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Electrical parameters of transcranial direct current stimulation that effectively alter cerebral blood flow in experimental animals: a systematic review

Parâmetros elétricos da estimulação transcraniana por corrente contínua efetivos para alterações do fluxo sanguíneo cerebral de animais experimentais: uma revisão sistemática

Parámetros eléctricos de la estimulación transcraneal con corriente continua efectiva para cambios en el flujo sanguíneo cerebral en animales de experimentación: una revisión sistemática

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Abstract

Objective: To identify the electrical parameters of transcranial direct current stimulation **(**tDCS) that effectively alter cerebral blood flow in rats. Methodology: Six eletronic databases were searched with no time or language restrictions to identify experimental studies with rats using tDCS with anodal and/or cathodal stimulation with or without a comparison group. Internal validity was assessed via the following criteria: housing, lighting, temperature, water/food, groups randomization and ethical aspects. The 'Laboratory Systematic Review Center for Laboratory animal Experimentation' (SYRCLE) tool was used to assess risk of bias. The tDCS electrical parameters and cerebral blood flow were considered as primary outcomes and cerebral histological alterations as the secondary outcome. Results: Four articles were included. All four studies were considered to present a high level of scientific bias. The electrical tDCS parameters implemented were heterogeneous but overall, tDCS with anodal stimulation promoted an increase in cerebral blood flow while the cathodal stimulation decreased it. Cerebral histological alterations were assessed in two studies and tissue necrosis was reported in only one animal per study. Conclusion: The identification of tDCS electrical parameters that effectively alter cerebral blood flow in rats was not possible due to the heterogeneity of tDCS protocols being implemented in the literature. Considering the high risk of scientific bias in the included studies, the current available evidence regarding tDCS efficacy is insufficient and inconclusive.

Keywords: Transcranial direct current stimulation; Cerebrovascular circulation; Blood flow cerebral; Rats.

Resumo

Objetivo**:** Identificar os parâmetros da estimulação transcraniana por corrente contínua (ETCC) mais eficazes para promover alterações sobre o fluxo sanguíneo cerebral de ratos. Metodologia**:** Seis bancos de dados eletrônicos foram pesquisados sem restrição linguística e temporal para identificar estudos experimentais que utilizaram a ETCC anódica e/ ou catódica comparando-as a um grupo controle ou a um grupo sham, em ratos. Os critérios utilizados para avaliar a validade interna dos estudos experimentais foram: alojamento, iluminação, temperatura, água/dieta, randomização dos grupos e aspectos éticos. Para análise do risco de viés dos estudos, foi utilizada a ferramenta Laboratory Systematic Review Center for Laboratory animal Experimentation (SYRCLE). Os parâmetros elétricos e fluxo sanguíneo cerebral foram considerados como desfechos primários e a avaliação de alterações histológicas cerebrais como desfecho secundário**.** Resultados: Quatro artigos foram incluídos. Todos os artigos foram classificados como alto risco de viés. Os instrumentos de avaliação e os parâmetros elétricos aplicados foram heterogêneos, entretanto, viu-se que a estimulação anódica promoveu um aumento do fluxo cerebral sanguíneo e a estimulação catódica efeito contrário. Dois estudos realizaram avaliação histológica cerebral e destacaram a presença de necrose tecidual em apenas um animal de cada estudo. Conclusão**:** Diante da diversidade dos protocolos da ETCC, não foi possível determinar os parâmetros elétricos eficazes na promoção de alterações do fluxo sanguíneo cerebral em ratos. Devido ao alto risco de viés nos artigos incluídos, as evidências disponíveis sobre a eficácia da ETCC são insuficientes e inconclusivas.

Palavras-chave: Estimulação transcraniana por corrente contínua; Circulação cerebrovascular; Fluxo sanguíneo cerebral; Ratos.

Resumen

Propósito: Identificar los parámetros de estimulación de corriente continua transcraneal (tDCS) más efectivos para promover cambios en el flujo sanguíneo cerebral en ratas. Metodología: Se realizaron búsquedas en seis bases de datos electrónicas sin restricciones lingüísticas y temporales para identificar estudios experimentales que usaron tDCS anódico y/o catódico, comparándolos con un grupo de control o un grupo simulado, en ratas. Los criterios utilizados para evaluar la validez interna de los estudios experimentales fueron: alojamiento, iluminación, temperatura, agua/dieta, aleatorización de los grupos y aspectos éticos. Para analizar el riesgo de sesgo en los estudios se empleó la herramienta Laboratory Systematic Review Center for Laboratory animal Experimentation (SYRCLE). Los parámetros eléctricos y el flujo sanguíneo cerebral se consideraron como resultados primarios y la evaluación de los cambios histológicos cerebrales como resultado secundario**.** Resultados: Cuatro artículos fueron incluidos. Todos los artículos fueron clasificados como de alto riesgo de sesgo. Los instrumentos de evaluación y los parámetros eléctricos aplicados fueron heterogéneos, sin embargo, la estimulación anódica promovió un aumento del flujo sanguíneo cerebral y la estimulación catódica tuvo el efecto contrario. Dos estudios realizaron una evaluación histológica del cerebro y destacaron la presencia de necrosis tisular en solo un animal de cada estudio. Conclusión: dada la diversidad de protocolos de tDCS, no fue posible determinar los parámetros eléctricos efectivos para promover cambios en el flujo sanguíneo cerebral en ratas. Debido al alto riesgo de sesgo de los artículos incluidos, la evidencia disponible sobre la efectividad de la tDCS es insuficiente y no concluyente.

Palabras clave: Estimulación transcraneal de corriente directa; Circulación cerebrovascular; Flujo sanguíneo cerebral; Ratas.

1. Introduction

Transcranial direct current stimulation (tDCS) is a low-cost neuromodulation technique capable of altering cortical excitability and facilitating neuroplasticity (Bhattacharya et al., 2021; Nitsche & Paulus, 2000a; Michael et al., 2011). The proposed mechanisms of action underlying tDCS neuromodulatory effects such as changes in resting membrane action potential include its action upon Sodium and Calcium channels and N-methyl D-aspartate (NMDA) receptors(Ghanavati et al., 2022; Kim et al., 2010; Stagg & Nitsche, 2011). Furthermore, it is suggested in the literature that tDCS may also induce changes in blood flow in cortical and subcortical regions(Lang et al., 2005).

Presuming that tDCS application could lead to changes in cerebral blood flow (CBF), this technique could be of therapeutic relevance especially for conditions disrupting the cerebral vasculature such as strokes and migraines(Bornheim et al., 2020; Gorelick et al., 2011; Li & Morton, 2020; Moisset et al., 2020; Orrù et al., 2020). Therefore, considering its therapeutic potential and limited knowledge on its mechanisms of action, tDCS has been extensively explored in experimental research (Kim et al., 2010; Wachter et al., 2011).

The neuromodulatory effects observed with tDCS essentially depend on the electrical parameters being implemented (i.e., polarity, intensity, density, amplitude, duration, as well as electrode size and placement) and on individual anatomical

characteristics (e.g., cranium thickness)(Garnett et al., 2015; Liebetanz et al., 2009; Nitsche et al., 2008; Nitsche & Paulus, 2000a), age (Ghasemian-Shirvan et al., 2022), making it difficult to standardize protocols among different studies. Therefore, this systematic review aimed to identify the electrical parameters of transcranial direct current stimulation (tDCS) that effectively alter cerebral blood flow in rats.

2. Methodology

2.1 Study Selection

This systematic review was developed based on a previous study selection with the inclusion criteria consisting of experimental studies using tDCS with anodal and/or cathodal stimulation with or without a comparison group (control – no intervention, or *sham*) in male and/or female rats. Exclusion criteria consisted of studies with humans, *in vitro* studies, and studies implementing different stimulation techniques such as electrical-acupuncture, trigeminal/vagus nerve stimulation, peripherical nerve stimulation, transcranial magnetic stimulation, spinal cord stimulation, and transcutaneous electrical stimulation.

Primary outcomes of interest were the electrical tDCS parameters (i.e., intensity, size and placement of electrode, and duration and frequency of stimulation) and CBF. Secondary outcomes of interest were defined as the changes in cerebral histology.

2.2 Search Strategy

The following databases were searched with no time or language restrictions: CINAHAL, LILACS, MEDLINE/PubMed, Scielo, Scopus e Web of Science. The descriptors used in the search strategy followed MeSH and DeCS terms: Transcranial direct current stimulation, Electric stimulation therapy, tDCS, Transcranial electrical stimulations, Cathodal stimulation tDCS, Anodal stimulation tDCS, and rats. Index terms were then combined via the Boolean operator 'AND'. An initial search was conducted in December of 2015 and redone in July of 2020 to update the systematic review. Additionally, the reference lists of included studies were manually searched.

2.3 Data Extraction and Quality Appraisal

Data screenings were conducted by two independent reviewers (CCSA and EPR). Initially, title and abstracts were screened according to the inclusion and exclusion criteria. In the case of the article either meeting both inclusion and exclusion criteria or if the information from title and abstract was not clear, the full-text was obtained and screened. Studies were only included after a full-text screening. Any discrepancy regarding study eligibility was solved by either a consensus among the two reviewers or by a third reviewer (DAO).

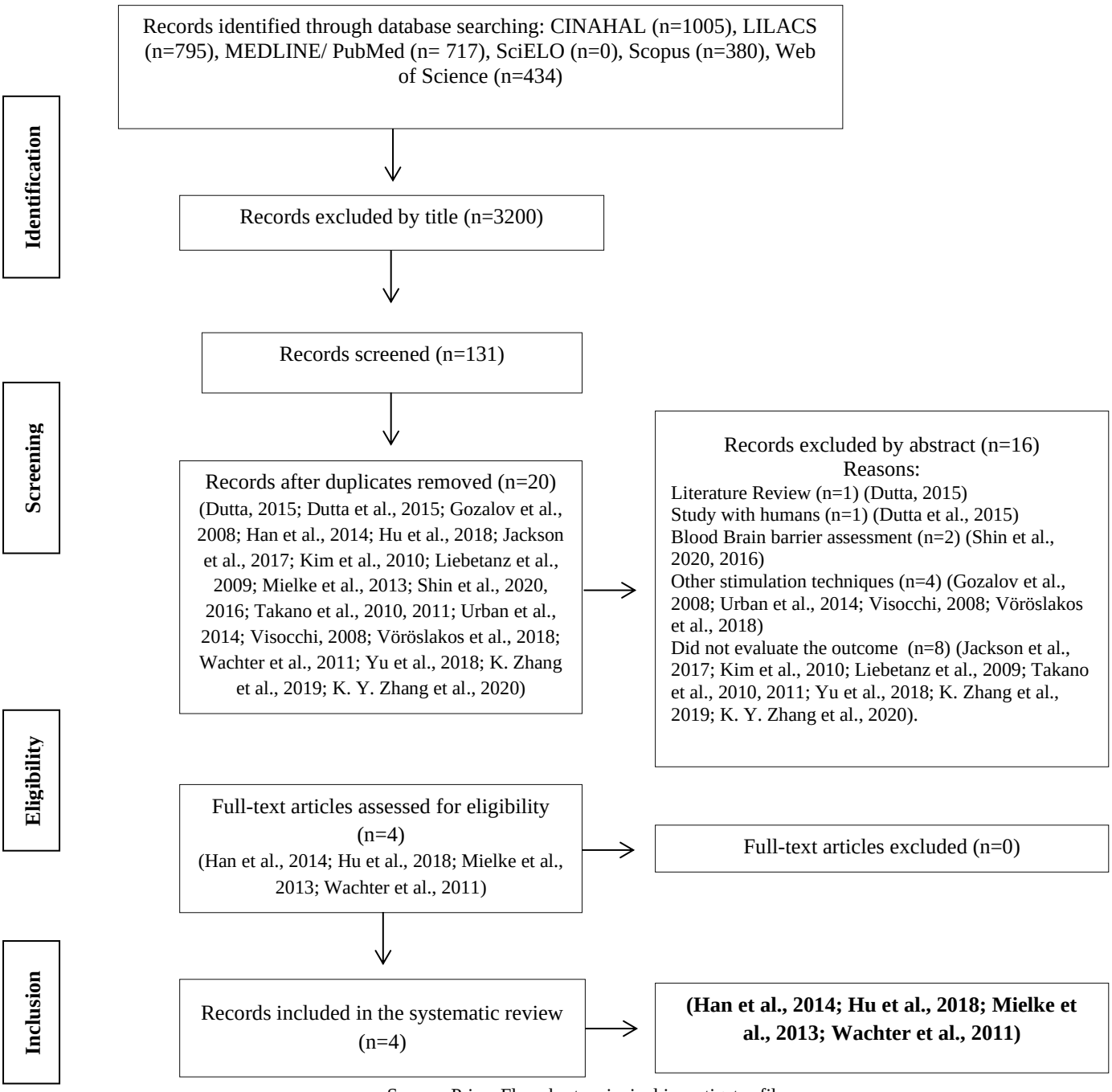
The methodological quality appraisal and the risk of bias analyses were conducted by two independent reviewers (CCSA and EPR) according to the specific criteria for internal validity of experimental studies according to Hooijmans *et al*., 2010, such as housing, lighting, temperature, water/food, group randomization and ethical aspects(Hooijmans, Leenaars, & Ritskes-Hoitinga, 2010). Additionally, 'Laboratory Systematic Review Center for Laboratory animal Experimentation' (SYRCLE) tool provided by the SYRCLE at Central Animal Laboratory(Hooijmans et al., 2014) was used to assess scientific bias.

Upon completion of data collection, the development of a metanalysis was evaluated however, its development was not feasible due to methodological differences among studies.

3. Results

The database search resulted in a total of 3331 studies of which 20(Dutta, 2015; Dutta et al., 2015; Gozalov et al., 2008; Han et al., 2014; Hu et al., 2018; Jackson et al., 2017; Kim et al., 2010; Liebetanz et al., 2009; Mielke et al., 2013; Shin et al., 2020; Shin et al., 2016; Takano et al., 2010, 2011; Urban et al., 2014; Visocchi, 2008; Vöröslakos et al., 2018; Wachter et al., 2011; Yu et al., 2018; Zhang et al., 2019; K. Y. Zhang et al., 2020) were selected for full-text screening resulting in four eligible studies(Han et al., 2014; Hu et al., 2018; Mielke et al., 2013; Wachter et al., 2011) as shown in the flowchart diagram (Figure 1). No studies were included based on the manual search of reference lists.

Figure 1. Study research and selection for systematic review in accordance wiht the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA).



Source: Prism Flowchart- principal investigator file.

Table 1 shows the assessment of the included studies according to the internal validity criteria for animal rooms as presented by Hooijmans *et al*., 2010. Of the four included studies, two reported 100% of the criteria items(Mielke et al., 2013; Wachter et al., 2011) while the other two studies reported only 20% of the criteria items related to the ethical aspects of the study(Han et al., 2014; Hu et al., 2018).

Table 1: Quality assessment of the bioterium.

Author / year	Housing	Lighting	Temperature	Water/food	Ethical aspects
WACHTER, et al., 2011	+	+	+	+	+
MIELKE et al., 2013	+	+	+	+	+
HAN et al., 2014	-	-	-	-	+
HU et al., 2018	-	-	-	-	+

(+) presente information; (-) missing information. **S**ource: Items highlighted by Hooijmans et al., 2010 - principal investigator file. Fonte: Authors.

According to the SYRCLE tool, the included studies were considered to present a high level of scientific bias (Han et al., 2014; Hu et al., 2018; Mielke et al., 2013; Wachter et al., 2011) (Table 2). Information regarding allocation sequence, group randomization, or blinding of research personnel during intervention, results and statistical analyses were not provided by any of the four studies.

Table 2. Bias risk assessment of the selected studies.

Questions	Studies			
	WACHTER, et al., 2011	MIELKE et al., 2013	HAN et al, 2014	HU et al., 2018
1) Was the allocation sequence adequately generated and applied?	no	no	no	no
2) Were the groups similar at baseline or were they adjusted for confounders in the analysis?	yes	no	no	yes
3) Was the allocation to the different groups adequately concealed during?	no	?	no	no
4) Were the animals randomly housed during the experiment?	no	no	no	no
5) Were the caregivers and/or investigators blinded from knowledge which intervention each animal received during the experiment?	no	no	no	no
6) Were animals selected at random for outcome assessment?	no	no	no	no
7) Was the outcome assessor blinded?	no	no	no	no
8) Were incomplete outcome data adequately addressed?	yes	yes	yes	?
9) Are reports of the study free of selective outcome reporting?	yes	yes	yes	yes
10) Was the study apparently free of other problems that could result in high risk of bias?	yes	yes	yes	yes

Yes = low risk of bias; no = high risk of bias; Unclear? = unclear risk of bias. Source:SYRCLE Tool by Hooijmans et al., 2014 – principal investigator file. Fonte: Authors.

The studies' characteristics are shown in Table 3 in a chronological order according to the publication date. All studies were conducted in male Sprague-Dawley rats; Three studies (Han et al., 2014; Mielke et al., 2013; Wachter et al., 2011) utilized isoflurane and one study (Hu et al., 2018) used sodium pentobarbital for anesthesia before tDCS intervention; The current polarization implemented in these studies were: anodal and cathodal stimulation (Wachter et al., 2011), cathodal stimulation Only (Mielke et al., 2013), and anodal stimulation Only (Han et al., 2014; Hu et al., 2018).

Table 3: Characteristics of selected studies, arranged in chronological order of publication

Author / year	Study design	Species	Sex	Mean body weight (g)	Anesthesia	tDCS
WACHTER et al., 2011	Cross-over	Sprague-Dawley	Male	310	Isoflurane (1.0-1.5%; flow of 1.8-2.0 l/ min)	Anodal Cathodal
MIELKE et al., 2013	Experimental	Sprague-Dawley	Male	290	Isoflurane (1.0-1.5%; flow of 1.8-2.0 l/ min)	Cathodal
HAN et al, 2014	Experimental	Sprague-Dawley	Male	300-350	Isoflurane	Anodal
HU et al., 2018	Experimental	Sprague-Dawley	Male	270	Sodium pentobarbital (3%, 5 mg/100g, i.p.)	Anodal

g (grams); l/min (liters/minute); tDCS (Transcranial direct current stimulation); mg/100g (miligram/ 100 grams); i.p. (intraperitoneal).
Source: Principal investigator file. Fonte: Authors.

The tDCS protocols implemented in the four included articles are summarized in Table 4. Two studies used similar protocols regarding the reference point for electrode placement and duration of stimulation(Mielke et al., 2013; Wachter et al., 2011). The remaining stimulation parameters such as intensity, current frequency, and electrode size varied among the four studies(Han et al., 2014; Hu et al., 2018; Mielke et al., 2013; Wachter et al., 2011).

Table 4: tDCS protocols used in selected studies, arranged in chronological order of publication.

Author / year	Groups (N)	Intensities	Electrodes				Duration	Frequency
			Area of contact		Localization			
			AE	RE	AE	RE		
WACHTER et al., 2011	Anodal (N= 8) Cathodal (N= 8)	25 µA 50 µA 100 µA	3.5mm^2	10.5 cm^2	Approximately 2 mm behind the coronal suture and 4 mm lateral to the sagittal	Ventral region of the rat thorax	15 min	6 sessions/ rat (48-hour interval between sessions)
MIELKE et al., 2013	Cathodal (N= 21)							
	E1 (n=9)	200 µA n(3) 400 µA n(3) 600 µA n(3)	3.5 mm^2 7.0 mm^2 10.5 mm^2	10.5 cm^2	Approximately 2 mm behind the coronal suture and 4 mm lateral to the sagittal suture	Ventral region of the rat thorax	15 min	1 session/ rat
	E2 (n=6)	400 µA n(3) 600 µA n(3)	10.5 mm^2 10.5 mm^2	10.5 cm^2	Approximately 2 mm behind the coronal suture and 4 mm lateral to the sagittal suture	Ventral region of the rat thorax	15 min	1 session/ rat
	E3 (n=6)	600 µA n(3) 700 µA n(3)	14.0 mm^2 14.0 mm^2	10.5 cm^2	Approximately 2 mm behind the coronal suture and 4 mm lateral to the sagittal suture	Ventral region of the rat thorax	15 min	1 session/ rat

HAN et al, 2014	Anodal (N=12)	200 µA	3.5 mm^2		Right cortex (2 mm posterior from bregma and 5 mm lateral from the medial point)	Ventral region of the rat thorax	10 min	
HU et al., 2018	Anodal (N=11)	15 µA	150 µm	3mm	Anteroposterior coordinates 3.5mm and mediolateral	Fixed on the skull after lambda	10s	1 session/ rat

N (total sample number); n (sample number in the group); AE (active electrode); RE (reference electrode); E1 (experiment 1); E2 (experiment 2); E3 (experiment 3); µA (microampère); mm (millimeter); mm^2 (square millimeter); µm (micrometer); cm^2 (square centimeter); min (minute); s (second). **S**ource: Principal investigator file. Fonte: Authors.

Table 5 provides a synthesis of the findings related to CBF and the histological analysis of cerebral tissue after tDCS intervention. The assessment tools used to investigate CBF varied among the four studies and included the use of laser doppler flowmetry(Wachter et al., 2011), laser doppler imaging(Mielke et al., 2013), near-infrared spectroscopy(Han et al., 2014), and laser speckle contrast imaging(Hu et al., 2018). Even though different assessment tools were used for CBF, similar findings regarding the effects of tDCS on CBF were found.

Table 5: Results found in tDCS protocols used in selected studies on cerebral blood flow, arranged in chronological order of publication

Author / year	**Assessment Instruments**	**Anodal tDCS**		**Cathodal tDCS**		
		Cerebral Blood Flow	**Histological Analysis**	**Cerebral Blood Flow**		**Histological Analysis**
WACHTER et al., 2011	Laser Doppler Flowmetry	25 µA 50 µA 100 µA	Ø ↑ **18% CBF** ↑ **25% CBF**	Unilateral lesion in the parieto-occipital cortex was observed adjacent to the electrode- signs of axonal degeneration, necrosis in one animal	25 µA 50 µA 100 µA	↓ **CBF** ↓ **25% CBF** ↓ **CBF**	Unilateral lesion in the parieto-occipital cortex was observed adjacent to the electrode- signs of axonal degeneration, necrosis in one animal
MIELKE et al., 2013	Laser Doppler blood perfusion imaging	NR		NR	E1 * I=600 µA; TEA= 10.5 mm^2 E2 - I=400 µA; TEA= 10.5 mm^2 E3 # I=700 µA; TEA= 14.0 mm^2	↓ **CBF** Ø ↓ **CBF**	No pathological findings such as gliosis, edema or hemorrhage, except for one animal that found axonal degeneration, necrosis.
HAN et al, 2014	Near-infrared Spectroscopy (NIRS)	**↑Oxy-Hb during tDCS** **↓Oxy-Hb after tDCS** *These changes may reflect changes in the CBF		NR	NR		NR
HU et al., 2018	Laser-Speckle Contrast Imaging	↑ **CBF** during the 10s of the tDCS and in the seconds after the tDCS as described below (M± SD) 30s 10.1 ± 5.1% 60s 3.3 ± 2.1% 90s 1.5 ± 1.1% 120s 1.1 ± 0.89%		NR	NR		NR

CBF (cerebral blood flow); Oxy-Hb (oxygenated hemoglobina); µA (microampère); mm^2 (square millimeter); s (second); NR (no rated); Ø No significant reduction compared to the other groups in the experiment; (M± SD) (mean± standard deviation).
*Significant reduction in this group in relation to the other groups in experiment 1; # Significant reduction of this group in relation to the other groups of the experiment. **S**ource: Principal investigator file.

The effects of current polarity on CBF collectively show that on the three studies using anodal stimulation(Han et al., 2014; Hu et al., 2018; Wachter et al., 2011) a significant increase in CBF was found following tDCS. On the other hand, the two studies using cathodal stimulation(Mielke et al., 2013; Wachter et al., 2011) reported a significant decrease in CBF.

The duration of tDCS-induced changes in CBF varied among studies. One study(Wachter et al., 2011) reported that higher intensities (50 e 100 µA) of tDCS anodal stimulation promoted an increase in CBF that lasted for 30 minutes after the intervention. Additionally, this study reported that the decrease of CBF following tDCS cathodal stimulation was also dependent on the current intensity being used with intensities around 100 µA reducing approximately 25% of CBF for at least 30 minutes after the intervention. For intensities between 25 µA and 50 µA, CBF reached baseline levels within the 30-minute window(Wachter et al., 2011).

Another study reported up to 50% reduction in CBF with the duration of effects lasting up to 90 minutes after tDCS cathodal stimulation (e.g., intensity of 600 µA; total area of active electrode of 10,5mm^2)(Mielke et al., 2013). The use of tDCS anodal stimulation on the study by Han *et al*.,(Han et al., 2014) showed an increase in CBF during the intervention (e.g., intensity of 200 µA; duration of 10 minutes) with a reduction in CBF being observed immediately after tDCS was ended.

Lastly, the study conducted by Hu and colleagues(Hu et al., 2018), aimed to monitor real-time tDCS anodal stimulation-induced changes in CBF. In this study, CBF was monitored for 160 seconds (s) of which 20 s were designated as baseline measurements before the intervention, 10 s of tDCS anodal stimulation, and 30 s, 60 s, 90 s, and 120 s of follow-up measurements after tDCS anodal stimulation. The authors concluded that the velocity of CBF increased during the first 30 s and gradually decreased in the period between 30 s and 90 s(Hu et al., 2018).

Two studies looking at histological changes in cerebral tissue(Mielke et al., 2013; Wachter et al., 2011) reported signs of axonal degeneration suggestive of necrosis in only one animal per study.

4. Discussion

Though the studies included in this systematic review show that tDCS promotes changes in CBF in rats, the heterogeneity of protocols implemented among these studies precluded the determination of optimal tDCS parameters to induce changes in CBF. Moreover, due to the high level of scientific bias the interpretation of these results regarding tDCS efficacy need to be taken with caution.

The internal validity assessment according to Hooijmans *et al.*, 2010, showed that two studies obtained the maximum score with all items being reported while two studies only reported items related to ethical aspects. The validity and reliability of experimental studies are maximized when the material, animals, and methods implemented are safe and reproducible. The housing conditions, lighting, temperature, water/diet directly influence animal behavior. Therefore, reporting the variables that may influence the animals' quality of life is a key determinant on obtaining reliable and tenable results according to the ethical aspects(Deguchi, Tamioso, & Molento, 2016).

In addition, a high level of scientific bias was identified by the SYRCLE tool in all four studies. Only one study implemented a cross-over design allowing animals to experience both control and intervention conditions(Ding et al., 2015). However, the remaining studies did not report the presence of a control group for comparison. The lack of a control group makes it difficult to determine if the effects observed with tDCS are truly due to the intervention itself(Pithon, 2013).

None of the studies reported sample randomization which is considered a key strategy to reduce selection bias and to consequently prevent direct interference on results observed with the intervention(Leal, Bezerra, & Lemos, 2012; Montori & Guyatt, 2001). Additionally, none of the studies reported blinding of research personnel during either intervention delivery or analysis of results. Blinding is also considered as a key strategy to minimize scientific bias especially in regards to

measurement and outcome assessment therefore, minimizing a direct interference of research personnel on the results observed with the intervention(Leal et al., 2012; Montori & Guyatt, 2001).

The methods used for CBF assessment varied among studies. Even though in three studies laser was used as an assessment tool, the techniques varied widely among studies(Hu et al., 2018; Mielke et al., 2013; Wachter et al., 2011). Laser doppler flowmetry, laser doppler imaging, and laser speckle contrast imaging (LSCI) all offer a quantitative and objective measure of CBF (Corrêa et al., 2010; Cyr et al., 2019). However, LSCI has some advantages when compared to laser doppler imaging due to its high resolution digitalization capabilities of a broader area in a shorter time-frame(Corrêa et al., 2010; Cyr et al., 2019). On the other hand, the near-infrared spectroscopy offers an indirect measure of CBF by looking at the variations in absolute hemoglobin concentration and in oxyhemoglobin (Lima & Bakker, 2011).

Overall, the experimental protocols implemented in the studies included in this systematic review show a lack of standardization of electrical parameters to induce immediate changes in CBF. Current intensity varied between 25 µA and 700 µA and the duration of stimulation varied between 10 s and 15 minutes. The placement and size of the ground electrode were similar in three out of the four studies. The area of contact for active electrode on the other hand varied between 150 µm a 14 mm^2. Such methodological variations can also be observed in clinical studies where reportedly the results of tDCS stimulation on the cerebral cortex are dependent on the current polarity, stimulation area, duration of stimulation, intensity, and density being used (M A Nitsche & Paulus, 2000a, 2000b; Woods et al., 2016).

It is important to highlight that even though these differences in assessment tools and intervention protocols were observed in the included studies, similar results of tDCS on CBF were found. Overall, the results showed that tDCS anodal stimulation promotes an increase in CBF in rats while tDCS cathodal stimulation promoted a reduction in CBF. These findings are in accordance with a clinical study conducted with 14 healthy participants where the authors reported a 17.1% increase in CBF following tDCS anodal stimulation and a -6.5% reduction in CBF following tDCS cathodal stimulation (Zheng et al., 2011).

The compiling evidence that tDCS can promote changes in CBF has increased the interest of its implementation in clinical practice. It is reported in the literature that conditions affecting the cerebral vasculature can lead to cumulative damage and consequently to the deterioration of cerebral function (Gorelick et al., 2011) and that the increase in the local blood flow and consequent increase in glucose and oxygen supply have been shown to improve cerebral function (Pulgar, 2015).

However, in order to expand the therapeutic applicability of this technique, overcoming the challenges of determining optimal stimulation parameters becomes essential.

5. Conclusion

The present systematic review was inconclusive in determining the tDCS electrical parameters that effectively alter CBF in rats due to the wide heterogeneity of protocols being used among the included studies. Considering the provided data, the studies included in this systematic review showed a high level of scientific bias. Therefore, future studies aiming to establish standard tDCS protocols should implement a rigorous methodology by implementing allocation concealment, blinding, and randomization strategies. Furthermore, future studies should provide enough detail regarding the protocol being implemented in order to maximize the reproducibility of protocols and the replicability of results.

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