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ORIGINAL PAPER

Cerebellar Transcranial Direct Current Stimulation (ctDCS) Ameliorates Phantom Limb Pain and Non-painful Phantom Limb Sensations

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Abstract

Phantom limb pain (PLP) is a disabling and intractable sensation arising in about 80% of patients after amputation. The aim of this study was to evaluate the possibility to modulate nociceptive processing and pain perception with cerebellar transcranial direct current stimulation (ctDCS) in patients suffering from painful and non-painful phantom limb sensations. Fourteen upper limb amputees underwent ctDCS (anodal or sham, 2.0 mA, 20 min per day, 5 days a week). Clinical scores and electrophysiological parameters were assessed before tDCS, at the end of the 5-day treatment, 2 and 4 weeks later. Laser-evoked potentials (LEPs) were obtained from the stump using a Nd:YAP laser by pulses with short duration (5 ms) and small diameter spots (5 mm). Changes in visual analogue scores (VAS) were evaluated (chronic pain, paroxysmal pain, stump pain, phantom movements, phantom sensations). Anodal polarization significantly dampened LEP amplitudes (N1, $p = 0.021$ and N2/P2, $p = 0.0034$), whereas sham intervention left them unchanged. Anodal ctDCS significantly reduced paroxysmal pain ($p < 0.0001$), non-painful phantom limb sensations ($p < 0.0001$) and phantom limb movements ($p = 0.0003$), whereas phantom limb and stump pain did not change compared to the sham condition. Anodal ctDCS significantly improves both paroxysmal pain and non-painful phantom limb sensations, which are likely induced by maladaptive changes in the sensorimotor network and posterior parietal cortex respectively.

Keywords Phantom limb pain · tDCS · Cerebellum · Cerebellar tDCS · Phantom pain treatment · Pain tDCS

Introduction

Phantom limb pain (PLP) is an intractable and disabling sensation arising after amputation and occurring in about 80% of patients [1–3]. Ambroise Paré was the first to describe the phenomenon in 1554, suggesting that both peripheral factors and central pain memory might contribute [4]. The pain is referred to the distal portion of the amputated limb, as stabbing, throbbing, burning, or cramping, and it is commonly associated with non-painful phantom limb sensations.

Central factors seem to mainly contribute to its pathogenesis, both at a cortical and spinal level, comprising functional reorganization of cortical sensorimotor maps [5–8] and spinal "phenotypic switch" in the expression of neuropeptides [2, 9]. In particular, studies reported that the severity of pain and the degree of topographic reorganization are positively correlated, suggesting that maladaptive changes may be primed by nociceptive input before the amputation [10–13]. More recently,

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the "maladaptive model" has been reviewed as some authors found a strong correlation between PLP severity and residual activity in the primary sensorimotor missing limb cortex [14, 15].

To date, PLP remains a challenge for clinicians and neuroscientists. The short- and long-term effectiveness of pharmacological interventions is still debated [1, 16]. Most of the studies were limited by their small sample sizes and different pharmacological effects on either painful or non-painful phenomena; also, invasive spinal cord stimulation (SCS) failed to demonstrate long-lasting effects, likely due to its poor somatotopic specificity [17, 18].

Recently, some studies have suggested that the cerebellum may exert a key role in the sensory-motor integration aimed at antinociceptive behavior [19] and in behavioral responses to nociceptive stimulation [20]. In papers from our laboratory, we have shown that cerebellar transcranial direct current stimulation (ctDCS) modulates pain perception in humans, probably by interfering with the inhibitory tone exerted by the cerebellum over cortical areas (cerebellar brain inhibition (CBI) [21, 22]); accordingly, in chronic pain syndromes, the modulation of CBI using non-invasive brain stimulation (NIBS) techniques seems to interfere with maladaptive motor patterns, improving pain and promoting motor skills acquisition [23]. Further support for the usefulness of ctDCS comes from a paper showing that anodal stimulation reduces lower extremity pain perception [24].

The aim of this study was to evaluate the effects of ctDCS on amputated patients with phantom limb pain and non-painful phantom limb sensations. Pain improvement was assessed by clinical scores and neurophysiological parameters (laser-evoked potentials (LEPs)). LEPs offer a unique opportunity to study the sensory-discriminative, as well as the affective-emotional dimension of pain, which are differently carried by medial and lateral spinal nociceptive systems and rely on the activation of distinct cortical areas [25, 26], whereas N1 refers to the activation of somatosensory areas, N2-P2 complex likely origins from a wide network, including caudal and rostral regions of the anterior cingulate cortex [27, 28].

Materials and Methods

Patients

Fourteen patients were recruited from the inpatient and outpatient population of the Pain Therapy Unit of the Cisanello Hospital in Pisa. All the patients suffered from unilateral upper limb amputation (eight left, six right). Table 1 reports a summary of the demographic and clinical data.

Inclusion criteria were as follows: (1) age 18 to 70 years; (2) normal score (> 24) at the Mini-Mental State Examination; (3) limb amputation at least 6 months before study enrolment; (4) stable presence of PLP for at least 2 months, (5) no coexistence of major neurologic, neuropsychological, and psychiatric diseases; and (6) stable pharmacological therapy during the month before the inclusion.

The Groningen Questionnaire Problems after Amputation [3] was used to assess time, side, level, and reason of amputation; duration of pain experienced before amputation; frequency of phantom sensation, phantom pain, and stump pain; amount of trouble and suffering experienced as a consequence of these sensations; type of phantom sensations; medical treatment received for phantom pain and/or stump pain and self-medication (Table 1). The questionnaire was administered before the experimental procedure.

Experimental Protocol

In a crossover, double-blind, sham-controlled design, each patient underwent sham and anodal ctDCS. All patients carried out the two experimental conditions, held at least 3 months apart to avoid carry-over effects. Each session, either anodal or sham, lasted 5 days a week (Monday to Friday, 20 min a day).

Both the electrophysiological assessment and clinical scores were assessed at baseline (T_0), immediately at the end of the stimulation week (T_1), 2 weeks (T_2) and 4 weeks (T_3) later.

Patients were enrolled by a physician with expertise in pain medicine (G.D.C.); clinical scores were administered by a psychologist (F.M.), whereas electrophysiological recordings were performed by a neurologist (T.B.), both blinded to the ctDCS condition.

Informed consent was obtained from all individual participants included in the study. The study was approved by the local ethical committee at the University of Pisa (formally named "Comitato Etico di Area Vasta Nord Ovest della Toscana"), in accordance with the tenets of Helsinki.

Clinical Evaluation

Patients were asked to rate their pain state on a 10-cm VAS at baseline (T_0), immediately at the end of the ctDCS week (T_1), 2 weeks (T_2) and 4 weeks (T_3) later [29, 30]. VAS scores were used to assess (1) PLP intensity (i.e., pain felt constantly in the phantom limb: 0 = absent, 10 = the worst possible PLP); (2) pain paroxysms (i.e., episodes of increased PLP above the background level); (3) stump pain; (4) non-painful phantom limb sensation, including the "telescoping phenomenon" (0 = lack of phantom sensation, 10 = the most intense phantom sensation possible); and (5) phantom limb movements (0 = immobilized phantom limb, 10 = limb easily moved). The order of the VAS ratings was randomized across patients and each evaluation session.

Table 1 Demographic and clinical assessment

Patient's number	Age/sex	Etiology of amputation	Site of amputation	Months from amputation	Phantom sensations	PLP	Prosthesis/physical therapy	Timeline of intervention
1	29/F	Traumatic	L lower arm	19	Movement, hot or cold	Squeezing, pressure	Yes/No	Anodal/sham
2	35/F	Traumatic	L lower arm	23	Tingling, cold	Electric discharge, squeezing	No/No	Sham/anodal
3	38/M	Traumatic	L upper arm	9	Movement, cold, itching	Pressure, stabbing	No/No	Anodal/sham
4	42/F	Traumatic	R lower arm	11	Itching, tingling,	Electric discharge, stabbing, pressure	Yes/No	Sham/anodal
5	32/F	Traumatic	R lower arm	16	Movement, hot	Electric discharge, pressure	Yes/Yes	Anodal/sham
6	52/M	Cancer	L upper arm	7	Tingling, cold	Electric discharge, squeezing	No/No	Sham/anodal
7	44/M	Traumatic	R lower arm	18	Movement, itching, hot	Electric discharge, pressure	Yes/No	Anodal/sham
8	43/M	Traumatic	R upper arm	14	Movement, itching, hot	Pressure, squeezing, electrical shock	No/No	Sham/anodal
9	40/F	Traumatic	L lower arm	13	Itching, hot or cold	Electric discharge, squeezing	Yes/No	Sham/anodal
10	55/M	Vascular	L lower arm	15	Movement, itching, tingling,	Electric discharge, pressure	No/No	Anodal/sham
11	24/F	Traumatic	L lower arm	10	Movement, hot	Electric discharge, squeezing, pressure	Yes/No	Anodal/sham
12	35/F	Traumatic	L lower arm	22	Movement, hot or cold	Squeezing, pressure	Yes/No	Sham/anodal
13	58/M	Vascular	L upper arm	10	Movement, tingling, hot or cold	Electric discharge, squeezing, pressure	No/No	Sham/anodal
14	36/F	Traumatic	L lower arm	9	Movement, tingling,	Pressure, squeezing, electrical shock	Yes/No	Anodal/sham

Laser-Evoked Potentials

A solid-state laser was used (neodymium: yttrium–aluminum–perovskite (Nd: YAP); wavelength 1.04 mm, pulse duration 2–20 ms, maximum energy 7 J: Stimul 1340VR, Electronical Engineering®, Florence, Italy). The laser beam was transmitted from the generator to the stimulating probe via a 10-m length optical fiber; signals were amplified, band pass filtered (0.1–200 Hz, time analysis 1000 ms) and fed to a computer for analysis [31–33].

The stump was stimulated by laser pulses (individual variability, 15.75–24.91 J/cm2) with short duration (5 ms) and small diameter spots (5 mm), inducing pinprick sensations [26]. Twenty stimuli, whose intensity was established on the basis of the perceptive threshold of each patient, were delivered: we used a fixed intensity set at two times the individual sensory threshold, defined as the lower stimulus intensity that elicited a distinct painful pinprick sensation. In order to reduce both skin lesions and fatigue of peripheral nociceptors, the laser beam was shifted slightly by ~ 10 mm in a random direction between consecutive pulses [31]. Patients were reclined on a couch, wore protective goggles, and were instructed to keep their eyes open and gaze slightly downwards; they were requested to mentally count the number of stimuli to keep their attention level constant. The interstimulus interval varied randomly between 15 and 30 s.

The main Aδ-LEP complex, N2/P2, and the earlier lateralized N1 component were recorded through standard disc, non-polarizable Ag/AgCl surface electrodes (diameter 10 mm; BiomedVR, Florence, Italy). N2 and P2 components were recorded from the vertex (Cz), referenced to the earlobes; the N1 component was recorded from the contralateral temporal leads (T3 or T4), referenced to Fz [33]. The baseline-to-peak and the peak-to-peak amplitudes of N1 and N2/P2 components, respectively, were evaluated. Blinks and saccades were recorded with an EOG electrode placed on the superolateral right canthus connected to the system reference. Ground was placed on the mid-forehead.

Skin impedance was kept below 5 kΩ. An automatic artifact rejection system excluded all trials contaminated by transient signals exceeding the average value by ± 65 μV on each recording channel, including the EOG.

Cerebellar Transcutaneous Direct Current Stimulation

Cerebellar transcutaneous direct current stimulation (ctDCS) was applied using a battery-driven constant current stimulator

(HDCStim, Newronika®, Italy) and a pair of electrode in two saline-soaked synthetic sponges with a surface area of 35 cm^2. Direct current was transcranially applied for 20 min with an intensity of 2.0 mA and constant current flow was measured by an ampere meter (current density ~ 0.08 mA/cm^2). These values are in line with those reported for cerebellar stimulation [34, 35] and are considered to be safe [36, 37]. Direct current stimulation strength remained below the sensory threshold throughout the experimental session. At the offset of tDCS, the current was decreased in a ramp-like manner, a method shown to achieve a high level of blinding between sessions [38, 39]. For anodal ctDCS, the anode was applied on the median line, 2 cm below the inion, with lateral borders about 1 cm medially to the mastoid apophysis, and the cathode over the right shoulder [21, 34, 35, 40]. For sham ctDCS, the current was turned on for 5 s and then turned off in a ramp-shaped fashion, thus inducing skin sensations similar to those produced by real ctDCS.

We stimulated the cerebellum bilaterally, as previous studies have shown that varying the position of the active electrode with ~ 1 cm induced negligible changes in the electrical field distribution [41, 42].

Patients were blinded to the tsDCS protocol and did not discriminate between anodal and sham condition. In order to report possible adverse effects, the questionnaire developed by Brunoni and colleagues was administered to each patient [43].

Statistical Analysis

Parametric analyses were used, as all datasets successfully passed the Shapiro-Wilk test for normality ($p > 0.05$). In order to verify the absence of biases due to intra-subject changes across ctDCS sessions, a t test was run to compare baseline data for anodal and sham ctDCS.

A two-way repeated measures (RM) ANOVA was performed, with "stimulation" (two levels: sham and anodal) and "time" (four levels: T_0, T_1, T_2, and T_3) as experimental factors; the Bonferroni t test was used for multiple post hoc comparisons, with a level of significance set at $\alpha = 0.025$. Pearson's correlation coefficient was used to compare possible changes in electrophysiological parameters with the clinical outcome.

Data were analyzed using SPSS v. 21.0 for Windows (SPSS Inc.).

Results

Laser-Evoked Potentials

The baseline laser-evoked potential (LEP) amplitudes did not change among different experimental sessions ($p > 0.3$ for all the comparisons made).

Patients showed a remarkable reduction of N1 and N2/P2 amplitudes following the anodal stimulation compared to the sham condition (N1: $F_{(3,\ 39)} = 10.9$, $p < 0.0001$; N2/P2: $F_{(3,\ 39)} = 9.6$, $p < 0.0001$, two-way ANOVA, with "stimulation" and "time" as experimental factors; N1: $p < 0.0001$; N2/P2: $p = 0.0001$, with "stimulation" as factor; Table 2). This reduction was significant at all time points, both for N1 (T_1, $p < 0.0001$; T_2, $p < 0.0001$; T_3, $p < 0.0001$) and N2/P2 responses (T_1, $p < 0.0001$; T_2, $p < 0.0001$; T_3, $p = 0.0002$, Bonferroni post hoc t test; Fig. 1 and Supplementary Table 1).

Clinical Assessment

The baseline VAS scores did not change among different experimental sessions ($p > 0.2$).

Patients showed a marked improvement in paroxysmal pain, with changes lasting up to 4 weeks after ctDCS completion ($F_{(3,\ 39)} = 14.9$, $p < 0.0001$, two-way ANOVA, with "time" and "stimulation" as experimental factors; $p < 0.0001$, with "stimulation" as factor); this improvement was significant at T_1, T_2, and T_3 when compared to the sham group ($p < 0.0001$, Bonferroni post hoc t test: Table 3 and Fig. 2). No difference was found both in phantom and stump pain scores, although the anodal group showed a tendency towards the improvement in phantom pain score (phantom limb pain: $F_{(3,\ 39)} = 2.3$, $p = 0.09$; stump pain: $F_{(3,\ 39)} = 1.4$, $p = 0.27$, two-way ANOVA, with "time" and "stimulation" as factors).

Phantom limb movements and sensations clearly improved following anodal stimulation ($F_{(3,\ 39)} = 5.3$, $p =$

Table 2 Laser-evoked potentials (LEPs). Amplitudes of N1 and N2/P2 components at baseline and their changes over time, following either the anodal or sham ctDCS. Values are expressed in mV, as mean values ± standard deviation (S.D.)

	Anodal ctDCS				Sham ctDCS			
	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3
N1	6.2 ± 2.2	4.1 ± 2.1	4.3 ± 1.8	4.6 ± 1.7	6.7 ± 2.1	6.4 ± 2.3	6.3 ± 2.4	6.7 ± 2.0
N2/P2	11.5 ± 4.0	8.0 ± 3.1	8.4 ± 3.0	8.9 ± 3.5	11.3 ± 3.8	11.1 ± 3.9	10.9 ± 4.4	11.2 ± 3.8

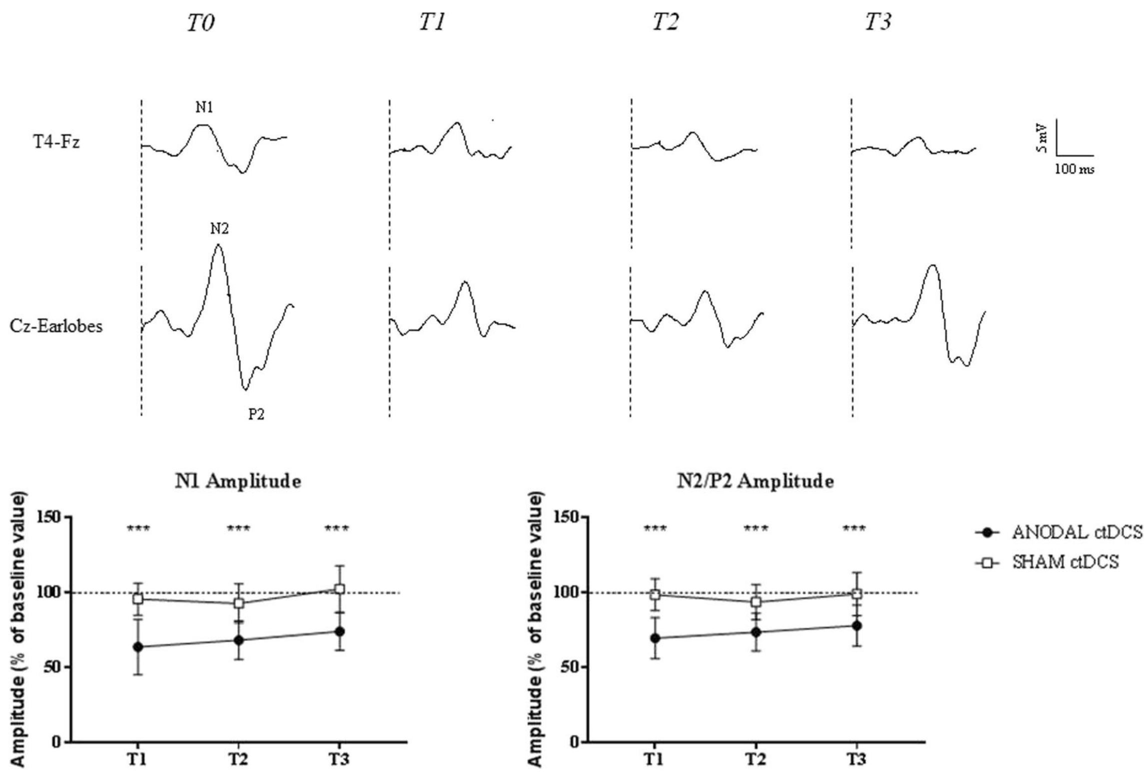

Fig. 1 LEP changes. **a** Exemplificative LEPs recorded at baseline (T_0), immediately after (T_1), at 1 week (T_2), and 3 weeks (T_3) following the completion of anodal ctDCS. Note the reduction of LEP amplitudes over time both for N1 and N2/P2 components. **b** Changes in LEP amplitudes between the anodal (black circles) and sham condition (white squares). Data are given as percentage of baseline value ± S.D. At each time interval, the statistical significance refers to the comparison between anodal (active) and sham (placebo) stimulation (***$p < 0.001$, Bonferroni post hoc comparison)

0.004; $F_{(3,\ 39)} = 8.7$, $p = 0.0002$, respectively, two-way ANOVA, with "time" and "stimulation" as experimental factors), and the reduction in VAS scores was significant at all time intervals when compared to the sham condition (Fig. 3 and Supplementary Table 1).

Changes in paroxysmal pain VAS scores linearly correlated with the reduction in LEP amplitudes. Indeed, patients with greater clinical improvement showed a more robust modulation of evoked responses ($p = 0.0004$, Pearson's correlation coefficient).

Discussion

Anodal ctDCS improves both paroxysmal pain and non-painful phantom limb sensations in subjects with upper limb amputations.

Previous studies have shown that tDCS applied over the motor cortex (M1) is a promising therapeutic tool in PLP, with effects likely arising from a transient restoration of the cortical representation of the phantom limb [29, 30, 44]; although the exact mechanisms of action are still

Table 3 Clinical assessment. VAS scores both for painful and non-painful phantom sensations, following either the anodal or sham ctDCS. Values are expressed as mean values ± standard deviation (S.D.)

	Anodal ctDCS				Sham ctDCS			
	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3
Phantom limb pain	6.6 ± 2.0	5.6 ± 2.1	5.9 ± 2.1	5.5 ± 2.2	6.5 ± 2.3	5.9 ± 2.4	6.1 ± 1.8	6.4 ± 2.4
Paroxysmal pain	7.8 ± 1.8	4.6 ± 2.0	4.5 ± 1.8	4.2 ± 2.1	7.9 ± 1.7	7.5 ± 1.5	7.7 ± 1.7	7.4 ± 1.6
Stump (residual) pain	6.4 ± 2.2	5.3 ± 1.9	5.5 ± 2.0	4.4 ± 2.2	6.5 ± 2.3	5.9 ± 2.0	6.1 ± 2.3	6.0 ± 2.2
Phantom movements	6.9 ± 2.0	4.5 ± 2.3	4.6 ± 1.9	3.9 ± 1.9	6.9 ± 2.0	6.1 ± 2.3	6.2 ± 2.5	5.9 ± 2.3
Phantom sensations	9.2 ± 1.0	6.0 ± 2.7	5.4 ± 2.2	5.4 ± 2.0	9.5 ± 0.8	8.6 ± 1.0	8.6 ± 1.1	8.4 ± 1.0

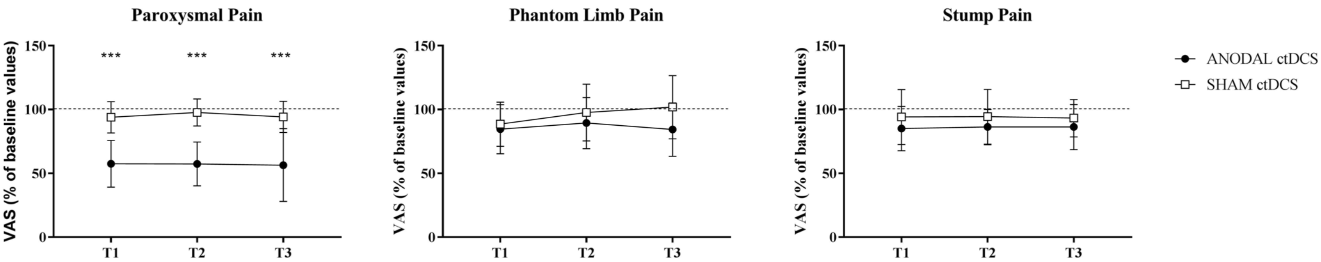

Fig. 2 Painful phantom limb phenomena. Changes in VAS scores over time (paroxysmal pain, phantom limb pain, and stump pain); note that anodal ctDCS (black circles) significantly improved paroxysmal pain compared to the sham condition (white squares). Data are reported as percentage of baseline value ± S.D. At each time interval, the statistical significance refers to the comparison between anodal (active) and sham (placebo) stimulation (***$p < 0.001$, Bonferroni post hoc)

debated, this reinforcement seems to play a role in PLP relief and behavioral therapies targeting motor representation have shown encouraging results [45, 46]. Also, high-frequency repetitive transcranial magnetic stimulation (rTMS) on the contralateral primary motor cortex was shown to improve PLP in land mine victims [47], although other studies reported conflicting results about the use of rTMS, either low or high frequency, in amputated patients [48–50].

Here, the reduction in N1 and N2/P2 amplitudes confirms data in healthy humans [21, 24] and suggests that anodal ctDCS is able to modulate both somatosensory and cingulated cortices, interfering with the sensory-discriminative and emotional dimensions of pain [25, 51].

Interestingly, our results proved ctDCS effects on painful and non-painful phantom limb phenomena, in accordance with previous papers supporting a cerebellar involvement in phantom limb sensations [52–54]. Others studies found a clinical dissociation between painful and non-painful phantom phenomena following tDCS applied over M1 [29]. The discrepancy between these results and our findings is probably due to different tDCS target, with anodal ctDCS leading to a sustained cerebellar-brain inhibition (CBI), not restricted to motor areas. Further support comes from a recent paper showing that PLP relief significantly correlated with reduced activity in both primary somatosensory and motor cortices (S1/M1): the authors proved that anodal tDCS applied over the S1/M1 missing hand cortex leads to PLP relief, longer than that induced by M1 stimulation alone, probably by restoring defective intracortical inhibitory processes [55].

Accordingly, an increased activity within S1/M1 areas has been recently recognized as a marker of chronic PLP [14, 15] and transcranial magnetic stimulation (TMS) studies have shown that motor thresholds and intracortical inhibition decrease for the muscle proximal to the amputation [56, 57]; although the relationship between increased motor excitability and PLP remains unclear, these changes are greater in amputees with PLP than in pain-free patients and positively correlated with the severity of pain [10].

As an alternative, not mutually exclusive explanation, we cannot rule out the possibility of a modulation of the lemniscal pathways, as suggested by the selective modulation of paroxysmal pain scores. Some studies have proposed that pain paroxysms are due to demyelination of non-nociceptive Abeta-fibers, whereas spontaneous constant pain arises from damage to nociceptive Adelta afferents [58, 59]. That may explain, at least in part, the lack of changes in PLP scores. Accordingly,

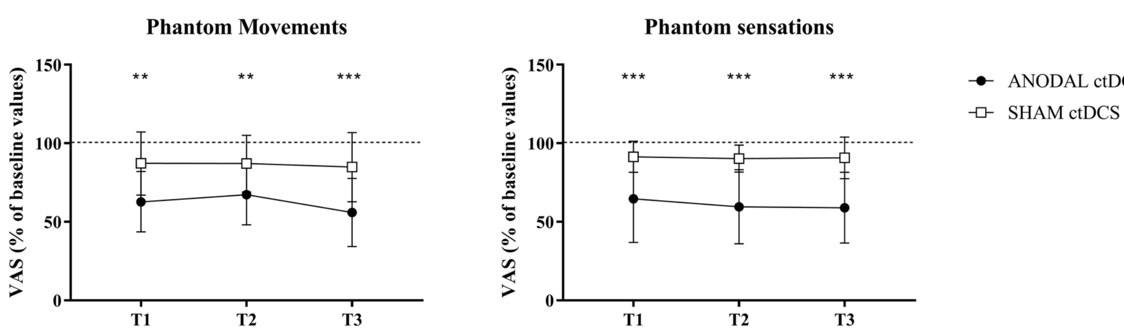

Fig. 3 Non-painful phantom limb phenomena. Changes in VAS scores; note that anodal ctDCS (black circles) remarkably reduced both phantom movements and sensations compared to the sham condition (white squares). Data are given as percentage of baseline value ± S.D. At each time interval, the statistical significance refers to the comparison between anodal (active) and sham (placebo) stimulation (**$p < 0.01$; ***$p < 0.001$, Bonferroni post hoc)

ctDCS may act not only on cortical networks but also on spinal neurons, influencing pain processing through top-down and bottom-up mechanisms [60].

Finally, the lack of changes in stump pain is not surprising, as it refers to the so-called residual limb pain (RLP), localized to the remaining body part after amputation and likely due to peripheral factors, comprising post-surgical neuromas and nociceptor sensitization, difficult to treat with non-invasive brain stimulation techniques [3, 61, 62].

Limitations of the Study

Our study has some limitations. First, only patients with upper limb amputations were enrolled; our sample included a large number of young patients, whose amputations mainly resulted from traumatic injuries. These patients do not usually undergo preoperative peripheral nerve block, a method shown to reduce the occurrence of PLP [63], in accordance with the hypothesis that phantom pain is primed by nociceptive input before the amputation [12, 13]. It would be of interest to complete the trial including patients with lower limb amputation.

Second, an extensive neurophysiological evaluation of peripheral nerves and sympathetic function has not been included. It may be useful for the study of mechanisms underlying phantom limb pain, residual phantom pain, and non-painful sensations.

Third, no patient underwent mirror therapy (MT); MT may enhance the effects of anodal ctDCS, as described for the stimulation of primary motor cortex [44].

Conclusions

To our knowledge, this is the first study describing analgesic effects of cerebellar direct current stimulation in neuropathic pain and the first extensively assessing LEP changes in amputees. Cerebellar tDCS improved painful and non-painful sensations, through different mechanisms of action, providing a novel therapeutic option for the treatment of PLP. Further studies are needed to improve this technique, possibly in association with behavioral strategies. Finally, ctDCS may be also used to reduce the pre-amputation pain before surgery, in order to prevent central and spinal sensitization that plays a critical role, especially for amputations due to vascular or neoplastic causes, where surgery may be planned weeks before, possibly avoiding maladaptive cortical changes priming pain before the amputation.

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