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Long-Lasting Effect of Transcranial Direct Current Stimulation in the Reversal of Hyperalgesia and Cytokine Alterations Induced by the Neuropathic Pain Model

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ABSTRACT

Background: Neuropathic pain (NP) is caused by an insult or dysfunction in the peripheral or central nervous system (CNS), the main symptoms being mechanical allodynia and hyperalgesia. NP often shows insufficient response to classic analgesics and its management remains a challenge. Transcranial direct current stimulation (tDCS) is a non-invasive method of cerebral stimulation and represents a promising resource for pain management.
Objective/hypothesis: We investigated the effects of tDCS on the nociceptive response and on IL-1β, IL-10, and TNF-α levels in CNS structures of rats with NP.
Methods: After induction of NP by chronic constriction injury (CCI) of the sciatic nerve, the rats received 20 min of bicephalic tDCS for 8 days. Hyperalgesia was assessed by the hot plate and von Frey tests and evaluated at baseline, 7 days, and 14 days after CCI surgery, and also immediately, 24 hours, and 7 days following tDCS treatment. The levels of IL-1β, IL-10 and TNF-α in the cortex, spinal cord, and brainstem were determined by ELISA at 48 hours and 7 days post-tDCS.
Results: The CCI model provoked thermal and mechanical hyperalgesia until at least 30 days post-CCI; however, bicephalic tDCS relieved the nociceptive behavior for up to 7 days after treatment completion.
Conclusions: Bicephalic tDCS is effective to promote antinociceptive behavior in neuropathic pain, which can be reflected by a spinal neuroimmunomodulation linked to pro- and anti-inflammatory cytokine levels observed in the long-term.

Introduction

Neuropathic pain (NP) arising from peripheral and central nerve damage and constitutes a significant clinical problem, which is often severely debilitating and largely resistant to treatment, partly due to the fact that its mechanisms are insufficiently understood [1]. The prevalence of NP is between 6.9% and 10% depending on the related dysfunction [2,3]. Its main symptoms may include both hypersensitivity types, thermal and mechanical hyperalgesia, defined as increased pain sensitivity to a thermal or mechanical nociceptive stimulus, respectively [4].

NP shows changes at different levels of the nervous system by peripheral and central sensitization [5–7]. The most prominent characteristic of the NP process is the combination of sensory loss due to damage in the transmission pathways and the development of adaptive and maladaptive neuroplastic changes in both the peripheral

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and central nervous system (CNS) [8,9]. Adaptive responses include stimulation of axonal growth, sprouting, and synaptic remodeling, and others [10,11]. On the other hand, the maladaptive process is involved with reduced threshold response of nociceptive neurons, ectopic impulse generation and reduced inhibition [9]. In addition, it has been described the crucial role of glutamate ligation to N-methyl-D-aspartate (NMDA) receptors and the depolarization of postsynaptic cell in chronic pain states, which are indispensable for the initiation of a long-lasting highly localized increase in synaptic strength, the long-term potentiation (LTP) [12,13]. Moreover, there is increasing evidence of neuroimmune modulators involvement in the initiation and the maintenance of pain states in the peripheral and in the central nervous system. Previous studies using neuropathic pain model showed increased levels interleukin-1 beta (IL-1β) and reduced expression of tumor necrosis factor alpha (TNF-α) at long lasting time in spinal cord, contributing to NP by nociceptive neuron activation [9,14].

Non-neuronal and neural-plastic changes involved in the nociceptive process and the limited efficacy of pharmacological approach in neuropathic pain states justify the need for an assessment of central nervous system-based non-invasive therapeutic tools as an adjuvant for pain treatment [8,15]. Also, recent studies suggest functional reorganization and hyper-excitability of the somatosensory and motor cortex are presented in neuropathic pain process [16,17]; thus neuromodulatory techniques can be useful to minimize these changes. In this context, a technique that non-focally modulates plastic changes induced by pain-related neural networks, such as transcranial direct current stimulation (tDCS), may have significant therapeutic effects. This technique modulates neural activity via weak electrical currents that, when applied as a direct current component, polarize neural tissue, thereby inducing significant changes in the resting membrane potential threshold [18] and subsequent modifications in synaptic plasticity [19–21]. The rationale use of tDCS in the reduction of pain is supported by the activation the descending inhibitory system [22]. The tDCS technique modulates different circuits, such as cortico-striatal and thalamocortical which is dependent from the type of stimulation; anodal tDCS promoted depolarization, while cathodal promoted hyperpolarization [20,23].

In addition, tDCS not only alters the activity of cortical areas located directly under the electrodes, but also in remote areas probably due to primary interconnections between the stimulated area and other brain structures [24]. This method of noninvasive brain modulation has shown significant results in different types of chronic pain in humans [25–27]. Furthermore, the neuromodulatory effects of tDCS have also been consistently demonstrated in animals, such as in rat models of focal epilepsy [28], memory [29], Parkinson's disease [30], and acute stroke [31]. Previous studies with rat models by our research group showed both immediate and long-lasting effects after repeated sessions of bicephalic tDCS on chronic inflammation [32] and hyperalgesia induced by chronic restraint stress models [33]. Considering the possible benefits of tDCS on pain management, we investigated the effects of tDCS on thermal and mechanical hyperalgesia induced by a NP model as well as its effect on central IL-1β, IL-10, and TNF-α levels.

Methods

Animals

A total of 84 adult male Wistar rats (55–65 days old; weight 200–250 g) were used. The animals were randomized by weight and housed in groups of three per polypropylene cage (49 cm × 34 cm × 16 cm) with sawdust-covered flooring. All animals were maintained in a controlled environment (22 ± 2 °C) under a standard light–dark cycle (lights-on at 7 a.m. and lights-off at 7 p.m.), with water and chow (Nuvital, Porto Alegre, Brazil) *ad libitum.* All experiments and procedures were approved by the institutional Animal Care and Use Committee (GPPG-HCPA protocol no. 120512) and performed in accordance with the *Guide for the Care and Use of Laboratory Animals*, 8th ed. The experimental protocol complied with the ethical and methodological standards of the ARRIVE guidelines [34]. The experiment used the number of animals necessary to produce reliable scientific data.

Experimental design

The rats were acclimated to the maintenance room for 1 week before the experiment began. The animals were divided into the following seven groups: control (CT), sham neuropathic pain (SN), sham neuropathic pain plus sham tDCS treatment (SNS), sham neuropathic pain plus tDCS treatment (SNT), neuropathic pain model (NP), neuropathic pain plus sham tDCS treatment (NPS), and neuropathic pain plus tDCS treatment (NPT). After the establishment of NP as assessed by nociceptive tests, the animals underwent a daily tDCS session for eight consecutive days. Nociceptive tests (von Frey and hot plate tests) were performed at baseline, 7 days, and 14 days after the CCI surgical procedure, and immediately after the last session of tDCS (or 22 days post-surgery), 24 hours after the last session of tDCS (or 23 days post surgery), and 7 days after the last session of tDCS (or 29 days post surgery). The rats were killed by decapitation 48 hours or 7 days after treatment completion. For all procedures (nociceptive tests and biochemical assays), the experimenter was blinded to the group of rats being tested.

Neuropathic pain model

The chronic constriction injury (CCI) of the sciatic nerve described by Bennett and Xie (modified) [35] was used as a model for the induction of NP. The rats were anesthetized with isoflurane (5% for induction, 2.5% for maintenance) and placed in the dorsal position for left thigh hair shaving and skin antisepsis with 2% iodine–alcohol. After skin incision in the middle third of the left hind limb to expose the biceps femoris muscle, the common sciatic nerve was exposed and three ligatures were tied (Vycril4.0) around it at 1 mm intervals; thus, the total length of nerve involved was approximately 5 mm. The ligatures reduced the diameter of the nerve by a barely noticeable amount without arresting epineurial blood flow. To ensure equal level of constriction, the same investigator performed the ligatures on all rats. Finally, the skin was sutured using Mononylon 4.0. For sham surgery, the sciatic nerve was exposed similarly to the CCI model but not ligated. Following surgery and anesthetic recovery, the animals were returned to their cages, where they remained until the day of death. The control group did not undergo the surgical procedure.

tDCS

After the establishment of NP, the animals in the real treatment groups underwent a 20-minute session of bicephalic tDCS every afternoon for 8 days, as described by Adachi et al. [36]. A constant direct current of 0.5 mA was delivered from a battery-powered stimulator using electrocardiogram electrodes with conductive adhesive hydrogel. The rats' heads were shaved for firmer adherence and the electrodes were trimmed to 1.5 cm^2 for better fit. The electrodes were fixed to the head with adhesive tape (Micropore™) and covered with a protective mesh to prevent removal.

The cathode was positioned at the midpoint between the lateral angles of both eyes (supraorbital area) and the anode was placed on the head using landmarks of the neck and shoulder lines as a