

## **Effects of transcranial direct current stimulation (tDCS) on pro-inflammatory cytokines: a systematic review**

**Efeitos da estimulação transcraniana por corrente contínua (ETCC) em citocinas pró-inflamatórias: uma revisão sistemática**

**Efectos de la estimulación de corriente continua transcranial (tDCS) sobre las citoquinas pro-inflamatorias: una revisión sistemática**

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**Lucas Eduardo Galvagni**

ORCID: <https://orcid.org/0000-0003-2459-3966>  
Universidade Federal do Rio Grande do Sul, Brazil  
E-mail: [lucasgalvagni@gmail.com](mailto:lucasgalvagni@gmail.com)

**Ingrid Navarro Andrade**

ORCID: <https://orcid.org/0000-0002-3709-1482>  
Universidade Federal do Rio Grande do Sul, Brazil  
E-mail: [ingridnavarro25@hotmail.com](mailto:ingridnavarro25@hotmail.com)

**Vanessa Brunheri**

ORCID: <https://orcid.org/0000-0002-1141-9988>  
Universidade Federal do Rio Grande do Sul, Brazil  
E-mail: [vanessabrunheri@hotmail.com](mailto:vanessabrunheri@hotmail.com)

**Etiane Micheli Meyer Callai**

ORCID: <https://orcid.org/0000-0003-0840-5881>  
Universidade Federal do Rio Grande do Sul, Brazil  
E-mail: [eticallai@gmail.com](mailto:eticallai@gmail.com)

**Luciana Santa Catarina**

ORCID: <https://orcid.org/0000-0001-8025-4454>  
Universidade Federal do Rio Grande do Sul, Brazil  
E-mail: [lufsntcaterina@hotmail.com](mailto:lufsntcaterina@hotmail.com)

**Daniela Müller de Quevedo**

ORCID: <https://orcid.org/0000-0003-2169-9781>  
Universidade FEEVALE, Brazil  
E-mail: [danielamq@feevale.br](mailto:danielamq@feevale.br)

**Deise Ponzoni**

ORCID: <https://orcid.org/0000-0003-2855-7495>  
Universidade Federal do Rio Grande do Sul, Brazil  
E-mail: [deise.ponzoni@ufrgs.br](mailto:deise.ponzoni@ufrgs.br)

**Alexandre Silva Quevedo**

ORCID: <https://orcid.org/0000-0001-5613-8015>  
Universidade Federal do Rio Grande do Sul, Brazil  
E-mail: [quevedoalexandre@hotmail.com](mailto:quevedoalexandre@hotmail.com)

### **Abstract**

Transcranial direct current stimulation (tDCS) is a neuromodulation technique that causes alterations in the synthesis of several proteins, including cytokines (e.g., Interleukins). Pro-inflammatory cytokines are associated with the presence of pain and their reduction occurs in several pathologies. The aim of this study was to investigate the effects of tDCS on the variation of tissue and serum blood levels of pro-inflammatory cytokines and its relationship with behavioral changes, through a systematic review. PubMed, Embase and Lilacs database searches were performed for articles published in all languages before October 1, 2020. The search was based on the keywords "Transcranial Direct Current Stimulation" or "tDCS" and "IL-1alpha" or "IL-1Beta" or "IL-6" or "IL-8" or "IL-17" or "Tumoral necrosis factor alpha" or "TNF-alpha". The systematic review protocol was registered in PROSPERO (CRD42021283417). Initially, 416 studies were identified in the electronic databases, of which 40 were eliminated because they were duplicates. Of the remaining 376, 358 were excluded after analyzing the title and abstract (selection stage) and 09 were excluded after a complete reading. Nine studies were considered for evaluation. The results demonstrate that tDCS can alter the levels of pro-inflammatory cytokines and modify behaviors in animals, however these findings are variable. Still, the cause and effect relationship between cytokine levels and behavioral changes found was not conclusive. Further studies are needed to establish the mechanisms involved in the action of tDCS on the levels of pro-inflammatory cytokines.

**Keywords:** TDCS; Animals; Citokines.

### Resumo

A estimulação transcraniana por corrente contínua (ETCC) é uma técnica de neuromodulação que induz alterações na síntese de várias proteínas, incluindo as citocinas (ex. interleucinas). As citocinas pró-inflamatórias, estão associadas à presença de dor, e a alteração dos seus níveis ocorre em diversas patologias. O objetivo desse estudo foi investigar os efeitos da ETCC na variação dos níveis teciduais e sanguíneos de citocinas pró-inflamatórias e sua relação com alterações comportamentais, através de uma revisão sistemática. Foram realizadas buscas nas bases de dados PubMed, Embase e Lilacs para artigos publicados em todos os idiomas antes de 1º de outubro de 2020. A busca foi baseada nas palavras-chave "Transcranial Direct Current Stimulation" ou "tDCS" e "IL-1alpha" ou "IL-1Beta" ou "IL-6" ou "IL-8" ou "IL-17" ou "Tumoral necrosis factor alpha" ou "TNF-alpha". O protocolo de revisão sistemática foi registrado no PROSPERO (CRD42021283417). Foram identificados 416 estudos nas bases de dados eletrônicas, 40 foram eliminados por serem duplicados. Dos 376 restantes, 358 foram excluídos após análise do título e do resumo (etapa de seleção) e outros 09 após a leitura completa. Desta forma, nove estudos foram considerados para avaliação. Os resultados sugerem que a ETCC pode alterar os níveis de citocinas pró-inflamatórias e modificar comportamentos em animais, no entanto estes achados são variáveis. Ainda, a relação de causa e efeito entre os níveis de citocinas e as alterações comportamentais encontradas não foram conclusivas. Novos estudos são necessários para que sejam estabelecidos os mecanismos envolvidos na ação da ETCC sobre os níveis de citocinas pró-inflamatórias.

**Palavras-chave:** ETCC; Animais; Citocinas.

### Resumen

La estimulación con corriente continua transcranial (tDCS) es una técnica de neuromodulación que provoca alteraciones en la síntesis de varias proteínas, incluidas las citocinas (por ejemplo, las interleucinas). Las citocinas proinflamatorias se asocian con la presencia de dolor y su reducción ocurre en diversas patologías. El objetivo de este estudio fue investigar los efectos de tDCS en la variación de los niveles tisulares y sanguíneos de citocinas proinflamatorias y su relación con los cambios de comportamiento, a través de una revisión sistemática. Se realizaron búsquedas en las bases de datos PubMed, Embase y Lilacs de artículos publicados en todos los idiomas antes del 1 de octubre de 2020. La búsqueda se basó en las palabras clave "Transcranial Direct Current Stimulation" o "tDCS" y "IL-1alpha" o "IL-1Beta" o "IL-6" o "IL-8" o "IL-17" o "Tumoral necrosis factor alpha" o "TNF-alpha". El protocolo de revisión sistemática se registró en PROSPERO (CRD42021283417). Inicialmente se identificaron 416 estudios en las bases de datos electrónicas, de los cuales 40 fueron eliminados por estar duplicados. De los 376 restantes, 358 fueron excluidos después del análisis del título y el resumen (etapa de selección) y 09 fueron excluidos después de la lectura completa. Nueve estudios fueron considerados para evaluación. Los resultados demuestran que tDCS puede alterar los niveles de citoquinas proinflamatorias y modificar comportamientos en animales, sin embargo, estos hallazgos son variables. Aún así, la relación de causa y efecto entre los niveles de citoquinas y los cambios de comportamiento encontrados no fue concluyente. Se necesitan más estudios para establecer los mecanismos implicados en la acción de tDCS sobre los niveles de citocinas proinflamatorias.

**Palabras clave:** TDCS; Animales; Citoquinas.

## 1. Introduction

Transcranial Direct Current Stimulation (tDCS) applies electrical energy to the cerebral cortex through electrodes over different brain regions. This neurostimulation technique uses sustained direct current (Paulus; et al., 2013), being a non-invasive alternative, and easy to apply. In addition, this treatment involves modulation of brain areas associated with nociceptive processing (Fregni et al., 2006b; Liebetanz et al., 2009). Therefore, tDCS is a promising tool for treating pathologies that involve the central nervous system (CNS) (e.g., chronic pain).

TDCS can use anodic or cathodic electrical currents depending on the treatment required. The anodal current causes a depolarization of the neuronal membrane, increasing the excitability of neurons (Nitsche et al., 2008). When using cathodal current, there is hyperpolarization of the cell membrane, inhibiting neuronal activity (Nitsche & Paulus, 2000; Nitsche et al., 2008). Due to the action of tDCS altering cortical excitability, the regions of interest vary according to the pathology/dysfunction. For example, patients with painful disorders can receive treatment with an electrical current directed to the prefrontal cortex or the primary motor cortex (M1) (De Oliveira et al., 2019; Lopes et al., 2020), cortical areas involved in pain processing and perception (Quevedo & Coghill, 2007). In addition to the direct effects on brain tissues, remote areas of

the CNS can be affected by tDCS through descending pathways (i.e., top-down effect), which may contribute to pain control (Lefaucheur, 2006).

Among the different effects of tDCS, the modulation of cytokine levels has been observed (Spezia Adachi et al., 2015). Furthermore, the expression of pro-inflammatory cytokines in nervous tissue is directly related to the presence of pain (Spezia Adachi et al., 2015). Different cytokines are related to pro-inflammatory effects such as Tumor Necrosis Factor alpha (TNF- $\alpha$ ), Interleukin 1 alpha (IL-1 $\alpha$ ), Interleukin 1 beta (IL-1 $\beta$ ), Interleukin 6 (IL-6), Interleukin 8 (IL-8), Interleukin 15 (IL-15), Interleukin 17 (IL-17), and Interleukin 18 (IL-18) (Campos Kraychete et al, 2006). TNF- $\alpha$  is responsible for initiating the activation cascade of other cytokines, stimulating the synthesis, secretion, and activity of interleukins. Furthermore, TNF- $\alpha$  is related to the chemotaxis of trophic factors related to support neuronal cell types (Coppack, 2001; Campos Kraychete et al, 2006).

Each family member of interleukins may play different roles in the inflammatory cascade. For example, IL-1 ( $\alpha$  and  $\beta$ ) induces the inflammatory response related to the acute phase of infections (Dinarello, 2011). IL-6 is considered a mixed cytokine, as it has pro-inflammatory (i.e., stimulating the immune response) (Lin et al, 2000) and anti-inflammatory effects (Oliveira, 2011). IL-8 is one of the primary mediators of the immune response to intracellular microorganisms. Furthermore, IL-8 is strongly associated with neutrophil chemotaxis and the activation of polymorphonuclear neutrophils (Baggiolini & Clark-Lewis, 1992; Curfs et al, 1997). On the other hand, IL-17 is associated with extracellular bacterial infections (Jaiswal, 2014).

The effectiveness of tDCS in pain management has been documented using different protocols (Nitsche et al., 2008). However, the ideal parameters for optimizing the use of tDCS (i.e., cortical areas, treatment time, and current intensity) are the subject of investigations (Knotkova et al., 2019). Furthermore, the pathophysiologies of several diseases, such as chronic pain, are not fully understood. However, it has been characterized that blocking pro-inflammatory cytokines in the CNS can effectively control pain (Wieseler-Frank et al.; 2005). In addition, the use of tDCS is effective in reversing behaviors related to pathological processes (e.g., pain, epilepsy, anxiety) (Cioato & Torres, 2014; De Oliveira et al., 2019; Regner et al., 2020; Santos et al., 2020) and improvement in cognitive performance (Guo et al., 2020). Therefore, through a systematic review, the present study aimed to investigate the effects of tDCS on pro-inflammatory cytokine levels in the CNS and serum and behavioral changes.

## 2. Methodology

The review protocol was registered with PROSPERO (registration number CRD42021283417) and followed PRISMA guidelines (Page et al., 2021)

### 2.1 Literature search

This systematic review searched three databases: Embase, PubMed (Public/Publisher MEDLINE), and Lilacs (Latin American and Caribbean Literature in Health Sciences). Embase was chosen because it is the most complete database. PubMed is the main reference database in the health area. Likewise, Lilacs is also widely known in this area. All searches were based on the same criteria. The strategy employed included the following keywords ("Mesh Terms") with the Booleans arranged as follows: "tDCS" or "Transcranial Direct Current Stimulation" and "IL-1alfa" or "IL-1Beta" or "IL-6" or "IL-8" or "IL-17" or "Tumor necrosis factor-alpha" or "TNF-alpha". These keywords and booleans must be contained in the title or abstract of the articles. The search found 43 studies on the PubMed platform, 373 on Embase, and 0 (zero) on Lilacs.

## 2.2 Study selection

The collection and analysis of the articles took place from 10/01/2020 to 12/10/2020. The search and inclusion of studies in this review were performed by two researchers independently (LEG and INA), and no discrepancies were found between them.

Initially, the duplication of articles was verified through the management of the Microsoft Office Access 2013 database, where forty duplicate articles were observed. Afterward, the selected articles were evaluated separately by the two evaluators using the title and abstract according to the eligibility criteria, excluding articles irrelevant to the objective of the present review. Next, the selected articles were read in full by the two evaluators individually for deliberation on their inclusion. Then, the researchers extracted the data separately, and after collection, a new consensus meeting was held to verify the degree of agreement between the authors. If no agreement was reached between the authors, a third party would be asked to reach an agreement. As there was 100% agreement between the two evaluators, requesting any evaluation from this third researcher was unnecessary.

## 2.3 Inclusion criteria

All publication types, except gray literature, were accepted. In addition, studies published in all languages, performed in animals, and using the tDCS technique involving at least one proinflammatory cytokine were included. Therefore, the eligible studies met the following criteria:

1. Experimental studies in animals that received tDCS as an intervention.
2. The primary or exploratory objective evaluates tDCS effects on the production or release of pro-inflammatory cytokines.
3. Studies that include behavioral assessment in addition to biochemical tests.
4. Any duration of tDCS treatment.
5. Different currents (cathodic, anodic, or bimodal).
6. Activated electrode over the cortical areas.

## 2.4 Exclusion criteria

Studies that did not meet the inclusion criteria, articles that used other stimulation techniques, evaluated in humans, or only considered anti-inflammatory interleukins, systematic reviews, clinical trials, abstracts, congress data, theses, dissertations, in vitro studies or even studies that associate some drug therapy with tDCS were excluded. Excluded studies were selected first by title, then by abstract, and then by full text.

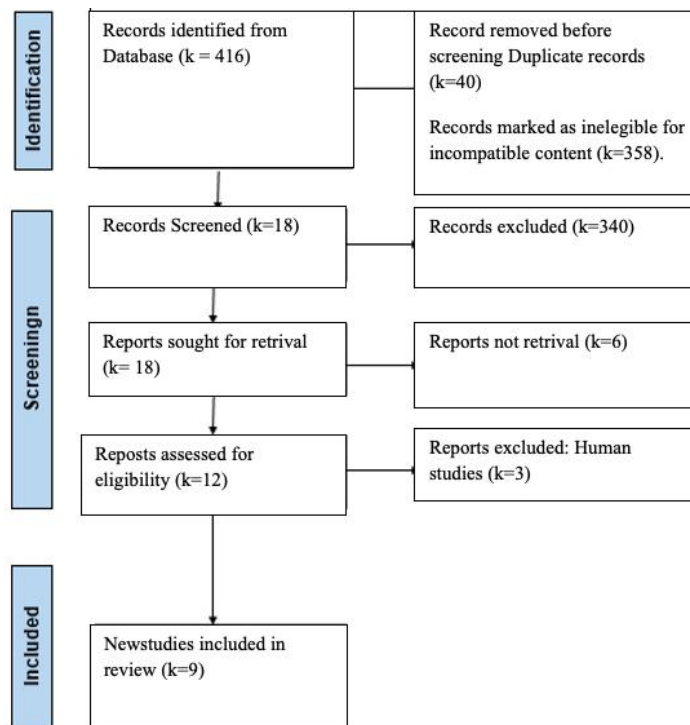
## 2.5 Analysis of the quality of studies

The detection of risk of bias was performed using the SYRCLE RoB tool, an adapted version of the Cochrane RoB tool for animal studies (Hooijmans et al., 2014)

## 3. Results

According to the defined search strategy, the search results of 416 studies found in the electronic databases were identified in the first step. Forty were excluded because they were duplicates. Of the remaining 376 articles, 358 were excluded after analyzing the title and abstract in the exhibition phase. Finally, nine articles were excluded after the complete reading. In addition, articles were excluded because they did not achieve the objectives of this study, used another electrotherapy, analyzed only anti-inflammatory cytokines, and studied humans. Therefore, nine studies were selected at the eligibility stage.

**Figure 1** - Flowchart of the studies selected for the systematic review.



Source: Authors.

The first reported study was published in 2012 (Spezia Adachi et al., 2012) (Table 1). Eight of the nine studies were developed by the same research group at the Federal University of Rio Grande do Sul (Callai et al., 2019; De Oliveira et al., 2019; Scarabelot et al., 2019; Cioato et al., 2016; Lopes et al., 2020; Spezia Adachi et al., 2012; Regner et al., 2020; Santos et al., 2020). All nine were scientific articles published in well-known journals, in English, with a predominance of three published in the Journal Brain Stimulation (Spezia Adachi et al., 2012; Cioato et al., 2016; Lopes et al., 2020). The others were published in different journals with high Impact Factors. The sample size ranged from 36 (Santos et al., 2020) to 156 (Lopes et al., 2020), evaluating animals and using heterogeneous methods regarding target areas, stimulation intensity, polarity, duration, and frequency.

**Table 1:** Basic Information of articles included in the review.

Article Title	Author / YEAR	Data Base	N – Sample rats	tDCS type - Anodal/Cathodal ou Bimodal	Application Location
Transcranial direct current stimulation (tDCS) and trigeminal pain: A preclinical study	Etiane Micheli Meyer Callai, 2019	EMBASE/PUBMED	151	Bimodal	Scalp
Transcranial direct current stimulation (tDCS) modulates biometric and inflammatory parameters and anxiety-like behavior in obese rats	Carla de Oliveira, 2019	EMBASE/PUBMED	40	Bimodal	Scalp
Transcranial direct-current stimulation reduces nociceptive behaviour in an orofacial pain model	Vanessa L Scarabelot, 2017	EMBASE/PUBMED	104	Bimodal	Scalp
Long-Lasting Effect of Transcranial Direct Current Stimulation in the Reversal of Hyperalgesia and Cytokine Alterations Induced by the Neuropathic Pain Model	Stefania Giotti Cioato, 2015	EMBASE/PUBMED	84	Bimodal	Scalp
Transcranial Direct Current Stimulation Ameliorates Cognitive Impairment via Modulating Oxidative Stress, Inflammation, and Autophagy in a Rat Model of Vascular Dementia	Tao Guo, 2020	EMBASE/PUBMED	60	Anodal	Skullcap
Reversal of chronic stress-induced pain by transcranial direct current stimulation (tDCS) in an animal model	Lauren Naomi Spezia Adachi, 2012	EMBASE/PUBMED	41	Anodal	Scalp
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	Bettega Costa Lopes, 2020	EMBASE/PUBMED	156	Bimodal	Scalp
Transcranial direct current stimulation (tDCS) affects neuroinflammation parameters and behavioral seizure activity in pentylenetetrazole-induced kindling in rats	Gabriela Gregory Regner, 2020	EMBASE/PUBMED	152	Bimodal	Scalp
Transcranial Direct Current Stimulation (tDCS) Induces Analgesia in Rats with Neuropathic Pain and Alcohol Abstinence	Daniela Silva Santos, 2020	EMBASE/PUBMED	36	Bimodal	Scalp

Source: Authors.

Table 1 shows that the application of bimodal tDCS was on the scalp in most investigations, while there was one study that used the skullcap. The number of animals used varied greatly (36 to 156), and the bases used for the search for articles were EMBASE/PUBMED.

Seven studies used bimodal stimulation (Table 1) (Callai et al., 2019; De Oliveira et al., 2019; Scarabelot et al., 2019; Cioato et al., 2016; Lopes et al., 2020; Regner et al., 2020; Santos et al., 2020) and two articles used anodal current (Spezia Adachi et al., 2012; Guo et al., 2020). In addition, the electrode position varied: seven articles used the area between the parietal cortex hemispheres (Scarabelot et al., 2019; Callai et al., 2019; Regner et al., 2020; Lopes et al., 2020; Santos et al., 2020; Cioato et al., 2016; Spezia Adachi et al., 2012), one study on the prefrontal cortex (De Oliveira et al., 2019), and one reported the application on the skullcap (Guo et al., 2020).

Eight studies used currents of 0.5 mA for 20 minutes (Table 2) (Callai et al., 2019; De Oliveira et al., 2019; Scarabelot et al., 2019; Cioato et al., 2016; Spezia Adachi et al., 2012; Lopes et al., 2020; Regner et al., 2020; Santos et al., 2020) and one study applied a current of 200  $\mu$ A for 30 minutes (Guo et al., 2020). The treatments consist of 8 (Callai et al., 2019; De Oliveira et al., 2019; Scarabelot et al., 2019; Cioato et al., 2016; Spezia Adachi et al., 2012; Lopes et al., 2020; Santos et al., 2020) or 10 tDCS sessions (Guo et al., 2020; Regner et al., 2020) (Table 2).

**Table 2:** Behavioral tests of articles included in the review.

Article Title	Author / YEAR	Current intensity	Application Time	Number of sessions	Pain / Pain Model	Behavioral test	Statistical test
Transcranial direct current stimulation (tDCS) and trigeminal pain: A preclinical study	Etiane Micheli Meyer Callai, 2019	0.5mA	20 min	8	Infraorbital Nerve Constriction	Von Frey	Friedman followed by Dunn test.
Transcranial direct current stimulation (tDCS) modulates biometric and inflammatory parameters and anxiety-like behavior in obese rats	Carla de Oliveira, 2019	0.5mA	20 min	8	Hypercaloric diet	Open field (OF) and elevated plus maze (LCE) apparatus	Kruskal-Wallis followed by Dunn test
Transcranial direct-current stimulation reduces nociceptive behaviour in an orofacial pain model	Vanessa L. Scarabelot, 2017	0.5mA	20 min	8	TMJ injection of Freund's Complete Adjuvant (FCA)	Von Frey and hot plate	Generalized Estimating Equations (GEE) followed by Bonferroni test
Long-Lasting Effect of Transcranial Direct Current Stimulation in the Reversal of Hyperalgesia and Cytokine Alterations Induced by the Neuropathic Pain Model	Stefania Giotti Cioato, 2015	0.5mA	20 min	8	Constriction of the sciatic nerve	Von Frey and hot plate	GEE followed by Bonferroni test
Transcranial Direct Current Stimulation Ameliorates Cognitive Impairment via Modulating Oxidative Stress, Inflammation, and Autophagy in a Rat Model of Vascular Dementia	Tao Guo, 2020	0.2mA	30 min	10	Bilateral common carotid artery occlusion	Morris water maze	One-way ANOVA followed by Bonferroni test
Reversal of chronic stress-induced pain by transcranial direct current stimulation (tDCS) in an animal model	Lauren Naomi Spezia Adachi, 2012	0.5mA	20 min	8	Physical restraint to generate stress	Von Frey and hot plate	One-way ANOVA followed by Tukey test
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	Bettega Costa Lopes, 2020	0.5mA	20 min	8	Constriction of the sciatic nerve	Von Frey and hot plate	GEE followed by Bonferroni test
Transcranial direct current stimulation (tDCS) affects neuroinflammation parameters and behavioral seizure activity in pentylenetetrazole-induced kindling in rats	Gabriela Gregory Regner, 2020	0.5mA	20 min	10	Pentylenetetrazole-induced kindling model	Adapted Racine scale	Fisher's exact probability test followed by the Bonferroni test
Transcranial Direct Current Stimulation (tDCS) Induces Analgesia in Rats with Neuropathic Pain and Alcohol Abstinence	Daniela Silva Santos, 2020	0.5mA	20 min	8	Constriction of the sciatic nerve	Hot plate	GEE followed by Bonferroni test

Source: Authors.

Most studies used the current intensity of 0.5mA and 20 minutes of application time. Several models were applied to study the effects of tDCS, including nerve and vascular manipulation. Likewise, the effects of tDCS were evaluated by different behavioral tests such as von Frey, Hot plate, and Open field. Statistical analysis was performed according to experimental design (i.e., intra- vs. inter-group analysis) or data distribution (i.e., parametric vs. non-parametric tests).

Regarding the animal model (Table 2), neuropathic pain was induced using the sciatic nerve (Cioato et al., 2016; Lopes et al., 2020; Santos et al., 2020) or infraorbital nerve constriction (Callai et al., 2019). Moreover, one study injected the Complete Freund's Adjuvant (CFA) into the temporomandibular joint to study inflammatory pain (Scarabelot et al., 2019). Finally, other animal models were used, such as Bilateral Carotid Artery Occlusion (Guo et al., 2020), physical restraint (Spezia Adachi et al., 2012), hypercaloric diet (De Oliveira et al., 2019), and kindling model of epilepsy (Regner et al. 2020).

To evaluate different behaviors, specific tests were used. For example, pain was assessed by the von Frey test (mechanical pain) associated with the Hot Plate test (thermal pain) (Table 2) (Scarabelot et al., 2019; Cioato et al., 2016; Spezia Adachi et al., 2012; Lopes et al., 2020). However, other studies evaluated only mechanical pain threshold (Callai et al., 2019) or thermal pain (Cioato et al., 2016). Moreover, one study evaluated anxiety and exploratory behavior/general activity using Elevated Plus Maze and Open Field, respectively (De Oliveira et al., 2016). Other behavior tests such as Morris Water Maze (Guo et al., 2020) and Racine Scale (Regner et al., 2020) were used during post-tDCS treatment. Two studies reported that the application of tDCS reduced mechanical and thermal hyperalgesia, causing an antiallodynic and analgesic effect, respectively (Table 3) (Callai et al., 2019; Scarabelot et al., 2019). The application of tDCS reduced the stress (Spezia Adachi et al., 2012), and when tDCS was associated with alcohol consumption, there was a reduction of hyperalgesia in animals

(Santos et al., 2020). Animals who were kept on a hypercaloric diet when they received tDCS sessions had lower body weight than stressed untreated animals (De Oliveira et al., 2019).

One study reported that bimodal tDCS, aerobic exercise, or both treatments combined promoted analgesic effects for neuropathic pain (Lopes et al., 2020). Cathodal tDCS did not change the convulsive behavior of rats (epileptic seizure occurrence or latency), while Anodal tDCS increased the number of epileptic seizures (Regner et al., 2020). A study stated that animals with induced pain (sciatic nerve constriction) showed less pain behavior from thermal and mechanical stimulation; however, there was no correlation between pain intensity (increase or reduction) and tDCS application (Cioato et al., 2016). One study performed several measurements in a water maze test and observed that animals treated with tDCS performed statistically significantly better when compared to untreated animals (Guo et al., 2020).

For the analysis of pro and anti-inflammatory mediators (Table 3), five studies performed the ELISA test (Callai et al., 2019; De Oliveira et al., 2019; Scarabelot et al., 2019; Cioato et al., 2016; Spezia Adachi et al., 2012), three studies used the “sandwich ELISA” test using monoclonal antibodies (Lopes et al., 2020; Regner et al., 2020; Santos et al., 2020), and one article used the Western blot test (Guo et al., 2020). Several cytokines have been associated with tDCS effects (Table 3), such as TNF- $\alpha$  (Callai et al., 2019; De Oliveira et al., 2019; Cioato et al., 2016; Guo et al., 2016; Guo et al., 2020; Spezia Adachi et al., 2012; Regner et al., 2020), IL-1 $\alpha$  (Regner et al., 2020), IL-1 $\beta$  (De Oliveira et al., 2019; Cioato et al., 2016; Guo et al., 2020; Spezia Adachi et al., 2012; Regner et al., 2020; Santos et al., 2020; Lopes et al., 2020) and IL-6 (Guo et al., 2020; Scarabelot et al., 2019).



**Table 3:** Outcomes and biochemical tests of articles included in the review.

Article Title	Author / YEAR	Behavior outcome	Statistical test	Biochemistry outcomes
Transcranial direct current stimulation (tDCS) and trigeminal pain: A preclinical study	Etiane Micheli Meyer Callai, 2019	Pain reduction in the test group (Rats submitted to infraorbital nerve constriction and treated with tDCS in the postoperative period).	Two-way Variance Analysis (ANOVA) Posthoc: Newman – Keuls (SNK) test.	TNF- $\alpha$ : = in the tDCS group NGF: $\downarrow$ in the tDCS group LDH: $\downarrow$ in the first 7 days / in the tDCS group *Brainstem and blood serum analysis
Transcranial direct current stimulation (tDCS) modulates biometric and inflammatory parameters and anxiety-like behavior in obese rats	Carla de Oliveira, 2019	Standard diet group had lower weight than the hypercaloric diet group. Within the hypercaloric group, the animals treated with active tDCS had lower weight when compared to the Sham-tDCS group.	One-way ANOVA Posthoc: SNK test.	BDNF: $\downarrow$ with a hypercaloric diet. IL-1 $\beta$ : $\downarrow$ with a hypercaloric diet. TNF- $\alpha$ : $\downarrow$ with a hypercaloric diet. The reduction ( $\downarrow$ ) was greater in the hypercaloric + tDCS group. *Analysis in the cerebral cortex
Transcranial direct-current stimulation reduces nociceptive behaviour in an orofacial pain model	Vanessa L Scarabelot, 2017	tDCS reduced mechanical and thermal hyperalgesia	Three-way ANOVA Posthoc: SNK test.	BDNF: $\uparrow$ in the tDCS group NGF: $\uparrow$ in the tDCS group IL-6: $\uparrow$ in the tDCS group *Analysis in the brainstem and blood serum.
Long-Lasting Effect of Transcranial Direct Current Stimulation in the Reversal of Hyperalgesia and Cytokine Alterations Induced by the Neuropathic Pain Model	Stefania Giotti Cioato, 2015	Animals with sciatic constriction felt less pain to thermal and mechanical stimulation	Three-way ANOVA Posthoc: SNK test.	IL-1 $\beta$ : $\uparrow$ in the pain group, IL-1 $\beta$ : $\downarrow$ in the pain + tDCS group, TNF- $\alpha$ : $\uparrow$ in the Pain + tDCS group * Analysis performed on cerebral cortex, spinal cord, and brainstem
Transcranial Direct Current Stimulation Ameliorates Cognitive Impairment via Modulating Oxidative Stress, Inflammation, and Autophagy in a Rat Model of Vascular Dementia	Tao Guo, 2020	Several tests performed and the tDCS group showed statistically significant better results	One-way ANOVA Posthoc: Bonferroni test.	IL-1 $\beta$ : $\downarrow$ tDCS group IL-6: $\downarrow$ tDCS group TNF- $\alpha$ : $\downarrow$ tDCS group *Analysis in the hippocampus
Reversal of chronic stress-induced pain by transcranial direct current stimulation (tDCS) in an animal model	Lauren Naomi Spezia Adachi, 2012	tDCS reduced stress in mice	One-way ANOVA Posthoc: Tukey test.	TNF- $\alpha$ : $\downarrow$ tDCS group/ hippocampus TNF- $\alpha$ : = tDCS group/ Corticosterone IL-1B: = in the serum
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	Bettega Costa Lopes, 2020	Post-surgical pain, but pain was relieved in the tDCS or Aerobic exercise or exercise + tDCS groups. Bimodal tDCS, aerobic exercise or both treatments combined promoted analgesic effects for neuropathic pain	One-way ANOVA Posthoc: Bonferroni test.	BDNF: $\uparrow$ tDCS E Physical exercises group IL-1B: $\downarrow$ tDCS E Physical exercises group *Analysis in the cerebral cortex, brainstem, and spinal cord
Transcranial direct current stimulation (tDCS) affects neuroinflammation parameters and behavioral seizure activity in pentylentetrazole-induced kindling in rats	Gabriela Gregory Regner, 2020	Cathodal tDCS did not change the convulsive behavior of the rats (epileptic seizure occurrence or latency). Anodal Tdcs increased the number of epileptic seizures.	One-way ANOVA Posthoc: SNK test.	IL-1 $\beta$ : $\downarrow$ Cathodal tDCS IL-1 $\beta$ : = Anodal tDCS. TNF- $\alpha$ : $\uparrow$ Cathodal tDCS TNF- $\alpha$ : = Anodal tDCS * Analysis in the hippocampus
Transcranial Direct Current Stimulation (tDCS) Induces Analgesia in Rats with Neuropathic Pain and Alcohol Abstinence	Daniela Silva Santos, 2020	Neuropathic pain groups exhibited thermal hyperalgesia. There was a reduction in hyperalgesia in the neuropathic pain + alcohol + tDCS group	One-way ANOVA Posthoc: SNK test.	IL-1 $\alpha$ : $\uparrow$ neuropathic pain + tDCS group (cerebral cortex) IL-1 $\alpha$ : $\uparrow$ neuropathic pain + alcohol + tDCS group (cerebral cortex) IL-1 $\beta$ : $\uparrow$ neuropathic pain + tDCS group (cerebral cortex) IL-1 $\beta$ : $\uparrow$ neuropathic pain + alcohol + tDCS group (cerebral cortex)

Source: Authors.

The effects of tDCS were assessed by behavioral changes (e.g., hyperalgesia, obesity, and allodynia). tDCS was able to alter the levels of TNF- $\alpha$  and several interleucins (e.g., IL-1 $\alpha$ , IL-1 $\beta$ , and IL-6) . In addition to cytokines, tDCS causes biochemical changes in levels of nerve growth factor (NGF), lactate dehydrogenase (LDH), and brain-derived neurotrophic factor (BDNF).

To investigate changes in the cytokine levels after tDCS, studies used the cerebral cortex (De Oliveira et al., 2019) and hippocampus (Guo et al., 2020; Spezia Adachi et al., 2012), (Table 3). After applying tDCS, three studies claimed a reduction in TNF- $\alpha$  levels (De Oliveira et al., 2019; Guo et al., 2020; Spezia Adachi et al., 2012). One study stated that TNF- $\alpha$  remained stable in the brainstem (Callai et al., 2019), and other studies indicated that there was an increase in this cytokine in the cerebral cortex, spinal cord, brainstem (Cioato et al., 2016), and hippocampus (Regner et al., 2020).

Five studies found a reduction in IL-1 $\beta$  levels in the cerebral cortex (De Oliveira et al., 2019), cerebral cortex, spinal cord, and brainstem (Cioato et al., 2016; Lopes et al., 2020); or hippocampus (Guo et al., 2020; Regner et al., 2020). However, one study indicated that IL-1 $\beta$  levels remained stable in the hippocampus (Spezia Adachi et al., 2012). Another article observed an increase in IL-1 $\beta$  levels in the cerebral cortex and brainstem (Santos et al., 2020). For IL-6, one study observed a reduction in the hippocampus (Guo et al., 2020), and in another study, there was an increase in this cytokine in the brainstem (Scarabelot et al., 2019). In addition, the IL-1 $\alpha$  levels were increased in the cerebral cortex and brainstem after the application of tDCS (Santos et al., 2020).

No study has evaluated pro-inflammatory interleukins IL-8, IL-15, IL-17, and IL-18 after tDCS application.

#### 4. Discussion

Pro-inflammatory cytokines are involved in pain processing, neuronal membrane depolarization, and hyperalgesia (Campos Kraychete et al., 2006). TDCS has been investigated as a modulatory factor of the CNS (Nitsche et al., 2008), which may help to inhibit or increase the synthesis/release of these cytokines. This systematic review analyzed the effects of tDCS on the pro-inflammatory cytokine levels in CNS and serum. Furthermore, behavioral changes associated with the variation in cytokine levels were investigated. Although there are methodological differences, studies show that tDCS may alter the synthesis of cytokines.

Bimodal stimulation was the most used method (Callai et al., 2019; De Oliveira et al., 2019; Scarabelot et al., 2019; Cioato et al., 2016, Santos et al., 2020; Lopes et al., 2020). This tDCS protocol was cited in the first time by Spezia and collaborators (Spezia Adachi et al., 2012) and has been reproduced by other authors (Callai et al., 2019; De Oliveira et al., 2019; Scarabelot et al., 2019; Cioato et al., 2016; Lopes et al., 2020; Regner et al., 2020; Santos et al., 2020). Spezia also determined tDCS parameters such as the application time, the current intensity, and the number of tDCS sessions (Spezia Adachi et al., 2012)

When nerves are damaged, microglia and astrocytes are activated and release pro-inflammatory cytokines, which might play an essential role in developing neuropathic pain (Dimming & Algorithm, 2014). In addition, these mediators generate a warning to the body about the potential risk through the activation of nociceptive fibers (Rocha et al., 2007, Cho et al., 2018; Kotani et al., 1999; Ouyang et al., 2011). The literature suggests that the initial response (protective), mainly pro-inflammatory, has harmful properties if it becomes a chronic process. Furthermore, there is a correlation between this inflammatory phase and the occurrence of pain after tissue damage (Thelin et al., 2020).

Besides being associated with allodynia/hyperalgesia, the presence of pro-inflammatory cytokines also can cause fever, increase protein synthesis by the liver, increase the release of corticosteroids, and reduce appetite. These changes occur to accelerate defensive enzymatic reactions, reducing the replication of pathogens and increasing the proliferation of immune cells to immobilize the injured area and conserve energy (Campos Kraychete et al., 2006). In addition, the presence of allodynia/hyperalgesia causes neuronal excitability. Those clinical findings are related to the mechanisms of maladaptation and chronicity (Ashmawi et al., 2016). In chronic pain, there are alterations in the nociceptive system, such as changes in information processing, neuroplastic rearrangement, and apoptosis of interneurons (Moore et al., 2002; Raghavendra et al., 2003). Accordingly, the presence of chronic pain causes a reduction in the patient's physical/mental condition and quality of life (Langley et al., 2013). The increase in pro-inflammatory cytokines among immune, neural or glial cells is essential for the development of pain, as these are the cytokines that are responsible for the establishment of chronic pain (Vanderwall et al., 2019).

One of the possible mechanisms involved in tDCS-induced analgesia is the modulation of levels of pro-inflammatory cytokines such as TNF- $\alpha$  (De Oliveira et al., 2019). This cytokine plays an essential role in inflammatory hyperalgesia and neuropathic pain. For example, in a model of induced pain in rats, there was a significant increase in the levels of TNF- $\alpha$  and IL-1 $\beta$  (Woolf et al., 1997). Furthermore, Cunha and collaborators demonstrated that the injection of TNF- $\alpha$  caused mechanical and thermal hyperalgesia (Cunha, 1992). Likewise, the injection of TNF- $\alpha$  directly into nerves induces Wallerian degeneration, a condition found in painful nerve injuries. Those data indicate a close relationship between increased TNF- $\alpha$  and pain. During the literature review, six studies evaluated the effect of tDCS on TNF levels (Spezia Adachi et al., 2015; Cioato et al., 2016; De Oliveira et al., 2019; Callai et al., 2019; Guo et al., 2020; Regner et al., 2020). Three articles showed decreased cytokine levels after using tDCS (Spezia Adachi et al., 2015; De Oliveira et al., 2019; Guo et al., 2020). One study stated that TNF- $\alpha$  remained stable (Callai et al., 2019), and two other studies indicated that there was an increase in this cytokine (Cioato et al., 2016; Regner et al., 2020).

Using a neuropathic pain model, Yana and collaborators demonstrated that IL-1 $\beta$  and IL-6 levels increase after peripheral nerve constriction (Yana et al., 1992). The increase in IL-1 $\beta$  is also associated with prostaglandins and substance P production in neurons and glial cells reinforcing the association of this interleukin with the presence of pain (Schweizer et al., 1988). Out of the seven studies that investigated the action of tDCS on IL-1 $\beta$  levels (Spezia Adachi et al., 2015; Cioato et al., 2016; De Oliveira et al., 2019; Guo et al., 2020; Regner et al., 2020; Lopes et al., 2020; Santos et al., 2020), five showed reduced levels of this cytokine after the treatment (Cioato et al., 2016; De Oliveira et al., 2019; Guo et al., 2020; Regner et al., 2020; Lopes et al., 2020).

IL-6 is involved in microglial and astrocytic activation (Jones et al., 1997), and its increase contributes to the development of neuropathic pain after peripheral nerve injury (Ramer et al., 1998). In a study using a model of neuropathic pain caused by freezing the sciatic nerve, an increase in IL-6 in the spinal cord was observed, demonstrating the association of this interleukin with the presence of pain (Joyce, 1996). One study observed a reduction of IL-6 after the application of tDCS (Guo et al., 2020), while the other study observed increased levels of this interleukin (Scarabelot et al., 2019). As for IL-1 $\alpha$ , which was analyzed in only one study (Santos et al., 2020), the level increased after treatment with tDCS. IL-17, considered a pro-inflammatory cytokine (Jaiswal, 2014), has not yet been analyzed in the studies selected for the review.

Behavioral tests are used to investigate the presence of allodynia or hyperalgesia. In addition, these procedures allow for the evaluation of the non-communicating subjects (Deuis et al., 2017). In addition, pain can change several behaviors, such as locomotion, and may cause depression and anxiety (Guo et al., 2020; Regner et al., 2020; Santos et al., 2020). There are controversies in the literature concerning the effects of tDCS on pain behavior, especially using von Frey and Hot Plate test. On the other hand, some authors observed that the tDCS application efficiently reduced mechanical and thermal pain (Cioato et al., 2016; Scarabelot et al., 2019; Spezia Adachi et al., 2012; Lopes et al., 2020). Other study demonstrated that the association of tDCS and alcohol caused analgesia, but tDCS alone did not reduce pain in rats (Santos et al., 2020). Moreover, a study failed to find analgesic effect of tDCS in neuropathic pain rat model (Callai et al., 2019). For anticonvulsant control, the cathodal tDCS also did not benefit the animals (Regner et al., 2020). Likewise, cathodal tDCS had no anticonvulsant action in an animal model. However, the use of tDCS improved cognitive impairment and stress effects in rats submitted to a rat model of vascular dementia (Guo et al., 2020).

## 5. Conclusion

The present literature review supports the idea that the application of tDCS can effectively modulate the levels of pro-inflammatory cytokines. However, even though there was a decrease in these cytokine levels in most studies, these results are not unanimous. For example, some studies found that tDCS might increase cytokine levels, and others were not able to find

any effects of tDCS on these biochemical factors. One of the possible explanations for the lack of effect is the methodological variability applied by different studies. Furthermore, it is worth noting that the duration of these effects of tDCS on cytokine levels is limited to a few hours (Liebetanz et al., 2009).

In animal models, it has been seen that tDCS may decrease cytokine levels and pain measurements. However, it is difficult to make a direct relationship (cause/effect) between the induced analgesia and variation in cytokine levels because these results may be independent. Future studies should use new approaches (e.g., different brain areas stimulated and new technologies to deliver varying intensities of currents). Therefore, it will be possible to investigate further the mechanisms involved in the effect of tDCS on the levels of pro-inflammatory cytokines and their consequences on pain.

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