The profile of dexamethasone combined with transcranial direct current stimulation in rats submitted to an arthritis model

O perfil da dexametasona combinada com estimulação transcraniana por corrente contínua em ratos submetidos a um modelo de artrite

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ABSTRACT

BACKGROUND AND OBJECTIVES: To pursue safer and more effective treatments for rheumatoid arthritis, the effect of dexamethasone treatment (DEX, 0.25mg/kg) combined with transcranial direct current stimulation (tDCS) in the behavior and neurochemical parameters of arthritic rats was evaluated.

METHODS: Thirty-six Wistar rats were divided into four groups: control+DEX (CTRL+DEX), arthritis+DEX (RA+-DEX), arthritis+DEX+sham-tDCS (RA+DEX+sham-tDCS) and arthritis+DEX+tDCS (RA+DEX+tDCS). The arthritic model (RA) was induced by complete Freund's adjuvant (CFA) paw administration. Paw edema and mechanical allodynia were assessed by plethysmometer and von Frey apparatus, respectively. Fourteen days after the CFA injection, rats received the treatment for eight days (DEX and/or tDCS). Behavioral parameters were measured with the Open-Field test. ELISA was used to evaluate hippocampal and spinal cord tumor necrosis factor (TNF-α) levels, cerebral cortex and brainstem BDNF levels.

RESULTS: In pre-treatment measurements, arthritic rats presented an increase in joint swelling and mechanical allodynia when compared to the control group, confirming chronic pain establishment. A slight antinociceptive effect of dexamethasone combined with tDCS in the pain model was observed. The pain model significantly induced an increase in the grooming behavior and a reduction in the spinal cord and hippocampal TNF-α levels; these effects were reverted in the sham- and active-tDCS--treated rats. However, no effects of DEX or tDCS were observed in the BDNF levels in the cerebral cortex and brainstem.

CONCLUSION: Despite the small effect observed, tDCS treatment cannot be discarded as a non-pharmacological adjuvant technique for inflammatory chronic pain treatment.

Keywords: Cerebral cortex, Hippocampus, Mechanical allodynia, Spinal cord.

RESUMO

JUSTIFICATIVA E OBJETIVOS: Para investigar métodos mais seguros e eficazes para o manejo da artrite reumatoide, avaliou-se o efeito do tratamento com dexametasona (DEX, 0,25 mg/kg) combinado com estimulação transcraniana por corrente contínua (ETCC) sobre parâmetros comportamentais e bioquímicos de ratos submetidos a um modelo de artrite reumatoide.

MÉTODOS: 36 ratos Wistar foram alocados em 4 grupos: controle+DEX (CTRL+DEX), artrite+DEX (AR+DEX), artrite+DEX+sham-ETCC (AR+DEX+sham-ETCC) e artrite+-DEX+ETCC (AR+DEX+ETCC). O modelo de artrite foi induzido pela administração de *complete Freund's adjuvant* (CFA) na pata. Edema na pata e a alodinia mecânica foram avaliadas por pletismômetro e teste de von Frey, respectivamente. 14 dias após injeção de CFA, ratos foram tratados por 8 dias (DEX e/ou ETCC). Atividade locomotora foi avaliada pelo teste do campo aberto. TNF-alfa (hipocampo e medula espinal) e BDNF (córtex e tronco) foram mensurados por ELISA.

RESULTADOS: Nas medições pré-tratamento, ratos com artrite exibiram um aumento de o inchaço articular e alodínia mecânica comparados ao grupo controle, confirmando o estabelecimento de modelo de dor crônica. Também se observou discreto efeito antinociceptivo da dexametasona combinada com ETCC no modelo de artrite. O modelo de dor induziu um aumento no comportamento de *grooming* e reduziu os níveis de TNF-alfa no hipocam-

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po; estes efeitos foram revertidos nos grupos *sham*- e ETCC ativo. Entretanto, não foram observados efeitos da DEX ou ETCC nos níveis de BDNF no córtex cerebral ou no tronco encefálico.

CONCLUSÃO: Apesar dos discretos efeitos observados, não se pode descartar a ETCC como uma abordagem terapêutica não farmacológica para o manejo da dor crônica inflamatória na artrite reumatoide.

Descritores: Alodínia, Córtex cerebral, Hipocampo, Medula espinal.

INTRODUCTION

Rheumatoid arthritis (RA) is associated with chronic pain characterized by maladaptive plasticity that is regulated by numerous modulatory mechanisms. These include effects at the nociceptor level, sympathetically mediated pain, the "wind-up" phenomenon, central sensitization, and changes in descending and ascending central modulatory mechanisms for the perception of pain[1]. Pro-inflammatory cytokines, e.g. tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-1 and IL-6, are increased in the synovium and synovial fluid in RA and play a pivotal role in RA's pathology[2,3].

Conventional anti-arthritic therapy comprises the use of steroidal and non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs (DMARD), and anti-cytokine drugs[4]. The efficacy of steroidal anti-inflammatory drugs in alleviating inflammatory disorders results from the pleiotropic effects of the glucocorticoid receptor on multiple signaling pathways that inhibit the synthesis of cytokines and inflammatory mediators[5]. Despite the efficacy of steroidal anti-inflammatory drugs, adverse effects such as growth retardation in children, immunosuppression, hypertension, inhibition of wound repair, osteoporosis and metabolic disturbances contraindicate prolonged therapy.

RA treatments that can modulate innate and adaptive immune cells are crucial to avoid pain and chronic inflammatory processes[6]. Preclinical and clinical studies have shown that both non-invasive stimulation[7-10] and invasive techniques, such as vagus nerve stimulation (VNS), are associated with an improvement in pain relief and an inflammatory modification profile in RA[11,12]. Transcranial direct current stimulation (tDCS) is a non-invasive technique that alters brain physiology as well as psychological, motor, and behavioral processes, and clinical symptoms in neurological and psychiatric diseases[13]. tDCS modulates the motor cortex, which is thought to influence pain perception indirectly via neural networks in pain-modulating areas, probably increasing cortical excitability[14]. Furthermore, tDCS not only changes neuronal activity in the desired cortical areas but also in distant ones, well beyond the sole change of neuronal excitability[15]. The tDCS effects are based on cortico-cortical interactions with some subcortical components (e.g., thalamic nuclei) in these circuits[16]. In humans, tDCS has shown significant effects on different types of chronic pain[17,18].

A previous study from the present authors showed long-lasting antinociceptive and neuroplastic effects of tDCS in a model of peripheral inflammation[8], supporting the use of the technique in conditions involving chronic pain and peripheral inflammation, such as RA. Another study also showed that tDCS in anesthetized rats modulated neuro-inflammation[19]. Besides that, it was demonstrated that tDCS has been effective in other pain preclinical models such as neuropathic pain[9,10], chronic restraint stress-induced hyperalgesia, and allodynia[7,20]. tDCS modulates the hyperalgesic response and inflammatory profile in rats subjected to neuropathic pain, changing BDNF, IL-1β, IL-4, and TNF-α levels in the CNS structures of rats[9,10].

The use of drugs, such as steroid anti-inflammatory, in RA treatment is often associated with several adverse effects that limit their usefulness. Thus, the use of no pharmacological tools, like tDCS associated with anti-inflammatory steroids to reduce the dosage and consequently cause less adverse effects while in synergy with the analgesic and anti-inflammatory effects can be an interesting strategy in the treatment of RA. Considering that the use of this combination has not been investigated yet, the current study, searching for safer and more effective RA treatments, aimed to evaluate the behavior and neurochemical parameters of dexamethasone (DEX, 0.25mg/kg) combined with tDCS in arthritic rats.

METHODS

Male Wistar rats (n=36; 60 days/old; weighting 280-300g) were allocated in home cages (49x34x16cm) with the floor covered with sawdust and kept in a controlled environment (22±2°C; 12h/12h light-dark cycle), with water and chow *ad libitum* (Nuvital, Porto Alegre/ Brazil). All the experiments and procedures had been previously approved by the Institutional Committee for Animal Care and Use (GPPG-HCPA protocol #20120220) and met ARRIVE guidelines[21].

Experimental design

Rats were assigned into four groups: control+dexamethasone (CTR-L+DEX, n=9) (0.25mg/kg); arthritis+dexamethasone (RA+DEX, n=9) (0.25mg/kg,); arthritis+dexamethasone (0.25mg/kg) + sham--tDCS (RA+DEX+sham-tDCS, n=9); and arthritis + dexamethasone (0.25mg/kg) + tDCS (RA+DEX+tDCS, n=9). The tDCS or sham and/or dexamethasone treatments were applied 14 days after the CFA injection.

The electronic Von Frey test was performed immediately at the pre--treatment (14 days after the CFA injection) and 24 hours after the end of treatment (last tDCS session and/or dexamethasone administration). The open field test was conducted 24 hours after the tDCS session and/or dexamethasone administration. The edema measures were performed at pre-treatment, 2, 4, 6 and 8 days after starting the treatments. The rats were killed by decapitation on day 23 after CFA injection (Figure 1).

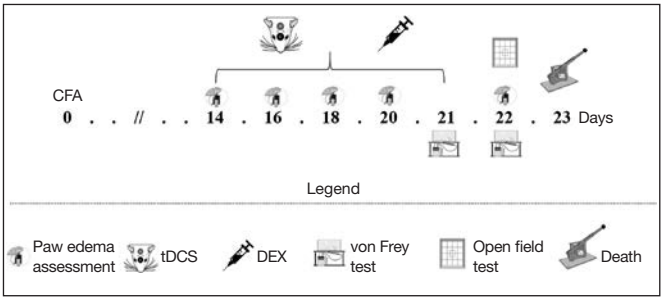

Figure 1. Experimental design

CFA = complete Freund's adjuvant; DEX = dexamethasone treatment; tDCS = transcranial Direct Current Stimulation.

Complete Freund's adjuvant (CFA)-induced arthritis
Isoflurane-anesthetized animals received an intradermal CFA injection into the right hind paw (1 mg·mL^{-1}; 100 µL; heat-killed and dried *Mycobacterium tuberculosis*, each milliliter of the vehicle containing 0.85 mL of paraffin oil and 0.15 mL of mannide monooleate), which was suspended in a 1:1 oil/saline emulsion (in a total volume of 200 µL). This model of arthritis needs approximately 14 days to be established[22].

Dexamethasone treatment
DEX (Nova Farma, Anápolis, [Goiás], [Brazil]) was dissolved in saline immediately before use and injected intraperitoneally (i.p., 0.25mg/kg) at 1 mL/kg once a day for eight days[23].

Transcranial direct current stimulation (tDCS)
After the establishment of arthritis, 14 days after CFA exposure, the animals in the active treatment groups underwent a 20-minute session of bicephalic tDCS every afternoon for eight days, as previously described[9,10]. 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 better adherence, electrodes were trimmed to 1.5 cm^2 for a better fit, and a constant current intensity of 0.5 mA was applied on their scalp. 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). The anode was placed on the head using neck landmarks and shoulder lines as guidance (the anterior and posterior regions in the midline between the two hemispheres of the parietal cortex). For sham stimulation, the electrodes were placed and fixed in the same position as for the actual stimulation. However, the stimulator was off throughout the procedure. The animals were carefully immobilized with a cotton towel to prevent their movements without causing discomfort for both the active treatment and the sham.

Paw volume measurement
The edema was measured using a plethysmometer (UGO Basile, Italy) and expressed in milliliters (mL).

Electronic von Frey test
Mechanical allodynia was assessed using an automatic von Frey aesthesiometer (Insight, São Paulo, Brazil). 24 hours before the test, the rats were habituated to the environment for 15 minutes to prevent novelty-induced analgesia[24]. The test consisted of poking the hind paw to provoke a flexion reflex followed by a clear flinch response. Pressure intensity was recorded automatically after paw withdrawal. Three successive von Frey readings were then averaged, and the interval between measures was of at least 5 seconds. The averages were used as the final measurements, and the paw withdrawal threshold was expressed in grams (g).

Open field test
Behavioral assessments were performed in a varnished wood cage lined with glass inside. Four measurements were taken during the 5-minute test sessions in the open field: (1) latency to leave the first quadrant (seconds); (2) total number of crossings (or number of total crossings) (i.e., horizontal activity in the inner and outer quadrants) (3) grooming behavior (in seconds); (4) number of rearing behaviors (i.e., vertical activity); and (5) number of fecal boluses. The number of line crossings (all paws crossed the boundary into a different marked-out area) was taken as a measure of locomotor activity. The latency to leave the first quadrant assessed anxiety-like behaviors.

Tissue collection
The rats were killed by decapitation 48 hours after the last tDCS session and/or dexamethasone administration and brain structures (hippocampus, spinal cord, cerebral cortex, and brainstem) were collected. The structures were kept frozen at -80°C until the assays were performed.

TNF-α and BDNF levels
Hippocampus and spinal cord TNF-α levels were determined via enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies specific for TNF-α (R&D Systems, Minneapolis, United States). The ELISA was made 48 hours after the last tDCS session and/or dexamethasone administration. BDNF analyses were performed on the brainstem and cerebral cortex using a commercially available enzyme-linked immunosorbent assay kit for rats (R&D Systems, Minneapolis, MN). All structures were homogenized with a handheld homogenizer using Protease Inhibitor Cocktail (Sigma® # P8340) in the ratio of 1:100 in phosphate-buffered saline (PBS) at pH 7.2. Each of the homogenates was centrifuged for 5 min at 10,000 rpm. Optical density - wavelength of 450 nm - was measured using an ELISA reader. The values were expressed in picograms per milliliter of tissue homogenate (pg/mL).

Statistical analysis
Generalized estimating equations (GEE) were used to analyze the group effects for joint swelling and mechanical allodynia at different assessment time points. One-way ANOVA followed by Student-Newman-Keuls (SNK) *post hoc* test was performed to compare group differences for behavioral parameters and biomarkers. Results are expressed as mean ± standard error of the mean (SEM). All statistical analyses were performed using SPSS, version 26.0 (SPSS, Chicago, IL, USA). The level of significance was set at $p<0.05$ for all statistical tests.

RESULTS

At baseline, measurements of joint swelling (GEE, $p<0.001$) (Figure 2) and hind paw withdrawal latency in the von Frey test (GEE, $p<0.05$) (Figure 3) were significantly different between the CTRL+DEX group and all three arthritis groups, confirming the efficacy of the pain model used (GEE, $p=0.001$).

Joint swelling

All immunized rats developed inflammatory monoarthritis. The peak incidence occurred on day 14 after immunization (pre-treatment) when bicephalic tDCS and DEX treatments started. The GEE analysis showed significant group effect (p=0.001), time effect (p=0.001), and group x time interaction (p=0.001) (Figure 2). Compared to baseline values, joint swelling immediately decreased in all three arthritic groups and 24 hours after the end of treatment (last tDCS session and/or DEX administration). On the other hand, there was no difference in joint swelling between arthritis groups in any of the assessment time points (p>0.05).

Mechanical allodynia

Mechanical allodynia was assessed by the von Frey test. The GEE analysis showed a significant group effect (p=0.001), time effect (p=0.001) and group x time interaction (p=0.002) (Figure 3). It was possible to observe immediately and 24 hours after the last bicephalic tDCS treatment session that pain rats subjected to DEX and tDCS slightly increased the paw threshold. m=Mechanical allodynia was also partially reversed by combined treatments (p>0.05).

Behavioral parameters by the open field test

A significant increase in grooming behavior was observed in arthritic groups, which was reverted by sham-tDCS treatment (one-way ANOVA-SNK, p=0.002). All arthritic groups showed a reduction in the number of crossings in the inner quadrant when compared to CTRL+DEX group (one-way ANOVA-SNK, p=0.01). No differences were found between groups regarding latency to leave the first quadrant, rearing behavior, crossings in the outer quadrant, total of quadrants, and the number of fecal boluses (one-way ANOVA-SNK, p>0.05 for all) (Table 1).

Biomarkers levels

Regarding the TNF-α levels, RA rats treated with DEX showed a decrease in spinal cord TNF-α levels (p=0.02) when compared to no pain rats. Immobilization (RA+-DEX+sham-tDCS group) intensified the reduction, and it was reversed by active tDCS in RA rats up until RA+-DEX levels (one-way ANOVA-SNK, p=0.02, Figure 4, panel A). RA rats treated with DEX showed a decrease in the hippocampus TNF-α levels (p=0.02) when compared to other groups (one-way ANOVA-SNK, p=0.02, Figure 4, panel B).

Concerning BDNF levels, there was no difference between groups in the BDNF levels in the cerebral cortex or brainstem (one-way ANOVA-SNK, p>0.05 for both; Figure 4, panel C and D, respectively).

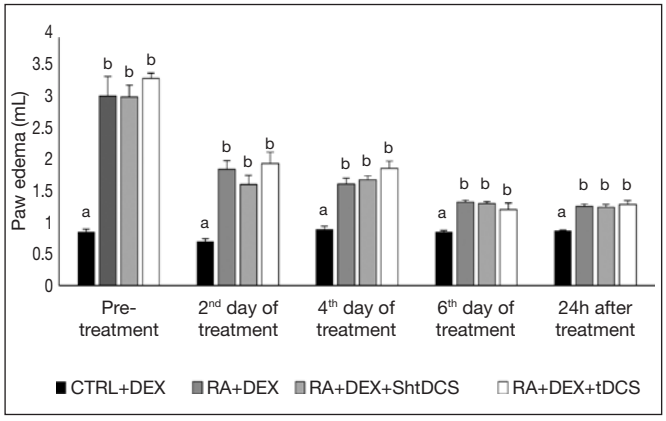

Figure 2. Paw edema measured at pre-treatment (14 days after CFA injection), 2,4 and 6 days of treatment, and 24h after the end of the treatment

(CTRL+DEX) = control+DEX; (RA+DEX) = arthritis+DEX; (RA+DEX+sham-tDCS) = arthritis+DEX+sham tDCS; (RA+DEX+tDCS) = arthritis+DEX+tDCS

Data are expressed as mean ± standard error of the mean (GEE/Bonferroni; p<0.05). Different letters mean statistical significance.

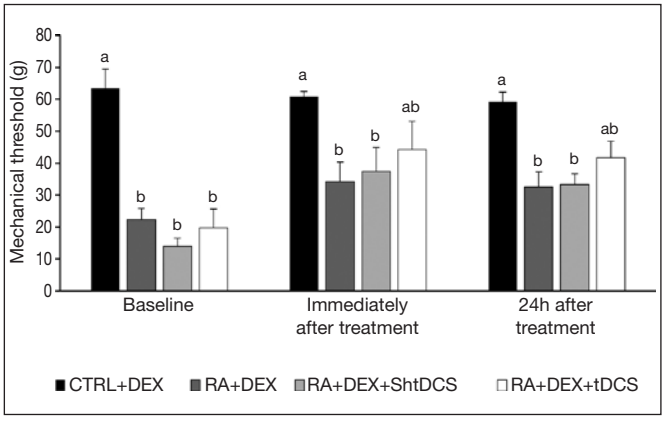

Figure 3. Mechanical threshold assessed by von Frey test

(CTRL+DEX) = control+DEX; (RA+DEX) = arthritis+DEX; (RA+DEX+sham-tDCS) = arthritis+DEX+sham tDCS; (RA+DEX+tDCS) = arthritis+DEX+tDCS

Data are expressed as mean ± standard error of the mean (GEE/Bonferroni; p < 0.05). Different letters mean statistical significance.

Table 1. An Open field test was performed 24 hours after the last tDCS session and/or dexamethasone administration

Open field test	CTRL+DEX	RA+DEX	RA+DEX+sham-tDCS	RA+DEX+tDCS
Latency (s)	3.71±1.12	2.83±0.47	4.66±1.30	6.71±1.49
Grooming (s)	2.33±1.56[a]	35.80±15.04[b]	1.83±1.22[a]	53.60±13.77[b]
Rearing (n)	32.42±4.60	22.57±7.59	35.00±5.53	23.14±6.06
Ext quad (n)	96.75±8.74	79.00±7.93	93.85±8.00	77.71±7.82
Int quad (n)	11.71±2.13[a]	4.83±0.87[b]	4.33±0.80[b]	5.28±2.00[b]
Total quad (n)	108.57±7.68	85.14±7.42	97.57±8.00	83.00±7.46
Fecal Bol (n)	2.00±0.87	3.42±1.13	4.00±0.44	2.83±0.60

Groups: CTRL+DEX, RA+DEX, RA+DEX+sham-tDCS, and RA+DEX+tDCS. Data are expressed as mean±standard error of the mean of number (n) or seconds (s). Different letters mean statistical significance (one-way ANOVA/SNK; p< 0.05).

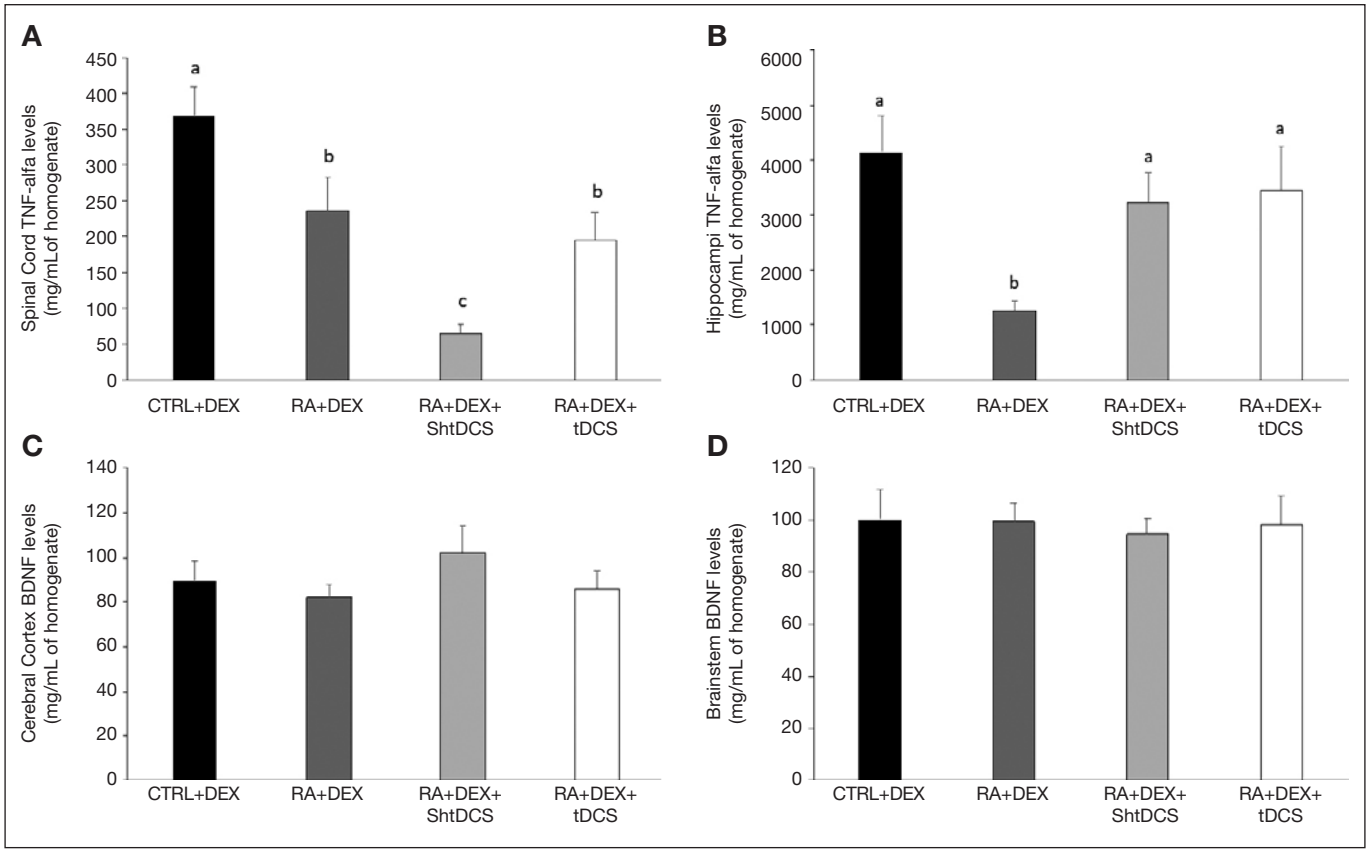

Figure 4. Biomarkers levels measured in central nervous structures of rats

(CTRL+DEX) = control+DEX; (RA+DEX) = arthritis+DEX; (RA+DEX+sham-tDCS) = arthritis+DEX+sham tDCS; (RA+DEX+tDCS) = arthritis+DEX+tDCS
Panel A. Spinal cord TNF-α levels. **Panel B.** Hippocampi TNF-α levels. **Panel C.** Cerebral cortex BDNF levels. **Panel D.** Brainstem BDNF levels. Data are expressed as mean ± standard error of the mean (one-way ANOVA/SNK; $p < 0.05$). Different letters mean statistical significance.

DISCUSSION

The current study demonstrated an immediate and long-lasting slight antinociceptive effect of DEX combined with bicephalic tDCS treatments in a rat model of arthritis. These effects were not observed after DEX administration alone, suggesting a specific effect of DEX combined with tDCS. Spinal cord TNF-α levels were reduced in arthritic rats, a result intensified by immobilization. tDCS reverted the immobilization effect. DEX associated with the arthritic rat model showed a decrease in the hippocampus TNF-α levels. Active and sham-tDCS were able to reverse this effect. There was an increase in grooming behavior in the arthritic rat model, which was reversed by sham-tDCS. Also, none of the DEX or tDCS effects were found in the cerebral cortex or brainstem BDNF levels.

For pain and inflammatory assessment, the rats were injected with CFA to develop the arthritic rat model. CFA triggers the release of inflammatory mediators, including cytokines and active oxygen species[25]. It also causes hyperalgesia and allodynia[25,26], which may persist for up to 28 days. The present study focused on the late phase of inflammation that induces immunological events. Peripheral afferent fibers synthesize an array of molecules that could potentially contribute to hyperalgesia in patients with chronic inflammatory diseases, such as RA. These include glutamate and other excitatory amino acids, neuropeptides like substance P, adenosine triphosphate, nitric oxide, and prostaglandins[27].

Steroidal anti-inflammatory drugs are one of the most commonly used for several pathologies. Still, there are several side effects associated with long-term or high-dose DEX[28]. Based on a previous study using a chronic inflammatory rat model (panniculitis) in which pain was completely reversed by DEX[23], now dexamethasone (0.25 mg/kg) was tested. In the current study, a discrete analgesic effect using an arthritis rat model was observed only when combined with bimodal tDCS in the arthritic rat model upon mechanical allodynia. Since arthritis is a more aggressive model than panniculitis, it's interesting to note that the inflammatory profiles linked to both disorders are different from the ones before shown in panniculitis[29] and arthritis[30]. In the current study, this analgesic effect was only observed in mechanical allodynia when DEX was combined with tDCS. A result that may be explained by the fact that panniculitis is a less severe inflammatory process than arthritic rats. It is important to note that, in a previous study, an arthritis model[30] used, in addition to histological changes including periarticular panniculitis in the tibiotarsal joint, also showed inflammatory infiltration containing giant cells, plasmocytes, lymphocytes, and mainly macrophages and neutrophils. Furthermore, the rats showed joint damage progression, revascularization, and synovial proliferation at day 14 post-CFA injection.

Previous preclinical studies from the present authors showed immediate and long-lasting effects of repeated sessions of bicephalic tDCS treatment on chronic inflammation[8], hyperalgesia in stress-induced chronic restraint[7], neuropathic pain model[9], and neuropathic pain model associated with repeated physical exercise[10]. Nonetheless, in the current study, a slight analgesic effect of bimodal tDCS was found only when combined with DEX. The role of central and peripheral levels of cytokines and neurotrophins associated with the antinociceptive effect of tDCS in different chronic pain models was shown. Still, in the present study, pain effect upon TNF-α levels without tDCS and/or DEX effects was found.

Furthermore, a previous study using mice injected with CFA in the footpad and with intrathecally injected TNF-α showed the central role of TNF-α in regulating synaptic plasticity (central sensitization), and that inflammatory pain was mediated via both TNF-α receptor 1 and TNF-α receptor 2[31]. In dorsal root ganglion neurons, TNF-α increases transient receptor potential subtype V1 and induces spontaneous discharge[32]. It's important to note that glucocorticoid receptors are expressed on microglia *in vitro* and microglia treated with steroidal drugs showed a decrease in the production of TNF-α and IFN-γ in response to lipopolysaccharide injection. Additionally, the administration of methylprednisolone reduces TNF-α expression after spinal cord injury in rats[33], corroborating the data of the present study that showed a decrease in central TNF-α levels in arthritic rats.

The present study also observed in the arthritic rats an increase in grooming behavior associated with a decrease in the time spent in the central part of the open field. Both can be interpreted as anxiogenic-like effects. A previous study has shown that the CFA model promoted increased anxiety-like behaviors, such as a decrease in time spent and in the number of entries in open arms of the elevated plus-maze and a reduction in the number of central squares visited in the open field[34]. The open field test is a method for measuring locomotor and exploratory activities, but it can also indicate some anxiety-like behaviors parameters[35]. Thus, it is possible to suggest that the arthritis-induced pain increased anxiety-like behavior and that it was not reversed by DEX and/or repeated bicephalic tDCS treatments. It's important to note that a previous study from the present research group demonstrated that tDCS reversed chronic neuropathic pain-induced behavioral alterations, indexed by changes in locomotor and exploratory activities and anxiety-like behaviors[36]. However, in the current study, tDCS was not able to reverse this behavior in a chronic inflammatory pain model.

This study has the following limitations: 1) Non-invasive tDCS is not focal in animal models. Given that in humans both electrodes are placed on the head, the attempt was to mimic similar electrode positions in the rats. However, it was not possible to avoid bicephalic stimulation because of their relatively small head size. 2) The experimental design did not include a control-saline, arthritis-saline, and arthritis-saline-tDCS group. Thus, the specific DEX effects were not evaluated by comparing them with potential saline effects. Nevertheless, the DEX effects on CFA-induced arthritis are well described[23,37]. Similarly, despite having a sham-tDCS group to compare the therein observed effects to the tDCS effects, a arthritis-saline-tDCS group was not included. 3) It was necessary to restraint the rats during tDCS because it was performed without anesthesia. Therefore, restraint-induced stress could not be avoided. 4) The choice to euthanize the rats 48 hours after the last tDCS session was made because all behavioral tests were performed in the afternoon 24 hours after the last tDCS session, which made euthanasia on the same day after the tests impractical. 5) The findings indicate the need to develop a new study using a low dose of DEX combined with tDCS. 6) Based on the previous study from this research group, the dose of DEX (0.25mg/kg) was considered low. However, data from literature diverge about the low and high dosage range for rats[38,39].

CONCLUSION

In sum, arthritic rats displayed an increase in grooming behavior and anxiety-like behavior indexed by decreased inner crossings; and decreased central TNF-α level. DEX combined with bimodal tDCS showed a slight analgesic effect upon mechanical allodynia, suggesting a possible use of tDCS as an adjuvant technique to reduce the dose of corticoids in the RA management. However, new studies are encouraged to investigate drug–tDCS combinations with the objective of reducing the effective dosage and increasing drug efficacy.

AUTHORS' CONTRIBUTIONS

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Statistical Analysis, Methodology, Writing - Review and Editing, Visualization

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Statistical analysis, Funding acquisition, Conceptualization, Resource management, Project management, Writing – Original preparation, Writing – Reviewing and editing, Supervision, Visualization

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