Brazilian Journal of Anesthesiology 2023;73(4): 514—518

Sociedade Brasileira de Anestesiologia

Brazilian Journal of ANESTHESIOLOGY

SHORT COMMUNICATION

Transcranial Direct Current Stimulation (tDCS) antinociceptive effect is not altered by isoflurane anesthesia in neuropathic pain rats

Lisiane Santos da Silva[a,b], Roberta Ströher Toledo[a], Dirson João Stein[a,b,c], Josimar Macedo de Castro [a,b,c], Wolnei Caumo[b,c], Iraci L.S. Torres [a,b,c,*]

[a] *Hospital de Clínicas de Porto Alegre, Laboratório de Farmacologia e Neuromodulação da Dor: Investigações Pré-clínicas, Porto Alegre, RS, Brazil*
[b] *Universidade Federal do Rio Grande do Sul, Faculdade de Medicina, Programa de Pós-Graduação em Medicina: Ciências Médicas, Porto Alegre, RS, Brazil*
[c] *Hospital de Clínicas de Porto Alegre, Núcleo Translacional: Farmacologia da Dor e Neuromodulação, Porto Alegre, RS, Brazil*

Received 12 September 2022; accepted 7 March 2023
Available online 14 March 2023

KEYWORDS
Neuropathic pain;
Isoflurane;
tDCS;
von Frey Test;
IL-10

Noninvasive neuromodulation techniques are on the rise as adjuvant alternatives for the treatment of chronic pain. In this way, Transcranial Direct Current Stimulation (tDCS) has been demonstrated as a viable non-pharmacological method for the treatment of Neuropathic Pain (NP) in humans. In a recent review, Caumo and colleagues have shown evidence that motor cortex stimulation can bring benefits for the treatment of NP, associated or not with other therapies such as mirror therapy or visual illusion.[1] Additional preclinical research is therefore required to clarify its mechanisms of action, enabling it to become a successful and widely employed therapeutic option. However, tDCS application in awake animals requires animal immobilization, which is considered a stressful procedure[2] related to Stress-Induced Analgesia (SIA), mediated by the activation of the descending inhibitory pain pathway.[3] Furthermore, repeated exposure to stressful events, such as restraint, is related to hyperalgesia and allodynia, as a result of peripheral and central sensitization, which is linked to chronic pain.[4,5] In this way, our research hypothesis is that anesthesia during tDCS application is a reasonable option to improve the quality of rat preclinical neuromodulation assessments. It is important

Abbreviations: An, Anesthesia; BDNF, Brain-Derived Neurotrophic Factor; CCI, Chronic Constriction Injury; IL-10, Interleukin 10; NP, Neuropathic Pain; P, Pain; PFC, Prefrontal Cortex; SIA, Stress-Induced Analgesia; SP, Sham Pain; tDCS, Transcranial Direct Current Stimulation; TNF-a, TNF-alpha.

* Corresponding author.
E-mail: iltorres@hcpa.edu.br (I.L. Torres).

https://doi.org/10.1016/j.bjane.2023.03.002

to highlight that no previous study has evaluated the impact of anesthetics during the application of tDCS in rodents. Among the most commonly used compounds for non- and minimally invasive procedures in preclinical studies, isoflurane is an ideal inhalation anesthetic that produces mild induction, excellent muscle relaxation, and rapid recovery, which can be an alternative to alleviate the effects of stress during containment;[6] however, how it interferes with the expected results during brain stimulation has to be investigated. Additionally, it has been demonstrated that tDCS modulates central inflammatory levels, which could account for its antinociceptive effects.[7] Considering the absence of studies on this theme, this investigation aimed to evaluate the effects of isoflurane anesthesia during tDCS application on the nociceptive response and the Prefrontal Cortex (PFC) IL-10 levels in rats submitted to a neuropathic pain model.

Forty-eight male Wistar rats (50—60 days), from the Laboratory Animal Breeding and Experimentation Center (CREAL/Universidade Federal do Rio Grande do Sul — UFRGS), were kept under strictly controlled environmental conditions (3 animals/polypropylene cage, 12 h light/dark cycle, 22 ± 2°C, water, and rodent chow *ad libitum*). The animals (n = 48, a number based on the sample size used in previous studies[4,7] and calculated using G*Power software to obtain a minimum effect size of 15%, with an alpha error of 0.05 and power of 80%) were housed in groups of three in Microisolator cages (49 × 34 × 16 cm) with sawdust-covered floors. Cages were environmentally enriched using pieces of wood, ropes, cardboard rolls, and shredded paper, increasing the animals' well-being, motivating them to perform new behaviors, and reducing stress, anxiety, and frustration. All experiments and procedures were approved by the Institutional Committee for Animal Care and Use (GPPG-HCPA protocol #20200689) and conformed to the Guide for the Care and Use of Laboratory Animals (8th ed. 2011) and law #11.794, which establishes procedures for the scientific use of animals in Brazil. All experimental procedures were approved by the Institutional Committee for Animal Care and Use (GPPG/HCPA protocol #2018–0025). The experimental protocol complied with the ethical and methodological standards of the ARRIVE guidelines. All efforts were made to minimize animal suffering.

The current study had as primary outcome the nociceptive response, and as secondary outcome, the prefrontal cortex (PFC) IL-10 levels in rats submitted to a neuropathic pain model. The animals were habituated to the environment for fourteen days and afterward randomly (baseline von Frey data) allocated to one of the following groups: Sham pain (Sp, n = 24) and Pain (P, n = 24). On the following day, the animals were submitted to surgery for Chronic Constriction Injury (CCI) of the left sciatic nerve and NP model induction, as described by Bennett and Xie[8] with minor modifications. Animals from the Sp group were submitted to surgery without sciatic nerve constriction. Fourteen days after surgery, the establishment of NP was confirmed by the von Frey test. Then, rats were allocated to one of the eight following subgroups: Sham pain (Sp, n = 6); Sham pain + Sham tDCS (SpSt, n = 6), Sham pain + tDCS (Spt, n = 6), Sham pain + tDCS + Anesthesia (SptAn, n = 6); Pain (P); Pain + Sham tDCS (PSt, n = 6); Pain + tDCS (Pt, n = 6); and Pain + tDCS + Anesthesia (PtAn, n = 6). Afterward, the bicephalic tDCS treatment was initiated. tDCS was applied once a day for eight consecutive days for 20 minutes. The animals in the anesthesia groups were anesthetized with isoflurane (5% for induction and 2.5% for maintenance). Twenty-four hours after the end of treatment, the animals were again submitted to the behavioral test (von Frey) and were killed by decapitation the following day, having the PFC removed and stored at -80°C for later analysis. Biochemical assays were performed using Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) for quantification of the levels of the anti-inflammatory Interleukin 10 (IL-10), using specific monoclonal antibodies (R&D Systems, USA). Data were expressed in $pg.mg^{-1}$ of protein. The timeline of the experimental protocol is demonstrated in Supplementary Figure 1.

The Shapiro-Wilk test revealed that the variables did not have a normal distribution ($p \leq 0.05$), which was confirmed by histograms. Thus, for repeated measures, the Generalized Estimating Equation (GEE) test was adopted, adjusting the comparisons for the following factors: Time (baseline vs. D14 vs. D22), CCI (exposed vs. not exposed to the model), tDCS (tDCS vs. shamtDCS vs. no intervention), and interactions between factors (Time vs. CCI vs. tDCS). In case of significant interaction, experimental groups were independently evaluated using the Bonferroni test. For IL-10 levels, all groups were compared using the Kruskal-Wallis test. Data were expressed as a median and interquartile range, considering significant differences with $p \leq 0.05$, and were analyzed using SPSS 20.0 (SPSS Inc., Chicago, IL).

All animals completed the experiment, and none were excluded from the analysis. According to the GEE, there was no difference between groups at baseline (Fig. 1, Panels 1A and 1B, n = 24/group); Time ($\chi^2(2) = 311.618$, $p < 0.001$) and CCI ($\chi^2(1) = 78.718$, $p < 0.001$) affected the animal's nociceptive threshold (Fig. 1, Panels 1A and 1C). Besides, an interaction was observed between Time and CCI ($\chi^2(2) = 159.783$, $p < 0.001$); animals exposed to CCI (P) displayed reduced nociceptive threshold, 14 (Bonferroni: -24.5g, $p < 0.001$, Fig. 1, Panel 1A, n = 24/group, Panel 1C, n = 24/group) and 22 (Bonferroni: -8.42g, $p < 0.001$, Fig. 1, Panels 1A and 1D, n = 6/group) days after surgery, compared to Sham pain (Sp) animals. An interaction was also observed between Time and tDCS ($\chi^2(4) = 20.025$, $p < 0.001$); in the intervention period, tDCS increased the nociceptive threshold in 5.51 g when compared to shamtDCS (Bonferroni: $p < 0.033$), and in 10.1 g when compared to no intervention (Bonferroni: $p < 0.001$) groups (Sp and P groups).

The Kruskal-Wallis test revealed significant differences between groups regarding PFC IL-10 levels ($\chi^2(7$, n = 48) = 38.687, $p < 0.001$, Fig. 2). The pairwise comparison showed that Sp was different from P ($\chi^2(1$, n = 12) = 33.500, $p < 0.001$), PSt ($\chi^2(1$, n = 12) = 34.500, $p < 0.001$), and PtAn ($\chi^2(1$, n=12) = 26.500, $p < 0.05$). SpSt was different from P ($\chi^2(1$, n = 12) = 29.000, $p < 0.01$), and from PSt ($\chi^2(1$, n = 12) = 30.000, $p < 0.01$). Spt was different from P ($\chi^2(1$, n = 12) = 27.000, $p < 0.05$), and PSt ($\chi^2(1$, n = 12) = 28.000, $p < 0.05$). Thus, untreated pain animals showed a significant increase in IL-10 levels,which was reversed by tDCS treatment. On the other hand, the association of tDCS and anesthesia led to a partial reversal of these effects, since it is not equivalent to the Sham pain group.

This study demonstrates that the association of isoflurane anesthesia does not alter the complete reversion of the mechanical allodynia induced by repeated tDCS in rats

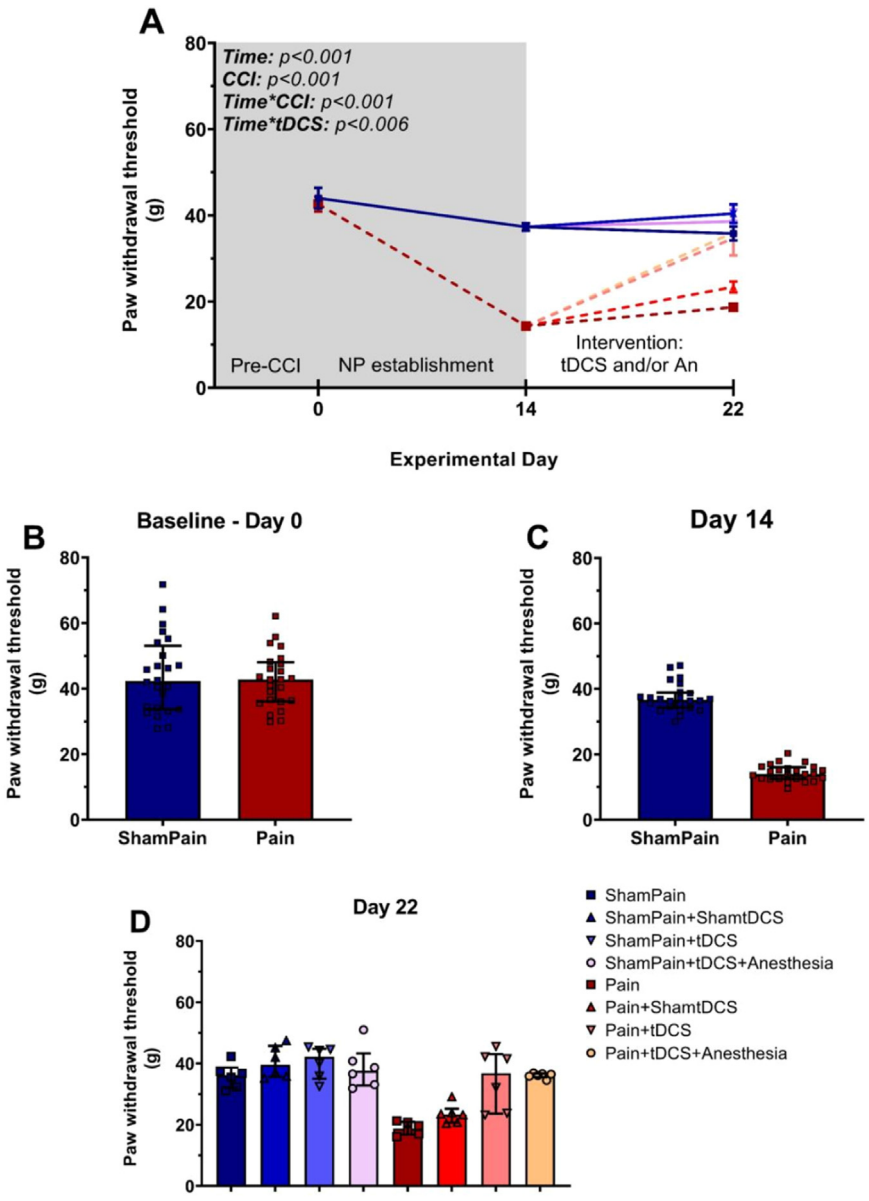

Figure 1 Data from the von Frey test. Panel A: von Frey test at baseline, 14 days after surgery and 24 hours after the end of treatment; Panel B: at baseline (D0), animals were allocated into two groups (Sham pain and Pain) with similar nociceptive thresholds; Panel C: fourteen days after surgery, animals allocated to the Pain group presented lower paw withdrawal threshold when compared to Sham operated rats; Panel D: on day twenty two, animals from the Pain group that received tDCS, showed restored nociceptive thresholds, regardless of having been anesthetized during treatment. An, Anesthesia; CCI, Chronic Constriction Injury; NP, Neuropathic Pain; Tdcs, Transcranial Direct Current Stimulation. Data are presented as mean ± SEM (n = 6/group).

submitted to the CCI model,[7] being pioneering in this field. A previous study from our group, using the same chronic pain model in restrained and awake animals during tDCS application showed partial or total reversal of mechanical allodynia.[7] Thus, this result is in agreement with our hypothesis that anesthesia during tDCS application is an option to improve the quality of rat preclinical neuromodulation assessments. In this way, isoflurane anesthesia provides more reliable preclinical studies in the search for the tDCS action mechanisms as an effective and widely used therapeutic technique.

Volatile anesthetic isoflurane appears to act on the lipid matrix of the neuronal cell membrane, increasing membrane fluidity and inhibiting signal transduction. In addition, it has been suggested that isoflurane suppresses pain activating a distinct population of GABAergic neurons in the central amygdala, which produces profound analgesia, including in a model of neuropathic pain.[6] Considering that tactile allodynia can be reduced, in part, by the descending modulation from higher areas, it is feasible to suggest that isoflurane potentiates the tDCS analgesic effect from neuromodulation of these areas, including the central

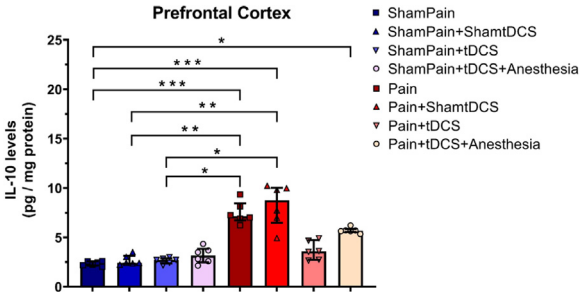

Figure 2 Data from the IL-10 levels in the PFC. Significant differences were observed between groups (Kruskal-Wallis test, $p < 0.001$). *$p < 0.05$; **$p < 0.01$; ***$p = 0.001$ (pairwise comparison). Data are expressed as a median and interquartile range of pg.mg^{-1} of protein (n = 6/group).

amygdala.[6] In addition, isoflurane promotes the release of GABA, increases glutamate reuptake, and alters cytokine levels, such as IL-6 and IL-10.[6]

Corroborating the isoflurane action altering the levels of cytokines, the present study showed that isoflurane associated with tDCS partially reverses the increase in the IL-10 levels induced by NP, attenuating the tDCS effect, which reversed this increase. In this way, it is possible to suggest that the analgesic effect of tDCS occurs through IL-10 level modulation in the PFC, since NP is also involved in chronic inflammatory processes.[9]

A previous study using a similar NP model (partial peripheral nerve ligation) showed a reduction in IL-10 levels in the dorsal root ganglion and an increase in the PFC, corroborating the involvement of the central nervous system in neuropathies.[9] The NP mechanism involves inflammation at the affected nerve, which initiates a cascade of events increasing and activating innate immune cells at the tissue injury site.[6] The release of immunoreactive markers such as cytokines promotes local actions resulting in a generalized immune response.[6] Previous studies have shown that the anti-inflammatory cytokine IL-10 plays a role in promoting the relief of the painful condition established by NP, by reducing the bioavailability of pro-inflammatory cytokines.[5] On the other hand, besides the classic anti-inflammatory role, IL-10 seems to be involved with the development of NP. Increased IL-10 expression and levels[10] were found after peripheral nerve injury for at least 6 weeks after NP model induction, corroborating the data of the current study. In addition, we showed that bicephalic tDCS decreases the nociceptive response and modulates PFC IL-10 levels, returning them to the levels of the sham groups. This effect was attenuated by isoflurane anesthesia.

Some limitations are important to consider in the present study: (i) Considering that in humans both electrodes are placed on the head during tDCS application, we set them in similar positions in animal models. However, the small head size of the rat contributes to bicephalic stimulation; (ii) The levels of IL-10 can be influenced by the time of brain collection. Animals were killed 48 hours after the last session of tDCS because the behavioral test at 24-hours was performed after the last session of treatment.

In summary, this study demonstrates that, even when animals are subjected to inhaled anesthetic during a brain stimulation procedure, they still benefit from its analgesic effects, even though isoflurane reduces the effects of tDCS in lowering IL-10 levels. Therefore, in the current study, we demonstrated that using an inhalation anesthetic could be a good option to avoid bias during repeated tDCS application in pain preclinical studies.

Register Number

GPPG/HCPA #2018-0025

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

This research was supported by the following Brazilian funding agencies: CNPq (Dr. ILS Torres; Dr. W Caumo); CAPES (88882.346191/2019-01, Dr. D. J. Stein); GPPG-HCPA (ILS Torres, Grant 2018—0025), FAPERGS (PRONEM 16/2551-0000249-5), CNPq — Universal (422866/2016-4). Projeto 0261/18 CHAMADA PÚBLICA MCTIC/FINEP/CT-INFRA 04/2018.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.bjane.2023.03.002.

References

1. Pacheco-Barrios K, Cardenas-Rojas A, Thibaut A, et al. Methods and strategies of tDCS for the treatment of pain: current status and future directions. Expert Rev Med Devices. 2020;17:879—98.
2. Torres ILS, Cucco SNS, Bassani M, et al. Long-lasting delayed hyperalgesia after chronic restraint stress in rats - effect of morphine administration. Neurosci Res. 2003;45:277—83.
3. Timmers I, Quaedflieg CWEM, Hsu C, Heathcote LC, Rovnaghi CR, Simons LE. The interaction between stress and chronic pain through the lens of threat learning. Neurosci Biobehav Rev. 2019;107:641—55.
4. Adachi LNS, Quevedo AS, Souza A, Scarabelot VL, Rozisky JR, de Oliveira C, et al. Exogenously induced brain activation regulates neuronal activity by top-down modulation: conceptualized model for electrical brain stimulation. Exp Brain Res. 2015;233:1377—89.
5. Vanderwall AG, Milligan ED. Cytokines in Pain: Harnessing Endogenous Anti-Inflammatory Signaling for Improved Pain Management. Front Immunol. 2019;10:3009.
6. Hua T, Chen B, Lu D, Sakurai K, Zhao S, Han BX, et al. General Anesthetics Activate a Potent Central Pain-Suppression Circuit in the Amygdala. Nat Neurosci. 2020;23:854—68.
7. Lopes BC, Medeiros LF, Souza VS, Cioato SG, Medeiros HR, Regner GG, et al. 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. Brain Stimul. 2020;13:774—82.
8. Bennett GJ, Xie YK. A Peripheral Mononeuropathy in Rat That Produces Disorders of Pain Sensation Like Those Seen in Man. Pain. 1988;34:87—107.
9. Hung AL, Lim M, Doshi TL. Targeting cytokines for treatment of neuropathic pain. Scand J Pain. 2017;17:287—93.
10. Okamoto K, Martin DP, Schmelzer JD, Mitsui T, Low PA. Pro- and anti-inflammatory cytokine gene expression in rat sciatic nerve chronic constriction injury model of neuropathic pain. Exp Neurol. 2001;169:386—91.