Brain Stimulation 13 (2020) 774–782

Contents lists available at ScienceDirect

Brain Stimulation

journal homepage: http://www.journals.elsevier.com/brain-stimulation

Transcranial direct current stimulation combined with exercise modulates the inflammatory profile and hyperalgesic response in rats subjected to a neuropathic pain model: Long-term effects

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ARTICLE INFO

Article history:
Received 16 October 2019
Received in revised form
6 February 2020
Accepted 19 February 2020
Available online 24 February 2020

Keywords:
Bimodal tDCS
Aerobic exercise
Neuropathic pain
Cytokines
Rats

ABSTRACT

Background: Behavioral alterations, like mechanical and thermal hyperalgesia, and modulation of biomarkers in the peripheral and central nervous systems (CNS) are markers of chronic pain. Transcranial direct current stimulation (tDCS) with exercise is a promising therapy for pain due to its neuromodulatory capacity.
Objective: To assess the individual effects of tDCS, exercise, and the two combined on the nociceptive response and BDNF, IL-1β, and IL-4 levels in the CNS structures of rats in a chronic pain model.
Methods: For 8 consecutive days after the establishment of chronic neuropathic pain by inducing a constriction injury to the sciatic nerve (CCI), the rats received tDCS, exercise, or both treatments combined (20 min/day). The hyperalgesic response was assessed by von Frey and hot plate tests at baseline, 7, and 14 days after CCI surgery and immediately, 24 h, and 7 days after the end of treatment. The BDNF, IL-1β, and IL-4 levels were assessed in the cerebral cortex, brainstem, and spinal cord by enzyme-linked immunosorbent assay at 48 h and 7 days after the end of treatment.
Results: The CCI model triggered marked mechanical and thermal hyperalgesia. However, bimodal tDCS, aerobic exercise, and the two combined relieved nociceptive behavior for up to 7 days following treatment completion.
Conclusions: Bimodal tDCS, aerobic exercise, or both treatments combined promoted analgesic effects for neuropathic pain. Such effects were reflected by cytokine modulation throughout the spinal cord-brainstem-cerebral cortex axis.

Introduction

Neuropathic pain (NP) arises from complex changes involving the peripheral and/or central nervous systems in response somatosensorial system injury [1]. Its main behavioral feature is the presence of hyperalgesia and allodynia, leading to impaired functional and cognitive capacities, motor deficits, and a reduction in

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https://doi.org/10.1016/j.brs.2020.02.025

the quality of life [2]. However, the underlying mechanisms remain unclear.

Increased nociceptive input occurs due to an imbalance between facilitatory and inhibitory synaptic mechanisms driven by upregulation and chronic activation of receptors [3]. Glial cells play an essential role for dealing with injury by changing their phenotype from a basal to a pro-inflammatory state by releasing several neuroimmune mediators, such as tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β), calcitonin gene-related peptide (CGRP), and substance P (SP) [4,5]. Furthermore, while the upregulation of key neurotrophic factors is needed to maintain cell integrity, they also promote sensitization of afferent terminals in chronic pain conditions, giving rise to aberrant nociceptive inputs that modulate pain signaling throughout the nociceptive pathways [6]. Therefore, neuromodulatory strategies for modulating hypernociceptive inputs are important in preventing and treating chronic pain conditions.

Transcranial direct current stimulation (tDCS) is a safe, non-invasive technique that modulates neuronal thresholds with anodal and cathodal currents that increase or decrease cortical excitability, respectively [7]. The effects of a weak electrical current delivered over the scalp depend on the brain regions, electrode diameters, and polarity applied; its effects can persist for hours or days [8]. While the short-term effects of tDCS are mediated by ionic channel modulation, the long-term effects are mediated by N-methyl-D-aspartate (NMDA) receptors [9]. Moreover, bimodal tDCS applied over the scalp has been reported to revert inflammatory and chronic stress-induced pain in rats [10,11].

Physical inactivity contributes to the development of cardiovascular diseases, diabetes, depressive symptoms, and chronic pain [12]. Systemic effects of exercise also play a neuroprotective role in rats in inflammatory and chronic pain models [13,14] and increase hippocampal volumes in older adults [15]. Even though the underlying mechanisms of the prophylactic and therapeutic effects of exercise remain unknown, opioid antagonists have been reported to alleviate exercise-induced analgesia, suggesting that the aerobic exercise effects are, at least partly, due to activation of the opioid system [16]. Furthermore, physical exercise induces IL-6 mRNA and protein production in the skeletal muscle, leading to an increase in the release of anti-inflammatory cytokines (IL-4 and IL-10) and the inhibition of IL-1β and TNF-α synthesis [17].

Despite these top-down and bottom-up neuromodulatory techniques need further elucidation, studies targeting to investigate the effects of tDCS or exercise have shown resembling effects mediated by both interventions, which includes, opioid, monoamines, endocannabinoids, glutamate releasing, and corticospinal tract modulation parameters [18–20]. Regarding these modulations of intracortical inhibition or facilitation parameters, Stagg et al. [21] have demonstrated that anodal tDCS applied upon motor cortex (M1) lead to a reduction of GABA levels, whilst cathodal stimulation reduced the glutamate levels. These results suggest that the effects provided by anodal or cathodal stimulation might have effects upon neuronal circuitry, similar those triggered by exercise.

Neuromodulation techniques combined with exercise may be an alternative non-invasive method to improve functional recovery [22]. However, little is known about the combined effects of tDCS and exercise on pain relief. We aimed to investigate if the association between tDCS and exercise provide more pronounced antinociceptive effects rather than each isolated treatment in a neuropathic pain model; and if the antinociceptive effects are involved with neurochemical biomarkers central levels.

Materials and methods

Animals

Male Wistar rats (n = 156; 8 weeks old, weight 280 ± 20 g) were randomly assigned by weight and housed in polypropylene cages (49 × 34 × 16 cm) with sawdust-covered flooring. All rats were maintained in a controlled environment (23 ± 2 °C) with a standard light-dark cycle (12 h/12 h) with water and chow *ad libitum*. The sample size was calculated to detect the statistical significance between means considering an alpha = 0.05 and power of 90% [23–25]. All experiments were approved by the Institutional Animal Care and Use Committee (GPPG-HCPA no. 20170061) and met the ethical and methodological ARRIVE guidelines [26].

Experimental design

Rats were initially divided into three groups: Control, Sham-Pain and Pain. In the 14th day the rats were subdivided into thirteen groups: Control-Group (C), Sham-Pain (S), Sham-Pain + Sham-tDCS (SS), Sham-Pain + Exercise (SE), Sham-Pain + tDCS (ST), Sham-Pain + Sham-tDCS + Exercise (SSE), Sham-Pain + tDCS + Exercise (STE) Pain-Group (P), Pain-Sham-tDCS (PS), Pain + Exercise (PE), Pain + tDCS (PT), Pain + Sham-tDCS + exercise (PSE), and Pain + tDCS + exercise (PTE). Nociceptive tests were assessed at baseline, 7, and 14 days after CCI procedure, and immediately, 24 h, and 7 days post-treatments. First, bimodal tDCS was performed; after that, rats were made to exercise on a treadmill from days 15–22 post-CCI. Rats were killed by decapitation at 48 h and 7 days after treatment completion (Fig. 1).

Neuropathic pain model

Animals were anesthetized with isoflurane (5% for induction, 2.5% for maintenance) and placed in the lateral decubitus for hair shaving and skin antisepsis with 2% alcohol as described in an earlier study [27]. After skin incision in the middle third of the left hind limb to expose the biceps femoralis muscle, the common sciatic nerve was exposed and three ligatures were tied (Vycril 4.0) with minor modifications [25]. For sham groups, the sciatic nerve was similarly exposed, but the nerve was not ligated. The same investigator performed the ligatures in all rats. After surgery, the wound was sutured using Mononylon 4.0 and the rats (Sham and CCI groups) received tramadol intraperitoneally during the first 3 days post-surgery (7:00 a.m. and 7:00 p.m.) to minimize excessive suffering.

tDCS protocol

Fourteen days post-surgery, the rats were submitted to a 20-min session of bimodal tDCS treatment for eight consecutive days in a constant direct current of 0.5 mA delivered from a battery-powered stimulation source, as described in a previous method [28]. Rats subjected to active or sham stimulation had their scalp shaved. The electrode size was trimmed to 1.5 cm^2 and a constant current intensity of 0.5 mA was applied upon the scalp. It provided a current density of 0.33 mA/cm^2 with no lesions previously reported [23,29]. The anode was placed on the head using landmarks of the neck and shoulder lines as a guide (the anterior and posterior regions of the midline between the two hemispheres of the parietal cortex), while the cathode was positioned at the midpoint between the lateral angles of both eyes (supraorbital area). For the sham groups, the electrodes were placed and fixed in the same position as for active

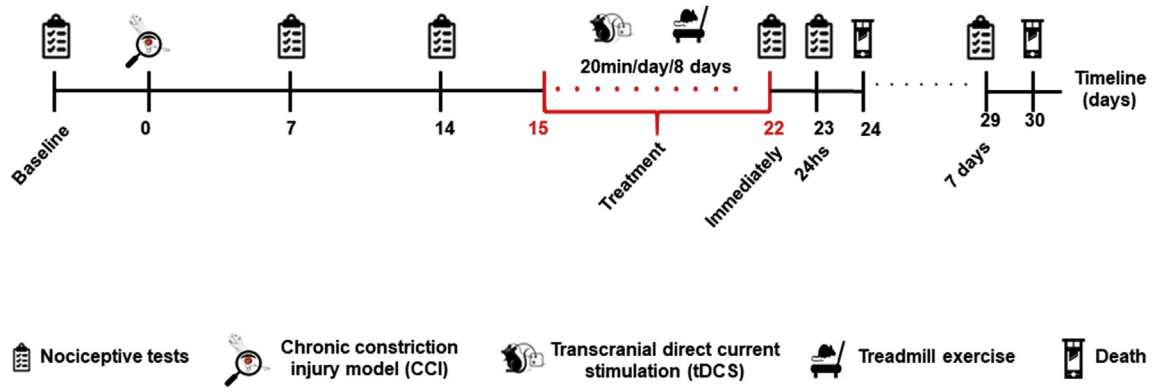

Fig. 1. Experimental Design.
Nociceptive tests were von Frey and hot plate to assess mechanical and thermal hyperalgesia, respectively. Bimodal tDCS and/or treadmill exercise was provided from 15th to 22nd days after CCI.

stimulation; however, the stimulator remained turned off throughout the experiments.

Exercise protocol

One week before surgery, the rats were habituated daily on a treadmill. On day 1, rats were placed for 20 min on a turned-off treadmill to recognize a new apparatus. Next, the rats were gradually conditioned to run by increasing the exposure time on the treadmill from 10 to 20 and 30 min at days 2, 3, and 4, respectively, at low speed (5 m/min). On day 5, we used an indirect method to determine the VO_{2max} [30]. Briefly, the rats ran at 7 m/min for 3 min, with an increase of speed at 5 m/min until the point of exhaustion, perceived as the incapacity to continue running. The time to fatigue (in min) and workload (in m/min) were obtained as indexes of aerobic capacity, which, in turn, were taken as VO_{2max}. The exercise protocol consisted of running sessions at 70% of VO_{2max}. The rats were subjected to one 20-min running session daily for 8 days (08:00–09:30 a.m.) on a 0° slope. No electric shocks were delivered to incite them to run.

Nociceptive tests

Mechanical allodynia

We used an automatic von Frey aesthesiometer (Insight, São Paulo, Brazil) to assess mechanical hyperalgesia. Rats were habituated in polypropylene cages (12 × 20 × 17 cm) with wire grid flooring 24 h prior and for 15 min before tests to minimize analgesia induced by novelty [31]. For testing, a polypropylene tip was applied underneath the floor grid and between the five distal foot pads, gradually increasing the pressure. Measurements were assessed in triplicate and means were expressed in grams (g) per paw withdrawal.

Thermal hyperalgesia

All rats were exposed to a hot plate (HP) for 5 min, 24 h prior to the test in order to avoid analgesia induced by novelty [31]. On the test day, the surface of the hot plate was pre-heated and kept at a constant temperature (55 ± 0.1 °C). The rats were placed on the heated surface surrounded by glass funnels. The time in seconds for the first behavioral response (foot-licking, jumping, or rapidly removing paws) was recorded as the latency of nociceptive response [32].

Tissue collection

At 48 h or 7 days after the last treatment, rats were killed by decapitation, and tissue samples (cerebral cortex, brainstem, and spinal cord) were harvested. The structures were kept at −80 °C until the assays were performed.

Biochemical assays

Cerebral cortex, spinal cord, and brainstem BDNF (DY248), IL-1β (DY501) and IL-4 (DY504) levels were determined by sandwich ELISA using monoclonal antibodies (R&D; Minneapolis, MN). The total protein was measured by the Bradford method using bovine serum albumin as a standard [33]. The results were corrected by total protein and expressed in pg/mg of protein.

Statistical analysis

Data were expressed as the mean ± standard error of the mean (S.E.M.). Generalized estimating equations (GEE) followed by Bonferroni testing was used to analyze behavioral data [34]. For biochemical assays, a four-way ANOVA followed by post-hoc Bonferroni correction was used, considering the interactions between independent variables (pain, tDCS, exercise, time). A p-value < 0.05 was considered significant. Data were analyzed with SPSS for Windows (version 20.0; IBM, Armonk, NY).

Results

Effects of isolated bimodal tDCS treatment or combined with aerobic exercise on mechanical hyperalgesia

We found no difference between groups at baseline ($p > 0.05$). There was an interaction between group and time in mechanical hyperalgesia as assessed in the von Frey test (Wald's $\chi^2 = 429{,}286$; $p < 0.05$, $n = 156$; Fig. 2A). From 7 to 14 days post-surgery, all CCI groups displayed marked mechanical hyperalgesia ($p < 0.05$) compared to control and sham groups. Immediately, 24 h, and 7 days post-treatment, the PE, PT, PSE, and PTE groups displayed increased nociceptive thresholds in response to treatment. Interestingly, at 7 days post-treatment, the PTE group displayed a slightly increase in the nociceptive threshold than the treatment alone (Wald's $\chi^2 = 1714{,}912$ for group effect and Wald's $\chi^2 = 10{,}512$ for time effect; $p < 0.05$, $n = 156$; Fig. 2C).

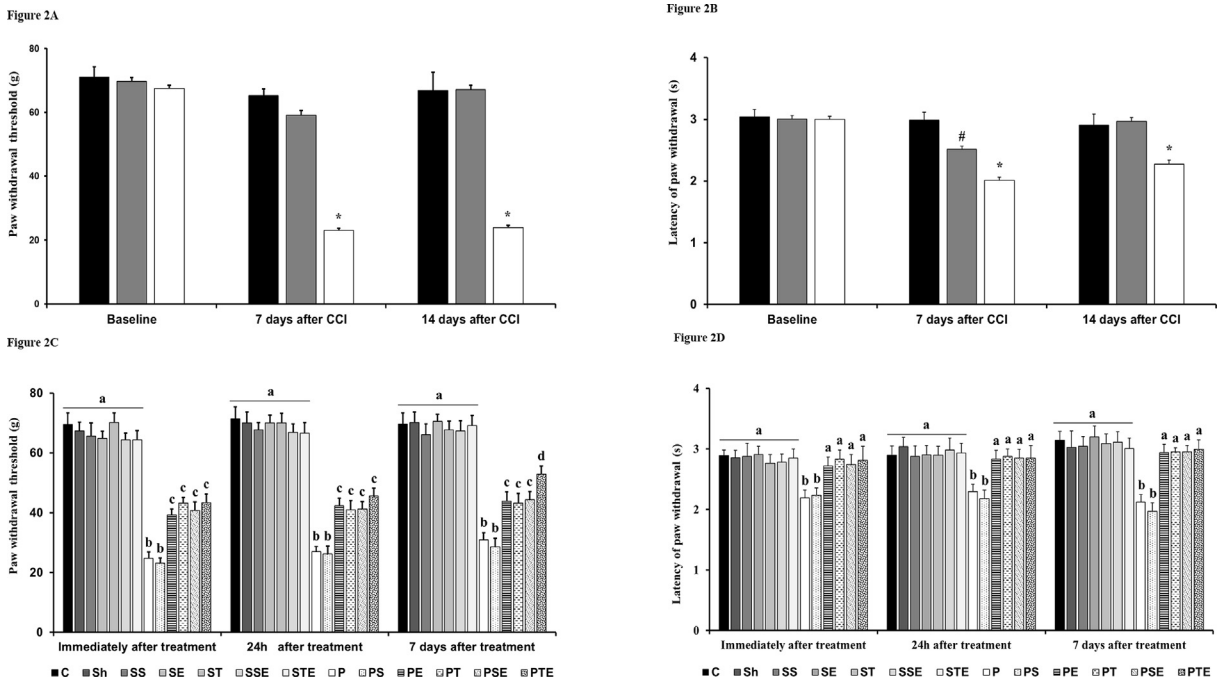

Fig. 2. Mechanical and thermal hyperalgesia assessed by von Frey and hot plate tests, at baseline, 7 and 14 days after the CCI model (n = 12 to control group, and 72 to Sham-Pain and Pain groups; Fig. 2A and B, respectively).
*. Mean statistically significant difference from Control, and Sham-Pain groups (GEE, $p < 0.05$). * and #. Mean statistically significant difference between all 3 groups (GEE, $p < 0.05$). Mechanical and thermal hyperalgesia assessed by von Frey and hot plate tests at immediately, 24 h, and 7 days after bimodal tDCS treatment and/or treadmill exercise (n = 12 per group; Fig. 2C and D, respectively). Different letters mean statistically significant difference between the groups (GEE, $p < 0.05$). Data presented as mean standard error of the mean (SEM) of paw withdrawal threshold (g). Control-Group (C), Sham-Pain (S), Sham-Pain + Sham-tDCS (SS), Sham-Pain + Exercise (SE), Sham-Pain + tDCS (ST), Sham-Pain + Sham-tDCS + Exercise (SSE), Sham-Pain + tDCS + Exercise (STE) Pain-Group (P), Pain-Sham-tDCS (PS), Pain + Exercise (PE), Pain + tDCS (PT), Pain + Sham-tDCS + Exercise (PSE) and Pain + tDCS + Exercise (PTE).

Effects of isolated bimodal tDCS treatment or combined with exercise on thermal hyperalgesia

We found no difference between groups at baseline ($p > 0.05$). There was an interaction between group and time in thermal hyperalgesia (Wald's $\chi^2 = 88{,}462$; $p < 0.05$, n = 156, Fig. 2B). At 7 days post-surgery, the Sham-Pain groups displayed decreased thermal withdrawal latency, which was even pronounced in the Pain groups. At 14 days post-surgery, only the Pain groups displayed marked thermal hyperalgesia compared to the Control and Sham-Pain groups ($p < 0.05$). From immediately to 7 days after the last treatment, all groups subjected to tDCS or exercise alone and to combined treatment showed no thermal hyperalgesia, compared to the P and PS groups (Wald's $\chi^2 = 153{,}658$ for group effect; $p < 0.05$, n = 156, Fig. 2D).

Cerebral cortex BDNF, IL-1β, and IL-4 levels

Regarding cerebral cortex BDNF levels, there was an interaction between pain, tDCS, exercise, and time (four-way ANOVA/Bonferroni, $F_{(2,120)} = 5.064$, $p < 0.01$; Table 1). At 48 h post-treatment, the PT group showed increased BDNF levels compared to the ST group. At 7 days post-treatment, the PSE group displayed decreased BDNF levels compared to the SSE group. At 7 days post-treatment, the PTE group displayed increased BDNF levels compared to the STE group. Additionally, there were interactions between exercise, tDCS, and time ($F_{(2,120)} = 4.168$, $p < 0.02$); exercise and time ($F_{(1,120)} = 6.154$, $p < 0.02$); tDCS, exercise, and time ($F_{(2,120)} = 6.255$, $p < 0.01$); and pain and stimulation ($F_{(2,120)} = 10.734$, $p < 0.001$). There were main effects of tDCS ($F_{(2,120)} = 6.249$, $p < 0.01$), exercise ($F_{(1,120)} = 36.409$, $p < 0.001$), and time ($F_{(1,120)} = 3495.979$, $p < 0.001$).

Regarding cerebral cortex IL-1β levels, there was a main effect of pain (four-way ANOVA/Bonferroni, $F_{(1,120)} = 12.168$, $p < 0.01$; Table 1). The pain groups displayed increased IL-1β levels in the cerebral cortex compared to the Sham-Pain groups. There was a main effect of time ($F_{(1,120)} = 25.444$, $p < 0.01$). Compared to 48 h post-treatment, the Pain groups displayed increased IL-1β levels at 7 days post-treatment.

Regarding IL-4 levels, there was an interaction between pain, tDCS, and exercise (four-way ANOVA/Bonferroni, $F_{(2,120)} = 5.354$, $p < 0.01$; Table 1). The PTE group displayed reduced IL-4 levels compared to the STE group. Moreover, there were interactions between pain, tDCS, and time ($F_{(2,120)} = 4.318$, $p < 0.02$); pain and tDCS ($F_{(2,120)} = 4.832$, $p < 0.02$); tDCS and exercise ($F_{(2,120)} = 3.852$, $p < 0.05$); exercise and time ($F_{(1,120)} = 6.440$, $p < 0.02$); and pain and time ($F_{(1,120)} = 7.429$, $p < 0.001$). There were main effects of pain ($F_{(1,120)} = 8.160$, $p < 0.001$), exercise ($F_{(1,120)} = 15.973$, $p < 0.001$), and time ($F_{(1,120)} = 998.533$, $p < 0.001$).

Brainstem BDNF, IL-1β, and IL-4 levels

Regarding BDNF levels, there was an interaction between pain, tDCS, and exercise (four-way ANOVA/Bonferroni, $F_{(2,120)} = 4.833$, $p < 0.05$; Table 2). The PT group displayed decreased BDNF levels compared to the ST group. Moreover, there were interactions between: pain, exercise, and time ($F_{(1,120)} = 8.488$, $p < 0.01$); pain and tDCS ($F_{(3,503)} = 4.833$, $p < 0.05$); pain and exercise ($F_{(1,120)} = 6.991$, $p < 0.01$); and tDCS and time ($F_{(2,120)} = 5.907$, $p < 0.01$). There was a main effect of time ($F_{(1,120)} = 29.549$, $p < 0.001$).

Regarding IL-1β levels, there was an interaction between pain, tDCS, and exercise (four-way ANOVA/Bonferroni, $F_{(2,120)} = 5.171$, $p < 0.01$; Table 2). The Pain groups displayed increased IL-1β levels

Table 1
BDNF, IL-1β and IL-4 levels in the cerebral cortex assessed at 48 h and 7 days after the bimodal tDCS treatment and/or treadmill exercise (n = 6 per group). Data presented as mean standard error of the mean (SEM) pg/mg of protein. Sham-Pain (S), Sham-Pain + Sham-tDCS (SS), Sham-Pain + Exercise (SE), Sham-Pain + tDCS (ST), Sham-Pain + Sham-tDCS + Exercise (SSE), Sham-Pain + tDCS + Exercise (STE) Pain-Group (P), Pain-Sham-tDCS (PS), Pain + Exercise (PE), Pain + tDCS (PT), Pain + Sham-tDCS + Exercise (PSE) and Pain + tDCS + Exercise (PTE).
BDNF: significant 4-way ANOVA interactions between: pain vs exercise vs tDCS vs time ($p < 0.01$); exercise vs tDCS vs time ($p < 0.02$); exercise vs time ($p < 0.02$); exercise vs tDCS vs time ($p < 0.01$); pain vs tDCS ($p < 0.001$). There were main effects of: exercise; ($p < 0.001$); tDCS ($p < 0.01$); and time ($p < 0.001$). IL-1β: significant 4-way ANOVA, pain and time main effects ($p < 0.01$ for both). IL-4: significant 4-way ANOVA, interactions between: pain vs tDCS vs exercise ($p < 0.01$); pain vs tDCS vs time ($p < 0.02$); exercise vs time ($p < 0.02$); exercise vs tDCS ($p < 0.05$); pain vs time ($p < 0.001$) and pain vs tDCS ($p < 0.02$). There were main effects of time ($p < 0.001$); exercise ($p < 0.001$) and pain ($p < 0.001$).

Cerebral Cortex

Biomarkers		BDNF levels (pg/mg of protein)		IL-1β levels (pg/mg of protein)		IL-4 levels (pg/mg of protein)	
Groups		48 h	7 days	48 h	7 days	48 h	7 days
Sham NP	Sham-Pain	7.57 ± 0.10	2.60 ± 0.08	8.57 ± 1.31	11.10 ± 1.18	2.88 ± 0.02	8.13 ± 0.35
	Sham-tDCS	8.09 ± 0.18	2.62 ± 0.21	10.47 ± 0.69	13.91 ± 1.19	2.82 ± 0.05	7.92 ± 0.43
	Exercise	7.72 ± 0.36	2.58 ± 0.14	12.78 ± 1.80	15.88 ± 1.13	2.80 ± 0.04	6.36 ± 0.70
	tDCS	7.58 ± 0.27	2.53 ± 0.15	11.36 ± 1.05	13.26 ± 0.89	3.24 ± 0.05	7.10 ± 0.29
	Sham-tDCS + Exercise	9.20 ± 0.37	3.42 ± 0.13	11.66 ± 0.73	16.79 ± 0.72	3.16 ± 0.03	7.11 ± 0.21
	tDCS + Exercise	8.62 ± 0.26	2.56 ± 0.11	13.28 ± 1.55	15.04 ± 1.44	3.50 ± 0.14	7.98 ± 0.58
NP	Pain	8.08 ± 0.26	2.43 ± 0.14	12.40 ± 1.96	16.50 ± 1.43	2.78 ± 0.07	5.55 ± 0.28
	Sham-tDCS	7.98 ± 0.13	2.90 ± 0.06	14.11 ± 2.09	16.88 ± 2.19	2.74 ± 0.01	7.35 ± 0.59
	Exercise	7.14 ± 0.11	2.86 ± 0.16	11.05 ± 1,00	16.78 ± 1.21	2.53 ± 0.08	6.03 ± 0.42
	tDCS	8.73 ± 0.38	2.97 ± 0.21	12.39 ± 0.87	17.68 ± 3.06	3.57 ± 0.05	6.80 ± 0.49
	Sham-tDCS + Exercise	8.81 ± 0.38	2.78 ± 0.19	14.89 ± 0.24	17.44 ± 2.41	3.39 ± 0.12	8.02 ± 0.14
	tDCS + Exercise	8.77 ± 0.30	3.58 ± 0.12	15.36 ± 0.72	14.53 ± 2.07	3.29 ± 0.15	6.58 ± 0.36

compared to the Sham-Pain groups. Moreover, the PS group displayed increased IL-1β levels compared to the SS group; the PE group also displayed increased IL-1β levels compared to the SE group. Furthermore, there were interactions between pain and exercise ($F_{(1,120)} = 11.121$, $p < 0.001$) and pain and time ($F_{(1,120)} = 5.578$, $p < 0.05$). There was a main effect of pain ($F_{(1,120)} = 17.092$, $p < 0.001$).

Regrading IL-4 levels, there was an interaction between pain, tDCS, and exercise (four-way ANOVA/Bonferroni, $F_{(2,120)} = 3.357$, $p < 0.05$; Table 2). The Pain, PS, and PT groups showed increased IL-4 levels compared to the Sham-Pain, SS, and ST groups. Moreover, the PTE group showed increased IL-4 levels compared to the STE group. Furthermore, there were interactions between pain, tDCS, and time ($F_{(2,120)} = 3.091$, $p < 0.05$); tDCS, exercise, and time ($F_{(2,120)} = 3.401$, $p < 0.05$); pain and exercise ($F_{(1,120)} = 6.889$, $p < 0.02$); and pain and time ($F_{(1,120)} = 5.778$, $p < 0.02$). There were main effects of pain ($F_{(1,120)} = 35.294$, $p < 0.001$); tDCS ($F_{(2,120)} = 6.369$, $p < 0.01$) and time ($F_{(1,120)} = 156.300$, $p < 0.001$).

Spinal cord BDNF, IL-1β, and IL-4 levels

Regarding BDNF levels, there was an interaction between pain, tDCS, exercise, and time (four-way ANOVA/Bonferroni, $F_{(2,120)} = 3.150$, $p < 0.05$; Table 3). At 48 h and 7 days post-treatment, the Pain group displayed increased BDNF levels compared to the Sham-Pain group. At 48 h post-treatment, the PS

Table 2
BDNF, IL-1β and IL-4 levels in the brainstem assessed at 48 h and 7 days after the bimodal tDCS treatment and/or treadmill exercise (n = 6 per group). Data presented as mean standard error of the mean (SEM) pg/mg of protein. Sham-Pain (S), Sham-Pain + Sham-tDCS (SS), Sham-Pain + Exercise (SE), Sham-Pain + tDCS (ST), Sham-Pain + Sham-tDCS + Exercise (SSE), Sham-Pain + tDCS + Exercise (STE) Pain-Group (P), Pain-Sham-tDCS (PS), Pain + Exercise (PE), Pain + tDCS (PT), Pain + Sham-tDCS + Exercise (PSE) and Pain + tDCS + Exercise (PTE).
BDNF: significant 4-way interactions between: pain vs exercise vs tDCS ($p < 0.05$); pain vs exercise vs time ($p < 0.01$); tDCS vs time ($p < 0.01$); painvs tDCS ($p < 0.05$) and pain vs exercise ($p < 0.01$). There was a main effect of time $p < 0.001$). IL-1β: significant 4-way interactions between: pain vs exercise vs tDCS ($p < 0.01$); pain vs time ($p < 0.05$); and pain vs exercise ($p < 0.001$). There was a main effect of pain ($p < 0.001$). IL-4: significant 4-way ANOVA interaction between: pain vs tDCS vs exercise ($p < 0.05$); exercise vs tDCS vs time ($p < 0.05$); pain vs tDCS vs time ($p < 0.05$); pain vs time ($p < 0.02$); and pain vs exercise ($p < 0.02$). There were main effects of time ($p < 0.001$); tDCS ($p < 0.01$); and pain ($p < 0.001$).

Brainstem

Biomarkers		BDNF levels (pg/mg of protein)		IL-1β levels (pg/mg of protein)		IL-4 levels (pg/mg of protein)	
Groups		48 h	7 days	48 h	7 days	48 h	7 days
Sham NP	Sham-Pain	16.28 ± 1.28	12.90 ± 0.39	78.15 ± 22.82	67.1 ± 6.06	10.86 ± 2.68	3.65 ± 0.40
	Sham-tDCS	15.68 ± 1.35	13.29 ± 0.28	50.67 ± 21.09	72.6 ± 4.17	11.42 ± 2.94	3.88 ± 0.43
	Exercise	14.96 ± 1.37	12.30 ± 0.38	38.92 ± 19.38	87.5 ± 9.39	9.31 ± 1.98	4.88 ± 0.49
	tDCS	18.84 ± 0.50	14.15 ± 0.35	106 ± 26.2	87 ± 2.35	13.92 ± 2.71	6.12 ± 1.13
	Sham-tDCS + Exercise	15.95 ± 1.15	13.66 ± 0.50	135.9 ± 42.84	131 ± 8.55	18.02 ± 2.49	7.55 ± 0.72
	tDCS + Exercise	13.40 ± 0.45	14.22 ± 0.87	77.79 ± 28.54	120 ± 19	14.12 ± 2.14	9.02 ± 1.16
NP	Pain	16.08 ± 1.01	12.58 ± 0.32	151.1 ± 16.14	102 ± 11.9	13.69 ± 2.02	8.70 ± 0.71
	Sham-tDCS	19.00 ± 0.70	13.51 ± 0.90	135.9 ± 32.66	94 ± 5.57	16.36 ± 3.04	9.66 ± 1.62
	Exercise	16.41 ± 1.82	14.36 ± 1.08	214.1 ± 26.03	133 ± 9.83	19.69 ± 1.16	9.17 ± 0.77
	tDCS	11.06 ± 0.50	14.43 ± 0.49	132.3 ± 33.86	130 ± 6.36	24.32 ± 2.21	9.26 ± 0.40
	Sham-tDCS + Exercise	17.08 ± 1.44	14.27 ± 1.20	112.6 ± 36.27	101 ± 1.25	20.91 ± 2.13	5.82 ± 0.77
	tDCS + Exercise	15.73 ± 1.39	14.76 ± 1.23	88.39 ± 30.87	98.4 ± 16.5	17.84 ± 0.81	7.40 ± 0.51

Table 3
BDNF, IL-1β and IL-4 levels in the spinal cord assessed at 48 h and 7 days after the bimodal tDCS treatment and/or treadmill exercise (n = 6 per group). Data presented as mean standard error of the mean (SEM) pg/mg of protein. Sham-Pain (S), Sham-Pain + Sham-tDCS (SS), Sham-Pain + Exercise (SE), Sham-Pain + tDCS (ST), Sham-Pain + Sham-tDCS + Exercise (SSE), Sham-Pain + tDCS + Exercise (STE) Pain-Group (P), Pain-Sham-tDCS (PS), Pain + Exercise (PE), Pain + tDCS (PT), Pain + Sham-tDCS + Exercise (PSE) and Pain + tDCS + Exercise (PTE).BDNF: significant 4-way ANOVA interactions between: pain vs exercise vs tDCS vs time ($p < 0.05$); pain vs exercise vs time ($p < 0.05$); pain vs exercise vs tDCS ($p < 0.001$); tDCS vs time ($p < 0.05$); exercise vs time ($p < 0.05$); exercise vs tDCS ($p < 0.02$); pain vs tDCS ($p < 0.01$) and pain vs exercise ($p < 0.01$). There were main effects of time ($p < 0.001$) and pain ($p < 0.01$).
IL-1β: significant 4-way ANOVA interactions between: pain vs tDCS ($p < 0.01$); tDCS vs time ($p < 0.001$) and pain vs time ($p < 0.001$). There were main effects of time ($p < 0.001$); tDCS ($p < 0.01$) and pain ($p < 0.001$). IL-4: significant 4-way ANOVA interactions between: pain vs exercise vs tDCS vs time ($p < 0.01$); exercise vs tDCS vs time ($p < 0.05$); tDCS vs time ($p < 0.01$); exercise vs time ($p < 0.001$); pain vs exercise ($p < 0.01$). There were main effects of time ($p < 0.01$); tDCS ($p < 0.01$); exercise ($p < 0.05$) and pain ($p < 0.01$).

Spinal Cord

Biomarkers		BDNF levels (pg/mg of protein)		IL-1β levels (pg/mg of protein)		IL-4 levels (pg/mg of protein)	
Groups		48 h	7 days	48 h	7 days	48 h	7 days
Sham NP	Sham-Pain	2.92 ± 0.22	3.43 ± 0.45	53.58 ± 8.08	51.35 ± 3.37	11.69 ± 1.35	12.85 ± 2.26
	Sham-tDCS	3.34 ± 0.48	3.19 ± 0.23	67.30 ± 4.05	59.09 ± 5.14	14.01 ± 1.28	12.66 ± 1.67
	Exercise	3.73 ± 0.38	3.48 ± 0.07	79.28 ± 4.17	68.14 ± 9.13	15.31 ± 0.81	12.58 ± 0.51
	tDCS	3.24 ± 0.35	5.34 ± 0.42	63.66 ± 5.53	94.79 ± 0.63	17.39 ± 1.33	17.82 ± 0.84
	Sham-tDCS + Exercise	4.93 ± 0.40	4.16 ± 0.35	67.06 ± 1.74	73.32 ± 4.19	12.70 ± 0.92	15.35 ± 2.49
	tDCS + Exercise	2.82 ± 0.44	6.07 ± 0.48	61.49 ± 2.76	92.93 ± 9.57	11.41 ± 0.57	19.75 ± 1.63
NP	Pain	6.64 ± 0.24	6.89 ± 0.59	80.60 ± 3.98	104.99 ± 5.51	14.28 ± 0.41	7.69 ± 0.79
	Sham-tDCS	3.53 ± 0.57	4.27 ± 0.61	68.21 ± 1.60	115.22 ± 13.47	14.51 ± 1.19	19.36 ± 0.59
	Exercise	6.26 ± 0.42	4.41 ± 1.02	75.70 ± 8.36	85.45 ± 18.88	13.08 ± 1.77	14.36 ± 1.96
	tDCS	2.86 ± 0.48	4.44 ± 0.98	63.51 ± 6.79	120.08 ± 5.32	18.91 ± 0.85	17.19 ± 2.95
	Sham-tDCS + Exercise	2.50 ± 0.28	5.63 ± 1.42	77.39 ± 3.38	89.43 ± 11.58	15.67 ± 1.33	17.14 ± 1.45
	tDCS + Exercise	3.16 ± 0.39	5.55 ± 1.09	80.43 ± 4.36	122.84 ± 3.07	14.87 ± 1.77	26.73 ± 1.86

group displayed increased BDNF levels compared to the SS group; the PSE group displayed decreased BDNF levels compared to the SSE group. Furthermore, there were interactions between pain, tDCS, and exercise ($F_{(2,120)} = 4.687$, $p < 0.001$); pain, exercise, and time ($F_{(1,120)} = 4.634$, $p < 0.05$); pain and tDCS ($F_{(2,120)} = 8.595$, $p < 0.01$); pain and exercise ($F_{(1,120)} = 9.909$, $p < 0.01$); tDCS and exercise ($F_{(2,120)} = 4.744$, $p < 0.02$); tDCS and time ($F_{(2,120)} = 8.421$, $p < 0.05$); and exercise and time ($F_{(1,120)} = 4.541$, $p < 0.05$). There were main effects of time ($F_{(1,120)} = 13.780$, $p < 0.001$), and pain ($F_{(1,120)} = 10.577$, $p < 0.01$).

Regarding IL-1β levels, there was an interaction between pain and tDCS (four-way ANOVA/Bonferroni, $F_{(2,120)} = 5.767$, $p < 0.01$; Table 3). The PT groups displayed increased IL-1β levels compared to the ST groups. Moreover, there were interactions between pain and time ($F_{(1,120)} = 16.458$, $p < 0.001$) and tDCS and time ($F_{(2,120)} = 12.925$, $p < 0.001$). There were main effects of pain ($F_{(1,120)} = 49.720$, $p < 0.001$), tDCS ($F_{(2,120)} = 6.728$, $p < 0.01$), and time ($F_{(1,120)} = 44.936$, $p < 0.001$).

Regarding IL-4 levels, there was an interaction between pain, tDCS, exercise, and time (four-way ANOVA/Bonferroni, $F_{(2,120)} = 5.100$, $p < 0.01$; Table 3). At 7 days post-treatment, the Pain group displayed decreased IL-4 levels compared to the Sham-Pain group. Moreover, at 7 days post-treatment, the PE group displayed increased IL-4 levels compared to the SE group; the PTE group displayed increased IL-4 levels compared to the STE group. Moreover, there were interactions between tDCS, exercise, and time ($F_{(2,120)} = 3.904$, $p < 0.05$); pain and exercise ($F_{(1,120)} = 11.118$, $p < 0.01$); tDCS and time ($F_{(2,120)} = 6.630$, $p < 0.01$); and exercise and time ($F_{(1,120)} = 23.886$, $p < 0.001$). There were main effects of pain ($F_{(1,120)} = 7.598$, $p < 0.01$), tDCS ($F_{(2,120)} = 20.603$, $p < 0.01$), exercise ($F_{(1,120)} = 8.147$, $p < 0.05$), and time ($F_{(1,120)} = 7.134$, $p < 0.01$).

Discussion

In this study, we showed that bimodal tDCS, aerobic exercise, or the combined treatments reverted thermal hyperalgesia in rats subjected to CCI. This effect was associated by a remarkable increase of the nociceptive threshold in all times assessed. tDCS or aerobic exercise alone partially increased the mechanical threshold immediately and 24 h after the last treatment. However, the combined treatments only displayed a slight improvement in the mechanical threshold at 7 days after the last treatment. We also showed modulations of central biomarker levels indexed by interactions observed among independent variables (pain, tDCS, exercise, and time) or main effects. The combination of tDCS and exercise has been poorly investigated as an adjuvant treatment against chronic pain. To our knowledge, this is the first study evaluating the combined effect of tDCS and aerobic exercise on nociceptive behavior in a neuropathic chronic pain model.

The differences found in the analgesic response induced by treatments may be related to different pathways activated by mechanical or thermal stimuli; while the first test activates Aβ fibers, the second mainly activates the Aδ and C fibers [35]. Current data corroborate our previous study, which showed that tDCS abolished the thermal hyperalgesia induced by CCI but was less effective on mechanical hyperalgesia [25]. It should be stressed that the central sensitization process triggered by neuropathic pain modulates the activity of wide dynamic range (WDR) neurons in the deepest laminae of the dorsal horn. This occurs in response to injury, contributing to behavioral and electrophysiological changes in chronic pain models [36]. Therefore, we hypothesized that synaptic transmission in neurons of pain pathways is mostly affected by nerve injury.

Numerous studies have used tDCS to treat neurological disorders [37,38], and chronic pain [39,40]. Although the main action mechanism of tDCS remains unclear, its analgesic effects comprise the modulation of a wide range of neurotransmitters, receptors, and ionic channels, including glutamatergic, serotoninergic, GABAergic, cannabinoid, and adenosinergic pathways [9,18]. Moreover, long-term effects triggered by tDCS are related to the facilitation of long-term potentiation phenomena [41].

However, exercise can provide both beneficial or detrimental effects [42]. The antinociceptive effects of exercise are mediated by opioid and non-opioid mechanisms, or an interaction between them [16,43]. Wheel running for 5 days before inducing muscle

pain has been reported to prevent the development of activity-induced hyperalgesia through modulation of serotonin transporters, mediated by mu-opioid receptors [44]. Cobianchi et al. [45] showed that short- (from 3 to 7 days post-CCI), but not long-lasting treadmill running, fully reverted mechanical allodynia. In our study, 7 days post-surgery, the Sham-Pain groups still exhibited a mild response to thermal stimulus, implying that inflammatory pain signaling was still occurring.

Consistent with our previous study [46], the neuromodulatory effects of tDCS on central biomarker levels depended on the structure assessed. For instance, bimodal tDCS increased BDNF in the cerebral cortex but decreased BDNF in the brainstem. Several connections among midbrain structures might act to affect the pronociceptive process. Such studies hold that descending facilitation involving BDNF/trkB and p38/MAPK signaling pathways throughout the periaqueductal grey (PAG), rostroventromedial medulla (RVM), and spinal cord increases NMDAR phosphorylation to yield a pronociceptive effect [47,48].

The top-down effects triggered by tDCS have the ability to modulate peripheral and central cytokine levels. For example, IL-1β and IL-10 levels were changed by tDCS in a model of chronic pain [25], while in chronically stressed rats, tDCS modulated hippocampal TNF-α levels [28]. Moreover, tDCS decreased mostly interleukin (IL-4, IL-6, IL-10) levels in the serum in depressed patients [49]. These effects demonstrate the ability of tDCS to modulate cytokines levels in cortical and subcortical structures, beyond a humoral response, highlighting that their potential effects might be implicated in biomarker development.

Additionally, exercise reverted the increase in pain-induced BDNF levels in the spinal cord. This corroborates a previous study showing that neurotrophic factors and interleukins are modulated differently in response to exercise [50,51]. BDNF released from pain-activated glial cells in the spinal cord has been reported to drive a neuronal gradient shift inducing sensitization of primary afferent neurons, which is restored by a BDNF antagonist [52] or exercise [53].

Our exercise protocol increased IL-4 levels in the brainstem and spinal cord but not in the cerebral cortex. Consistently, Bobinski et al. [54] showed that treadmill exercise increased IL-4 levels in the spinal cord in mice subjected to peripheral nerve injury. The anti-inflammatory and protective properties of IL-4 levels might be related to the preservation of spinal motor pathways through upregulation of arginase-1, IL-10, and CD206+ cells [55]. However, no effect was observed on IL-4 levels in the muscles and nerves in a axonotmesis mouse model after eccentric treadmill exercise [56]. Therefore, we hypothesize that the pattern of IL-4 expression depends on the exercise protocol (duration and intensity), pain model, and central structure analyzed. Furthermore, we also showed that IL-1β levels in the brainstem remained high after treadmill exercise. Contrastingly, Bobinski et al. [57] found decreased IL-1β levels in mice subjected to treadmill exercise. However, our exercise protocol began 15 days post-injury and lasted for 8 days, whereas their protocol began 3 days post-surgery and lasted for 10 days.

We also showed that exercise and/or tDCS modulates the hyperalgesic response and inflammatory profile in rats subjected to neuropathic pain. The reduction of the hypernociceptive response occurred from immediately to 7 days after the end of treatment (21 days post-surgery). However, we found no synergic effects between tDCS and aerobic exercise on the mechanical threshold in the short-term, but only a slight improvement in the long-term. Contrastingly, we showed a full reversal in thermal hyperalgesia. These discrepancies might be due to unknown mechanisms by which both treatments interact when applied in association [58]. Another explanation for such results is a ceiling effect, probably involving similar mechanisms between treatments [18,59]. Beyond that, it should be noted that exercise imposes stress upon the hypothalamic-pituitary-adrenal axis [60]. Therefore, we cannot

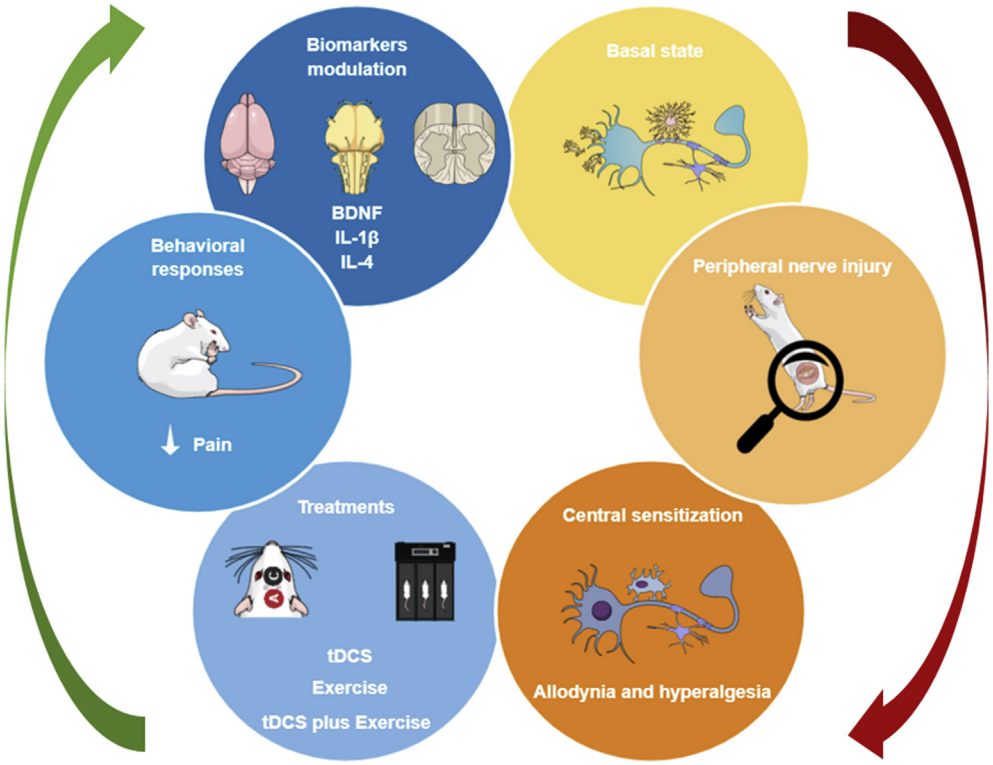

Fig. 3. Schematic representation of neuroimmunomodulatory effects of tDCS and/or exercise on neuropathic pain model in rats.

rule out that our employed protocol (treadmill exercise) had a stressor component, and that it may have interfered with the short-term effects. Contrastingly, a randomized placebo-controlled clinical trial has shown that tDCS to the primary motor cortex (M1) simultaneously applied with aerobic exercise reduced pain-related outcomes in human females with fibromyalgia [61]. We also found that the combined treatments increased BDNF and IL-4 levels in the rat cerebral cortex and brainstem, respectively. However, the combined treatments decreased IL-4 levels in the cerebral cortex. An explanation for that might be attributed to a structure-dependent function or interaction among pathways involved in exercise and/or tDCS mechanisms. More studies are required to elucidate the underlying mechanisms of combining these therapies and their effect on pain-related outcomes.

Our study has some limitations. Firstly, we only assessed the outcomes related to chronic pain in males, not females. Secondly, our protocol for VO_{2max} determination was based on an indirect method, and it is possible that some animals ran slightly above or below the maximal lactate steady state. Thirdly, the small head size of the rat and the restraint required for tDCS application allowed for only bimodal stimulation.

Conclusions

Our study showed that tDCS and aerobic exercise can treat chronic pain. According with Fig. 3, it is possible to observe the neuroimmunomodulatory effects of the therapies applied. Both methods are safe, inexpensive, and accessible strategies in chronic pain management by reducing the side effects of a sedentary lifestyle, and providing whole-body benefits. To date, most studies have focused on non-specific drugs that trigger several side effects in addition to not treating the pathology. Therefore, tDCS or exercise treatments should take into account the situation of each individual, while adjusting parameters to produce more efficient results (stimulation intensity vs. exercise intensity; time to stimulation vs. exercise). More studies are required to elucidate how tDCS and exercise might be applied more efficiently and whether both interventions may be used as a treatment approach.

Declaration of competing interest

None.

CRediT authorship contribution statement

Bettega Costa Lopes: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Liciane Fernandes Medeiros:** Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Vanessa Silva de Souza:** Investigation, Writing - review & editing. **Stefania Giotti Cioato:** Investigation, Writing - review & editing. **Helouise Richardt Medeiros:** Investigation, Writing - review & editing. **Gabriela Gregory Regner:** Investigation, Writing - review & editing. **Camila Lino de Oliveira:** Investigation, Writing - review & editing. **Felipe Fregni:** Writing - review & editing. **Wolnei Caumo:** Writing - review & editing. **Iraci L.S. Torres:** Conceptualization, Methodology, Formal analysis, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Acknowledgements

This study was supported by the following Brazilian funding agencies: the National Council for Scientific and Technological Development (CNPq; I.L.S. Torres, W. Caumo); the Committee for the Improvement of Higher Education Personnel (CAPES; B. C. Lopes, V.S. de Souza, H.R. Medeiros, G.G. Regner, C. L. de Oliveira); the Graduate Research Group of the Hospital de Clínicas de Porto Alegre (GPPG; I.L.S. Torres, Grant 20170061); MCT/FINEP – COENG/2013 (I.L.S. Torres, W. Caumo); Grant Doc-Fix FAPERGS/CAPES/04–2018; Grant Pesquisador Gaúcho/FAPERGS/02/2017 - 17/2551-0001.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2020.02.025.

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