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RESEARCH ARTICLE

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Transcranial direct current stimulation alleviates nociceptive behavior in male rats with neuropathic pain by regulating oxidative stress and reducing neuroinflammation

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Abstract

Transcranial direct curent stimulation (tDCS) and trans-spinal direct current stimulation (tsDCS) are promising therapies for pain that can alter the excitability of neuronal activity in cerebral cortex. The aim of the study is to investigate the therapeutic effects of direct current stimulation (DCS) over the spinal cord and cerebral cortex on oxidative stress and neuroinflammation in rats with chronic constriction injury (CCI). Male Wistar rats were randomly divided into four experimental groups: Sham, CCI, CCI + tDCS and CCI + tsDCS. The neuropathic pain model was induced by using the CCI model. Rats with neuropathy were treated with cathodal tDCS and tsDCS stimulations consisting of 0.5 mA for 30 min a day for 7 days from day 8 onwards. Locomotor activity was measured by open-field test and nociceptive behavior was assessed by hot-plate, tail-flick and Randall–Selitto tests. Following the behavioral experiments, total oxidant capacity (TOC), total antioxidant capacity (TAC) and proinflammatory cytokine levels were evaluated in spinal cord and cerebral cortex tissues. The CCI model induced significant mechanical and thermal hyperalgesia. Nociceptive behaviors in rats with CCI were reversed by DCS treatment. Higher TOC and lower TAC levels were detected in the spinal cord and cerebral cortex tissues of the CCI rats compared to the control. tsDCS treatment amended oxidant/antioxidant status. Moreover, tsDCS modulated the central levels of Tumor necrosis factor-α (TNF-α), interleukin 1-beta (IL-1β), IL-6 and IL-18. tsDCS stimulation showed better therapeutic effect on neuropathic pain by regulating oxidant/antioxidant levels and reducing neuroinflammation. DCS, especially at spinal level, may be a promising therapeutic strategy that can be used alone or in combination with other effective treatments for alleviating neuropathic pain.

KEYWORDS
chronic constriction injury, neuroinflammation, neuropathic pain, tDCS

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1 | INTRODUCTION

Neuropathic pain (NP) is a type of chronic pain usually resulting from damage to the somatosensory system (Ngernyam et al., 2013). Approximately 20% of the adult population worldwide suffers from chronic pain each year. Neuropathic pain occurs due to an insult or dysfunction resulting from damage to the peripheral or central nervous system. Two fundamental mechanisms play a role in neuropathic pain pathophysiology, including peripheral and central sensitization (Ngernyam et al., 2013). Peripheral sensitization involves increased peripheral nociceptor responsiveness occurring after tissue injury, while central sensitization involves hyperexcitability in central nociceptive pathways resulting in abnormal responses to normal stimuli (Latremoliere & Woolf, 2009). Those mechanisms contribute to the main clinical symptoms of neuropathic pain, including spontaneous pain, allodynia, and hyperalgesia (Xu et al., 2022).

The pathophysiological mechanisms underlying neuropathic pain are currently unknown; however, neuroinflammation seems to have an important role in the development of neuropathic pain (Wu et al., 2022). Neuroinflammation is a complex process between immune cells, neurons and glial cells (Deal et al., 2022). Once the peripheral nerve is damaged, immune cells infiltrate at the site of injury and trigger the release of proinflammatory mediators such as cytokines and chemokines (Deal et al., 2022). Inflammatory signals can be transmitted to supraspinal areas via primary afferent fibers projecting to dorsal horn neurons of the ascending pain pathways, which in turn project to supraspinal regions (Fiore & Austin, 2016). In the brain, activation of glial cells contributes to releasing proinflammatory cytokines causing the sensitization of somatosensory neurons (T. Zhang et al., 2022). Tumor necrosis factor-α (TNF-α) and interleukin 1-beta (IL-1β), important proinflammatory cytokines, play a critical role in pathological pain (Gruber-Schoffnegger et al., 2013). They cause neurodegeneration, impair synaptic plasticity, and block long-term potentiation (LTP) induction in the hippocampus (Gruber-Schoffnegger et al., 2013; Ji et al., 2014). In contrast, they may act as neuromodulators in the spinal cord, inducing synaptic plasticity (e.g., LTP) and triggering inflammatory and neuropathic pain (Ji et al., 2014).

Neuropathic pain usually exhibits an inadequate response to classical analgesics, and the current treatment options are limited by potential side effects (Hu et al., 2022). Although antidepressants and anticonvulsants are first line in the treatment of neuropathic pain, there is an urgent need for alternative treatment options because of poor response, low patient compliance, and potential side-effects of drugs (Cavalli et al., 2019; Hu et al., 2022). Neuromodulation treatments such as repetitive transcranial magnetic stimulation, transcranial direct current stimulation (tDCS) and deep brain stimulation are applied to treat such neuropathic pain diseases (Vranken, 2009). tDCS is a non-invasive brain stimulation method that can alter the excitability of neuronal activity in the cerebral cortex (Akcay & Derin, 2022). tDCS causes neuroplastic changes in the central nervous system (Akcay & Derin, 2022). The neuromodulatory effects of tDCS have been demonstrated as a therapeutic effect in rat models of focal epilepsy, dementia, Parkinson's disease, and acute stroke (Bornheim et al., 2020; Guo et al., 2020; Liu et al., 2021; Yang et al., 2020). Depending on the stimulus type, the tDCS method modulates the stimulus causing anodal tDCS depolarization and cathodal tDCS hyperpolarization in structures such as cortico-striatal and thalamocortical (Akcay & Derin, 2022). Neuromodulation therapies are promising in treating pain resulting from central or peripheral sensitization. Peripheral sensitization following peripheral nerve injury activate and sensitize peripheral sensory neurons further inceasing release of the pronociceptive mediators from the terminals of the spinal cord (Si-Qi et al., 2019). Spinal cord stimulation (SCS) is known to activate ascending non-nociceptive AB fibers to close the gate for C-fiber nociceptive transmission (Bazzari & Bazzari, 2022). The peripheral sensitization triggers the central sensitization due to spinal projection to supraspinal structures via ascending pathways (Kocot-Kępska et al., 2021). tDCS treatment has been shown to normalize the effects of central sensitization by affecting descending pain modulatory network (Meeker et al., 2019). Therefore, we aimed to investigate the therapeutic effects of tDCS and transspinal direct current stimulation (tsDCS) on the neuroinflammation in chronic construction injury-induced neuropathic pain model in rats, separately.

Significance

Neuropathic pain is a type of chronic pain resulting from damage to the somatosensory system. Although antidepressants and anticonvulsants are the first line in the treatment of NP, new treatment strategies are needed because the traditional ones do not respond adequately. Neuromodulatory techniques, such as transcranial direct current stimulation (tDCS), is a promising therapy for pain that can alter the excitability of neuronal activity in the cerebral cortex. Cortical and spinal cathodal tDCS stimulation showed therapeutic effect on neuropathic pain by regulating oxidant/antioxidant levels and reducing neuroinflammation. tDCS, especially at the spinal level, may be a promising therapeutic strategy that can be used alone or in combination with other effective treatments for alleviating neuropathic pain.

2 | MATERIALS AND METHODS

2.1 | Animals

The study was conducted in compliance with the European Community Directive (86/609 ECC) for the care and use of laboratory animals and the Regional Animal Care Committee (Akdeniz University, 18.10.2021/Approval no: 143). 12 weeks old

male Wistar Albino rats (250–300 g) was obtained from Akdeniz University Experimental Animals Application and Research Center. The animals were housed in separated cages and kept under a standard environmental condition (23–25°C, 50 ± 5% relative humidity, 12 h light/dark cycle) with free access to food and water. The animals were acclimatized to the environment before the experiments and then were randomized and divided into four groups as (i) Sham, (ii) chronic constriction injury (CCI), (iii) CCI + brain cathodal tDCS (CCI + tDCS) and (iv) CCI + foot cathodal tsDCS (CCI + tsDCS) ($n = 10$ per group). 10 rats in each group were allocated to behavioral test groups and then were used. Biochemical analysis. Researchers were blinded to the experimental groups. The size of experimental groups was determined by considering the accuracy and reproducibility of methods based on our previous data sets.

2.2 | CCI surgery

The chronic construction injury (CCI) model described by Bennett and Xie (1988) was used for the induction of neuropathic pain (Bennett & Xie, 1988). In brief, rats were anesthetized with isoflurane (5% for induction, 2.5% for maintenance), and a 1 cm incision was made along the longitudinal axis of the right hind leg distal to the hip, 3–4 mm below the femur. Then, four loose ligatures (4/0 chromic catgut) were tied proximal to the sciatic trifurcation approximately 1 mm apart. Ligations were loosened to minimize nerve constriction and allow epineural blood flow. After the procedure, the surgical incision was immediately sutured, povidone-iodine solution was applied externally, and rats were housed in separate cages for 4 hours after CCI surgery were allowed to recover for 1 week before treatments. For the Sham group, the sciatic nerve would be exposed, similar to the CCI model, but no ligatures were placed. On the 14th day, the pain tests were completed and the cortex and spinal cord tissues of the subjects were taken.

2.3 | Experimental design

Thermal and mechanical thresholds of all animals were measured prior to surgery and recorded as pre-operation values. The development of neuropathy was assessed by measuring thermal and mechanical thresholds 7 days after surgery or sham operation and these measurements were also recorded as pre-treatment and post-operation values. Animals with evidence of neuropathy were treated with cathodal tDCS or tsDCS therapy consisting of constant low-intensity current (0.5 mA) for 30 min a day for 8 days from day 7 onwards. At the end of the treatments, locomotor activity (open-field test), thermal hyperalgesia (hot-plate and tail-flick tests) and mechanical allodynia (Randall–Selitto) were evaluated by behavioral tests (see Figure 1 for experimental design). After the behavioral experiments were performed on the 7th and 14th days, TOC and TAC levels were evaluated in the spinal cord and cerebral cortex tissues. TNF-α, IL-1β, IL-6 and IL-18 levels were quantified in the same tissues by ELISA.

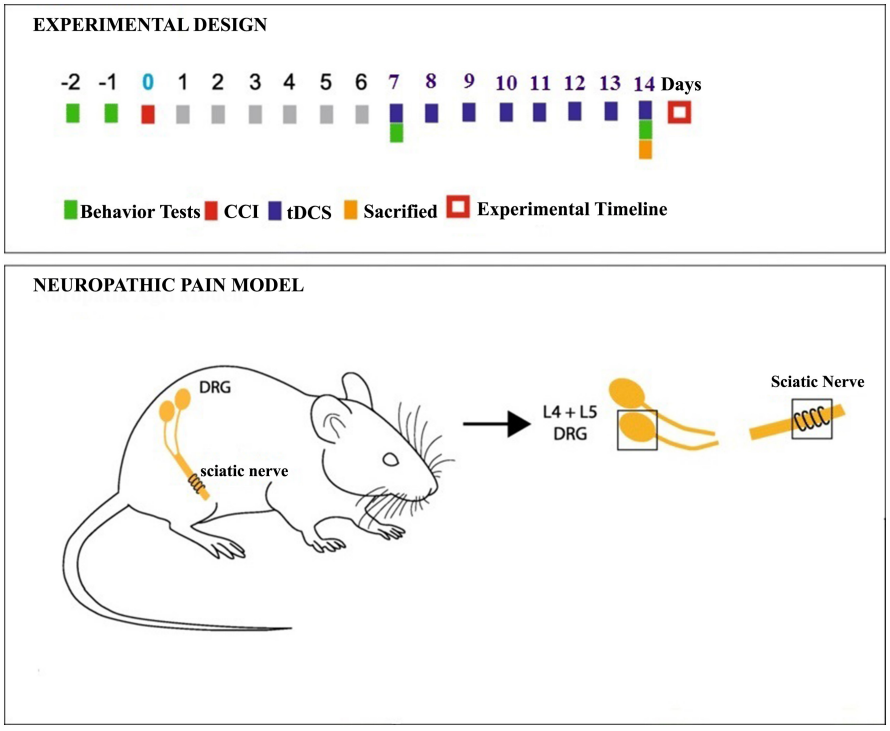

FIGURE 1 Experimental design.

2.4 | tDCS application

In the DCS and combination groups, cathodal tDCS and tsDCS were performed under anesthesia during the experiments. To investigate the effects of tDCS, the anodal electrode was positioned between the ears, from the neck of the rat (parietal cortex), while the cathodal electrode was positioned at the midpoint of the lateral angle on the eyes (supraorbital area) (Lopes et al., 2020). Rats subjected to active or sham stimulation had their scalp shaved. A rectangular-shaped stainless steel electrode, 1.5 cm × 1.5 cm, was used. To investigate the effect of tsDCS, the cathodal electrode was positioned over the vertebral column from T10-L4, close to the spinal cord levels where nociceptive primary afferent fibers originating from the foot dorsum synapse with spinothalamic neurons in the dorsal horn. A similar reference electrode was positioned over the lateral aspect of the abdominal muscles (Lenoir et al., 2018). The electrodes were placed on the skin in a similar manner to that used in human studies of tDCS or tsDCS for pain (Lenoir et al., 2018; Spezia Adachi et al., 2012). tDCS protocols were adopted from the study of Lopes et al. (2020). Seven days post-surgery, the rats were subjected to a 30-min session of cathodal tDCS treatment for eight consecutive days in a constant direct current of 0.5 mA delivered from a battery-powered stimulation source. For the sham groups, the electrodes were placed in the same positions as for real stimulation; however, the stimulator was remained turned off throughout the experiments so the animals could maintain continuity of the physical sensation of real tDCS conditions.

2.5 | Behavioral tests

2.5.1 | Open-field test

Behavioral experiments were performed 6 hours after tDCS and tsDCS applications. Locomotor activity was quantified in the open-field test on days 7 and 14 of the experiment. This test was carried out in a square-shaped, black matte-bottomed setup with a wall height of 40 cm and a base of 80 × 80 cm. The area was divided into 16 equal squares of 20 cm^2 in size. The rats were placed in the center of the area to explore for 5 min. The movements were recorded by a video tracking system (Noldus Ethovision XT System, The Netherlands). Total distance traveled (cm) was calculated (Akcay & Derin, 2022).

2.5.2 | Hot plate test

The hot-plate test, evaluating the thermal hyperalgesia, was carried out to confirm the neuropathic pain induction and to assess the effects of tDCS on the thermal nociceptive threshold (Cioato et al., 2016). This test was conducted at baseline, 7 and 14 days after surgery. The surface of the hot plate apparatus was pre-heated and kept at a constant temperature of 55 ± 0.1°C. The animals were placed inside glass funnels on the heated surface, and the time between the placement of the rat and the first response (foot-licking, jumping, or rapidly removing paws) was recorded as the paw withdrawal latency (PWL). The cut-off time was 20 s to prevent tissue damage. The hind paw retraction time (sec) was measured for each rat (Santos et al., 2020).

2.5.3 | Tail flick test

Thermal hyperalgesia was also evaluated by the tail-flick test in which the animal's tail is exposed to a heat source (Hacısüleyman et al., 2022). When the animal feels uncomfortable, it automatically raises its tail. Briefly, the 2 cm portion of the distal tail was immersed into a 52.5 ± 0.2°C water bath. The time the rats took to flick the tail was recorded as tail-flick latency; the cut-off latency was 15 s to avoid the injury of tissues of the tail (Arslan et al., 2018).

2.5.4 | Randall-Selitto test

The mechanical hyperalgesia was measured using the Randall–Selitto paw pressure test apparatus (Ugo-Basile 37215 Analgesy-meter, Italy). Randall–Selitto test involves applying an equally increasing mechanical pressure to the animal's paw (Zhang et al., 2021). This pressure causes pain that leads to an escape reaction. This test was conducted at baseline, 7 and 14 days after surgery. Briefly, rats were immobilized and grasped with one hand. Their hindpaws were submitted to a lineal incremental pressure until the paw was withdrawn or vocalizations were elicited. The force (grams) at which paw withdrawal appeared was recorded. 3–4 consecutive measurements with 5 min intervals were performed and the withdrawal threshold for each rat was calculated by mean the force at which the animal withdrew its paw. The cut-off force was 250 g. The average of three consecutive tests with an inter-stimulus interval of 1 min was considered as the muscle pressure threshold (De la Luz-Cuellar et al., 2023).

2.5.5 | Tissue collection

The animals were killed by decapitation 14 days after the last session of DCS treatment. Total cerebral cortex and and ipsilateral or contralateral spinal cord (T7/8-L5) were dissected, washed with normal saline and kept frozen at −80°C until use. Tissues were homogenized in phosphate buffer saline (PBS, pH 7.4) centrifuged at 12,000 rpm at 4°C for 20 min, and the supernatants were used in biochemical assays.

2.6 | Biochemical analysis

2.6.1 | Determining total antioxidant capacity

Total antioxidant capacity (TAC) in cerebral cortex and spinal cord tissues was evaluated using the OxiSelect™ TAC Assay kit (Cell Biolabs, Inc., San Diego, CA, USA). The TAC assay is based on the reduction of copper (II) to copper (I) by antioxidants such as uric acid and reaction with a chromogen, determining the absorbance at 490 nm (de la Cruz Cortés et al., 2021). TAC levels were expressed as nmol/mg protein.

2.6.2 | Determining total oxidant capacity

Total oxidant capacity (TOC) determination in cerebral cortex and spinal cord tissues was measured using the OxiSelect™ In Vitro reactive oxygen species (ROS) and reactive nitrogen species (RNS) Assay Kit (Cell Biolabs, Inc., San Diego, CA, USA) This method is based on the oxidation of dichlorodihydrofluorescin (DCFH) by ROS/RNS within the samples into the fluorescent form 2′, 7′-dichlorodihydrofluorescin (DCF). Samples were measured fluorometrically against a hydrogen peroxide or DCF standard at 480 nm excitation and 530 nm emission. The free radical content of oxidant molecules present in the sample was determined by comparison with a predetermined DCF or hydrogen peroxide standard curve, and concentrations were calculated by linear regression (Härtel et al., 2022).

2.6.3 | Enzyme-linked immunosorbent assay (ELISA)

The levels of TNFα, IL-1β, IL-6 and IL-18 were quantified using ELISA kits. Commercially available ELISA kits (R&D Systems, Minneapolis, USA) for rat TNF-α (EK710127), IL-1β (EK710260), IL-6 (EK710281) were performed according to the manufacturer's instructions. TNF-α, IL-1β and IL-6 concentrations in the samples were calculated from their corresponding absorbance values via the standard curve. Data were normalized to total tissue protein and expressed as pg·mg^{-1} tissue protein.

2.6.4 | Protein measurements

Protein concentrations were measured in the cerebral cortex and spinal cord tissues at 595 nm by a modified Bradford assay using Coomassie Plus reagent with bovine serum albumin as standard (Pierce Chemical Company, Rockford, IL, USA).

2.7 | Statistical analysis

Analysis of the data was carried out using the SPSS 20.0 program. One-way ANOVA test was used in the analysis of the data conforming to the normal distribution in the evaluation made with the Shapiro–Wilk test, and the post-hoc Tukey test was used in the pairwise comparison between the groups. The analysis of the data that did not fit the normal distribution was made with the Kruskal–Wallis test. The Mann–Whitney-U test was used for the pairwise comparison of the groups Data were expressed as the mean ± standard error of mean (*SEM*). Values with $p < .05$ were considered statistically significant.

3 | RESULTS

3.1 | Assessment of neuropathy

The development of neuropathy has been shown in Figure 2. Mechanical and thermal thresholds were decreased on the 7th day when nerve damage occurred. Two rats without neuropathy were excluded from the experiment. The development of neuropathy was not observed in the sham group as expected. Thresholds and latencies of response against painful stimuli were decreased significantly ($p < .05$) in all operated groups compared to sham group (Figure 2).

3.2 | Evaluation of locomotor activity

There was no difference between sham and CCI groups in locomotor activity total distance (cm) values on day 0. The total distance (cm) values, the determinant of locomotor scores, were significantly decreased in the CCI group on the 7th day compared to the sham group ($F(3,38) = 270{,}062$; $p < .01$ Figure 3b), On the 14th day ($F(4,40) = 6784$; $p < .01$ Figure 3c), an increase was observed in both CCI + tDCS and CCI + tsDCS groups compared to the CCI group; however, this increase was significant only in the CCI + tsDCS group ($p < .05$).

3.3 | Effects of tDCS and tsDCS on thermal and mechanical hyperalgesia

There was a significant decrease in the PWL of the CCI rats compared to the sham group in hot plate test on the 14th day ($F(3,34) = 34{,}415$; $p < .01$ Figure 4a). tsDCS treatment markedly increased the PWL of CCI rats on the 14th day ($p < .05$). tDCS treatment also improved the PWL of CCI rats, however, this effect was not significant ($p > .05$).

Similar to hot plate test, tail-flick latency of CCI rats were significantly reduced in tail-flick test on the 14th day, as compared to the sham group ($F(3,34) = 16{,}753$; $p < .01$ Figure 4b). tsDCS treatment markedly increased the tail-flick latency of CCI rats on the 14th day ($p < .05$). tDCS treatment also improved the tail-flick latency of CCI rats, however, this effect was not significant ($p > .05$).

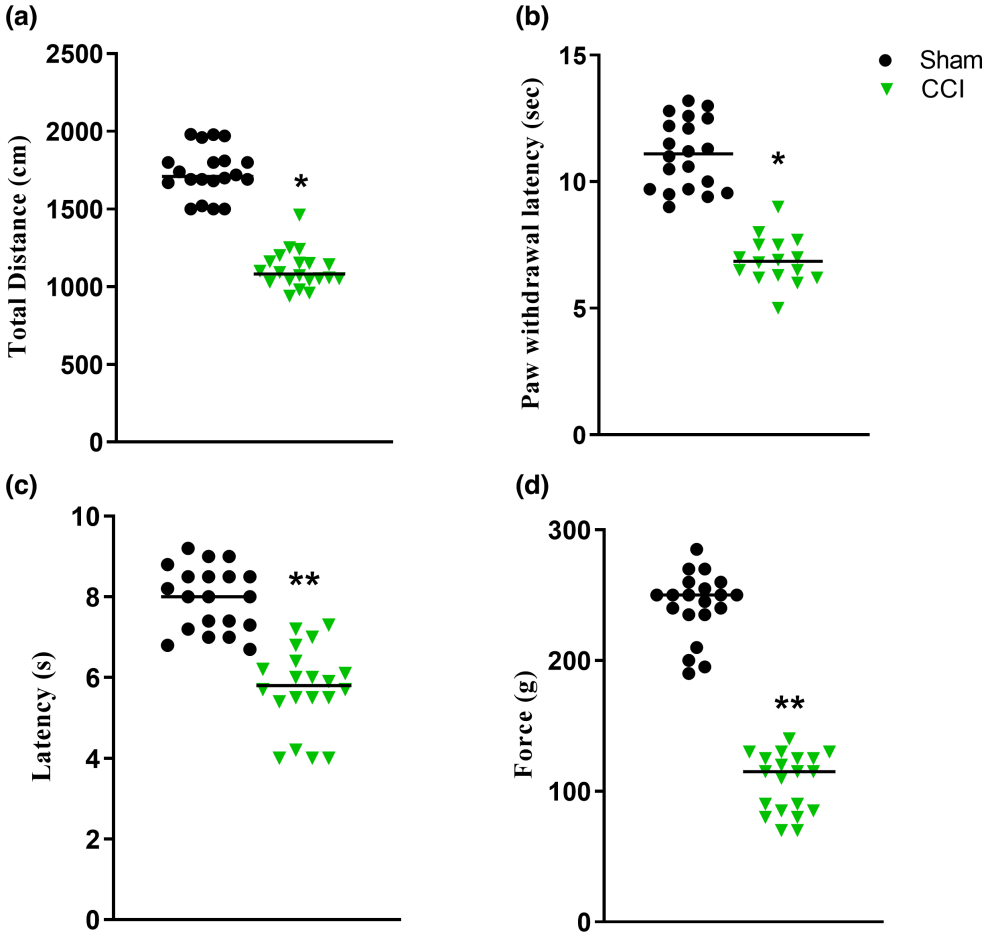

FIGURE 2 Assessment of neuropathy on 7th day. (a) Open filed total distance results, (b) Hot-plate test results, (c) Tail-flick test results, (d) Randall–Selitto test results. $^{*}p < .05$, $^{**}p < .01$ shows the difference according to the sham group.

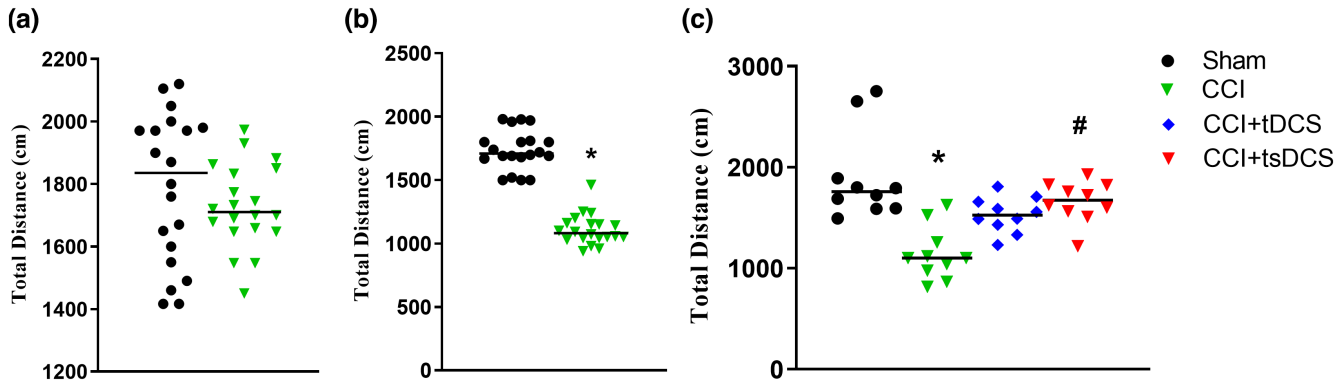

FIGURE 3 Open field test results. (a) 0th-day baseline locomotor activity results, (b) 7th-day locomotor activity results, (c) 14th-day locomotor activity results. ($n = 10$, for each group; $^{*}p < .05$ shows the difference according to the Sham group. $\#p < .05$ shows the difference according to the CCI group, one-way ANOVA test, followed by Tukey post hoc test). All data are presented as means ± *SEM*.

There was a significant decrease in muscle pressure threshold of the CCI rats compared to the sham group in Randall–Selitto test on the 14th day ($F(3,34) = 100,560$; $p < .01$ Figure 4c). tsDCS treatment markedly increased the muscle pressure threshold of CCI rats on the 14th day ($p < .001$). tDCS treatment also improved the muscle pressure threshold of CCI rats, however, this effect was not significant ($p > .05$).

3.4 | TAC and TOC levels in the cerebral cortex and spinal cord tissues

TAC levels detected in the cerebral cortex ($F(3,36) = 41,319$; $p < .01$ Figure 5a), and spinal cord ($F(3,36) = 17,999$; $p < .01$ Figure 5b), tissues are shown in Figure 5. There was a significant decrease in the

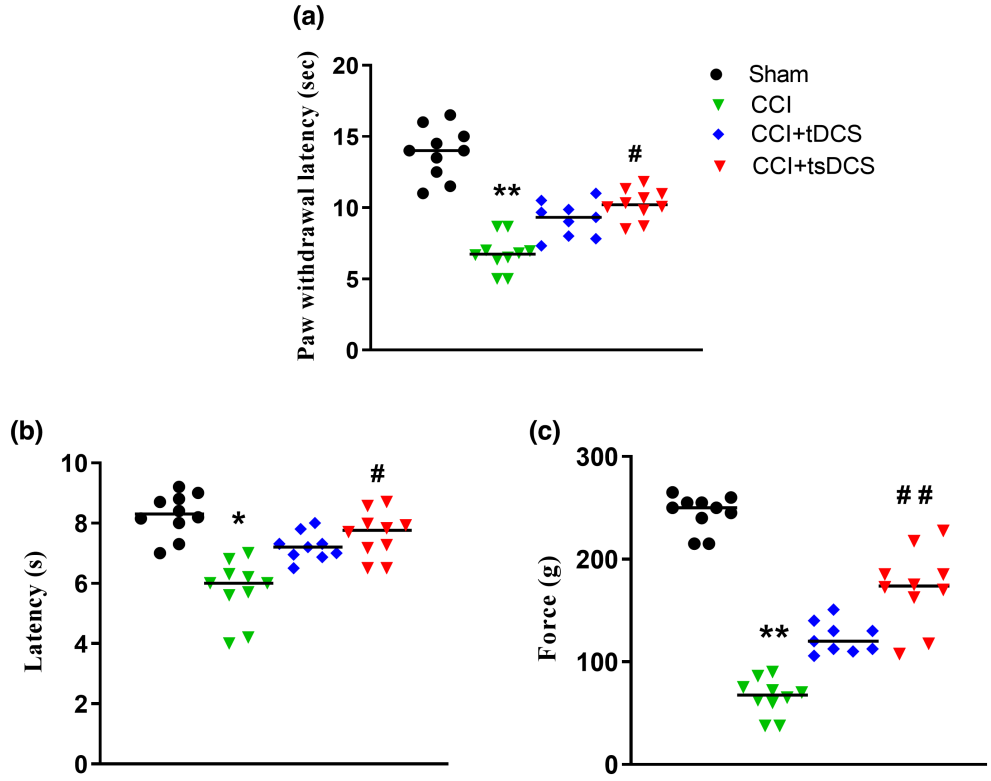

FIGURE 4 14th day results. (a) Hot-plate test results, (b) Tail-flick test results, (c) Randall–Selitto test results. ($n = 10$, for each group; $^{*}p < .05$, $^{**}p < .01$ shows the difference according to the Sham group. $\#p < .05$, $\#\#p < .01$ shows the difference according to the CCI group, one-way ANOVA test, followed by Tukey post hoc test). All data are presented as means ± *SEM*.

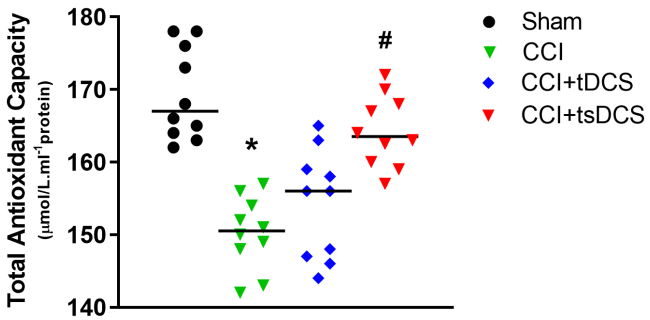

FIGURE 5 Total antioxidant capacity results. (a) TAC results in cerebral cortex, (b) TAC results in spinal cord. ($n = 10$, for each group; $^{*}p < .05$ shows the difference compared to the Sham group, $\#p < .05$ shows the difference compared to the CCI group, one-way ANOVA test, followed by Tukey post hoc test). All data are presented as means ± *SEM*.

TAC values in the cerebral cortex and spinal cord tissues of the CCI rats compared to the sham group ($p < .05$). tsDCS treatment significantly increased the TAC in both tissues of CCI rats on the 14th day ($p < .05$), while there was no difference with tDCS treatment ($p > .05$).

TOC levels detected in the cerebral cortex ($F(3,36) = 17{,}020$; $p < .01$ Figure 6a) and spinal cord ($F(3,36) = 13{,}460$; $p < .01$ Figure 6b), tissues are shown in Figure 6. There was a significant increase in the TOC values in the cerebral cortex and spinal cord tissues of the CCI rats compared to the sham group ($p < .01$ for both). tsDCS treatment significantly decreased the TOC in both tissues of CCI rats on the 14th day ($p < .05$), while there was no difference with tDCS treatment ($p > .05$).

3.5 | TNFα, IL-1β, IL-6 and IL-18 levels in the cerebral cortex and spinal cord tissues

TNF-α ($F(3,35) = 6168$; $p < .01$ Figure 7a), IL-1β ($F(3,35) = 5433$; $p < .01$ Figure 7b), IL-6($F(3,35) = 5817$; $p < .01$ Figure 7c) and IL-18 ($F(3,35) = 4468$; $p < .05$ Figure 7d) levels in the cerebral cortex and spinal cord are shown in Figures 7 and 8. TNF-α, IL-1β, IL-6 and IL-18 levels in the cerebral cortex of the CCI group were significantly increased compared to the sham group ($p < .05$) (Figure 6). tsDCS treatment significantly decreased all the proinflammatory cytokine levels of CCI rats on the 14th day ($p < .05$ for all), while tDCS treatment significantly decrease only IL-6 and IL-18 levels in the cerebral cortex ($p < .05$ for all). TNF-α ($F(3,35) = 8797$; $p < .01$ Figure 8a), IL-1β ($F(3,35) = 5395$; $p < .01$ Figure 8b), IL-6($F(3,35) = 5584$; $p < .01$ Figure 8c) and IL-18 ($F(3,35) = 4346$; $p < .05$ Figure 8d) levels in the spinal cord of the CCI group were also significantly increased compared to the sham group. Both tsDCS and tDCS treatments significantly decreased all the proinflammatory cytokine levels of CCI rats in the spinal cord on the 14th day ($p < .05$ for all).

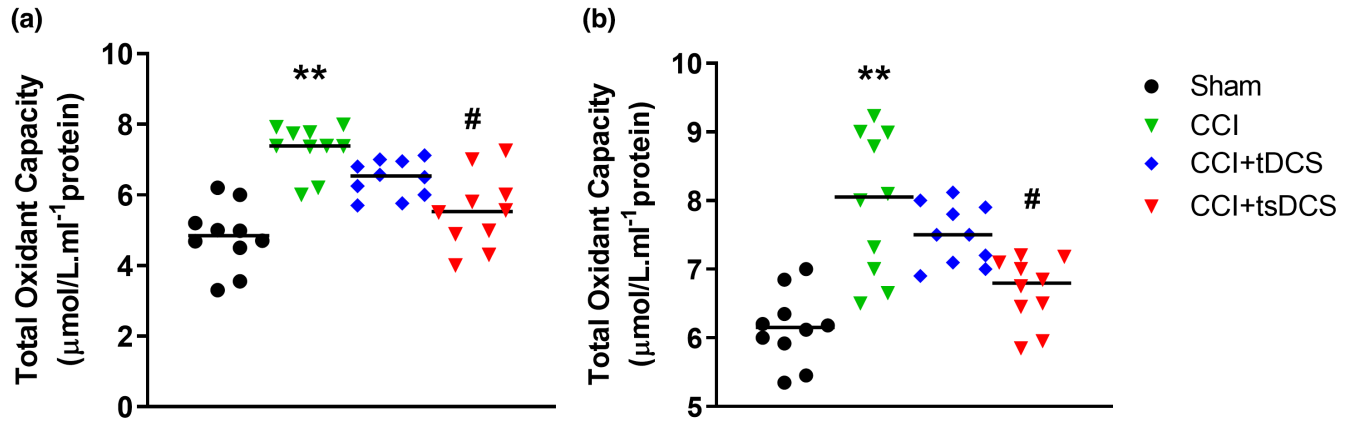

FIGURE 6 Total oxidant capacity (TOC) results. (a) TOC results in cerebral cortex, (b) TOC results in spinal cord. ($n = 10$, for each group; $^{**}p < .01$ shows the difference compared to the Sham group, $\#p < .05$ shows the difference compared to the CCI group, one-way ANOVA test, followed by Tukey post hoc test). All data are presented as means ± *SEM*.

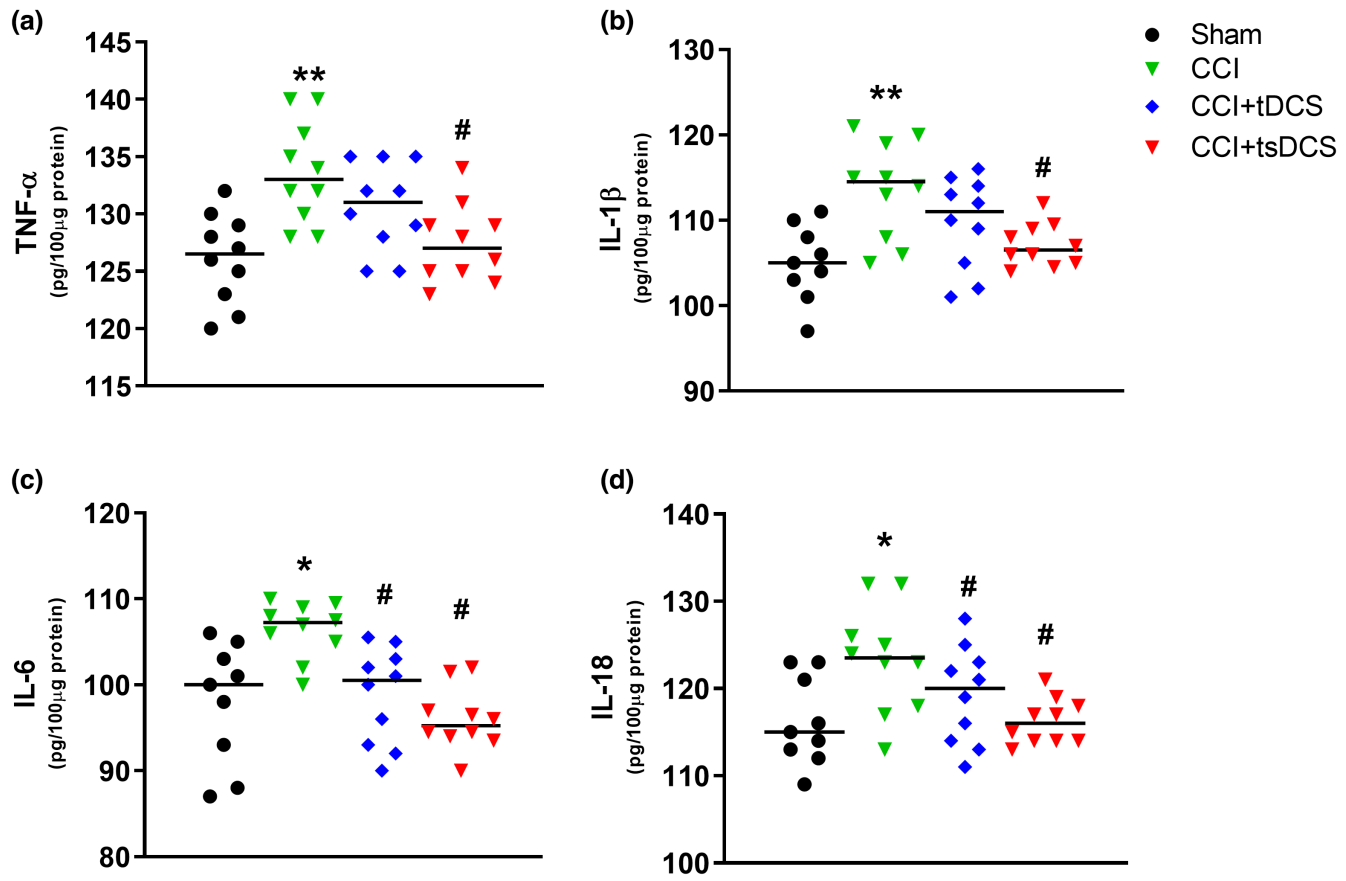

FIGURE 7 Neuroinflammation results in cerebral cortex. (a) TNF-α levels, (b) IL-1β levels, (c) IL-6 levels, (d) IL-18 levels. ($n = 10$, for each group; $^{*}p < .05$, $^{**}p < .01$ shows the difference compared to the Sham group, $\#p < .05$ shows the difference compared to the CCI group, one-way ANOVA test, followed by Tukey post hoc test). All data are presented as means ± *SEM*.

4 | DISCUSSION

This study reported the novel finding that tsDCS treatment alleviates pain behaviors in CCI rats by reducing oxidative status and proinflammatory cytokine production in cortex and spinal cord tissues. The possible role of tsDCS treatment in oxidative status and inflammatory markers was reported in vivo for the first time with the CCI model of neuropathic pain. Clinical management of neuropathic pain is still challenging because of its long-lasting nature and refractory to drug treatments (Wen et al., 2017). Non-invasive brain stimulation, including tDCS and tsDCS, is increasingly drawing attention as a potential therapy for chronic pain status (Li et al., 2022). Clinical

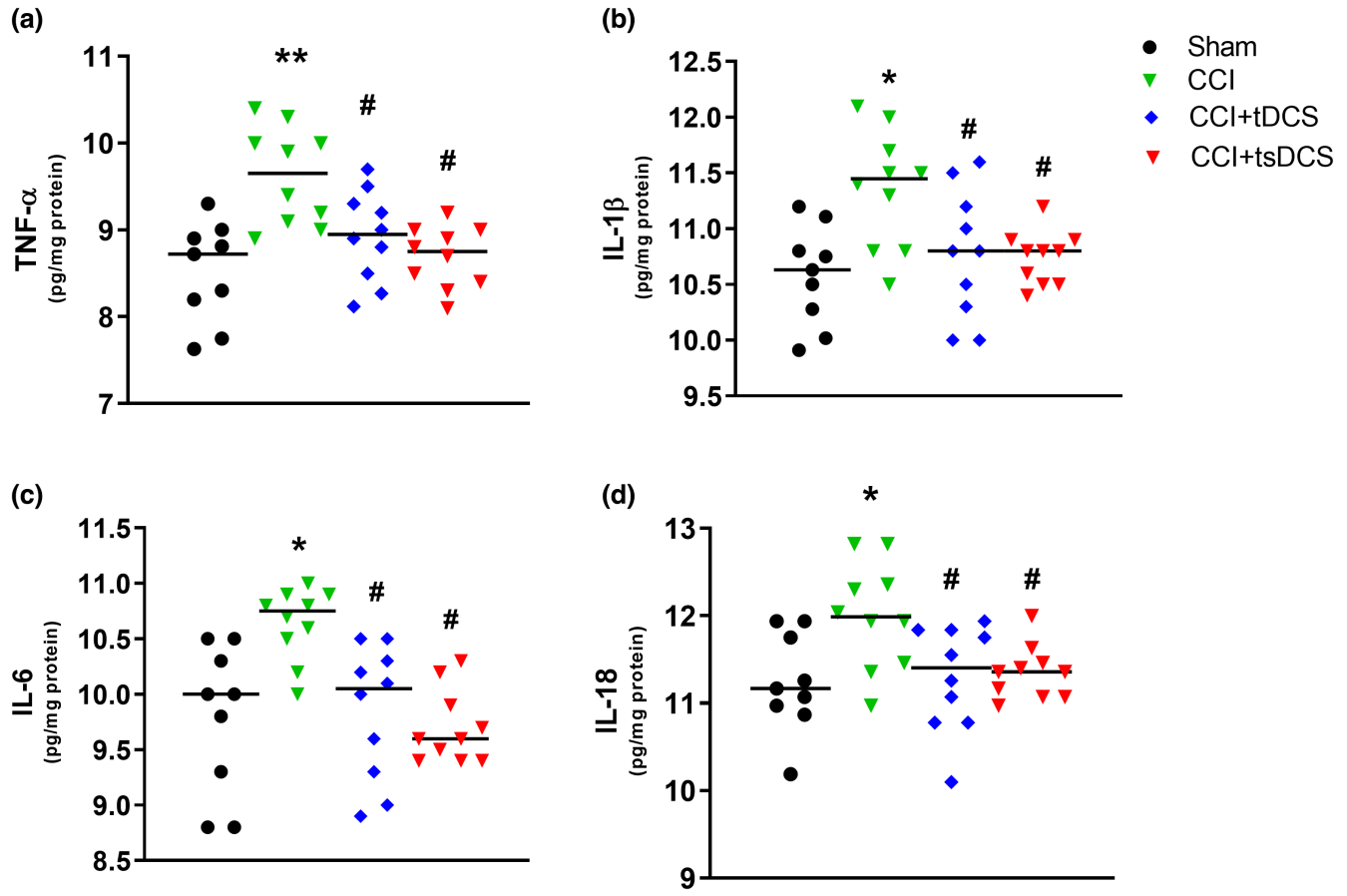

FIGURE 8 Neuroinflammation results in spinal cord. (a) TNF-α levels, (b) IL-1β levels, (c) IL-6 levels, (d) IL-18 levels. ($n = 10$, for each group; $^{*}p < .05$, $^{**}p < .01$ shows the difference compared to the Sham group, $\#p < .05$ shows the difference compared to the CCI group, one-way ANOVA test, followed by Tukey post hoc test). All data are presented as means ± *SEM*.

studies showed that tDCS and tsDCS modulate central nervous system excitability and plasticity (Monte-Silva et al., 2013; Nitsche & Paulus, 2000). tDCS is well known to modulate cerebral cortex activity by changing cortical excitability (Cogiamanian et al., 2012). It has the potential to find a role in the treatment of various neurological and psychiatric disorders (Flöel, 2014). On the other hand, using tsDCS to modulate spinal cord function has not yet gained as much attention as tDCS. However, direct current stimulation over the spinal cord may modulate supraspinal activities because of the complex projections between the brain and spine (Cogiamanian et al., 2012). Invasive electrical SCS has been used for over 30 years to treat chronic pain conditions (Mailis-Gagnon et al., 2004). Non-invasive tsDCS was used in most of the studies and was shown to suppress muscle activity (Meyer-Frießem et al., 2015; Perrotta et al., 2016; Truini et al., 2011), and cathodal tsDCS was shown to facilitate motor evoked potentials by motor cortex stimulation (Yamaguchi et al., 2020). There is no single protocol for tsDCS treatment. The stimulation intensity and polarity, placing electrodes in proper positions, and selecting target stimulation may affect the treatment outcomes (Williams et al., 2022). Modulating the spinal pathway by direct current stimulation over the thoracic and cervical spinal cord suggests tsDCS's effect on somatosensory, motor, and nociceptive spinal cortices (Aguilar et al., 2011; Ahmed, 2011; Ahmed & Wieraszko, 2012). During tsDCS, current flows between two electrodes, passing through the brain to complete the circuit (Thair et al., 2017). In our study, we choose the cathodal tsDCS treatment because anodal stimulation depolarizes the neurons thus increasing cortical excitability, whereas cathodal stimulation hyperpolarizes neurons thus decreasing cortical excitability (Thair et al., 2017). Although tDCS appears to be a promising tool for the treatment of chronic pain conditions, its mechanism of action and parameters or stimulation are still uncertain and needed to be clarified with basic research (Dedoncker et al., 2016) Similar to tDCS, the physiological mechanisms underlying the effects of tsDCS still need to be completely understood (Fava de Lima et al., 2022). This study investigated the antinociceptive effects and the mechanism of actions of tDCS and tsDCS in CCI-induced neuropathic pain behavior. Our findings showed that tsDCS stimulation on the cortex or spinal cord relieves mechanical and thermal hyperalgesia of CCI rats by reducing oxidative stress and inflammation.

We chose CCI model to induce neuropathic pain in rats, because it is a reliable and easily reproducible model. This model finely imitates traumatic mechanical injury in humans and demonstrates many pathophysiological characteristics of chronic neuropathic pain

(Austin et al., 2012). Following the CCI surgery in our study, the rats showed abnormal posture and the licking phenomenon of injured hind legs. These pain behaviors were similar to clinical neuropathic symptoms arising from nerve injury in chronic pain patients (Cheng et al., 2021). Because pain behaviors are evident from day 3 to day 14 after CCI and peaked at day 7 (Cheng et al., 2021), we assessed the nociceptive behaviors using a time course (7 and 14 days after the surgical procedure) (Cioato et al., 2016). We determined that mechanical and thermal hyperalgesia were developed on the 7th day in CCI rats as shown in hot-plate, tail-flick and Randall–Selitto tests. These findings are consistent with previous studies showing increased nociceptive behaviors of sciatic nerve injury in rats following CCI (Chen et al., 2018; Cheng et al., 2021; Wen et al., 2021). In our study, tDCS treatment provided slight improvements in nociceptive behaviors of CCI rats; however, these improvements were significant with tsDCS Although Lopes et al. (2020) reported that tDCS less effectively abolished the thermal hyperalgesia induced by CCI than mechanical hyperalgesia, our results showed that tDCS were less effective in abolishing both thermal and mechanical hyperalgesia. Decreased locomotor activity is also characteristic of persistent neuropathic pain mode (Grégoire et al., 2012). In our study, tsDCS improved locomotor activity better than tDCS. This effect may be related to the impact of tsDCS on the motor unit. Schlaier et al. (2007) showed in their study that invasive SCS was effective in intracortical facilitation in neuropathic pain patients suggesting increased recruitment of cortical motor neurons. SCS was also effective in restoring locomotion in animal models of Parkinson's disease (Fuentes et al., 2009). Parallel with those studies and our data, Bocci et al. (2014) showed that cathodal tsDCS improves motor unit recruitment by making motoneurons more responsive to synaptic activation. Recently, Kamali et al. (2023) showed that cathodal tsDCS improved motor function potentially through the effect of tsDCS over the spinal interneurons.

Oxidative stress and proinflammatory cytokines play crucial roles in the development and maintenance of NP (Haranishi et al., 2022). Release of free radicals and production of ROS in nerve and spinal cord after peripheral nerve injury involve the generation of nociceptive behaviors (Kim et al., 2004). Studies have shown that treatments that reduce oxidative stress relieves hyperalgesia and allodynia in NP models (Abbaszadeh et al., 2018; Komirishetty et al., 2016). Caillaud et al. (2018) reported that curcumin treatment decreased the production of ROS and increased antioxidant capacity in sciatic nerve damage (Caillaud et al., 2018). Di Naso et al. also indicated that the increased production of oxidants or decreased endogenous antioxidant activity markedly affects the diabetic neuropathy (Di Naso et al., 2011). Moreover, Safakhah et al. reported that forced exercise attenuates neuropathic pain in CCI rats by increasing TAC (Safakhah et al., 2017). Zhang et al. reported that isoorientin, natural flavonoid-like compound, treatment significantly improved hyperalgesia and allodynia and increased sensory nerve conduction velocities in mice with sciatic nerve injury. In addition, they showed that antioxidant parameters significantly increased TAC, superoxide dismutase and catalase levels, while decreasing malondialdehyde concentrations, which are oxidative stress markers (Zhang et al., 2019). Although tDCS was shown to ameliorate cognitive impairment via modulating oxidative stress and inflammation in models of dementia, ischemia and Parkinson's disease (Guo et al., 2020; Kaviannejad et al., 2022; Leffa et al., 2018), there is only one recent study, probably carried out at the same time as our research showing its effect on the oxidative parameter in the CCI model of neuropathic pain (Centeno Crespo et al., 2023). They reported that tDCS increases the pain threshold of CCI. Moreover, they observed tDCS treatment did not modify reactive oxygen species levels nitrite levels were decreased with tDCS treatment, whereas reactive oxygen species and thiobarbituric acid-reactive substances levels and superoxide dismutase and catalase activities remained unaltered. On the other hand, total sulfhydryl content increased with tDCS treatment in CCI rats suggesting the degradation of molecules maintaining hyperexcitability and hyperalgesia caused by the lesion (Centeno Crespo et al., 2023) In our study, we analyzed different parameters of oxidative status than Centeno Crespo et al. 2023 We here report that there was no significant treatment effect with tDCS while tsDCS provided a significant increase in total antioxidant capacity and decrease in total oxidant capacity both cortex and spinal cord tissues. This is one of the first reports revealing the effect of tsDCS over oxidative stress parameters in pathological conditions. Various studies using tDCS to treat chronic pain (Antal et al., 2010; Young et al., 2020) revealed the possibele mechanism of action of tDCS as modulation of neurotransmitters, receptor and ion channels seems to contribute its analgesic effects (Lopes et al., 2020). We may speculated that tsDCS might exert similar roles in alleviateting chronic pain behaviors by modulating the corticomotoneuronal synaps.

Neuropathic pain is correlated with the glial cell activation and release of proinflammatory cytokine secretion in the spinal dorsal horn (Miao & Wang, 2020). TNF-α is well-known and one of the most potent proinflammatory cytokines be expressed by microglia, astrocytes and primary sensory dorsal root ganglion neurons (Ji et al., 2014). IL-1β is another crucial inflammatory cytokine expressed by both microglia and astrocytes in the spinal cord (Ji et al., 2014). Studies showed that TNF-α and IL-1β induce neuropathic pain in rats (Zelenka et al., 2005) and anticytokine therapy targeted downstream signaling molecules may be promising in neuropathic pain management (Schafers & Sommer, 2007). Moreover, it is known that inflammatory changes in macrophages leads to secretion of IL-18 as well as IL-1β in both the central nervous system and the site of injury (peripheral nervous system) following CCI (Vasudeva et al., 2015; Zhang et al., 2013). Cheng et al. showed that CCI damage increased IL-1β and IL-18 in spinal cord and also decreased paw withdrawal latency and paw withdrawal threshold. They also reported that loganin, an iridoid glycoside, treatment reduced IL-1β and IL-18 in spinal cord (Cheng et al., 2021). Similarly, Wen et al. found that CCI damage significantly decreased paw withdrawal latency and paw withdrawal threshold on the 7th and 14th days, while TNF-α, IL-1β and IL-6 levels in the spinal cord increased significantly (Wen et al., 2021).

tDCS treatment is known to induce modulation of central cytokine levels. Ciaoto et al. showed that 0.5 mA 20 min cathodal tDCS

following CCI surgery reduced nociceptive behavior in the CCI model and was also effective in modulating the central cytokine levels (Cioato et al., 2016). They also reported that central nervous system stimulation plays an important role in the management of neuropathic pain (Cioato et al., 2016). In their study, Lopes et al. treated CCI rats with 0.5 mA cathodal tDCS brain stimulation for 20 min for 8 days (Lopes et al., 2020). They reported that tDCS treatment reduced mechanical and thermal hyperalgesia by modulating peripheral and central cytokine levels (Lopes et al., 2020). Consistent with those reports, our study showed CCI damage significantly increased TNF-α, IL-1β, IL-6 and IL-18 levels in the cerebral cortex and spinal cord. tDCS and tsDCS applied for 7 days reduced TNF-α, IL-1β, IL-6 and IL-18 levels in spinal cord tissue of CCI rats. On the other hand, in the cerebral cortex, tDCS treatment only slightly reduced TNF-α and IL-1β levels whereas peripherally applied tsDCS did it significantly. Still, both tDCS and tsDCS treatments reduced the IL-6 and IL-18 levels markedly in that tissue McCarthy and McCrory (2014) reported significant reductions in IL-6, TNFα, and vascular endothelial growth factor (VEGF) in CSF of neuropathic pain patients after invasive SCS. Tao et al. (2021) showed that SCS might relieve neuropathic pain behaviors by interfering neuroinflammation and reducing the IL-1β levels in CSF and serum of rats with spared nerve injury. Sun et al. (2022) observed pain relief in CCI rats with SCS by attenuating microglial activation. SCS-mediated pain relief is thought to act through modulation of the maladaptive aggregate response to local injury, neuroinflammation and central sensitization at both the segmental and supraspinal levels (Caylor et al., 2019). It is clear that spinal neuromodulation seems promising for the management of pain, and tsDCS, a non-invasive method to active neural circuits in the human spinal cord, may have the potential to be a complementary therapy in chronic pain management. Our results show that tsDCS treatment may be more effective than tDCS treatment due to its ability to modulate supraspinal or cortical network and block pain signals from transmitting to the brain alongside its local anti-inflammatory effects in the spinal cord. Our study has some limitations. We only used male rats, not females. It is known that sex differences are important contributors to pain sensitivity and analgesic efficacy of treatments. Another important limitation is the absence of a group of anodal tDCS or tsDCS stimulation. Anodal or cathodal stimulation may change the effectiveness of the treatment. Additionally, the lack of a control group of the tDCS or tsDCS stimulation seems like a limitation. However, our previous reports revealed no difference between control, tDCS or tsDCS treatment-only groups. Therefore, this group was not included in our study to avoid excessive use of animals considering the 3R rules (Replacement, Reduction, Refinement)..

5 | CONCLUSION

The findings showed that 7-day tsDCS stimulation attenuated nociceptive behavior, reducing thermal and mechanical hyperalgesia caused by sciatic nerve injury. tDCS and tsDCS stimulations showed therapeutic effect on neuropathic pain by regulating oxidant and antioxidant levels. In our study, tsDCS stimulation over T4-L10 altered nociceptive behaviors more significantly. With this aspect, we can conclude that peripheral stimulation via tsDCS treatment may be a better alternative to inhibit the neuropathic pain behaviors in CCImodel rather than tDCS treatment. Optimized DCS strategies are needed to be used alone or in combination with other effective treatments for alleviating neuropathic pain in future.

In conclusion, it can be said that tDCS is a potential method that can be used alone or in combination with other effective treatments for alleviating neuropathic pain.

DECLARATION OF TRANSPARENCY

The authors, reviewers and editors affirm that in accordance to the policies set by the *Journal of Neuroscience Research*, this manuscript presents an accurate and transparent account of the study being reported and that all critical details describing the methods and results are present.

AUTHOR CONTRIBUTIONS

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Conceptualization*, G.A. and D.N.S.; *Methodology*, G.A.; N.D. and D.N.S.; *Investigation*, G.A. and D.N.S.; *Formal Analysis*, G.A. and D.N.S.; *Resources*: G.A. and D.N.S.; *Writing – Original Draft*, G.A. and D.N.S.; *Writing – Review & Editing*, G.A. and D.N.S.; *Visualization*, G.A. and D.N.S.; *Supervision*, G.A. and D.N.S.; *Funding Acquisition*, G.A.

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CONFLICT OF INTEREST STATEMENT

Authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

All supporting data are included within the main article. For the original data, please contact the corresponding author.

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SUPPORTING INFORMATION

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Figure S1.

Transparent Science Questionnaire for Authors

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