Received: 16 June 2018 | Revised: 4 December 2018 | Accepted: 21 December 2018

DOI: 10.1111/odi.13038

ORIGINAL ARTICLE

WILEY ORAL DISEASES

Transcranial direct current stimulation (tDCS) and trigeminal pain: A preclinical study

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Funding information
FIPE/Graduate Research Group (GPPG) of Hospital de Clínicas de Porto Alegre – HCPA, Grant/Award Number: 14-0329

Abstract

Objective: Our objective was to evaluate the Transcranial direct current stimulation (tDCS) effect on facial allodynia induced by chronic constriction of the infraorbital nerve (CCI-ION) and on the brainstem levels of TNF-α, NGF, IL-10, and serum LDH in rats.

Methods: Rats were exposed to the CCI-ION model. Facial allodynia was assessed by von Frey filaments test at baseline, 3, 7, 10, and 14 days postsurgery and 24 hr and 7 days after the bimodal tDCS sessions for 20 min/day/8 days.

Results: Chronic constriction of the infraorbital nerve induced a significant decrease in the mechanical threshold 14 days after surgery. This effect was reversed by tDCS treatment, with the mechanical threshold returning to basal levels at 24 hr after the end of the treatment and it persisted for 7 days after the end of the treatment. tDCS also decreased LDH serum levels compared to those in the control group. There was an interaction between pain and treatment with respect to brainstem levels of NGF, TNF-α, and IL-10.

Conclusion: Chronic constriction of the infraorbital nerve model was effective in establishing trigeminal neuropathic pain on 14 days after surgery, and tDCS reduced allodynia and LDH serum levels and promoted alterations in NGF, TNF-α, and IL-10 brainstem levels. Thus, we suggest that tDCS may be a potential therapy in the trigeminal pain treatment.

KEYWORDS
CCI-ION, pain, rat, tDCS, trigeminal neuralgia

1 | INTRODUCTION

Trigeminal neuralgia (TN) is the most prevalent neuropathic orofacial pain, and it is accompanied by allodynia and recurrent paroxysmal attacks of pain in the trigeminal area (Haviv et al., 2016; Zakrzewska & McMillan, 2011). Patients with TN usually describe their pain as an electric shock, burning, sharp, shooting, and lancinating sensation (Yang et al., 2016). TN etiology is associated with neurovascular compression, demyelinating disease (Leclercq, Thiebaut, & Heran, 2013), or injury of the nerve structure caused by trauma, for example, dental treatment (Rodriguez-Lozano, Sanchez-Perez, Moya-Villaescusa, Rodriguez-Lozano, & Saez-Yuguero, 2010).

The therapeutic approach is usually based on pharmacological treatment, and anticonvulsants are the first-line drugs (Zakrzewska & McMillan, 2011). However, the adverse effects of anticonvulsants are not well tolerated by most of the patients (Edlich, Winters, Britt, & Long, 2006). We highlight that this pathology mainly affects old-age individuals (Zakrzewska & McMillan, 2011) who usually take multiple drugs, increasing the risk of drug interactions. In case of non-responsiveness to pharmacological treatment, surgical approach may be necessary, especially in cases of trigeminal neuralgia resulting from neurovascular compression (Leclercq et al., 2013); however, dependent upon the health conditions of the patients, it may not be possible to perform the surgical procedure. Thus, investigation of new therapeutic options is especially important to improve the quality of life of TN patients.

Transcranial direct current stimulation (tDCS) is a non-invasive method of cerebral stimulation that may represent a promising tool for modulating trigeminal nociceptive processes and decreasing the pain response (Hagenacker et al., 2014). It has been used in the treatment of several pain conditions in patients, such as central post-stroke pain (Bae, Kim, & Kim, 2014); it has also been tested in animal pain models, including mechanical allodynia in chronically stressed rats (Spezia Adachi et al., 2012) and neuropathic (Cioato et al., 2016) or inflammatory pain (Laste et al., 2012).

Transcranial direct current stimulation is able to promote relief of pain symptoms, both short and long term (at least up to 7 days) after the end of the tDCS treatment according to our previous studies (Cioato et al., 2016; Laste et al., 2012; Spezia Adachi et al., 2012). It is known that tDCS modulates the resting membrane potential of neurons in the stimulated region, and it promotes subsequent protein synthesis (Stagg & Nitsche, 2011). Previous studies suggest that the effects of tDCS involve a top-down mechanism in which cortical stimulation modulates remote areas, such as the spinal cord and brainstem. This modulation seems to be involved with changes in neurotrophin levels, such as brain-derived neurotrophic factor (BDNF; Spezia Adachi et al., 2015). Similarly, nerve growth factor (NGF) could be involved in the mechanism of tDCS (Mizumura & Murase, 2015).

Transcranial direct current stimulation may possibly induce changes in biomarker levels, with a central role in pain processes and inflammation, such as cytokines, important mediators released from injured tissue. These mediators may present pro- or anti-inflammatory activity. Tumor necrosis factor alpha (TNF-α) is particularly associated with pain and activates other cytokines (Choi et al., 2015; Leung & Cahill, 2010). Additionally, the release of interleukin-10 (IL-10), an anti-inflammatory cytokine, inhibits synthesis of both, nuclear factor kappa B (NFκB) and pro-inflammatory cytokines, such as interleukin-1β (IL-1β) and TNF-α, minimizing tissue damage (Khan et al., 2015; Ouyang, Rutz, Crellin, Valdez, & Hymowitz, 2011). An important marker for cell damage is lactate dehydrogenase (LDH), an intracellular enzyme. Elevated serum levels of LDH are positively correlated with cell damage (Adeva-Andany et al., 2014).

Thus, the neuroplastic changes involved in the nociceptive process and the limited efficacy of pharmacological approaches to treat neuropathic pain states justify the search for a more effective, tolerable, and safe therapy for TN. Therefore, the aim of this study was to evaluate the effects of tDCS on the facial mechanical hyperalgesia of rats submitted to an orofacial neuropathic pain model. In addition, we evaluated the effects of TN and tDCS treatment on the brainstem levels of TNF-α, NGF, and IL-10, as well as serum LDH levels in rats.

2 | MATERIAL AND METHODS

In this study, we used 151 male Wistar rats (60–70 days old), weighing between 270 and 340 g, from the Center for Reproduction and Animal Experimentation of the Institute of Basic Health Sciences of Federal University of Rio Grande do Sul, Brazil. Rats were housed in polypropylene cages (49 × 34 × 16 cm), with sawdust-covered floors, and a maximum number of four animals per box. Rats were maintained on a standard 12:12 light–dark cycle (lights on at 07:00 hr and lights off at 19:00 hr), at room temperature (22 ± 2°C), with water and chow (Nuvital, Porto Alegre, Brazil) available ad libitum. All experiments and procedures were approved by the Institutional Committee for Animal Care and Use (GPPG-HCPA protocol No 14-0329) and conformed to the Guide for the Care and Use of Laboratory Animals 8th ed. 2011. The maintenance of the animals followed law 11.794 (Brasil, 2008; MCTIa, 2013; MCTIb, 2013), which establishes procedures for the scientific use of animals. The experimental protocol complied with the ethical and methodological standards of the ARRIVE guidelines (Kilkenny, Browne, Cuthill, Emerson, & Altman, 2010). Vigorous attempts were made to minimize the number of animals required to generate reliable scientific data and mitigate external sources of pain and discomfort. The rats were acclimated to the maintenance room for 2 weeks before the experiment began.

They were randomized by weight and then divided into two experimental protocols. In the first protocol, a pilot project was conducted with 21 rats divided into two groups (pain and sham pain) to determine the establishment of orofacial neuropathic pain, without the influence of postoperative pain. Facial allodynia was evaluated by the von Frey filaments test (VFFT) at baseline and at 3, 7, 10, and 14 days after surgery. Rats were euthanized after the behavioral test, 14 days after surgery (Table 1).

In the second experimental design (Protocol 2), the effect of tDCS on a trigeminal neuralgia model was tested. One hundred and thirty rats were divided into seven experimental groups: control (C), sham pain (S), sham pain + sham tDCS (SS), sham pain + tDCS (ST), pain (P), pain + sham tDCS (PS), and pain + tDCS (PT). The tDCS treatment was initiated 14 days after surgery. It was performed for 20 min/day for eight consecutive days of treatment. The von Frey facial filament test was applied at baseline, 14 days after surgery, 24 hr after the end of the tDCS treatment, and 7 days after the end of the tDCS treatment (Table 2). The euthanasia of animals occurred in two time periods: phase 1—24 hr after the end of tDCS treatment and phase 2—7 days after the end of the treatment (Figure 1).

TABLE 1 von Frey test protocol 1: Data were reported as percentage of the baseline (median ± interquartile range), (n = 4–6 animal/group)

Days after surgery	3rd	7th	10th	14th
Sham pain group	100 (94.44–107.14)[a]	88.88 (86.66–107.14)[a]	88.88 (76.66–114.28)[a]	100 (72.20–107.14)[a]
Pain group	100 (88.88–111.15)[a]	100 (87.63–109.37)[a]	100 (81.25–112.15)[a]	76.20 (64.03–84.86)[b]

Note. Different superscript letters (a and b) indicate a statistically significant difference between the intragroup timepoints analyzed (Friedman test/Dunn, p = 0.000)

2.1 | Orofacial neuropathic pain model

The pain model used to induce trigeminal neuralgia involved chronic constriction of the infraorbital nerve (CCI-ION) and was adapted from the model proposed previously (Imamura, Kawamoto, & Nakanishi, 1997). Anesthesia was induced using ketamine (50 mg/kg i.p.) and xylazine (10 mg/kg i.p.). Anesthesia was maintained with isoflurane (2% inhalation). Surgical access was made by an intraoral incision on the left side 0.5 mm from the insertion of the first molar and extended along the vestibular mucosa for 1 cm in the anterior direction. The tissues were removed, and the infraorbital nerve was exposed and isolated (Figure 2). Two ligatures were made with a 2 mm distance between them using Vicryl 4-0 thread. After the ligation was performed, the incision was sutured. The animals were housed in a heated incubator until recovery from anesthesia.

Rats from the sham pain group underwent the same procedure of anesthesia, surgical access, and suture, but without nerve constriction. After surgery, tramadol hydrochloride (5 mg/kg i.p.) was administered at 12-hr intervals for the first 24 hr for relieving pain to minimize the animal's discomfort.

2.2 | Transcranial direct current stimulation

Transcranial direct current stimulation treatment was initiated 14 days after surgery. Animals in the treatment groups underwent 20-min daily sessions of bimodal tDCS for 8 days, as described by Cioato et al. (2016). A constant direct current of 0.5 mA was delivered from a battery powered stimulator using electrocardiogram electrodes with conductive adhesive hydrogel. The rats' heads were shaved for firmer adherence, and round electrodes of 1.5 cm diameter were used for a better fit. The electrodes were fixed to the head with adhesive tape (Micropore™) and covered with a protective mesh to prevent removal. The cathode was positioned at the midpoint between the lateral angles of both eyes (supraorbital area), and the anode was placed on the head using landmarks of the neck and shoulder

TABLE 2 von Frey test protocol 2. Data were reported as median ±interquartile range for each timepoint

	Baseline	14 days after surgery	24 hr after treatment	7 days after treatment
C	153.33 (74.33–300.00)[a]	110.66 (58.33–201.00)[a]	85 (52.66–226.66)[a]	60.00 (37.37–70.00)[a]
S	136.66 (58.33–193.33)[a]	45.33 (22.33–105.46)[a]	180 (48.66–3,000)[a]	40.25 (32.00–60.00)[a]
SS	110.16 (75.41–173.33)[a]	27.66 (5.16–110.50)[a]	240 (100.83–300)[a]	15.00 (9.66–28.66)[a]
ST	126.66 (69.5–300.00)[a]	141 (31.25–193.33)[a]	87.66 (23.58–154.16)[a]	26.00 (11.75–32.75)[a]
P	199.16 (95–250.00)[a]	12.23 (9.7–60.16)[b]	48.83 (10.16–83.33)[b]	43.00 (16.00–60.50)[ab]
PS	86.66 (70.66–166.66)[a]	9.8 (5.13–50.66)[b]	58.33 (37.33–73.33)[c]	12.50 (8.50–18.74)[abc]
PT	88.66 (53.33–160.00)[a]	12.66 (4.4–26.90)[b]	43.33 (14.90–50.50)[ab]	7.00 (16.50–21.87)[ab]

Notes. C: control; P: pain; PS: pain + sham tDCS; PT: pain + tDCS. (n = 4–6 animals/group); S: sham pain; SS: sham pain + sham tDCS; ST: sham pain + tDCS.
Different superscript letters (a, b, and c) indicate a statistically significant difference between the intragroup timepoints analyzed (Friedman/Dunn, p = 0.004).

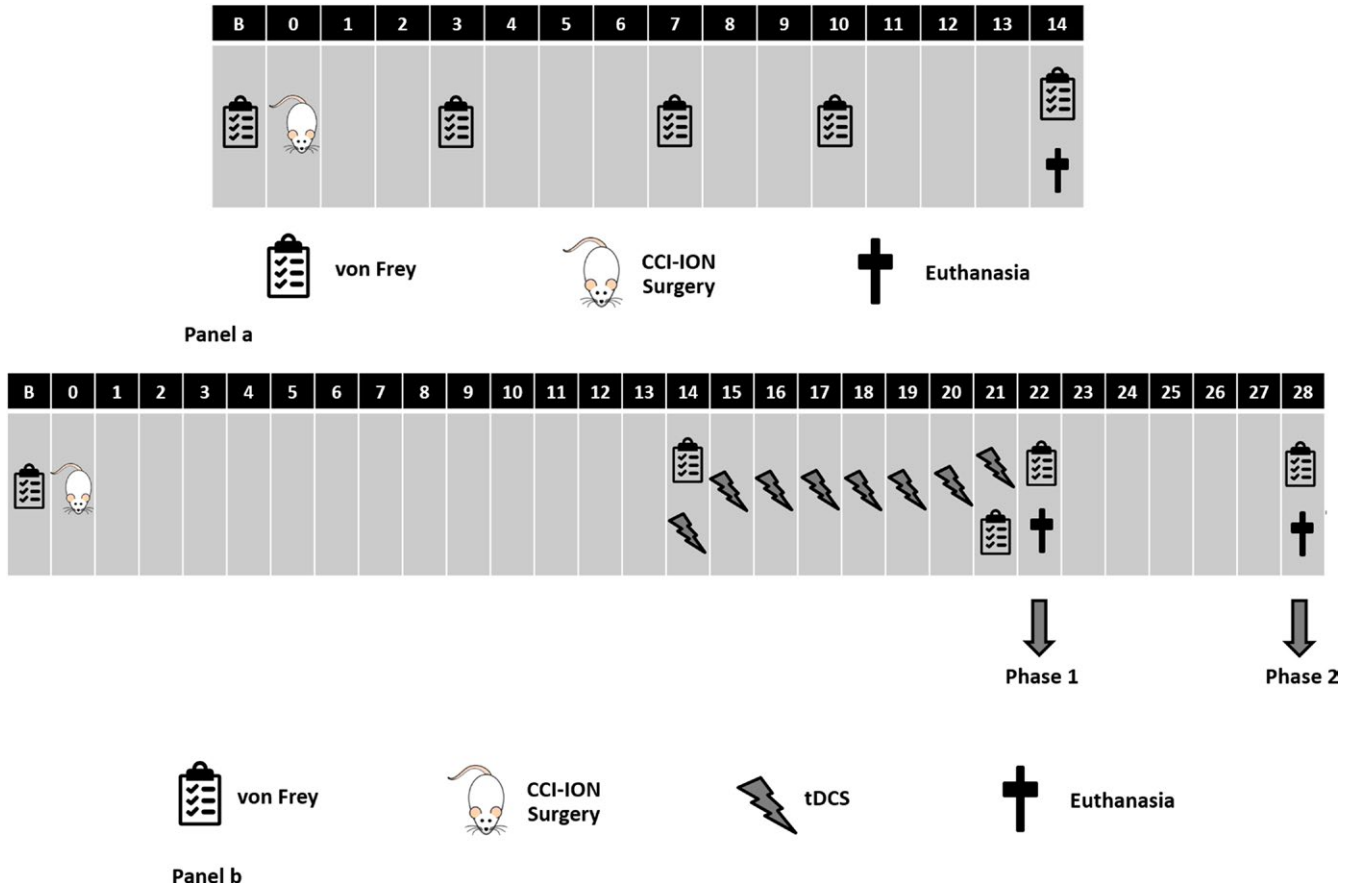

FIGURE 1 Experimental design. (a) Protocol 1. (b) Protocol 2. von Frey test. CCI-ION: chronic constriction injury infraorbital nerve. tDCS: transcranial direct current stimulation. Euthanasia [Colour figure can be viewed at wileyonlinelibrary.com]

lines as guides (the anterior and posterior regions in the midline between the two hemispheres of the parietal cortex, as previously described (Takano et al., 2011; Figure 3)). This technique mirrors human tDCS protocols used in pain treatment (Antal, Nitsche, & Paulus, 2006; Fregni et al., 2006; Nitsche et al., 2008; Rosen, Ramkumar, Nguyen, & Hoeft, 2009) and has been applied by our research group showing long-lasting effects on pain relief and an antihyperalgesic response in rats with inflamed paws (Laste et al., 2012). For sham tDCS, the electrodes were placed and fixed in the same position as for actual stimulation; however, the stimulator remained in the "off" position throughout the procedure (Cioato et al., 2016).

2.3 | von Frey filaments test

Allodynia was assessed using the orofacial filament von Frey test. This test entails perpendicular pressure made with a plastic filament over the vibrissae area. Each filament presents a gradation equivalent of the force in grams required to bend it. The force required for an animal to exhibit pain-like behavior with face withdrawal was registered, according to the protocol described previously (Vos, Strassman, & Maciewicz, 1994). Three measures were averaged in intervals of 5 min. The pain threshold is

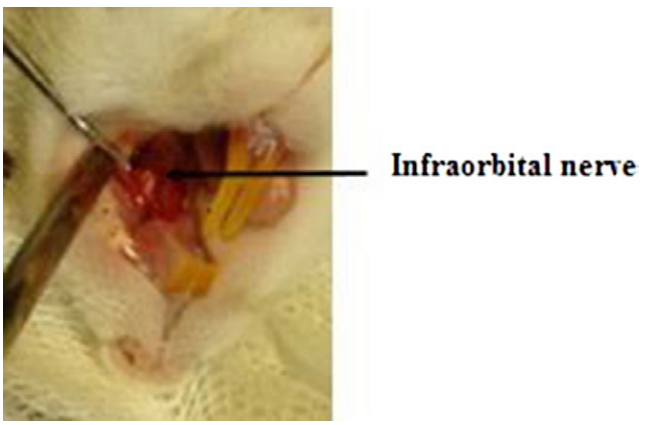

FIGURE 2 CCI-ION: Infraorbital nerve is exposed [Colour figure can be viewed at wileyonlinelibrary.com]

equivalent to the median of the three measures and is expressed in grams.

2.4 | Tissue collection

Rats were euthanized by decapitation, and the brainstem and blood were collected for biochemical analysis in two phases: phase 1—rats

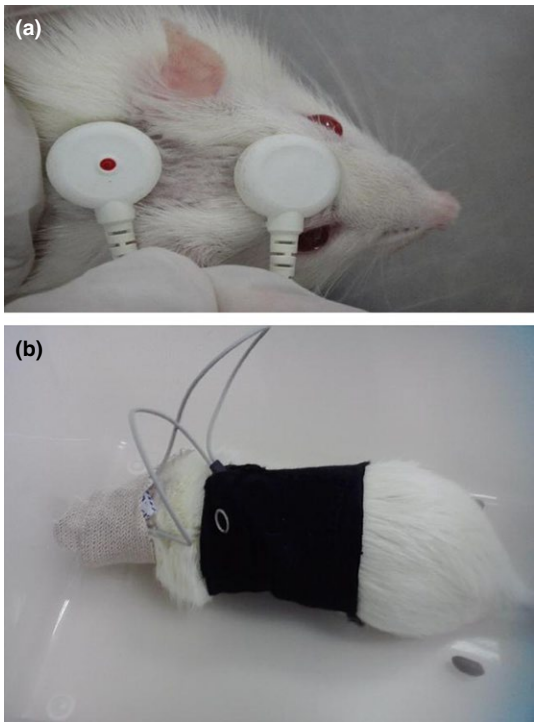

FIGURE 3 (a) tDCS placement. Anodal electrode was positioned at midline, adjacent to the neck, and cathodal electrode was positioned at midline along a line passing between the middle of the eyes. (b) tDCS session procedure. The rat was kept immobilized to avoid device displacement (Spezia Adachi et al., 2012). With authorization [Colour figure can be viewed at wileyonlinelibrary.com]

were euthanized after VFFT measured at 24 h after the end of tDCS and phase 2—rats were euthanized 8 days after the end of the tDCS treatment. The samples collected were stored at −80°C until the assays were performed.

2.5 | Biochemical assays

The brainstem levels of NGF, TNF-α, and IL-10 were analyzed using a commercial ELISA kit (mouse enzyme assay; R&D Systems, Minneapolis, USA). Brainstem tissue was homogenized in a commercial solution with antiproteinases (Sigma® # P8340) in the ratio of 1:10 and centrifuged for 5 min at 20,200 x *g*. Total protein was measured by the method of Bradford (1976). The results were expressed as pg/mg protein for the brainstem and serum. Additionally, LDH serum levels were measured using a spectrophotometer commercial kit and expressed in U/L (BioLab).

2.6 | Statistical analysis

Analysis of the von Frey facial filaments test data was performed using a Friedman test followed by a Dunn post hoc test. Biochemical data analyses were performed with two-way ANOVA, followed by Student–Newman–Keuls (SNK). Results were considered statistically significant if $p < 0.05$. Data were expressed as mean ± standard error of the mean (*SEM*). The SPSS 20.0 program was used for all statistical analysis.

3 | RESULTS

3.1 | Facial allodynia

3.1.1 | Protocol 1

Facial allodynia was confirmed in the pain group (P) on the 14th day after surgery, indicated by the significant reduction in the nociceptive threshold in the pain group between the timepoints analyzed, which was not observed in the sham pain group (Friedman test/Dunn, $p = 0.000$; Table 1).

3.1.2 | Protocol 2

We observed lower facial mechanical thresholds 14 days after surgery in the pain group (P), considering intragroup comparisons over time in relation to baseline measures (Friedman test/Dunn, $p = 0.004$). This difference persisted for up to 23 days after surgery (Friedman test/Dunn, $p = 0.004$). However, this effect was not observed 29 days after the surgery (Friedman test, $p > 0.05$; Table 2).

Facial mechanical allodynia was significantly lower than at the baseline measurement (Friedman test/Dunn, $p = 0.004$) at the 14th day after surgery in the pain + tDCS group (PT). This difference was partially reversed after 24 hr following the end of the tDCS treatment (Friedman test, $p > 0.05$; Table 2).

3.2 | NGF brainstem levels

Nerve growth factor levels were evaluated in the brainstem 24 hr after the last tDCS session, and an interaction was found between pain and tDCS treatment (two-way ANOVA/SNK; $F_{2,38} = 5.48$, $p = 0.009$). At 7 days after the end of the tDCS treatment, there was an interaction between pain and tDCS treatment and a significant tDCS effect (two-way ANOVA/SNK; $F_{2,44} = 9.9$, $p = 0.000$ and $F_{2,44} = 3.27$, $p = 0.049$; Figure 4a).

3.3 | TNF-α brainstem levels

Twenty-four hours after the end of the tDCS treatment, there was no difference in TNF-α brainstem levels between groups (two-way ANOVA, $p > 0.05$). There was an interaction between pain and treatment 7 days after the end of the tDCS treatment (two-way ANOVA/SNK, $F_{2,45} = 3.9$, $p = 0.027$; Figure 4b).

3.4 | IL-10 brainstem levels

No difference in brainstem IL-10 levels was observed 24 hr after the end of the tDCS treatment (two-way ANOVA, $p > 0.05$). Seven days after the end of the tDCS treatment, there was an interaction

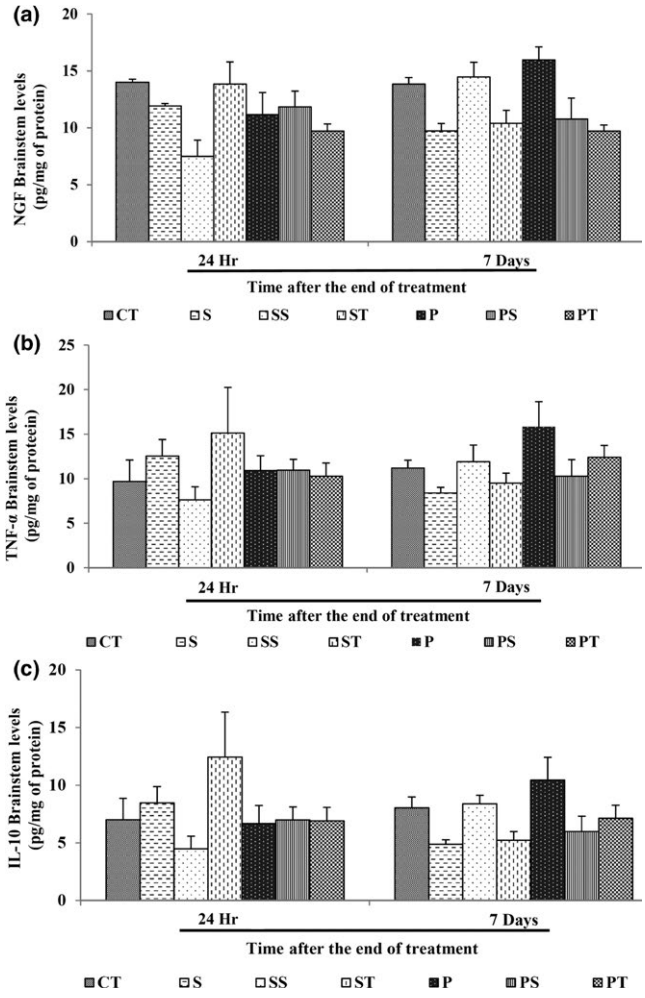

FIGURE 4 Biochemical analysis. Data are presented as mean ± standard error of mean. Total control group (CT), sham surgery group (S), sham surgery + sham tDCS group (SS), sham surgery + tDCS group (ST), pain group (P), pain + sham tDCS group (PS), and pain + tDCS group (PT). (a) Brainstem NGF levels. There was an interaction between pain and tDCS treatment at 24 hr and 7 days after the end of the tDCS treatment (two-way ANOVA/SNK, $F_{2,38} = 5.48$, $p = 0.009$; $F_{2,44} = 9.9$, $p = 0.000$, respectively). (b) Brainstem TNF-α levels. There was an interaction between pain and tDCS treatment at 7 days after the end of the tDCS treatment (two-way ANOVA/SNK, $F_{2,45} = 3.9$, $p = 0.027$). (c) Brainstem IL-10 levels. There was an interaction between pain and tDCS treatment at 7 days after the end of the tDCS treatment (two-way ANOVA/SNK $F_{2,45} = 6.21$, $p = 0.005$)

between pain and treatment (two-way ANOVA/SNK $F_{2,45} = 6.21$, $p = 0.005$; Figure 4c).

3.5 | LDH serum levels

There were significant effects of pain and treatment on the serum LDH levels (two-way ANOVA/SNK, $F_{2,46} = 13.64$, $p = 0.000$; $F_{2,46} = 3.69$, $p = 0.034$, respectively) at 24 hr after the last tDCS session. There was no significant difference between the groups 7 days after the last tDCS session (two-way ANOVA, $p > 0.05$; Figure 5).

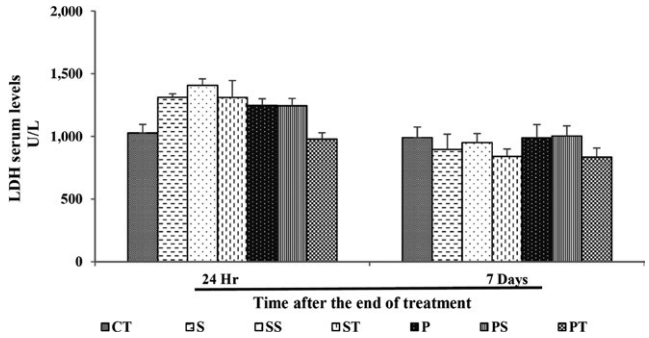

FIGURE 5 Effect of tDCS upon serum LDH levels. Data are presented as mean ± standard error of mean. Total control group (C), sham surgery group (S), sham surgery + sham tDCS group (SS), sham surgery + tDCS group (ST), pain group (P), pain + sham tDCS group (PS), and pain + tDCS group (PT). (*) At 24 hr after the end of the treatment, there was a significant effect of pain and treatment, separately (two-way ANOVA/SNK $F_{2,46} = 13.64$, $p = 0.000$; $F_{2,46} = 3.69$, $p = 0.034$, respectively)

4 | DISCUSSION

In this study, we observed that 14 days after surgery is the time required for the establishment of neuropathic pain indexed by allodynia development. This result corroborates a previous study conducted by our group in a model of sciatic nerve constriction neuropathy (Cioato et al., 2016). It is important to note that after the surgical procedure, to promote nerve constriction, it is necessary to discriminate between inflammatory and neuropathic pain. However, there is considerable divergence in the literature regarding the length of this period. It can vary from 3 days in a sciatic nerve constriction model (Riffel et al., 2016), to a period between 4 and 8 weeks for an infraorbital constriction model by the technique proposed by Kernisant (Kernisant, Gear, Jasmin, Vit, & Ohara, 2008). A similar result was found by Vos and colleagues (Vos et al., 1994) using an infraorbital nerve constriction model, which demonstrated a significant increase in response to mechanical stimulation by von Frey test filaments from the 12th to 15th days after surgery. On the other hand, a previous study reports a decrease in the mechanical nociceptive threshold in the Semmes-Weinstein monofilament test at 2 weeks after constriction surgery (Henry, Freking, Johnson, & Levinson, 2007). We emphasize that the sciatic nerve differs from the infraorbital because it is a nerve with motor and sensory components. However, we believe that similar results found in sciatic and trigeminal neuropathy are very important since the stimuli from the two nerves share ascending pathways from the medial lemniscus toward the reticular formation and thalamus (Merighi et al., 2008).

On the other hand, Imamura's study showed a significant increase in heat thermal hypersensitivity beginning on the 4th day after the surgical procedure, with peak pain between the 8th and 12th days after surgery (Imamura et al., 1997). The difference between the installation periods of thermal and mechanical neuropathic pain may be explained by the fact that different nociceptive stimuli involve different types of fiber conduction. Thermal allodynia depends on

the plasticity of fibers of smaller caliber and slow conduction (C fibers), whereas mechanical allodynia depends on changes in fibers of greater caliber and faster conduction (fibers Aδ and Aβ; Imamura et al., 1997). Moreover, the afferent pathway for the two types of stimuli differs; C fibers ascend through the paleospinothalamic tract, whereas Aδ and Aβ fibers ascend by the neospinothalamic tract (Merighi et al., 2008).

In addition, in the second protocol, we showed that bimodal tDCS treatment partially reverted mechanical allodynia induced by a neuropathic pain model at 24 hr in the pain + tDCS group. However, at 7 days after the end of the tDCS treatment, all pain groups (independently of treatment) presented similar nociceptive thresholds to the control group. The mechanism proposed for tDCS effects is related to neuromodulation promoted by the low-intensity electric current flow (Nitsche et al., 2008). The explanation of the pain relief may be an indirect action of the motor cortex, which is the target of tDCS treatment in humans for pain conditions. It is suggested that stimulation of projections from the motor cortex to the thalamus and the anterior cingulate cortex is involved in the inhibition of pain sensation or alteration in the affective aspects related to pain (Drouot, Nguyen, Peschanski, & Lefaucheur, 2002; Lima & Fregni, 2008). The duration of membrane electrical modifications is limited to minutes after the end of the tDCS application. However, repeated sessions, such as were applied in the current study (eight consecutive daily sessions), may prolong the tDCS effects for up to several weeks (Reis et al., 2009). The long-lasting effects are attributed to changes in gene expression, protein synthesis, and synaptic modulation, which are related to alteration in the resting potential of the plasma membrane (Nitsche et al., 2003). However, we were not able to show this effect in the current study because the pain groups did not present anymore allodynia induced by infraorbital nerve constriction. It is known that the type of wire used to execute the ligature influences the intensity of pain (Robinson & Meert, 2005). In this study, Vicryl was used; it is a monofilament wire that loses tension over nerve fibers with the passage of time. Since stability of symptoms is dependent on suture material (Robinson & Meert, 2005), we could conclude that the decrease in symptoms was due to this. However, this may not be the only explanation, since in a previous study by our group using sciatic constriction together with a reabsorbable wire, the pain was maintained for a period of at least 29 days after surgery. Thus, another possibility could be that fewer nerve fibers have been reached because, in our model, the constriction was performed in the distal part of the nerve, after its emergence in the infraorbital foramen of the maxilla.

There was an interaction between pain and tDCS with respect to NGF levels at 24 hr and at 7 days after the last tDCS treatment. NGF is associated with survival, development, and trophism of the central and peripheral neural cells (Levi-Montalcini & Angeletti, 1968) and synaptic plasticity (Berry, Bindocci, & Alleva, 2012). In the state of pain, NGF participates in sensitization and neuroplastic changes (Mizumura & Murase, 2015). A previous study demonstrated that NGF injection in rats produces hyperalgesia (Lewin, Rueff, & Mendell, 1994). However, there is a conflict between studies that evaluated NGF tissue levels and neuromodulation therapies. In contrast to our results, a study using a sciatic neuropathy model treated with tDCS reported increased NGF levels in the cerebral cortex and spinal cord (Cioato et al., 2016). Conversely, Brunoni and colleagues observed no changes in the plasma NGF levels of depressive patients treated with tDCS (Brunoni et al., 2015). However, pain models that trigger tissue damage for a long period of time induce constant nociceptive signaling and lead to plastic alterations in pain processing (Costigan, Scholz, & Woolf, 2009). Thus, we can suggest that the tDCS action mechanism, which reduces allodynia in rats on the 22nd day after infraorbital constriction (or 24 hr after the end of the tDCS treatment), may be related to reduction in brainstem NGF levels.

There was an increase in the TNF-α and IL-10 brainstem levels induced by the pain model 7 days after the end of tDCS; this effect was reversed by tDCS treatment. It is interesting to note that TNF-α is a cytokine related to the pathogenesis of peripheral and central neuropathic pain. In a chronic sciatic constriction model study in rats, TNF-α was detected at the lesion site and underwent overstimulation over time (Leung & Cahill, 2010). It is important to highlight that the tDCS action mechanism may be linked to cytokine levels in the brainstem, according to our results. In addition, chronic sciatic constriction neuropathic pain in rats presents elevation of these cytokine levels in central structures such as the hippocampus and locus coeruleus (Covey, Ignatowski, Knight, & Spengler, 2000; Covey et al., 2002). Moreover, Wei Guo Zou Ren and Dubner (2008) demonstrated an increase in brainstem TNF-α and IL-1β levels in rats submitted to infraorbital nerve constriction, with increased *N*-methyl-D-aspartate (NMDA) receptor NR1 subunit phosphorylation (Wei et al., 2008).

Interleukin-10 is an anti-inflammatory cytokine released at a late stage of the inflammatory process. It has a protective function, preventing excessive tissue damage caused by inflammation (Ouyang et al., 2011; Tabas & Glass, 2013), and it is related to neuropathic pain (Khan et al., 2015). Previous studies by our research group have shown an increase in the brainstem levels of these cytokines 7 days after tDCS treatment compared to 24 hr in the orofacial inflammatory pain model induced by complete Freund's adjuvant (CFA) injection at the temporomandibular joint (TMJ) in rats (Scarabelot et al., 2015). In a sciatic neuropathic pain model, a decrease in IL-10 spinal cord levels occurred 7 days after tDCS treatment (Cioato et al., 2016). It seems paradoxical, but we found that decreased brainstem IL-10 levels, which have an analgesic function, and tDCS caused a decrease in the nociceptive threshold. Thus, we suggest the involvement of central cytokine levels in the tDCS action mechanism in the orofacial neuropathic pain model in rats.

The infraorbital neuropathic pain model used in the current manuscript mimics secondary trigeminal neuralgia in relation to nociceptive behavior, and it is appropriate for short duration studies (until 22 days after surgery), according to our protocol. In addition, we have demonstrated that tDCS treatment was able to increase the mechanical nociceptive threshold in rats exposed to the orofacial neuropathic model until baseline levels. Furthermore, tDCS induces

a decrease in brainstem TNF-α and IL-10 levels in neuropathic pain in rats, suggesting a long-lasting, state-dependent effect of the tDCS treatment.

In addition, LDH is an intracellular enzyme and high serum LDH levels may indicate cell death by membrane lysis (Adeva-Andany et al., 2014). In the current study, we observed pain and treatment effects on serum LDH levels. However, we highlight that we measured peripheral LDH levels, and the tDCS action mechanism was not linked directly to its levels.

Among the strengths of this study, we emphasize that the pain model used is interesting because it is easy to perform; it is less invasive and presents a lower risk of injury to adjacent structures, such as the eyeball or ethmoidal bone, than other trigeminal neuralgia models (Jacquin & Zeigler, 1983; Kernisant et al., 2008). In addition, the vibrissae region remains intact, which is important for performing the von Frey facial test. However, it is important to point out some study limitations. One of them is the development of a cicatricial area inside the mouth close to the area to be tested. This area consists of an intraoral region behind the vibrissae, which is compressed by the filament during the von Frey test. Soft tissue in this area is very thin in rats. Because they are rodents, the laboratory rats are fed pelleted chow, which is hard, and the friction during feeding or gnawing could weaken the wire tension of nerve constriction. The second limitation is that we used only male rats, avoiding hormonal alterations that are implicated in changes in nociceptive response. Also, it can be noticed that in the pain groups, allodynia was not present any longer than 29 days after surgery. This made it difficult to evaluate the tDCS effect on the reversion of allodynia in the pain group. Another relevant point is that the infraorbital nerve is an exclusively sensitive nerve and no movement occurs during the constriction, making it difficult to standardize surgeries. This detail may increase the number of animals that do not develop neuropathic pain after surgery, despite constriction. In this study, two rats were excluded from the study after 14 days of behavior testing for this reason.

5 | CONCLUSION

The pain model used in this study was effective in the establishment of neuropathic pain simulating secondary trigeminal neuralgia 14 days after surgery, which is after the presence of inflammatory pain resulting from surgery subsides. This model is of great validity when used in studies of shorter duration. It should be noted that in a CCI-ION model like this, Vicryl wires should be avoided because of lack of stability of results. tDCS alters the nociceptive and cellular responses in rats submitted to the orofacial pain model; in addition, it altered central levels of neuromodulators (NGF) and pro-and anti-inflammatory cytokines, suggesting an anti-inflammatory effect secondary to the decrease in nociceptive signaling. In conclusion, we consider that this study has excellent translational scientific relevance because it provides technical guidance for other preclinical investigations of trigeminal neuralgia, it also supports the use of tDCS as a non-pharmacological therapy for orofacial pain in clinical studies.

ACKNOWLEDGMENTS

This work was supported by the following Brazilian funding agencies: Brazilian Federal Agency for Support and Evaluation of Graduate Education—CAPES (M.M.E. Callai), CAPES/PNPD (Dr. C. de Oliveira, Edital N° 07/2016); National Council for Scientific and Technological Development—CNPq (Dr. ILS Torres; Dr. W. Caumo); and the Graduate Research Group (GPPG) of Hospital de Clínicas de Porto Alegre—HCPA (grant number 14-0329). We also want to thank the Engineering Division from the HCPA for having developed the SMS stimulator; the Graduate Research Group of the Hospital de Clínicas de Porto Alegre—GPPG (I.L.S. Torres; MCT/FINEP—COENG/2013). PRONEM/FAPERGS 003/2011.

CONFLICT OF INTEREST

None of the authors had any financial or commercial interest in the outcome of this study.

AUTHOR CONTRIBUTIONS

EMC, LFM: designed and developed the study, analyzed the data, drafted the paper; VLS: designed the study, analyzed the data, drafted the paper; AS: designed and developed the study; CO, SGC, FF: developed the study; ICM: designed study; WC, SQ: analyzed the data, drafted the paper; ILST: analyzed the data, drafted the paper, coordinated the study.

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REFERENCES

Adeva-Andany, M., Lopez-Ojen, M., Funcasta-Calderon, R., Ameneiros-Rodriguez, E., Donapetry-Garcia, C., Vila-Altesor, M., & Rodriguez-Seijas, J. (2014). Comprehensive review on lactate metabolism in human health. *Mitochondrion*, *17*, 76–100. https://doi.org/10.1016/j.mito.2014.05.007

Antal, A., Nitsche, M. A., & Paulus, W. (2006). Transcranial direct current stimulation and the visual cortex. *BrainResearch Bulletin*, *68*(6), 459–463. https://doi.org/10.1016/j.brainresbull.2005.10.006

Bae, S. H., Kim, G. D., & Kim, K. Y. (2014). Analgesic effect of transcranial direct current stimulation on central post-stroke pain. *The Tohoku Journal of Experimental Medicine*, *234*(3), 189–195. https://doi.org/10.1620/tjem.234.189

Berry, A., Bindocci, E., & Alleva, E. (2012). NGF, brain and behavioral plasticity. *Neural Plasticity*, *2012*, 784040. https://doi.org/10.1155/2012/784040

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry.*, *72*, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Brasil. (2008). Presidência da República. Lei nº 11.794 de 8 de outubro de 2008. Regulamenta o inciso VII do parágrafo 1º do artigo 225 da Constituição Federal, estabelecendo Procedimentos para o Uso Científico de Animais. Retrieved from http://bit.ly/1WV52wP; 2008 [Accessed 10 January 2018]. 2010;8(6):1-6.

Brunoni, A. R., Machado-Vieira, R., Zarate, C. A., Vieira, E. L., Valiengo, L., Bensenor, I. M., & Teixeira, A. L. (2015). Assessment of non-BDNF neurotrophins and GDNF levels after depression treatment with sertraline and transcranial direct current stimulation in a factorial, randomized, sham-controlled trial (SELECT-TDCS): An exploratory analysis. *Progress in neuro-psychopharmacology & Biological Psychiatry*, *2*(56), 91–96. https://doi.org/10.1016/j.pnpbp.2014.08.00

Choi, B. M., Lee, S. H., An, S. M., Park, D. Y., Lee, G. W., & Noh, G. J. (2015). The time-course and RNA interference of TNF-alpha, IL-6, and IL-1beta expression on neuropathic pain induced by L5 spinal nerve transection in rats. *Korean Journal of Anesthesiology*, *68*(2), 159–169. https://doi.org/10.4097/kjae.2015.68.2.159

Cioato, S. G., Medeiros, L. F., Marques Filho, P. R., Vercelino, R., de Souza, A., Scarabelot, V. L., … Torres, I. L. (2016). Long-lasting effect of transcranial direct current stimulation in the reversal of hyperalgesia and cytokine alterations induced by the neuropathic pain model. *BrainStimulation*, *9*(2), 209–217. https://doi.org/10.1016/j.brs.2015.12.001

Costigan, M., Scholz, J., & Woolf, C. J. (2009). Neuropathic pain: A maladaptive response of the nervous system to damage. *Annual Review of Neuroscience*, *32*, 1–32. https://doi.org/10.1146/annurev.neuro.051508.135531

Covey, W. C., Ignatowski, T. A., Knight, P. R., & Spengler, R. N. (2000). Brain-derived TNF alpha: Involvement in neuroplastic changes implicated in the conscious perception of persistent pain. *BrainResearch*, *859*(1), 113–122.

Covey, W. C., Ignatowski, T. A., Renauld, A. E., Knight, P. R., Nader, N. D., & Spengler, R. N. (2002). Expression of neuron-associated tumor necrosis factor alpha in the brain is increased during persistent pain. *Regional Anesthesia and Pain Medicine*, *27*(4), 357–366.

Drouot, X., Nguyen, J. P., Peschanski, M., & Lefaucheur, J. P. (2002). The antalgic efficacy of chronic motor cortex stimulation is related to sensory changes in the painful zone. *BrainA Journal of Neurology*, *12*(7), 1660–1664. https://doi.org/10.1093/brain/awf161

Edlich, R. F., Winters, K. L., Britt, L., & Long, W. B. (2006). Trigeminal neuralgia. *Journal of Long-Term Effects of Medical Implants*, *16*(2), 185–192.

Fregni, F., Gimenes, R., Valle, A. C., Ferreira, M. J., Rocha, R. R., Natalle, L., & Boggio, P. S. (2006). A randomized, sham-controlled, proof of principle study of transcranial direct current stimulation for the treatment of pain in fibromyalgia. *Arthritis & Rheumatism*, *54(12)*, 3988–3998. https://doi.org/10.1002/art.22195

Hagenacker, T., Bude, V., Naegel, S., Holle, D., Katsarava, Z., Diener, H. C., & Obermann, M. (2014). Patient-conducted anodal transcranial direct current stimulation of the motor cortex alleviates pain in trigeminal neuralgia. *Journal of Headache Pain*, *25*(15), 78. https://doi.org/10.1186/1129-2377-15-78

Haviv, Y., Khan, J., Zini, A., Almoznino, G., Sharav, Y., & Benoliel, R. (2016). Trigeminal neuralgia (part I): Revisiting the clinical phenotype. *CephalalgiaAn International Journal of Headache*, *36*(8), 730–746. https://doi.org/10.1177/0333102415611405

Henry, M. A., Freking, A. R., Johnson, L. R., & Levinson, S. R. (2007). Sodium channel Nav1.6 accumulates at the site of infraorbital nerve injury. *BMC Neuroscience*, *8*, 56.

Imamura, Y., Kawamoto, H., & Nakanishi, O. (1997). Characterization of heat-hyperalgesia in an experimental trigeminal neuropathy in rats. *Experimental Brain Research*, *116*, 97–103.

Jacquin, M. F., & Zeigler, H. P. (1983). Trigeminal orosensation and ingestive behavior in the rat. *Behavioral Neuroscience*, *97*(1), 62–97. https://doi.org/10.1037/0735-7044.97.1.62

Kernisant, M., Gear, R. W., Jasmin, L., Vit, J. P., & Ohara, P. T. (2008). Chronic constriction injury of the infraorbital nerve in the rat using modified syringe needle. *Journal of Neuroscience Methods*, *172*(1), 43–47. https://doi.org/10.1016/j.jneumeth.2008.04.013

Khan, J., Ramadan, K., Korczeniewska, O., Anwer, M. M., Benoliel, R., & Eliav, E. (2015). Interleukin-10 levels in rat models of nerve damage and neuropathic pain. *NeuroscienceLetters*, *10*(592), 99–106. https://doi.org/10.1016/j.neulet.2015.03.001

Kilkenny, C., Browne, W. J., Cuthill, I. C., Emerson, M., & Altman, D. G. (2010). Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. *PLoS Biology*, *8*(6), e1000412. https://doi.org/10.1371/journal.pbio.1000412

Laste, G., Caumo, W., Adachi, L. N., Rozisky, J. R., de Macedo, I. C., Filho, P. R., … Torres, I. L. (2012). After-effects of consecutive sessions of transcranial direct current stimulation (tDCS) in a rat model of chronic inflammation. *Experimental Brain Research*, *221*(1), 75–83. https://doi.org/10.1007/s00221-012-3149-x

Leclercq, D., Thiebaut, J. B., & Heran, F. (2013). Trigeminal neuralgia. *Diagnostic and Interventional Imaging*, *94*(10), 993–1001. https://doi.org/10.1016/j.diii.2013.08.002

Leung, L., & Cahill, C. M. (2010). TNF-alpha and neuropathic pain–a review. *Journal of Neuroinflammation*, *16*(7), 27. https://doi.org/10.1186/1742-2094-7-27

Levi-Montalcini, R., & Angeletti, P. U. (1968). Nerve growth factor. *Physiological Reviews*, *48*(3), 534–569. https://doi.org/10.1152/physrev.1968.48.3.534

Lewin, G. R., Rueff, A., & Mendell, L. M. (1994). Peripheral and central mechanisms of NGF-induced hyperalgesia. *The European Journal of Neuroscience*, *6*(12), 1903–1912. https://doi.org/10.1111/j.1460-9568.1994.tb00581.x

Lima, M. C., & Fregni, F. (2008). Motor cortex stimulation for chronic pain: Systematic review and meta-analysis of the literature. *Neurology*, *70*(24), 2329–2337. https://doi.org/10.1212/01.wnl.0000314649.38527.93

MCTIa. (2013). Ministério da Ciência Tecnologia e Inovação. Diretriz Brasileira para o Cuidado e a Utilização de Animais para Fins Científicos e Didáticos e DBCA, Portaria nº 465 de 23 de maio de 2013. Retrieved from http://www.cobea.org.br/arquivo/ [Accessed 10 January 2018].

MCTIb. (2013). Ministério da Ciência Tecnologia e Inovação. Diretrizes da Prática de Eutanásia do Concea, Portaria n. 596 de 25 de junho de 2013. Retrieved from http://www.cobea.org.br/download/download?ID_DOWNLOAD¼14 [Accessed 10 January 2018].

Merighi, A., Salio, C., Ghirri, A., Lossi, L., Ferrini, F., Betelli, C., & Bardoni, R. (2008). BDNF as a pain modulator. *Progress in Neurobiology*, *85*(3), 297–317. https://doi.org/10.1016/j.pneurobio.2008.04.004

Mizumura, K., & Murase, S. (2015). Role of nerve growth factor in pain. *Handbook of Experimental Pharmacology*, *227*, 57–77. https://doi.org/10.1007/978-3-662-46450-2_4

Nitsche, M. A., Cohen, L. G., Wassermann, E. M., Priori, A., Lang, N., Antal, A., … Pascual-Leone, A. (2008). Transcranial direct current stimulation: State of the art 2008. *BrainStimulation*, *1*(3), 206–223. https://doi.org/10.1016/j.brs.2008.06.004

Nitsche, M. A., Fricke, K., Henschke, U., Schlitterlau, A., Liebetanz, D., Lang, N., & Paulus, W. (2003). Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. *The Journal of Physiology*, *553*(1), 293–301. https://doi.org/10.1113/jphysiol.2003.049916

Ouyang, W., Rutz, S., Crellin, N. K., Valdez, P. A., & Hymowitz, S. G. (2011). Regulation and functions of the IL-10 family of cytokines in inflammation and disease. *Annual Review of Immunology*, *29*, 71–109. https://doi.org/10.1146/annurev-immunol-031210-101312

Reis, J., Schambra, H. M., Cohen, L. G., Buch, E. R., Fritsch, B., Zarahn, E., & Krakauer, J. W. (2009). Noninvasive cortical stimulation enhances motor skill acquisition over multiple days through an effect on consolidation. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(5), 1590–1595. https://doi.org/10.1073/pnas.0805413106

Riffel, A. P., de Souza, J. A., Santos, M., Horst, A., Scheid, T., Kolberg, C., & Partata, W. A. (2016). Systemic administration of vitamins C and E attenuates nociception induced by chronic constriction injury of the sciatic nerve in rats. *Brain Research Bulletin*, *121*, 169–177. https://doi.org/10.1016/j.brainresbull.2016.02.004

Robinson, I., & Meert, T. F. (2005). Stability of neuropathic pain symptoms in partial sciatic nerve ligation in rats is affected by suture material. *NeuroscienceLetters*, *373*(2), 125–129. https://doi.org/10.1016/j.neulet.2004.09.078

Rodriguez-Lozano, F. J., Sanchez-Perez, A., Moya-Villaescusa, M. J., Rodriguez-Lozano, A., & Saez-Yuguero, M. R. (2010). Neuropathic orofacial pain after dental implant placement: Review of the literature and case report. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics*, *109*: e8–e12.

Rosen, A. C., Ramkumar, M., Nguyen, T., & Hoeft, F. (2009). Noninvasive transcranial brain stimulation and pain. *Current Pain and Headache Reports*, *13*(1), 12–17.

Scarabelot, V. L., Medeiros, L. F., de Oliveira, C., Adachi, L. N. S., de Macedo, I. C., Cioato, S. G., & Torres, I. L. (2015). Melatonin alters the mechanical and thermal hyperalgesia induced by orofacial pain model in rats. *Inflammation*, *39*(5), 1649–1659. https://doi.org/10.1007/s10753-016-0399-y

Spezia Adachi, L. N., Caumo, W., Laste, G., Fernandes Medeiros, L., Ripoll Rozisky, J., de Souza, A., … Torres, I. L. (2012). Reversal of chronic stress-induced pain by transcranial direct current stimulation (tDCS) in an animal model. *BrainResearch*, *13*(1489), 17–26. https://doi.org/10.1016/j.brainres.2012.10.009

Spezia Adachi, L. N., Quevedo, A. S., de Souza, A., Scarabelot, V. L., Rozisky, J. R., de Oliveira, C., … Torres, I. L. (2015). Exogenously induced brain activation regulates neuronal activity by top-down modulation: Conceptualized model for electrical brain stimulation. *Experimental Brain Research*, *233*(5), 1377–1389. https://doi.org/10.1007/s00221-015-4212-1

Stagg, C. J., & Nitsche, M. A. (2011). Physiological basis of transcranial direct current stimulation. *The Neuroscientist: A Review Journal Bringing Neurobiology, Neurology and Psychiatry*, *17*(1), 37–53. https://doi.org/10.1177/1073858410386614

Tabas, I., & Glass, C. K. (2013). Anti-inflammatory therapy in chronic disease: Challenges and opportunities. *Science (New York, N.Y.)*, *339*(6116), 166–172. https://doi.org/10.1126/science.1230720

Takano, Y., Yokawa, T., Masuda, A., Niimi, J., Tanaka, S., & Hironaka, N. (2011). A rat model for measuring the effectiveness of transcranial direct current stimulation using fMRI. *NeuroscienceLetters*, *491*(1), 40–43. https://doi.org/10.1016/j.neulet.2011.01.004

Vos, B. P., Strassman, A. M., & Maciewicz, R. J. (1994). Behavioral evidence of trigeminal neuropathic pain following chronic constriction injury to the rat's infraorbital nerve. *The Journal of Neuroscience: the Official Journal of the Society for Neuroscience*, *14*(5 pt 1), 2708–2723.

Wei, F., Guo, W., Zou, S., Ren, K., & Dubner, R. (2008). Supraspinal glial-neuronal interactions contribute to descending pain facilitation. *The Journal of Neuroscience: the Official Journal of the Society for Neuroscience*, *28*(42), 10482–10495. https://doi.org/10.1523/JNEUROSCI.3593-08.2008

Yang, K. Y., Kim, M. J., Ju, J. S., Park, S. K., Lee, C. G., … Ahn, D. K. (2016). Antinociceptive effects of botulinum toxin type A on trigeminal neuropathic pain. *Journal of Dental Research*, *95*(10), 1183–1190. https://doi.org/10.1177/0022034516659278

Zakrzewska, J. M., & McMillan, R. (2011). Trigeminal neuralgia: The diagnosis and management of this excruciating and poorly understood facial pain. *Postgraduate Medical Journal*, *87*(1028), 410–416. https://doi.org/10.1136/pgmj.2009.080473

How to cite this article: Callai EMM, Scarabelot VL, Fernandes Medeiros L, et al. Transcranial direct current stimulation (tDCS) and trigeminal pain: A preclinical study. *Oral Dis*. 2019;25:888–897. https://doi.org/10.1111/odi.13038