Neurochemical Research (2020) 45:2653–2663
https://doi.org/10.1007/s11064-020-03116-w

ORIGINAL PAPER

Transcranial Direct Current Stimulation (tDCS) Induces Analgesia in Rats with Neuropathic Pain and Alcohol Abstinence

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Received: 13 February 2020 / Revised: 31 July 2020 / Accepted: 15 August 2020 / Published online: 25 August 2020

Abstract

Neuromodulatory techniques have been studied to treat drug addiction or compulsive eating as well as different chronic pain conditions, such as neuropathic and inflammatory pain in the clinical and preclinical settings. In this study, we aimed to investigate the effect of transcranial direct current stimulation (tDCS) on the association of alcohol withdrawal with neuropathic pain based on nociceptive and neurochemical parameters in rats. Thirty-six adult male Wistar rats were randomized into five groups: control, neuropathic pain, neuropathic pain + tDCS, neuropathic pain + alcohol, and neuropathic pain + alcohol + tDCS. The neuropathic pain model was induced by chronic constriction injury (CCI) to the sciatic nerve. Rats were then exposed to alcohol (20%) by oral gavage administration for 15 days (beginning 24 h after CCI). tDCS was started on the 17th day after surgery and lasted for 8 consecutive days. The nociceptive test (hot plate) was performed at baseline, 16 days after CCI, and immediately and 24 h after the last session of tDCS. Rats were killed by decapitation, and structures were removed and frozen for biochemical analysis (nerve growth factor and interleukin (IL-1α, IL-1β, and IL-10 measurements). Neuropathy-induced thermal hyperalgesia was reversed by tDCS, an effect that was delayed by alcohol abstinence. In addition, tDCS treatment induced modulation of central levels of IL-1α, IL-1ß, and IL-10 and neurotrophic growth factor. We cannot rule out that the antinociceptive effect of tDCS could be related to increased central levels of IL-1α and IL-10. Therefore, tDCS may be a promising non-pharmacological therapeutic approach for chronic pain treatment.

Keywords tDCS · Alcohol withdrawal · Neuropathic pain · Analgesia · Rats

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Introduction

Neuropathic pain (NP) is a relevant clinical problem since it is severely debilitating and largely resistant to treatment because its mechanisms are poorly understood [1]. Similarly, alcohol abuse is an extremely serious public health problem because alcohol is the most widely used addictive substance worldwide [2]. Alcohol consumption is also a risk factor for many chronic diseases, with its effects dependent on volume, alcohol content, and frequency of use [2, 3]. Taken together, these conditions change neurotransmitter levels, leading to changes in widespread modulation of neuronal activity [4].

Chronic alcohol exposure or withdrawal may cause detrimental impairment of the nociceptive system. A systematic review and meta-analysis [5] reported an association between chronic pain and alcohol use, which can be related to dysfunction in the neuro-immuno-endocrine circuitry, leading to greater susceptibility to substance abuse, including alcohol abuse. It is related to dopaminergic imbalance in the mesocorticolimbic pathway [6] and changes in the reward system through dopaminergic signaling pathways between the ventral tegmental area, nucleus accumbens, and medial prefrontal cortex as observed in conditioned place preferences for pain in rats [7]. This modulation is similar to those reported in brain areas activated by alcohol-induced analgesic effects [8, 9]. This hypothesis is supported by reports showing that 25% of individuals who use alcohol aim to relieve some type of pain [10–13]. The use of alcohol induces analgesia in humans and animals due to changes in the central and peripheral nervous systems. However, these effects can lead to positive feedback loops and contribute to alcohol abuse [14]. It is important to note that the previous preclinical study of the research group demonstrated that protracted alcohol withdrawal produced an analgesic effect indexed via an increased nociceptive threshold, which can be related to the increased central levels of BDNF and IL-10 [15]. On the other hand, some studies showed that alcohol withdrawal can trigger hyperalgesia as a component of withdrawal syndrome [16, 17]. The side effects of alcohol abstinence (hyperexcitability, anxiety, sleep disorders, and dysphoria, among others) also contribute to alcohol abuse as well as relapse [12, 18, 19]. In such instances, alcohol is consumed in increasing quantities to alleviate the motivational symptoms triggered by withdrawal [20, 21]. Paradoxically, hyperalgesic alcoholics respond better to the analgesic effects of alcohol than non-users, and this can be attributed to the belief that alcohol normalizes perceptions of pain and discomfort [22]. Additionally, a greater tendency toward familial alcoholism in the presence of chronic pain has been suggested [23].

It should also be stressed that both chronic pain and alcohol exposure/withdrawal lead to modifications in both central and peripheral neuroinflammatory patterns [2, 15, 24]. For example, previous studies have reported altered patterns of cytokines and neurotrophic factors following inflammatory and chronic pain injury models throughout the cortex-brainstem-spinal cord axis [25, 26]. Additionally, binge drinking and binge-like alcohol exposure induced the production of several cytokines, such as interleukins IL-10, IL-1α, and IL-1β, showing that both interventions can cause broad neuroimmune signaling throughout the peripheral and central nervous systems [2, 15, 27, 28]. Thus, it should be emphasized that an intermingled relationship between neuroimmunomodulatory and behavioral changes is involved in neuropathic pain and symptoms of alcohol withdrawal, with new therapeutic approaches required to better understand and treat these conditions.

`Non-invasive brain stimulation techniques (NIBS) have been used to treat different conditions such as inflammatory and neuropathic pain in clinical [29] and preclinical settings [26, 30]. These neuromodulatory techniques can reduce cravings in individuals with drug addiction [31] or compulsive eating [32, 33]. Transcranial direct current stimulation (tDCS) is notable in that it is considered a safe, low-cost, and an easily applied technique for modulating the neuronal membranes' resting potential using a weak electrical current applied through the scalp [34, 35]. The analgesic effects of tDCS have also been demonstrated in clinical studies by reducing pain scores and the frequency of analgesic use in different chronic pain states [29, 36] probably due to its ability to modulate cortical and subcortical structures directly or indirectly involved in inhibitory descending pain control [29, 32, 37]. The neuromodulatory features of tDCS corroborate its use as a non-pharmacological approach modulating behavior and neuroimmune alterations induced by chronic pain and alcohol abstinence.

Thus, we aimed to investigate the effect of tDCS treatment on the association between neuropathic pain and alcohol withdrawal based on the nociceptive and neurochemical parameters of rats. Furthermore, we hypothesized that the use of tDCS as a neuromodulatory tool would lead to modification of pain thresholds accompanied by changes in central biomarker levels.

Methods

Animals

Thirty-six male Wistar rats (weight 200–250 g) were randomized by weight and kept in groups of three or four animals per home cage (49 × 34 × 16 cm). Rats were maintained in a room under controlled temperature (22 ± 2 °C), on a standard 12 h light/dark cycle (lights on at 7 a.m.), with access

to water and chow ad libitum during the whole experiment. All experiments and procedures were approved by the Institutional Animal Care and Use Committee (GPPG-HCPA protocol no. 20,150,501). The experimental protocol complied with the ethical and methodological standards of the ARRIVE guidelines [38].

Experimental Design

The rats were assigned into five groups: control (CT), neuropathic pain (NP), neuropathic pain plus transcranial direct current stimulation (NPtDCS), neuropathic pain plus alcohol (NPAL), and neuropathic pain plus alcohol plus tDCS (NPAL tDCS). During the establishment of NP (from 1 to 15th after the surgery procedure), the rats were given oral alcohol gavage. After that, the rats were subjected to a daily tDCS session for eight consecutive days. The nociceptive test (hot plate) was performed at baseline, 16 days after the CCI surgical procedure, immediately after the last session of tDCS, and 24 h after the last session of tDCS. The rats were killed by decapitation 48 h post-treatment (Fig. 1). For all procedures (nociceptive and biochemical assays), the experimenter was blinded to the group of rats being tested.

Transcranial Direct Current Stimulation

The rats were subjected to bimodal tDCS (0.5 mA) for 20 min per day for 8 days under immobilization from the 17th - to 24th -day post-CCI surgery [37, 39]. 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 lines as a guide (the anterior and posterior regions in the midline between the two hemispheres of the parietal cortex, as described by Takano et al. [40]. Adapted electroencephalogram electrodes (1.5 cm^2) with a conductive hydrogel were fixed to their heads with an adhesive tape to prevent removal and connected to a battery-driven stimulator to deliver a constant electrical current. The rats had their heads shaved for better adherence. To deliver the current, animals had to be immobilized using a soft cloth during stimulation. The stimulation was performed at the same time of day (11 a.m.) by the same researcher. This technique has been applied by our research group and has been found to show long-lasting effects and is able to mirror human tDCS protocols used in pain treatment [35, 41].

Neuropathic Pain Model: Chronic Constriction Injury (CCI) of the Sciatic Nerve

Chronic constriction injury (CCI) was induced as described by Bennett [42]. Briefly, each rat was anesthetized by isoflurane inhalation (5% for induction and 2.5% for maintenance). The common sciatic nerve was then exposed and freed from the adherent tissue at the mid-thigh by blunt dissection of the biceps femoris muscle. Three loose ligatures were placed 1 mm apart using a chromic gut suture vicryl 4.0. After the procedure, the wound was closed with non-absorbable mono nylon yarn 4.0. Rats undergoing surgery received intraperitoneal tramadol (5 mg/kg) for pain relief immediately after surgery and once every 12 h for 2 days after CCI induction [43].

Model of Exposure to Alcohol

For alcohol administration, the ethanol was diluted daily with distilled water to prepare a 20% v/v solution. It was then delivered by oral gavage in a volume of 4 g/kg body weight according to previous studies [15, 44]. Administrations were performed from the 1st until the 15th day after the surgical procedure, between 8 a.m. and 10 a.m. The rats were weighed every 3 days to allow for adjustment of the volume/weight administered.

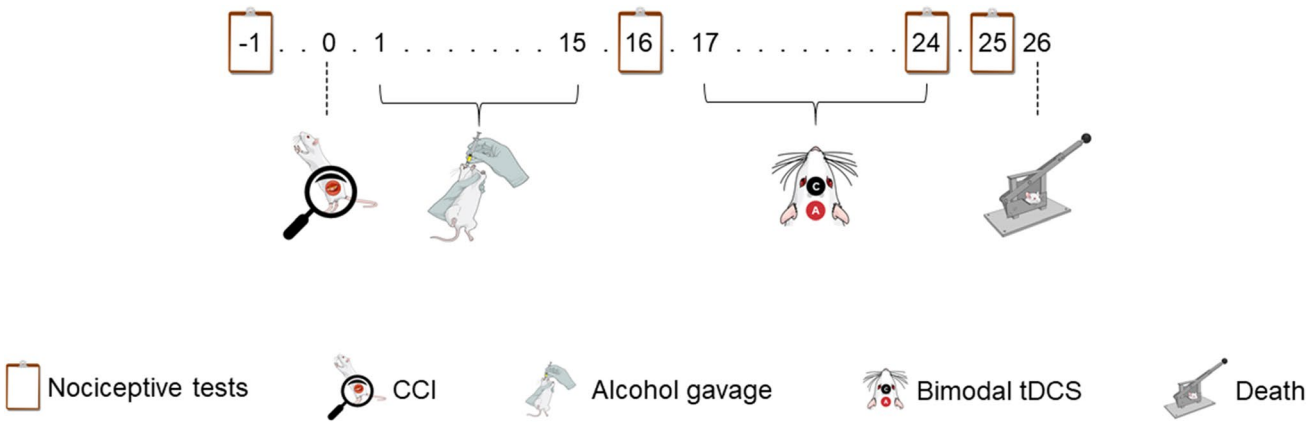

Fig. 1 Experimental design. *CCI* chronic constriction injury, *tDCS* transcranial direct current stimulation

Behavioral Tests

The behavior test (hot plate) was performed at baseline, 16 days post-surgery, as well as immediately and 24 h after the last tDCS session.

Thermal Hyperalgesia (Hot Plate Test)

This test was carried out to determine changes in latency such as licking or jumping responses, which resulted from supraspinal sensory integration and indicate modifications in the supraspinal process [45–47]. The rats were acclimated 24 h prior to the test for a period of 5 min inside the switched apparatus. During the test, the plate temperature was maintained at 55 °C ± 0.1. The rats were placed in a transparent polypropylene funnel on the heated surface. The time between placing the animals and the beginning of paw withdrawal or "tapping" was recorded as response latency in seconds, with each being a single measurement in each evaluation period [48, 49].

Sample Collection

The rats were killed by decapitation 48 h after the last treatment session with tDCS, and the central nervous system structures (cerebral cortex and brainstem) were removed and frozen at − 80 °C for further analysis.

Biochemical Assays

Nerve growth factor (NGF), IL-1α, IL-1β, and IL-10 levels were determined by sandwich ELISA using monoclonal antibodies specific for each measurement (R&D Systems, Minneapolis, United States). Total protein was measured using the Bradford's method using bovine serum albumin as a standard [50] Results were expressed as pg/mg of protein.

Statistical Analysis

The behavioral tests were analyzed using generalized estimated equations (GEE) by Bonferroni. The biomarkers data were analyzed through a one-way ANOVA followed by a Student-Newman-Keuls test. P-values < 0.05 were considered statistically significant. SPSS 19.0 for Windows was used for statistical analyses.

Results

Thermal Hyperalgesia

There was no difference between the groups at baseline (GEE: Wald $\chi 2 = 79.99$, $P = 0.60$). There was an interaction between group and time (GEE/Bonferroni; Wald $\chi 2 = 79.99$, $P = 0.001$). The neuropathic pain groups displayed thermal hyperalgesia 16 days after CCI surgery as indexed by a decrease in the nociceptive threshold (Fig. 2), thus confirming the effectiveness of neuropathic pain induction. This effect was immediately reversed 24 h after the end of tDCS treatment. However, this analgesic tDCS effect was only observed 24 h post tDCS treatment in the neuropathic pain + alcohol + tDCS group (Fig. 2).

Central NGF Levels

There was an increase in cerebral cortex NGF levels in the neuropathic pain + alcohol group, when compared to the control group. In addition, neuropathic pain + tDCS and neuropathic pain + alcohol + tDCS groups displayed increased NGF levels compared to the control and neuropathic pain groups (one-way ANOVA/SNK, $F_{(4,27)} = 7.876$, $P = 0.001$; Fig. 3, Panel A). There were no differences among the

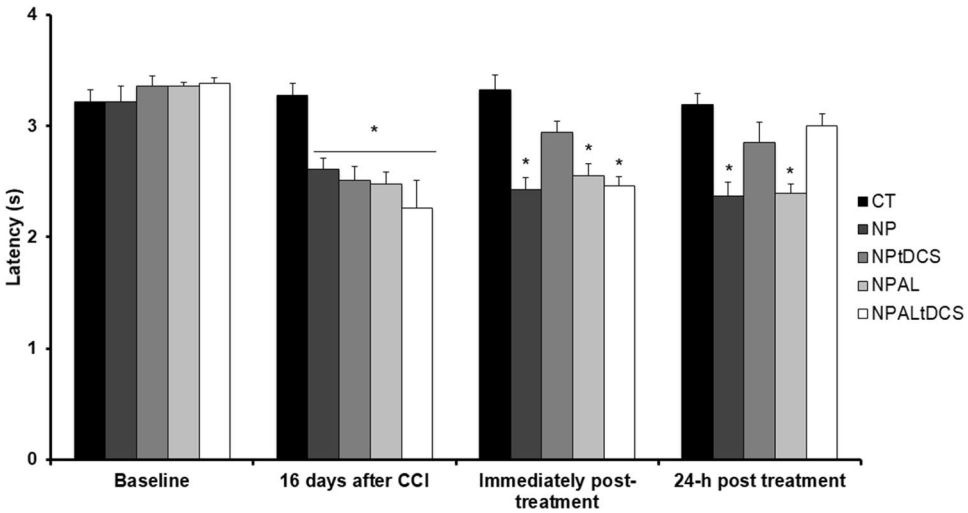

Fig. 2 Thermal hyperalgesia assessed by hot plate test at baseline, 16 days after the CCI model, immediately, and 24 h after bicephalic tDCS treatment (n = 36). Data are presented as mean ± standard error of the mean (SEM) of paw withdrawal latency (s). Control-Group (CT), Neuropathic pain (NP), Neuropathic pain + tDCS (NPtDCS), Neuropathic pain + Alcohol (NPAL) and Neuropathic pain + Alcohol + tDCS (NPALtDCS). *There was an interaction between group and time (GEE/Bonferroni, Wald $\chi 2 = 79.99$, $P = 0.001$)

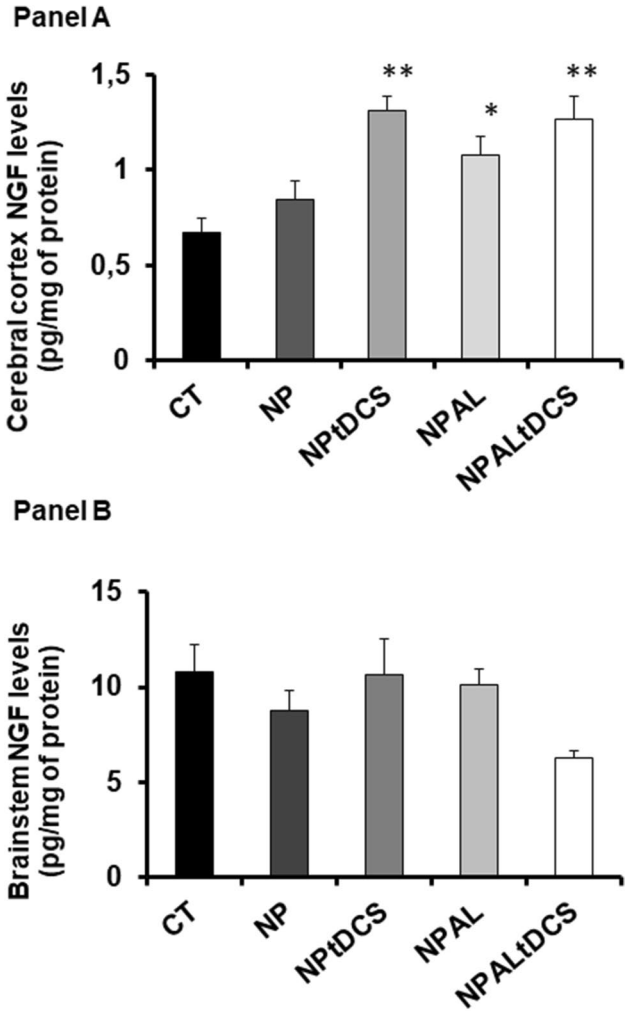

Fig. 3 NGF levels in the cerebral cortex (Panel A) and brainstem (Panel B) of rats subjected to CCI and alcohol abstinence as well as tDCS treatment. Data are presented as mean ± standard error of the mean (SEM) of pg/mg of protein. Control-Group (CT), Neuropathic pain (NP), Neuropathic pain + tDCS (NPtDCS), Neuropathic pain + Alcohol (NPAL) and Neuropathic pain + Alcohol + tDCS (NPALtDCS). Panel A: There were significant differences among groups in terms of cerebral cortex NGF levels (one-way ANOVA/SNK, P = 0.001).*—significant difference from CT group, and **—significant difference from CT and NP group Panel B: There were no differences among the groups in terms of brainstem NGF levels (one-way ANOVA, $F_{(4,27)} = 2.371$; P = 0.07)

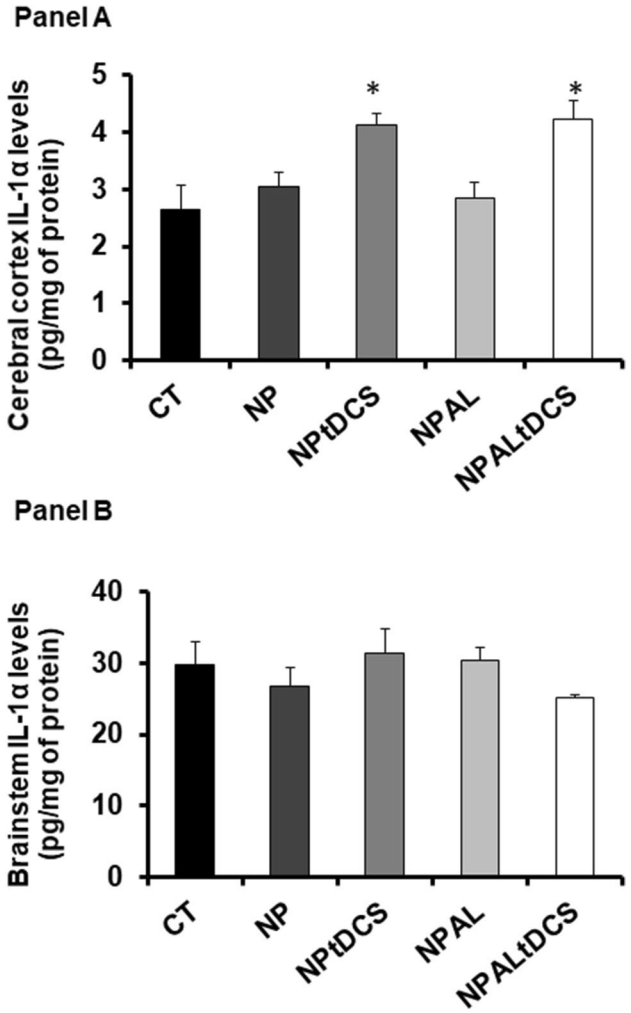

Fig. 4 IL-1α levels in the cerebral cortex (Panel A) and brainstem (Panel B) of rats subjected to CCI and alcohol abstinence as well as tDCS treatment. Data are presented as mean ± standard error of the mean (SEM) of pg/mg of protein. Control-Group (CT), Neuropathic pain (NP), Neuropathic pain + tDCS (NPtDCS), Neuropathic pain + Alcohol (NPAL) and Neuropathic pain + Alcohol + tDCS (NPALtDCS). Panel A: There was a significant difference in IL-1α levels in the cerebral cortex between groups (one-way ANOVA/SNK, $F_{(4,27)} = 5.364$, P = 0.003). *Significant difference from CT, NP, and NPAL groups. Panel B: There was no difference among the groups in terms of IL-1α levels in the brainstem (one-way ANOVA, $F_{(4,27)} = 1.035$, P = 0.40)

groups in terms of NGF levels in the brainstem (one-way ANOVA, $F_{(4,27)} = 2.371$; P = 0.07, Fig. 3, Panel B).

Central IL-1α Levels

There was an increase in IL-1α levels in the cerebral cortex in the neuropathic pain + tDCS and neuropathic pain + alcohol + tDCS groups compared to the other groups (one-way ANOVA/SNK, $F_{(4,27)} = 5.364$, P = 0.003; Fig. 4, Panel A). There were no differences among groups in terms of IL-1α levels in the brainstem (one-way ANOVA, $F_{(4,27)} = 1.035$, P = 0.40; Fig. 4, Panel B).

Central IL-1β Levels

There was increase in IL-1β levels in the cerebral cortex in the neuropathic pain + tDCS and neuropathic pain + alcohol + tDCS groups compared to the control group (one-way ANOVA/SNK, $F_{(4,27)} = 3.391$, P = 0.02; Fig. 5, Panel A). There were no differences among groups in terms of IL-1β

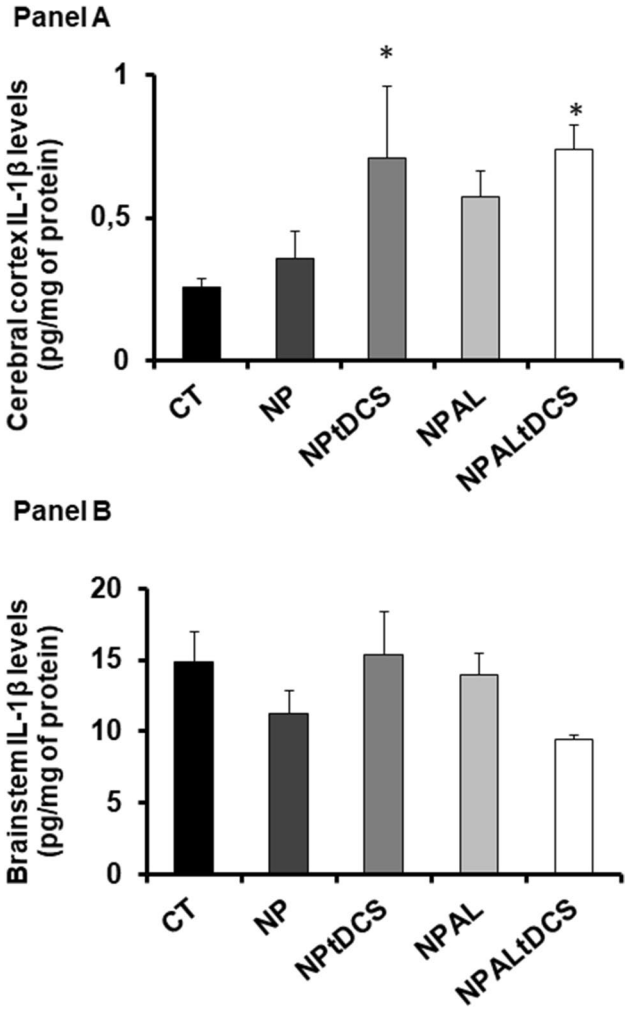

Fig. 5 IL-1β levels in the cerebral cortex (Panel A) and brainstem (Panel B) of rats subjected to CCI and alcohol abstinence as well as tDCS treatment. Data are presented as mean ± standard error of the mean (SEM) of pg/mg of protein. Control-Group (CT), Neuropathic pain (NP), Neuropathic pain + tDCS (NPtDCS), Neuropathic pain + Alcohol (NPAL) and Neuropathic pain + Alcohol + tDCS (NPALtDCS). Panel A: There were differences among the groups in terms of IL-1β levels in the cerebral cortex (one-way ANOVA/SNK, P = 0.02). *Statistically significant difference from the CT group. Panel B: There were no differences among the groups in terms of IL-1β levels in the brainstem (one-way ANOVA, $F_{(4,27)} = 1.776$, P = 0.16)

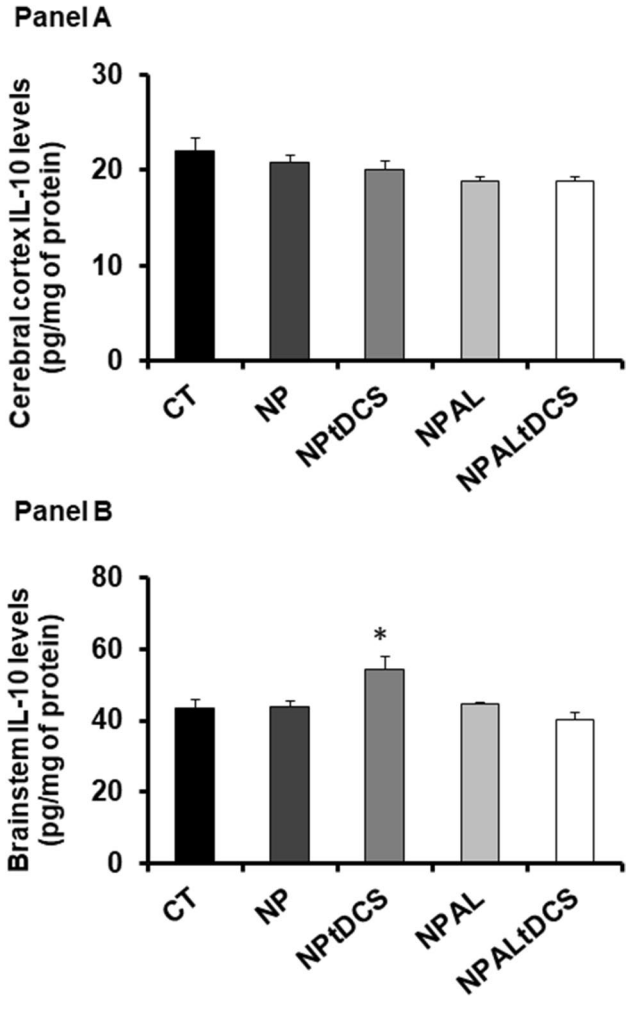

Fig. 6 IL-10 levels in the cerebral cortex (Panel A) and brainstem (Panel B) of rats subjected to CCI and alcohol abstinence as well as tDCS treatment. Data are presented as mean ± standard error of the mean (SEM) of pg/mg of protein. Control-Group (CT), Neuropathic pain (NP), Neuropathic pain + tDCS (NPtDCS), Neuropathic pain + Alcohol (NPAL) and Neuropathic pain + Alcohol + tDCS (NPALtDCS). Panel A: There were no differences among the groups in terms of IL-10 levels in the cerebral cortex (one-way ANOVA, $F_{(4,27)} = 2.335$, P = 0.08). Panel B: There was a significant difference in IL-10 levels in the neuropathic pain + tDCS group compared to other groups (one-way ANOVA/SNK, $F_{(4,27)} = 5.686$, P = 0.002). *Significant difference from CT, NP, NPAL, and NPALtDCS groups

levels in the brainstem (one-way ANOVA, $F_{(4,27)} = 1.776$, P = 0.16; Panel B).

Central IL-10 Levels

There were no differences among the groups in terms of IL-10 levels in the cerebral cortex (one-way ANOVA, $F_{(4,27)} = 2.335$, P = 0.08; Fig. 6, Panel A). In the brainstem, there was an increase in IL-10 levels in the neuropathic pain + tDCS group compared to other groups (one-way ANOVA/SNK, $F_{(4,27)} = 5.686$, P = 0.002; Fig. 6, Panel B).

Discussion

The present study showed that bimodal tDCS induced short- and long-term antinociceptive effects in rats with neuropathic pain. However, when alcohol abstinence was associated with CCI, only long-term effects were observed. These

results corroborate our previous research which showed that tDCS may have analgesic effects in different chronic pain models [26, 37, 39]. Besides, tDCS treatment induced modulation of central levels of interleukins (IL-1α, IL-1ß, and IL-10). Cerebral cortex NGF levels were increased by tDCS treatment and alcohol withdrawal, with the effect of the latter intensified when simultaneously administered with the former.

This study interestingly found that tDCS-induced analgesia was delayed by alcohol withdrawal. It is well known that alcohol consumption induces depressant effects in the central nervous system, modulates pain thresholds, and displays analgesic and anti-inflammatory effects [51, 52]. As such, its withdrawal associated with chronic pain drives an imbalance between excitatory/inhibitory neurotransmission in different cortical and subcortical brain regions, such as the medial prefrontal cortex, nucleus accumbens, and amygdala, which are connected to important brain regions related to nociception/analgesia, including the periaqueductal gray and rostral ventromedial medulla. The delayed tDCS-induced analgesia may be due to its neuromodulatory role, after-effects, as well as its non-specific modes of action [53]. Alcohol is a psychoactive substance that acts on multiple important neurotransmitter systems, including the GABAergic, glutamatergic, serotonergic, and opioidergic systems [54–58]. Indeed, $GABA_A$ and NMDA receptors participate in alcohol-induced analgesia and alcohol withdrawal-induced hyperalgesia/hyperexcitability [59–64]. In this way, we can suggest that the imbalance between these neurotransmitter systems could be involved in the delayed response observed in tDCS-induced analgesia.

Despite a previous study showing an increase in the thermal nociceptive threshold induced by alcohol withdrawal in rats not experiencing pain as evaluated by the tail-flick latency test [15], effects of alcohol withdrawal effect upon thermal hyperalgesia were not observed in the current study. This difference can be interpreted in two ways: (a) different status of the animals, with a group experiencing pain and the other not experiencing pain; and (b) differences in evaluated behavior, nociceptive thresholds, and the degree of hyperalgesia. While the nociceptive threshold is the latency of response to the nociception stimulus, hyperalgesia is an abnormal increase in the sensitivity to nociceptive stimuli, including different activation of fibers and supraspinal responses triggered by each test [65]. In addition, our data are in agreement with those of previous studies, which suggested that alcohol withdrawal exacerbates the symptoms of mechanical hyperalgesia without affecting the thermal response [66]. In contrast, previous studies have shown mechanical and thermal hypersensitivity in Sprague-Dawley rats subjected to alcohol withdrawal [67, 68]. On the other hand, studies have suggested pain relief induced by alcohol, but the mechanisms of action as well as the variables involved are still unclear [69–72]. It is important to note that the inconsistencies found in the literature may be related to the protocol for alcohol use, withdrawal times, as well as the strains and baseline status of the animals. Currently, it is believed that neurotransmitter and inflammatory systems are common pathways involved in the pathologies of both chronic pain [24, 37] and alcohol withdrawal [15, 73]. In this study, we showed that tDCS improved thermal hyperalgesia and modulated central biomarker levels, corroborating previous studies from our research group [24, 30, 32, 36, 37]. tDCS treatment increased IL-1α in the cerebral cortex in rats with chronic pain and alcohol abstinence. Previous studies have shown that IL-1α has antiallodynic and antihyperalgesic effects in a rat neuropathic pain model [74]. Thus, it is likely that an increase in central IL-1α levels might be the mechanism underlying the pain relief induced by tDCS treatment.

It is important to note that the increased brainstem IL-10 levels found in the current study in the neuropathic pain group may have also contributed to the tDCS-induced antinociceptive effect once it takes effect on key centers for pain modulation [25, 37, 75]. In contrast, alcohol abstinence attenuated the effects of tDCS on IL-10 levels without leading to changes in long-lasting tDCS antinociceptive effects. On the other hand, a previous study using a different alcohol protocol showed an increase of IL-10 levels in the hippocampus, prefrontal cortex, and brainstem in rats after alcohol abstinence [15]. Altogether, these findings highlight an important interaction between the immune system and alcohol exposure/withdrawal.

It is interesting to note that IL-1α and IL-1ß act on the same receptor to differentially influence nociceptive transmission and neuropathic pain responses [74, 76, 77]. As such, neurochemical measures were performed 48 h after the end of tDCS treatment or 26 days after CCI Model induction in the current study. This corroborates our previous findings that the levels of substances associated with neuropathic pain did not change when measured at the same time point. However, 29 days after CCI, neuropathic pain rats showed an increase in IL-1ß levels in the cerebral cortex. [37]. On the other hand, the tDCS group showed an increased level of IL-1 ß in rats with neuropathic pain independent of alcohol abstinence. Previous studies have shown that intrathecal IL-1ß administration in normal and inflamed rats led to different effects [78] without changing the latencies of paw withdrawal in normal rats while producing significant antinociception when administered intrathecally in rats with peripheral inflammation (carrageenan model). Considering the dual effect of IL-1ß, we cannot disregard the involvement of this interleukin in the observed antinociceptive effect in the current study. Analysis of the results of interleukin modulation should thus be related to the neuroimmunomodulatory effects of tCDS.

NGF mediates neuronal activity as well as the synaptic plasticity of neurons [79]. In the current study, tDCS was able to cause analgesia in rats with neuropathic pain, with this effect linked to elevated levels of NGF in the cerebral cortex. In addition, we found that alcohol withdrawal also increased NGF levels in the cerebral cortex of rats with neuropathic pain, with the alcohol withdrawal effect intensified when associated with tDCS. A previous study showed that chronic exposure to ethanol decreased NGF levels but that this effect was time- and site-dependent, with effects varying depending on the length of alcohol exposure and structures analyzed [80]. Besides, chronic consumption of high amounts of alcohol in rats leads to a transient increase in NGF levels in distinct brain regions [81]. We also highlight that the changes in NGF levels observed in the study may have been influenced by the length of alcohol exposure or withdrawal. tDCS also triggers a central neuromodulatory effect once it modulates NGF levels independent of the alcohol withdrawal effect.

Conclusions

The rationale of the current study was that pain from chronic conditions can be relieved by alcohol consumption. However, this substance is highly addictive for humans and animals. In this context, it is important to understand the central effects induced by alcohol exposure or withdrawal. In the same line, tDCS as a central neuromodulatory technique may benefit patients suffering both from alcohol abuse and chronic neuropathic pain. Besides, alcohol abuse and neuropathic pain treatments are oftentimes refractive to pharmacological treatment. Thus, tDCS may be a promising non-pharmacological therapeutic approach for both chronic conditions. This study showed that bimodal tDCS was able to effectively induce analgesia in rats with neuropathic pain, which was delayed by alcohol abstinence. We suppose that the analgesic effect of tDCS might be related to increased central levels of IL-1α, IL-10, and NGF, since its antinociceptive role has been well described in key pain pathways likely due to its capacity to neuromodulate immune signaling. Concerning alcohol exposure/withdrawal, the increase in central NGF levels suggests that alcohol-induced neuroplasticity might contribute to this dependence taking into consideration its broad interference upon biological processes. Overall, further research is needed to improve and broaden existing knowledge regarding tDCS and the effects of alcohol on pain.

Acknowledgements The authors thank the Biomedical Engineering group from the Hospital de Clínicas de Porto Alegre (HCPA) for the development of the tDCS equipment used in this study.

Author Contributions DSS and ILST were responsible for the study concept and design. DSS, BCL, LSS, AS, and JA contributed to the acquisition of data. DSS, LFM, JA, and ILST were responsible for data analysis. DSS, LFM, FF, WC, and ILST drafted the manuscript. All authors revised and edited the manuscript as well as approved the final version.

Funding This work was supported by the following Brazilian funding agencies: Brazilian Federal Agency for Support and Evaluation of Graduate Education—CAPES/MD-PhD (D.S. Santos, B.C. Lopes, L.S. Santos); National Council for Scientific and Technological Development—CNPq (Dr. I.L.S. Torres, Dr. W. Caumo); Graduate Research Group of the Hospital de Clínicas de Porto Alegre—GPPG (I.L.S. Torres, Grant No.: 15–0501) MCT/FINEP—COENG/2013; and the Support Program for Emerging Nuclei PRONEM (Paulo Roberto Sanches, Grant No. 16/2551-0000249-5).

Compliance with Ethical Standards

Conflict of interest All authors declares that they have no conflict of interest.

Ethical Approval All experiments and procedures were approved by the Institutional Animal Care and Use Committee of the Hospital de Clínicas de Porto Alegre/HCPA (GPPG-HCPA protocol no. 15.0501). The experimental protocol complied with the ethical and methodological standards of the ARRIVE guidelines (Kilkenny et al. 2013).

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