra Malavera,*,† Federico Arturo Silva,* Felipe Fregni,† Sandra Carrillo,* and Ronald G. Garcia*,‡,§

**Neurovascular Science Group, Fundación Cardiovascular de Colombia, Floridablanca, Colombia.*
†Spaulding Neuromodulation Center, Spaulding Rehabilitation Hospital, Harvard Medical School, Boston, Massachusetts.
‡MASIRA Research Institute, School of Medicine, Universidad de Santander, Bucaramanga, Colombia.
§Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital, Harvard Medical School Boston, Massachusetts.

Abstract: **We evaluated the effects of repetitive transcranial magnetic stimulation (rTMS) in the treatment of phantom limb pain (PLP) in land mine victims. Fifty-four patients with PLP were enrolled in a randomized, double-blinded, placebo-controlled, parallel group single-center trial. The intervention consisted of real or sham rTMS of M1 contralateral to the amputated leg. rTMS was given in series of 20 trains of 6-second duration (54-second intertrain, intensity 90% of motor threshold) at a stimulation rate of 10 Hz (1,200 pulses), 20 minutes per day, during 10 days. For the control group, a sham coil was used. The administration of active rTMS induced a significantly greater reduction in pain intensity (visual analogue scale scores) 15 days after treatment compared with sham stimulation (−53.38 ± 53.12% vs −22.93 ± 57.16%; mean between-group difference = 30.44%, 95% confidence interval, .30–60.58; *P* = .03). This effect was not significant 30 days after treatment. In addition, 19 subjects (70.3%) attained a clinically significant pain reduction (>30%) in the active group compared with 11 in the sham group (40.7%) 15 days after treatment (*P* = .03). The administration of 10 Hz rTMS on the contralateral primary motor cortex for 2 weeks in traumatic amputees with PLP induced significant clinical improvement in pain.**

Perspective: ***High-frequency rTMS on the contralateral primary motor cortex of traumatic amputees induced a clinically significant pain reduction up to 15 days after treatment without any major secondary effect. These results indicate that rTMS is a safe and effective therapy in patients with PLP caused by land mine explosions.***

Key words: *Phantom limb pain, land mine victims, rTMS, neuropathic pain, noninvasive brain stimulation.*

Land mines are one of the world's most disabling public health hazards causing devastating injuries such as traumatic limb amputations and associated psychological disorders.[13,45] The exact number of worldwide land mine victims is currently unknown because there is no systematic collection of reliable data. However, it is widely estimated that land mines result in 15,000 to 25,000 victims each year.[43] After trauma-related limb amputation for land mine injury, one of the significant causes of disability is the presence of phantom limb pain (PLP).[38,42,48] PLP is a neuropathic syndrome characterized by pain felt in the patients' remaining perception of the amputated limb after partial or complete deafferentation. This pain is usually described as a stabbing, throbbing, burning, or cramping sensation.[14,24,33] PLP is present in up to 87% of all amputees[24] and is considered a challenging condition because of its negative effect on quality of life and lack of treatment response, particularly in patients with traumatic-related amputations.[1,15]

Received July 8, 2015; Revised April 21, 2016; Accepted May 6, 2016.
This study was partially supported by a grant from the Colombian Science and Technology Institute (COLCIENCIAS, project code: 6566-49-326169). Felipe Fregni is the principal investigator at Spaulding Rehabilitation Hospital of a research grant funded by NIH (5R01HD082302-02).
The authors have no conflicts of interest to declare.
Trial registry: www.clinicaltrials.gov NCT01872481.
Address reprint requests to Ronald G. Garcia, MD, PhD, Martinos Center for Biomedical Imaging, Building 149, 13th Street, Suite 2301, Charlestown, MA 02129. E-mail: rgarcia@nmr.mgh.harvard.edu
1526-5900/$36.00

http://dx.doi.org/10.1016/j.jpain.2016.05.003

Maladaptive plasticity seems to play a major role in the mechanisms of PLP. Reorganization of the primary sensorimotor cortex, including changes in motor cortex excitability and peripheral factors such as nociceptive inputs from the residual limb have been implicated in the development of this condition.[1,16,39] Additionally, psychological factors may affect pain duration and severity.[23] The high prevalence of PLP after amputation and its lack of treatment response have resulted in major efforts to develop interventions to decrease the pain in affected patients.[11] In light of PLP mechanisms, repetitive transcranial magnetic stimulation (rTMS) has been tested in this condition as a tool to block the maladaptive plasticity in the sensorimotor cortex.[1] rTMS applied daily over the primary motor cortex (M1) has shown pain relief effects in other neuropathic pain syndromes such as poststroke pain and spinal cord injury pain.[22,28,49] Some previous reports have also suggested analgesic effects of rTMS in subjects with PLP.[1,10] There have been only 3 trials testing rTMS in PLP—2 of them were small pilot studies[10,46] and the other was a randomized clinical trial (RCT) with 27 subjects.[1] The RCT showed that 5 consecutive sessions of rTMS induced a significant analgesic effect compared with sham rTMS, lasting up to 2 months in 39% of the subjects. However, a recent meta-analysis judged this trial as a high risk of bias study due to a deficient randomization method, which led to an unbalanced distribution between the intervention groups.[36] Furthermore, the conclusion of the cited meta-analysis, after including 56 trials using noninvasive brain stimulation techniques for chronic pain treatment, was that although single doses of high-frequency rTMS of the motor cortex may have small short-term effects on chronic pain, these effects do not meet the predetermined threshold of minimal clinical significance, and there is therefore a need for larger, rigorously designed studies, particularly of longer courses of stimulation.

In light of these results, we aimed to assess in a larger sample size study and properly designed RCT, the immediate and sustained effects of a larger dose of real rTMS of M1—10 sessions—on PLP compared with sham rTMS in land mine victims. We hypothesized that 10 Hz rTMS for 2 weeks over M1 contralateral to the PLP could significantly decrease the level of pain compared with sham stimulation.

Methods

Study Design

This was a single-center, double-blinded, sham-controlled, randomized, parallel-group trial that consisted of 3 main phases: 1) a baseline evaluation consisting of a week period of observation to establish baseline measurements for pain levels, depression, and anxiety symptomatology, 2) a treatment phase consisting of daily sessions with active or sham rTMS for 5 days a week during 2 consecutive weeks, and 3) a follow-up evaluation after 15 and 30 days of treatment completion. In the baseline evaluation, we recorded demographic data, medical history, medications, and other therapies used for the treatment of PLP.

Study Population

Fifty-four patients (mean age, 33.9 ± 8.41 years; 4 female patients) were included in the study. The participants were prospectively selected from the rehabilitation department of the Regional Military Hospital and local nongovernmental organizations in Bucaramanga, Colombia. Patients were included if they fulfilled the following criteria: adults aged 18 years or older, who had amputation at any level of 1 lower limb by antipersonnel land mines with symptoms compatible with PLP. PLP was defined as a sensation of shooting, stabbing, boring, squeezing, throbbing, or burning or paresthesia or any other pain sensation in a limb that did not exist anymore.[34]

We excluded patients with a diagnosis of complex regional pain syndrome, any pathology that could alter the course of PLP (diagnosis of cancer, immunological disorders, renal insufficiency requiring dialysis treatment, etc), pregnancy, neuropsychiatric disorders that can affect the patient ability to consent to the study participation and contraindications to rTMS, such as cardiac pacemaker, medical pumps, or implanted metals in the scalp.[47] This study was performed in accordance with the Declaration of Helsinki (1964).[8] Written informed consent was obtained from each participant before inclusion in the study, which was approved by the local institutional review board.

Intervention: rTMS

Patients received rTMS on the primary motor cortex (M1) contralateral to the amputated leg using a figure-of-eight coil connected to a Magstim Rapid[2] magnetic stimulator, which provides a biphasic pulse (Magstim Company Ltd, Whitland, UK). The coil was positioned tangentially to the scalp, approximately at a 45° angle from the midline. The resting motor threshold (RMT) (of the first dorsal interosseous) was defined as the minimal intensity to induce motor evoked potentials of 50 μV peak-to-peak amplitude in at least 5 of 10 trials. Twenty trains of 6 seconds each (intertrain interval 54 seconds), using an intensity of 90% of RMT and 10 Hz frequency, were applied in each patient for 10 days during a 2-week period. For the sham treatment group, stimulation parameters were the same (location and duration), but a sham coil (Magstim Company Ltd) was used. This coil has similar appearance to the active coil in shape and weight, and produces a similar sound artifact but does not induce a scalp skin sensation nor emit a magnetic pulse within the cortex.[30] All sessions were administered by only 1 investigator who was not blinded to the intervention and did not participate in the outcome assessments. Participants and investigators who performed the pain assessments were blinded to treatment allocation.

Randomization

A computer-generated randomization method with a permuted block size of 6 was used to allocate subjects to the sham or active rTMS interventions. The randomization code was only given to the treating investigator on the first day of treatment session by an independent investigator not involved with any other aspect of the trial.

The blinding integrity was assessed at the end of the study. Participants were asked to guess their treatment allocation. We did not assess blinding in 2 patients because of early trial withdrawal from the study. The blinded investigators were also asked to guess the patient allocation.

Study Outcomes

All evaluations were performed by an investigator blinded to treatment allocation. The primary end point of the study was the score change in the visual analogue scale (VAS) for pain. Response was defined as a reduction of ≥30% compared with baseline (at 15 and 30 days after treatment).[12,21] The other outcome measures were considered secondary.

Pain Measurement

VAS for Pain

The response to the stimulation was evaluated by measuring the pain intensity using the VAS. This self-evaluation scale ranges from 0 to 10 as visually described in centimeter units, 0 cm indicates no pain and 10 cm the worst pain possible. This scale has been widely used in studies that evaluate pain as an outcome, and validity and reproducibility have been shown.[18] Because we expected daily variability in pain levels, pain was self-assessed daily at baseline for 1 week before treatment, and twice during the follow-up period (at 15 and 30 days after completing the treatment scheme). The patients were asked to continue their routine medication regimen during the study. If a patient required a change in medication dose, it was recorded and considered in the analysis.

Anxiety and Depression Symptomatology

Because depression and anxiety might be confounders of pain relief, we measured these 2 domains by using the instruments in the following sections.

Zung Self-Rating Depression Scale

This is a 20-item self-report scale that measures the 4 common characteristics of depression: pervasive affect, physiological equivalents, other disturbances, and psychomotor activities. The minimum score is 20 and the maximum score is 80. Four categories ranging from "normal" to "severely depressed" are on the basis of specific ranges of the score.[51]

Zung Self-Rating Anxiety Scale

This is a 20-item questionnaire on the basis of scoring in 4 groups of manifestations: cognitive, autonomic, motor, and central nervous system symptoms. The total scores range from 20 to 80, meaning normal range to extreme anxiety levels.[50] Depressive and anxiety symptoms were measured once at baseline, and twice during the follow-up period (at 15 and 30 days after completing the treatment scheme).

Sample Size and Statistical Analysis

A sample size of 54 patients (27 in each arm) was calculated expecting that 60% of subjects in the active group would obtain a significant pain reduction (decrease >30% in pain level) after finishing the intervention compared with 20% in the sham group. This was on the basis of the results of a previous study.[27] It was considered a power of 80%, type I error of .05 (double-sided), and an adjustment for a dropout rate of 5%.

The data are presented as mean and SD and also proportion of responders in each group. Continuous variables were subjected to a Shapiro–Wilk test to determine whether the data fitted normal distribution. Baseline characteristics of patients randomized to active and sham therapies were compared using Student t-test or Wilcoxon rank sum test for continuous variables and χ^2 test or Fisher exact test for categorical data. We analyzed the end point of the study using the intention-to-treat method including patients who attended at least 1 of the rTMS sessions. The missing data were considered at random, thus we used a regression imputation method to handle this issue. Such technique allowed us to substitute missing VAS values at the first (15 days after treatment, 2 subjects) and second (30 days after treatment, 6 subjects) follow-up visits, for values derived from a regression model using baseline variables as well as VAS scores from all other participants.

Risk ratios were calculated to evaluate statistically significant differences between treatment groups in the proportion of subjects attaining a clinical important pain reduction (>30%) 15 and 30 days after finishing the treatment protocol. The differences between groups in the proportion of subjects attaining a substantial clinical benefit (pain reduction >50%) were also explored. We also conducted additional analyses treating pain as a continuous variable and also for the other secondary continuous outcomes (depression and anxiety scores). For these analyses, a repeated-measure analysis of variance was performed using a 2-group (active vs sham) according to a 3 time periods (baseline, and 15 and 30 days after finishing treatment) design. Post hoc comparisons were carried out using a Scheffe test for multiple comparisons. Statistical significance was defined as $P < .05$. All analyses were conducted using Stata statistical software, release 11.0 (Stata Corp, College Station, TX).

Results

Fifty-four patients (n = 27 in the active group and n = 27 in the sham group) were included in the study.

A participant flow diagram is shown in Fig 1. There were no significant differences in demographic and clinical characteristics at baseline between the groups (Table 1). All patients tolerated the rTMS without experiencing any serious adverse effect. Some patients experienced minor adverse effects such as headache (11.1%), neck pain (5.5%), and sleepiness (18.5%) without significant differences between groups. There were also no differences in relation to the current use of nonsteroidal anti-inflammatory drugs (28.5% vs 37.0%, $P = .12$), and

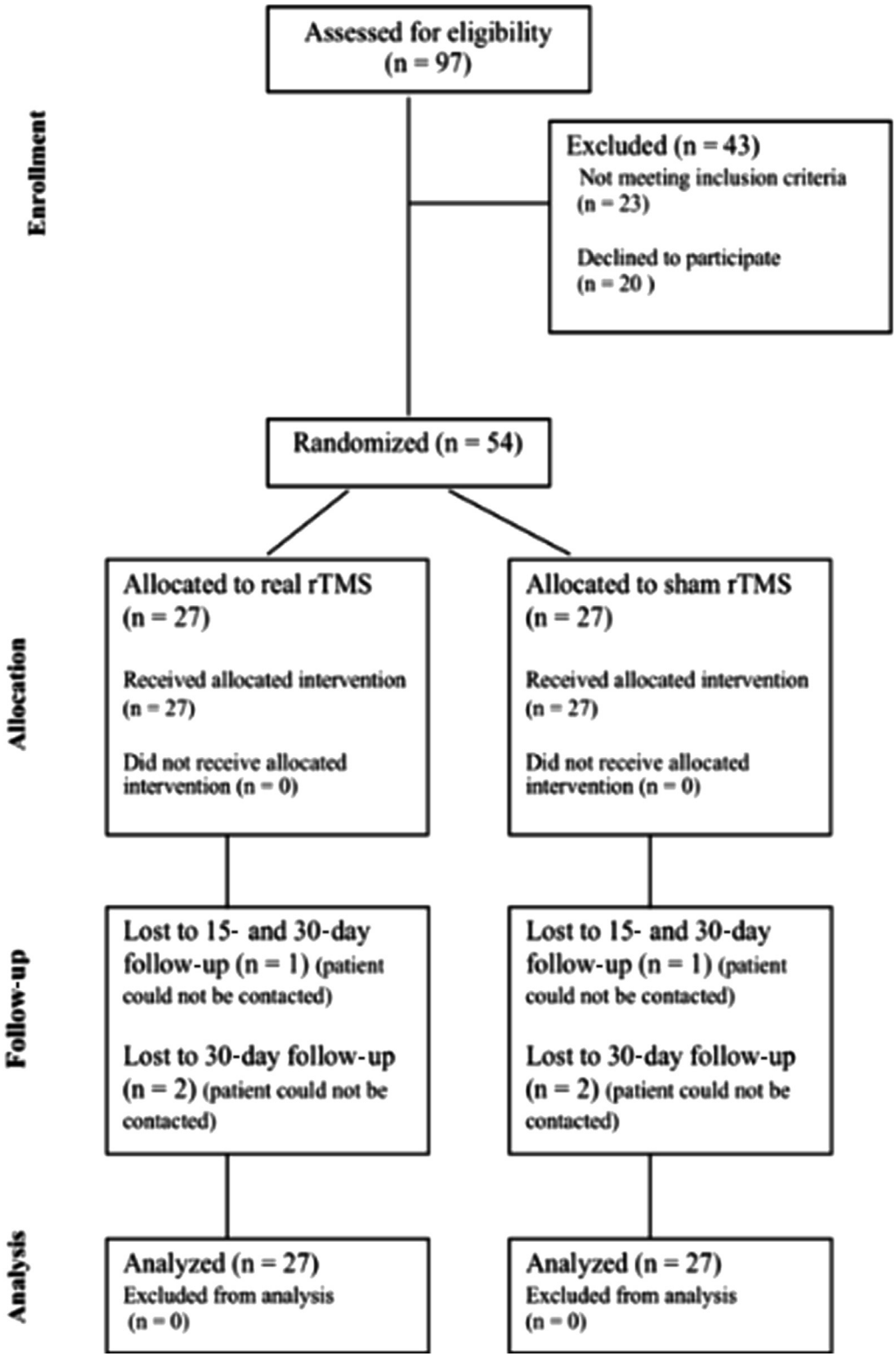

Figure 1. Consolidated Standards for Reporting Trials 2010 patient flow diagram.

Table 1. Baseline Characteristics of the Study Participants

Variable	Active rTMS Group (n = 27)	Sham rTMS Group (n = 27)
Age, y	33.1 ± 6.6	34.7 ± 9.9
Sex (female/male)	2/25	2/25
Years since amputation	7.4 ± 5.6	8.2 ± 6.3
Zung depression scale*	26.7 ± 5.7	25.6 ± 6.8
Zung anxiety scale†	27.8 ± 7.7	26.9 ± 9.3
VAS baseline‡	4.9 ± 1.9	4.8 ± 1.9
BMI	25.6 ± 4.4	25.2 ± 3.5

Abbreviation: BMI, body mass index calculated as weight (kg)/height (m^2).
NOTE. Data are expressed as mean ± SD.
*Depression scores range from 20 to 80, with higher scores indicating more severe anxiety symptoms.
†Anxiety scores range from 20 to 80, with higher scores indicating more severe anxiety symptoms.
‡Scores range from 0 to 10, with higher scores indicating more severe symptoms.

the participation in a physical rehabilitation program (88.8% vs 85.1%, P = .68) or psychological therapy (88.8% vs 77.7%, P = .27).

A significantly greater mean percentage reduction in pain intensity (VAS score) was found 15 days after treatment in the active group compared with the sham stimulation group (−53.38 ± 53.12% vs −22.93 ± 57.16%; mean between-group difference = 30.44%, 95% confidence interval [CI], .30–60.58; P = .03). However, no significant differences between groups were found 30 days after treatment (−37.74 ± 52.39% vs −14.97 ± 53.88%; mean between-group difference = 22.76%; 95% CI, −6.25 to 51.79; P = .12).

Nineteen subjects (70.3%) attained a significant clinical response (pain reduction > 30%) in the active group compared with 11 (40.7%) in the sham group 15 days after treatment (risk ratio [RR] = 1.72; 95% CI, 1.03–2.89). However, no statistically significant between-group difference was found 30 days after treatment (15 [55.5%] vs 9 [33.3%]; RR = 1.66; 95% CI, .88–3.13). A higher proportion of subjects obtaining a substantial clinical benefit (pain reduction >50%) was also found in the active treatment group compared with the sham stimulation group 15 days after treatment (17 [62.9%] vs 9 [33.3%]; RR = 1.88; 95% CI, 1.02–3.46). This difference also showed a statistical trend toward significance when evaluated 30 days after treatment (13 [48.1%] vs 6 [22.2%]; RR = 2.16; 95% CI, .96–4.85).

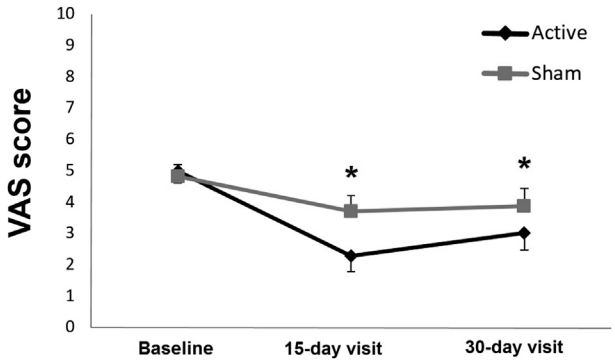

Figure 2. Variations in VAS score according to treatment group. *P < .05 compared with baseline score. The error bars represent standard errors.

Table 2. Average Scores of Pain, Depression, and Anxiety Scales in Subjects Included in the Study

Scale	Baseline	15-Day Visit	30-Day Visit
VAS score*			
Active rTMS	4.98 ± 1.97	2.28 ± 2.51	3.02 ± 2.64
Sham rTMS	4.82 ± 1.98	3.71 ± 2.97	3.88 ± 2.68
Zung depression scale†			
Active rTMS	26.7 ± 5.72	25.1 ± 5.87	24.9 ± 9.05
Sham rTMS	25.6 ± 6.82	24.2 ± 4.39	23.2 ± 2.99
Zung anxiety scale‡			
Active rTMS	27.8 ± 7.71	25.8 ± 7.02	23.8 ± 7.27
Sham rTMS	26.9 ± 9.32	25.1 ± 5.52	24.4 ± 4.24

NOTE. Data are expressed as mean ± SD.
*Scores range from 0 to 10, with higher scores indicating more severe symptoms.
†Depression scores range from 20 to 80, with higher scores indicating more severe anxiety symptoms.
‡Anxiety scores range from 20 to 80, with higher scores indicating more severe anxiety symptoms.

For our secondary analyses, we assessed the effects of rTMS on pain level using a repeated measure analysis of variance. We found a significant main effect of group of treatment and time ($F_{1,104}$ = 7.54, P < .01; $F_{2,104}$ = 19.49, P < .0001). The analysis also showed a significant interaction term (group per time; $F_{2,104}$ = 3.25, P = .04). Post hoc tests revealed a significant decrease in VAS scores 15 and 30 days after finishing the intervention in the active group, whereas no significant change was noted with sham stimulation (Fig 2). No statistically significant between-group difference was found when comparing the absolute VAS scores at day 15 (mean between-group difference = 1.42; 95% CI, −.07 to 2.93; P = .06) or day 30 (mean between-group difference = .86; 95% CI, −.59 to 2.31; P = .24) after treatment (Table 2). In relation to the scores of the depression and anxiety scales a main effect of time was found ($F_{2,104}$ = 4.55, P = .01; $F_{2,104}$ = 7.91, P < .0001), without significant group of treatment effects ($F_{1,104}$ = .11, P = .7; $F_{1,104}$ = .07, P = .7) or interactions terms ($F_{2,104}$ = 1.32, P = .27; $F_{2,104}$ = .2, P = .81). No statistically significant between-group difference was found when comparing the absolute scores of the depression and anxiety scales at day 15 or day 30 after treatment (Table 2).

Subjects and investigators did not guess correctly the treatment allocation beyond chance (P = .704; P = .571).

Discussion

The present study showed that the treatment with 10 Hz rTMS of contralateral M1 during 2 weeks in traumatic amputees with PLP induced a clinically significant pain reduction up to 15 days after treatment compared with sham stimulation. In addition, no serious adverse effects were found during the study indicating that rTMS

was a safe and effective therapy in patients with PLP caused by land mine explosions.

Previous studies had shown some beneficial effects of rTMS on PLP.[1,10,46] These reports have evaluated either the effects of low frequency rTMS (<1 Hz), which have been shown to decrease cortical network excitability, or high frequency (>1 Hz), which may induce an opposite effect.[2,40] In an initial case report study, Töpper et al[46] evaluated the effect of rTMS series on phantom pain-like syndrome in 2 patients with long-lasting brachial plexus avulsion, who underwent 10 and 1 Hz rTMS during 15 days, separated by 4 and 6 weeks, respectively (at 110% of RMT, 12-minute duration) over the contralateral posterior parietal cortex to the injured limb. The authors reported a maximum pain reduction of approximately 60% and 23.6%, during the rTMS treatment compared with baseline; however, the pain decrease was not maintained in the long-term. Similarly, Di Rollo and Pallanti[10] in 1 patient with PLP of traumatic origin, applied 15 sessions of low-frequency rTMS (thirty 20-second trains at 80% of RMT, 15 minutes) over the ipsilesional motor cortex, showing a pain reduction of 33.3% at the end of the third week of treatment and a slight decrease (16.6%) at the follow-up visit (3 weeks after the last session). In a recent clinical trial, Ahmed et al[1] evaluated the analgesic effect of rTMS for chronic PLP by assigning subjects to active (n = 17) or sham (n = 10) stimulation of the contralateral M1 for 5 consecutive days (200 pulses at 20 Hz, 10-second trains, at 80% of RMT). The authors reported a significant reduction on VAS in the real stimulation group immediately after the fifth session (55%) that was maintained after 2 months (39%) compared with the sham group. Although these studies showed promising effects of rTMS in PLP, there were some methodological limitations that could have affected their results, such as a low sample size, unbalanced distribution in the treatment groups, low number of sessions (ie, 5 sessions) and the lack of standardized criteria for placebo stimulation.[30] In addition, the population included in these studies was heterogeneous, with different amputation locations and etiologies, which is of particular importance because these factors could be related with different pathophysiological mechanisms and/or treatment responses.[9,31,37] Because of the challenges in recruiting a population with PLP with similar characteristics, our study included, in a 10-day stimulation protocol, a homogeneous population consisting of 100% of subjects with traumatic lower limb amputation caused by land mine explosions. Thus our findings extend beyond previous rTMS studies on PLP and provide a more reliable estimate of effect size. The number needed to treat for 30% pain reduction with rTMS compared with sham rTMS was 4. This result indicates that 1 patient in every 4 treated with rTMS will benefit from this treatment compared with sham treatment. This effect size (number needed to treat of 4; 95% CI, 1.8–23.1) is similar to tricyclic antidepressants for the treatment of central pain.[14]

The pain relief found in the present study could be explained by the potential effect of rTMS over the central pathophysiological mechanisms related to PLP. After a traumatic amputation, the main factors associated with PLP include maladaptive reorganization of the sensorimotor cortex, which involves a reduction in intracortical inhibition mechanisms, an imbalance between inhibitory and excitatory amino acids (gamma-aminobutyric acid and glutamate) and an increase in the excitability of corticospinal neurons.[6,32,48] It has been hypothesized that the administration of high-frequency rTMS over the motor cortex enhances its excitability leading to indirect activation of inhibitory projections toward the thalamus, resulting in a modulation of ascending nociceptive signal pathways.[3,20] Additionally, the modulation of thalamic activity generated by the enhancement of motor cortex excitability may influence other brain pain-related networks such as the orbitofrontal, anterior cingulate gyri, and the periaqueductal gray matter, which are related with the affective–emotional components of nociception.[7,26,32,48]

Besides rTMS, other noninvasive and invasive brain stimulation methods have been explored for the treatment of chronic PLP. Transcranial direct current stimulation (tDCS) is a noninvasive method that modulates spontaneous neuronal activity with anodal stimulation enhancing cortical excitability and cathodal inducing an opposite effect.[35] Recently, tDCS has been explored as a neurorehabilitory tool for the treatment of chronic PLP.[3-5] Bolognini et al[4,5] studied the effect of anodal tDCS (1.5 mA, 15 minutes for 5 days) over M1 contralateral to the amputated limb in 8 patients with unilateral lower and upper limb amputation of different etiologies. The authors reported a pain relief immediately after the 5 sessions and up to 1 week of the last stimulation session (−41%, $P = .04$). Among the invasive stimulation methods, epidural motor cortex stimulation (MCS) has emerged as an alternative therapy for refractory neuropathic pain.[25,29] However, its analgesic effects in patients with PLP have been described only in case series.[41] Fontaine et al[17] performed a systematic review of the effects of MCS on chronic neuropathic pain. The authors reported that 4 of 10 of the patients (40%) with PLP reported pain relief (>70%). In our study we found that 63% of the patients with PLP experienced pain relief >50%, results that are comparable with those reported in MCS studies; furthermore, these effects were present 15 days after finishing the treatment, which extend beyond the findings in patients using tDCS. It is worth noting that a recent meta-analysis evaluating the use of rTMS for the treatment of chronic pain reported significant heterogeneity, reporting a short-term analgesic effect but failing to reach pre-established criteria for a minimal clinically important difference.[36] One strength of our study was the inclusion of a very homogeneous population and the administration of the stimulation for 10 days, which could have contributed to the lasting and clinically significant pain reduction.

We also observed a significant reduction of depressive and anxiety symptoms after 30 days after the intervention without any differences between treatment groups, indicating an effect not attributable to rTMS. A recent meta-analysis showed that high-frequency rTMS over

the dorsolateral prefrontal cortex is associated with clinically relevant antidepressant effects with a safe profile, whereas no consistent effects have been found when stimulating the motor cortex,[19] which could explain the lack of differential treatment effects in our study.

This study has some limitations. Although our main outcome was the proportion of subjects attaining a clinically significant pain reduction, other nonpainful phantom phenomena such as phantom limb awareness, telescoping, and phantom sensations were not assessed and could have been confounding factors in the evaluation of rTMS effects. Despite this, our results support the notion that rTMS induces a clinically significant pain relief in subjects with PLP after traumatic amputation. In addition, although we found a significant difference between groups when analyzing mean percentage reduction (difference in pain scores from baseline), there was no statistically significant between-group differences when analyzing absolute VAS scores after treatment. Although randomization in theory allows for a balanced baseline measure in both treatment groups, this balance is often not seen in small randomized trials such as ours. Therefore, analyses of differences can correct for baseline imbalances, providing a more precise estimation of treatment effects in small randomized trials.[44] In addition, as for any small RCT, our results need to be confirmed in large randomized trials. An additional limitation is that we stimulated the motor cortex corresponding to the first dorsal interosseous muscle of the hand contralateral to pain instead of the area corresponding to the lower limb. However, previous studies using rTMS over the hand motor cortex have reported analgesic effects in patients with chronic neuropathic pain of diverse anatomical origin,[25,26] results that were confirmed by our study. Although the sham coil used in this trial might induce a slightly different scalp sensation compared with the real stimulation coil, it is noteworthy that both coils are similar in appearance (shape and weight) and auditory artifacts. In addition, although the investigator performing the stimulation was not blinded to the intervention, she did not participate in the outcome evaluation of the subjects; therefore, it is unlikely that this fact could have influenced the obtained results. Finally, this study consisted of a small sample size, which can compromise the generalization of the results. This was in part because of the challenges of recruiting such a homogeneous study population. In addition, although we found a clinically significant effect of the stimulation on pain reduction 15 days after finishing the treatment, further studies will be necessary to determine if longer rTMS stimulation protocols could derive in even more long-lasting and maintained analgesic effects in patients with PLP.

References

1. Ahmed MA, Mohamed SA, Sayed D: Long-term antalgic effects of repetitive transcranial magnetic stimulation of motor cortex and serum beta-endorphin in patients with phantom pain. Neurol Res 33:953-958, 2011

2. Andre-Obadia N, Peyron R, Mertens P, Mauguiere F, Laurent B, Garcia-Larrea L: Transcranial magnetic stimulation for pain control. Double-blind study of different frequencies against placebo, and correlation with motor cortex stimulation efficacy. Clin Neurophysiol 117:1536-1544, 2006

3. Bolognini N, Olgiati E, Maravita A, Ferraro F, Fregni F: Motor and parietal cortex stimulation for phantom limb pain and sensations. Pain 154:1274-1280, 2013

4. Bolognini N, Spandri V, Ferraro F, Salmaggi A, Molinari AC, Fregni F, Maravita A: Immediate and sustained effects of 5-day transcranial direct current stimulation of the motor cortex in phantom limb pain. J Pain 16:657-665, 2015

5. Bolognini N, Spandri V, Olgiati E, Fregni F, Ferraro F, Maravita A: Long-term analgesic effects of transcranial direct current stimulation of the motor cortex on phantom limb and stump pain: A case report. J Pain Symptom Manage 46:e1-e4, 2013

6. Chen A, Yao J, Kuiken T, Dewald JP: Cortical motor activity and reorganization following upper-limb amputation and subsequent targeted reinnervation. Neuroimage Clin 3: 498-506, 2013

7. Davis KD, Kiss ZH, Luo L, Tasker RR, Lozano AM, Dostrovsky JO: Phantom sensations generated by thalamic microstimulation. Nature 391:385-387, 1998

8. Declaration of Helsinki. Recommendations guiding doctors in clinical research. Adopted by the World Medical Association in 1964. Wis Med J 66:25-26, 1967

9. Di Pino G, Guglielmelli E, Rossini PM: Neuroplasticity in amputees: Main implications on bidirectional interfacing of cybernetic hand prostheses. Prog Neurobiol 88:114-126, 2009

10. Di Rollo A, Pallanti S: Phantom limb pain: Low frequency repetitive transcranial magnetic stimulation in unaffected hemisphere. Case Rep Med 130751:2011, 2011

11. Fang J, Lian Y, Xie K, Cai S: Pharmacological interventions for phantom limb pain. Chin Med J (Engl) 126: 542-549, 2013

12. Farrar JT, Young JP, LaMoreaux L, Werth JL, Poole RM: Clinical importance of changes in chronic pain intensity measured on an 11-point numerical pain rating scale. Pain 94:149-158, 2001

13. Ferguson AD, Richie BS, Gomez MJ: Psychological factors after traumatic amputation in landmine survivors: The bridge between physical healing and full recovery. Disabil Rehabil 26:931-938, 2004

14. Finnerup NB, Otto M, Jensen TS, Sindrup SH: An evidence-based algorithm for the treatment of neuropathic pain. MedGenMed 9:36, 2007

15. Flor H: Phantom-limb pain: Characteristics, causes, and treatment. Lancet Neurol 1:182-189, 2002

16. Flor H, Nikolajsen L, Staehelin Jensen T: Phantom limb pain: A case of maladaptive CNS plasticity? Nat Rev Neurosci 7:873-881, 2006

17. Fontaine D, Hamani C, Lozano A: Efficacy and safety of motor cortex stimulation for chronic neuropathic pain: Critical review of the literature. J Neurosurg 110:251-256, 2009

18. Fregni F, Boggio PS, Lima MC, Ferreira MJ, Wagner T, Rigonatti SP, Castro AW, Souza DR, Riberto M,

Freedman SD, Nitsche MA, Pascual-Leone A: A sham-controlled, phase II trial of transcranial direct current stimulation for the treatment of central pain in traumatic spinal cord injury. Pain 122:197-209, 2006

19. Fregni F, Marcolin MA, Myczkowski M, Amiaz R, Hasey G, Rumi DO, Rosa M, Rigonatti SP, Camprodon J, Walpoth M, Heaslip J, Grunhaus L, Hausmann A, Pascual-Leone A: Predictors of antidepressant response in clinical trials of transcranial magnetic stimulation. Int J Neuropsychopharmacol 9: 641-654, 2006

20. Garcia-Larrea L, Peyron R: Motor cortex stimulation for neuropathic pain: From phenomenology to mechanisms. Neuroimage 37(Suppl 1):S71-S79, 2007

21. Haanpaa M, Attal N, Backonja M, Baron R, Bennett M, Bouhassira D, Cruccu G, Hansson P, Haythornthwaite JA, Iannetti GD, Jensen TS, Kauppila T, Nurmikko TJ, Rice AS, Rowbotham M, Serra J, Sommer C, Smith BH, Treede RD: NeuPSIG guidelines on neuropathic pain assessment. Pain 152:14-27, 2011

22. Khedr EM, Kotb H, Kamel NF, Ahmed MA, Sadek R, Rothwell JC: Long-lasting antalgic effects of daily sessions of repetitive transcranial magnetic stimulation in central and peripheral neuropathic pain. J Neurol Neurosurg Psychiatry 76:833-838, 2005

23. Knotkova H, Cruciani RA, Tronnier VM, Rasche D: Current and future options for the management of phantom-limb pain. J Pain Res 5:39-49, 2012

24. Kooijman CM, Dijkstra PU, Geertzen JH, Elzinga A, van der Schans CP: Phantom pain and phantom sensations in upper limb amputees: An epidemiological study. Pain 87:33-41, 2000

25. Lefaucheur JP: Pain. Handb Clin Neurol 116:423-440, 2013

26. Lefaucheur JP, Drouot X, Ménard-Lefaucheur I, Keravel Y, Nguyen JP: Motor cortex rTMS restores defective intracortical inhibition in chronic neuropathic pain. Neurology 67:1568-1574, 2006

27. Leo RJ, Latif T: Repetitive transcranial magnetic stimulation (rTMS) in experimentally induced and chronic neuropathic pain: A review. J Pain 8:453-459, 2007

28. Leung A, Donohue M, Xu R, Lee R, Lefaucheur JP, Khedr EM, Saitoh Y, André-Obadia N, Rollnik J, Wallace M, Chen R: rTMS for suppressing neuropathic pain: A meta-analysis. J Pain 10:1205-1216, 2009

29. Lima MC, Fregni F: Motor cortex stimulation for chronic pain: Systematic review and meta-analysis of the literature. Neurology 70:2329-2337, 2008

30. Loo CK, Taylor JL, Gandevia SC, McDarmont BN, Mitchell PB, Sachdev PS: Transcranial magnetic stimulation (TMS) in controlled treatment studies: Are some "sham" forms active? Biol Psychiatry 47:325-331, 2000

31. Montoya P, Ritter K, Huse E, Larbig W, Braun C, Töpfner S, Lutzenberger W, Grodd W, Flor H, Birbaumer N: The cortical somatotopic map and phantom phenomena in subjects with congenital limb atrophy and traumatic amputees with phantom limb pain. Eur J Neurosci 10:1095-1102, 1998

32. Navarro X, Vivó M, Valero-Cabré A: Neural plasticity after peripheral nerve injury and regeneration. Prog Neurobiol 82:163-201, 2007

33. Nikolajsen L: Postamputation pain: Studies on mechanisms. Dan Med J 59:B4527, 2012

34. Nikolajsen L, Jensen TS: Phantom limb pain. Br J Anaesth 87:107-116, 2001

35. Nitsche MA, Cohen LG, Wassermann EM, Priori A, Lang N, Antal A, Paulus W, Hummel F, Boggio PS, Fregni F, Pascual-Leone A: Transcranial direct current stimulation: State of the art 2008. Brain Stimul 1:206-223, 2008

36. O'Connell NE, Wand BM, Marston L, Spencer S, Desouza LH: Non-invasive brain stimulation techniques for chronic pain. Cochrane Database Syst Rev CD008208, 2011

37. Pascual-Leone A, Peris M, Tormos JM, Pascual AP, Catalá MD: Reorganization of human cortical motor output maps following traumatic forearm amputation. Neuroreport 7:2068-2070, 1996

38. Perkins ZB, De'Ath HD, Sharp G, Tai NR: Factors affecting outcome after traumatic limb amputation. Br J Surg 99(Suppl 1):75-86, 2012

39. Reilly KT, Sirigu A: The motor cortex and its role in phantom limb phenomena. Neuroscientist 14:195-202, 2008

40. Rossi S, Hallett M, Rossini PM, Pascual-Leone A: Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol 120:2008-2039, 2009

41. Roux FE, Ibarrola D, Lazorthes Y, Berry I: Chronic motor cortex stimulation for phantom limb pain: a functional magnetic resonance imaging study: Technical case report. Neurosurgery 48:681-687, 2001

42. Schans CP, Geertzen JH, Schoppen T, Dijkstra PU: Phantom pain and health-related quality of life in lower limb amputees. J Pain Symptom Manage 24:429-436, 2002

43. Shabila NP, Taha HI, Al-Hadithi TS: Land mine injuries at the Emergency Management Center in Erbil, Iraq. Confl Health 4:15, 2010

44. Senn SJ: Covariate imbalance and random allocation in clinical trials. Stat Med 8:467-475, 1989

45. Smith WK, Wu Y, Pitkin M: Rehabilitation after landmine injury. Pain Med 7(Suppl 2):S218-S221, 2006

46. Töpper R, Foltys H, Meister IG, Sparing R, Boroojerdi B: Repetitive transcranial magnetic stimulation of the parietal cortex transiently ameliorates phantom limb pain-like syndrome. Clin Neurophysiol 114:1521-1530, 2003

47. Wassermann EM: Risk and safety of repetitive transcranial magnetic stimulation: Report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5-7, 1996. Electroencephalogr Clin Neurophysiol 108:1-16, 1998

48. Willoch F, Rosen G, Tölle TR, Oye I, Wester HJ, Berner N, Schwaiger M, Bartenstein P: Phantom limb pain in the human brain: Unraveling neural circuitries of phantom limb sensations using positron emission tomography. Ann Neurol 48:842-849, 2000

49. Yilmaz B, Kesikburun S, Yasar E, Tan AK: The effect of repetitive transcranial magnetic stimulation on refractory neuropathic pain in spinal cord injury. J Spinal Cord Med 37:397-400, 2014

50. Zung WW: A rating instrument for anxiety disorders. Psychosomatics 12:371-379, 1971

51. Zung WW: A self-rating depression scale. Arch Gen Psychiatry 12:63-70, 1965