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AVALIAÇÃO DOS EFEITOS DO TIPO *TOPDOWN* DA ESTIMULAÇÃO TRANSCRANIANA COM CORRENTE CONTÍNUA NAS ALTERAÇÕES NEUROTRÓFICAS CAUSADAS PELO ESTRESSE INDUZIDO POR IMOBILIZAÇÃO

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Sumário

Lista de Abreviaturas

BDNF – *Brain Derived Neurotrophic Factor*

CORT - Corticosterona

ETCC - Estimulação Transcraniana com Corrente Contínua

GABA - *Gamma-AminoButyric Acid*

HPA – *Hypothalamic-pituitary-adrenocortical*

IEG – *Immediate Early Gene*

SNC – Sistema Nervoso Central

5-HT - 5-hidroxitriptamina ou Serotonina

TrkB - Tyrosine kinase receptor B

ERM – Espectroscopia por Ressonância Magnética

Resumo

Apesar das respostas ao estresse serem necessárias para adaptações do organismo ao meio, a sua presença favorece o surgimento de diversas doenças e alterações sensoriais como a percepção de dor. Além disto, o estresse pode alterar os níveis de vários fatores neuroquímicos como o fator neurotrófico derivado do cérebro (BDNF) no sistema nervoso central. Como não é possível eliminar completamente os estímulos estressores, é necessário estudar novas técnicas terapêuticas para reduzir impactos do estresse na saúde. Uma alternativa para o controle dos efeitos do estresse pode ser a estimulação transcraniana por corrente contínua (ETCC) que tem demonstrado efetividade no tratamento de alterações neurológicas como depressão, Alzheimer e dor crônica. Esta terapia, além dos componentes locais (intracortical), foi sugerida por possuir ação nas vias dolorosas descendentes (efeito *topdown*). Desta forma, o presente estudo teve por objetivo a avaliação dos efeitos do tipo *topdown* da ETCC na reversão/prevenção de alterações neuroquímicas (níveis de BDNF na medula espinal) e comportamentais (sensibilidade mecânica) em ratos estressados. No primeiro dia, a sensibilidade mecânica de ratos Wistar, machos, de 2 meses de idade (n=100) foi aferida usando o teste de von Frey. No sétimo dia, os animais foram imobilizados por 20 min. Os animais foram divididos em dois grupos: um deles recebeu uma sessão de ETCC (500 μA), e outro recebeu simulação de tratamento (*sham*-ETCC) durante a imobilização. Esses dois grupos foram divididos em 4 grupos, de acordo com o tempo que foram eutanasiados após a estimulação verdadeira ou *sham*. Os animais foram testados novamente após a intervenção de acordo com os seus grupos (30, 60, 12 min ou 24 h) e eutanasiados imediatamente após. Os animais do grupo controle não receberam nenhum tratamento. Desta forma, os animais foram alocados em 10 grupos: ETCC 30, ETCC 60, ETCC 120, ETCC 24h, *Sham* ETCC 30, *Sham* ETCC 60, *Sham* ETCC 120 e *Sham* ETCC 24h. Os testes bioquímicos (BNDF) foram realizados por ELISA. As análises estatísticas utilizadas foram: ANOVA de medidas repetidas, ANOVA de 1 via e correlação de Pearson. O projeto foi

aprovado pela CEUA/HCPA (projeto 2019-0126). De acordo com os resultados, notou-se aumento do limiar de dor mecânica em 30 e 60 min nos grupos ETCC. Do mesmo modo, a sensibilidade mecânica não foi alterada nos animais que foram submetidos somente ao estresse. Houve redução nos níveis de BDNF na medula espinal nos grupos que receberam o estresse sem tratamento ativo (60min, 120min e 24h). Os animais que receberam ETCC não diferiram dos animais controle em relação aos níveis de BDNF. Observou-se uma correlação negativa entre os níveis de BDNF na medula espinal e a sensibilidade mecânica dos animais tratados com ETCC. Os presentes dados indicam que uma única sessão de ETCC tem efeito analgésico em animais, por até 60 minutos. O estresse por restrição de movimentos induz a diminuição dos níveis de BDNF na medula espinal de ratos, e a ETCC, através de vias descendentes, pode reverter/prevenir estas alterações neurotróficas. Neste contexto, a ETCC apresenta-se como alternativa de tratamento para certas mudanças causadas pelo estresse, diminuindo o impacto da agitada vida moderna, reduzindo gastos e aumentando a qualidade de vida da sociedade.

Palavras-chaves: Fator Neurotrófico Derivado do Encéfalo; Estresse Fisiológico; Imobilização; Estimulação Transcraniana por Corrente Contínua

Abstract

Although responses to stress are necessary for adaptations of the organism to the environment, their presence favors the appearance of several pathologies and sensory changes (*e.g.* perception of pain). In addition, stress can alter the levels of various neurochemical factors such as the brain-derived neurotrophic factor (BDNF) in the central nervous system. As it is not possible to eliminate stressful stimuli completely, it is necessary to study new therapeutic techniques to reduce the impact of stress on health. An alternative to control the effects of stress may be Transcranial Direct Current Stimulation (tDCS), which has been shown to be effective in the treatment of neurological disorders such as depression, Alzheimer's disease and chronic pain. This therapy, in addition to the local components (intracortical), was suggested to have action in the descending pathways (topdown effect). Thus, the present study aimed to evaluate the effects like topdown of the tDCS on reversing / preventing neurochemical (BDNF levels in the spinal cord) and behavioral (mechanical sensitivity) changes in stressed rats. On the first day, the mechanical sensitivity of 2-month-old male Wistar rats (n = 100) was measured using the von Frey test. On the seventh day, the animals were immobilized for 20 minutes. They were divided into two groups: those who received a session of tDCS (500 μA), and another received treatment simulation (sham-tDCS) during immobilization. These two groups were divided into 4 groups, according to the time they were euthanized after the real stimulation or sham.The animals were tested again after the intervention according to their groups (30, 60, 12 min or 24 hours) and euthanized soon after. The animals in the control group did not receive any treatment. Thus, the animals were allocated into 10 groups: tDCS 30, tDCS 60, tDCS 120, tDCS 24h, Sham tDCS 30, Sham tDCS 60, Sham tDCS 120 and Sham tDCS 24h. Biochemical tests (BNDF) were performed by ELISA. To evaluate the effects of ETCC, repeated measures ANOVA, 1-way ANOVA and Pearson correlation were used. The project was approved by CEUA / HCPA (project 2019-0126). An increase in the mechanical pain threshold was observed

in 30 and 60 minutes in the tDCS groups. Mechanical sensitivity was not altered in animals that were subjected only to stress. There was a reduction in BDNF levels in the spinal cord in the groups that received stress without active treatment (60min, 120min and 24h). The animals that received tDCS did not differ from the control animals in relation to BDNF levels. A negative correlation was seen between BDNF levels in the spinal cord and the mechanical sensitivity of animals treated with tDCS. The present data indicate that a single session of tDCS has analgesic effect in animals, for up to 60 minutes. Stress due to movement restriction induces a decrease in BDNF levels in the spinal cord of rats, and tDCS, through descending pathways, can reverse / prevent these neurotrophic changes. In this context, tDCS proved to be an alternative treatment for certain changes caused by stress, reducing the impact of busy modern life, reducing expenses and increasing the quality of life in society. Future studies should address the specific mechanisms of this action.

Key words: Brain-Derived Neurothrophic Factor; Stress, Physiological; Immobilization; Transcranial Direct Current Stimulation

Introdução

1 - INTRODUÇÃO

O estresse é considerado um dos males do século XXI, e atinge 90% da população mundial (GOMES; GARDIM; BERNARDO; PEFFARDINI *et al.*, 2013; HIRSCHLE; GONDIM, 2020; MAIA; DIAS, 2020). As respostas agudas ao estresse são indispensáveis à sobrevivência porque desencadeiam adaptações necessárias para a preservação da homeostasia diante de estressores internos e externos (DE CAMARGO; FURLAN, 2011; PACAK; MCCARTY, 2000). Entretanto, quando os estímulos estressantes extrapolam a capacidade de adaptação, as respostas podem trazer danos ao organismo (EPEL; CROSSWELL; MAYER; PRATHER *et al.*, 2018) e o estresse pode tornar-se um elemento chave na patogênese de desordens de mecanismos adaptativos (KOPTEV; VYNNYK, 2017). Sabe-se que o estresse favorece o surgimento de doenças neurodegenerativas, acelera os processos correlacionados ao envelhecimento e está relacionado com a patogênese de várias outras doenças (TANNO; MARCONDES, 2002). Como as mudanças necessárias para restringir os estímulos estressores nem sempre são possíveis em nossa sociedade, técnicas terapêuticas que reduzam seu impacto na saúde são importantes (SUBHANI; KAMEL; SAAD; NANDAGOPAL *et al.*, 2018). A Estimulação Transcraniana por Corrente Contínua (ETCC), que é uma técnica de neuroestimulação, influencia os mecanismos neurais que estão relacionados ao estresse psicossocial (ANTAL; FISCHER; SAIOTE; MILLER *et al.*, 2014) e tem sido estudada como tratamento para transtornos psiquiátricos (MOFFA; BRUNONI; NIKOLIN; LOO, 2018), (KEKIC; BOYSEN; CAMPBELL; SCHMIDT, 2016).

Estressores são situações do cotidiano que podem ser agrupados em acontecimentos diários menores, tensão crônica ou eventos de vida estressores (MARGIS; PICON; COSNER; SILVEIRA, 2003). A exposição repetida a essas situações potencialmente estressantes desencadeia a mobilização de sistemas neuroendócrinos, endócrinos e metabólicos (PACAK; MCCARTY, 2000), e do Sistema Nervoso Central (SNC), que é o principal sítio de ativação,

tanto de respostas fisiológicas, como comportamentais (GOMES; GARDIM; BERNARDO; PEFFARDINI *et al.*, 2013). O eixo hipotálamo-hipófise (HPA, do inglês *hypothalamic-pituitary-adrenocortical*) é a parte central do sistema neuroendócrino e sua desregulação é um dos mecanismos conhecidos para desenvolvimento de diferentes doenças, incluindo desordens neuropsiquiátricas (PARKER; SCHATZBERG; LYONS, 2003). Em resposta ao estresse, acontece a hipersecreção de alguns hormônios, dentre ele os glicocorticoides cortisol ou corticosterona em roedores, e prejuízo da retroalimentação negativa mediada por eles (SCHOENROCK; TARANTINO, 2014). Em roedores, uma das formas de se inferir o estresse é por meio da dosagem de corticosterona em diferentes tecidos como o sangue (KRISHNAN; HAN; GRAHAM; BERTON *et al.*, 2007). Devido à relevância que o estresse e seus potenciais danos têm na vida moderna, a pesquisa científica nessa área evoluiu muito. Nesse contexto, os modelos animais são essenciais para o estudo de mecanismos fisiológicos e avaliação de novas terapiasres (SCHOENROCK; TARANTINO, 2014). Na literatura, existem modelos bem estabelecidos de estresse (HEBERT; SEROVA; SABBAN, 2005). A imobilização é modelo laboratorial mais utilizado para indução de estresse em roedores (MO; RENOIR; HANNAN, 2019). Essa técnica tem protocolos para imobilização crônica e aguda, bem como suas vantagens e desvantagens, bem descritos na literat(DAVIS; SMITH, 2019; FINK, 2007; SON; YANG; KIM; LEE, 2019). Apesar da imobilização por restrição de movimento tratar-se de uma técnica indolor e não gerar fraqueza muscular significativa (BUYNITSKY; MOSTOFSKY, 2009), afeta parâmetros psicológicos, fisiológicos e bioquímicos em ratos (GRANDIN; DEESING, 2002). As alterações causadas por técnicas de imobilização em animais se estendem inclusive à expressão das neurotrofinas (ROTHMAN; MATTSON, 2013). Existem evidências de que o estresse por restrição de movimento é capaz

de alterar o limiar nociceptivo (ADACHI; DE OLIVEIRA; VERCELINO; DE MACEDO *et al.*, 2017) e reduzir os níveis de BDNF.

As neurotrofinas são proteínas que regulam a neuroplasticidade, o desenvolvimento, a sobrevivência e a manutenção da arborização dendrítica no sistema nervoso (LEVI-MONTALCINI, 1987; PACHECO, 2009). Em mamíferos, as principais são o NGF (Fator de crescimento de nervo, do inglês Nerve Grouth Factor), o BDNF (Fator neurotrófico derivado do cérebro, do inglês *brain-derived neurotrophic fator)*, o NT-3 e NT-4 (SEBBEN; COCOLICHIO; SCHMITT; CURRA *et al.*, 2011). Esses polipeptídeos são sintetizados como moléculas precursoras, que são convertidas em suas formas maduras por pró-hormônio convertases, furinas, plasminas ou metaloproteases (KANDRATAVICIUS; MONTEIRO; SILVA; LEITE, 2010). O BDNF, apesar de ser amplamente distribuído no SNC, também é encontrado em células endoteliais, musculares e no plasma do sangue, pois é armazenado nas plaquetas (LOMMATZSCH; ZINGLER; SCHUHBAECK; SCHLOETCKE *et al.*, 2005). Essa neurotrofina age por meio da autofosforilação dos receptores TrkB e desencadeia cascatas intracelulares que resultam em aumento da excitação de neurônios no corno dorsal da medula espinal (BARDE; EDGAR; THOENEN, 1982). Esse tipo de receptor é encontrado em neurônios do SNC, sistema nervoso periférico, em células da glia, em um subconjunto de células T periféricas e também há sua expressão em alguns tipos de tumores (BRUNETTO; PAGNUSSATO; GALIA, 2019; DE SANTI; ANNUNZIATA; SESSA; BRAMANTI, 2009). O estresse causa redução nos níveis de BDNF no hipocampo, e essa neurotrofina tem seus níveis aumentados após com o tratamento com antidepressivos (MONTEGGIA; BARROT; POWELL; BERTON *et al.*, 2004). Em conjunto com outros mecanismos, a deficiência de

BDNF contribui para o desenvolvimento de doenças neuropsiquiátricas (SCHOENROCK; TARANTINO, 2014).

Novas alternativas têm surgido como adjuvante no tratamento de desordens psiquiátricas e neurológicas. Dentre elas, as técnicas de neuroestimulação como a ETCC (BERLIM; DIAS NETO; TURECKI, 2009b; FREGNI; GIMENES; VALLE; FERREIRA *et al.*, 2006). Ela promove alterações na excitabilidade neuronal espontânea em regiões corticais (PLOW; PASCUAL-LEONE; MACHADO, 2012). Além disso, causa aumento dos níveis de glutamato (OKANO; FONTES; MONTENEGRO; FARINATTI *et al.*, 2015), que estimula a produção de BDNF (GULYAEVA, 2017). Seus efeitos dependem da polaridade da corrente (NITSCHE; COHEN; WASSERMANN; PRIORI *et al.*, 2008). Enquanto a estimulação anódica despolariza (aumenta a excitação), a catódica hiperpolariza (diminui a excitação) a membrana plasmática (NITSCHE; COHEN; WASSERMANN; PRIORI *et al.*, 2008). Mas isso nem sempre é uma regra, pois estudos mostram que essas alterações podem variar com o estado cerebral e com parâmetros de dose, como a duração e a intensidade da estimulação (BATSIKADZE; MOLIADZE; PAULUS; KUO *et al.*, 2013; STAGG; NITSCHE, 2011). Outra característica é que a estimulação pode ser bimodal, isto é, estímulo catódico e anodal podem ser aplicados simultaneamente devido à proximidade dos eletrodos (FITZGERALD; MCQUEEN; DASKALAKIS; HOY, 2014), devido à estimulação áreas de adjacentes às de interesse (NITSCHE; COHEN; WASSERMANN; PRIORI *et al.*, 2008). Além dos efeitos ligados a circuitos cortico-corticais e envolvendo outras áreas subcorticais, estudos mostram que os sistemas descendentes neuronais podem também ser ativados pela ETCC (ADACHI; DE OLIVEIRA; VERCELINO; DE MACEDO *et al.*, 2017). Estes mecanismos podem causar a modulação das vias ascendentes sensoriais (QUEVEDO; COGHILL, 2007).

O início do processamento nociceptivo inicia pela transdução de estímulos nocivos, que ocorre em terminações de fibras aferentes primárias (GARLAND, 2012). Elas se projetam para

a medula espinal, onde fazem sinapse com neurônios de segunda ordem, e transmitem o potencial de ação para regiões encefálicas, onde ocorre a percepção de dor (ALMEIDA; ROIZENBLATT; TUFIK, 2004). O processamento da informação sensorial em diferentes níveis do sistema nervoso ocorre por diferentes mecanismos, dentre os quais destaca-se o efeito topdown (ZHANG; WANG; WANG; LU *et al.*, 2013). Conforme esse mecanismo, vias descendentes, cujas fibras têm origem no encéfalo, projetam-se para a o tronco encefálico e medula espinal e fazem sinapses com neurônios e interneurônios (KOMAKI; HIKISHIMA; SHIBATA; KONOMI *et al.*, 2016). Dessa forma, o sistema cognitivo é capaz de modular a informação sensorial (KRAUS; ANDERSON, 2007).

Os trabalhos com ETCC utilizando roedores apresentam importantes limitações. No intuito de manter os eletrodos intactos na cabeça durante o período de estimulação, alguns protocolos indicam a aplicação com anestesia geral. No entanto, anestésicos podem influenciar os níveis de BDNF em estruturas do SNC (HEAD; PATEL; NIESMAN; DRUMMOND *et al.*, 2009). Outros, lançam mão de eletrodos implantados no crânio, tornando a técnica invasiva (LIEBETANZ; KLINKER; HERING; KOCH *et al.*, 2006). A metodologia eleita para a presente pesquisa foi a aplicação de ETCC de forma transcutânea, com o rato acordado e imobilizado com uma faixa de tecido (ADACHI; DE OLIVEIRA; VERCELINO; DE MACEDO *et al.*, 2017). Apesar deste modelo ter a vantagem de dispensar o uso de anestésicos e não ser invasivo, utiliza dos mesmos meios para produzir um modelo de estresse por imobilização de movimento (HEBERT; SEROVA; SABBAN, 2005). Desta forma, o presente estudo teve por objetivo a avaliação dos efeitos do tipo *topdown* da ETCC na reversão/prevenção de alterações neuroquímicas (níveis de BDNF na medula espinal) e comportamentais (sensibilidade mecânica) em ratos submetidos a um modelo de estresse por restrição de movimentos.

Objetivos

2 - OBJETIVOS

2.1 Objetivo geral

Este estudo tem o objetivo de avaliar sistemicamente os efeitos agudos de uma única sessão da ETCC em ratos submetidos a um modelo de estresse por restrição de movimentos.

2.2 Objetivos específicos

1) Identificar a ação de uma única sessão de ETCC na ativação do sistema modulador descendente, através da mensuração dos níveis de BDNF na medula espinal durante um evento estressante.

2) Avaliar, ao longo do tempo, as mudanças na sensibilidade mecânica de ratos submetidos a um modelo de estresse e a ação após uma sessão de ETCC usando o teste de von Frey.

Artigo

3 - ARTIGO

Manuscrito em preparação para submissão para a *Journal of Neuroscience*.

Single session of Transcranial Direct Current Stimulation reverses the effects of stress by topdown mechanisms

Abbreviated Title: Effects of tDCS on immobilization stress

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Conflict of interest

The authors declare that there are no conflicts of interest.

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Abstract

Stress is present in a modern lifestyle and can cause neurobiological, emotional, and behavioral changes. For example, stressful situations may change pain perception and the level of neurotrophic factors such as brain-derived neurotrophic factor (BDNF). Transcranial Direct Current Stimulation (tDCS) has demonstrated therapeutic effects in painful and stressful conditions through cortical circuits (local effects) and descending pathways (topdown effects). Therefore, the present study aimed to evaluate the acute effects of a single ETCC session on pain sensitivity and modulation of BDNF levels in the spinal cord of rats subjected to a stress model. In the present study, Wistar rats (n =100) were tested to assess mechanical sensitivity (von Frey test). Seven days later, the animals were subjected to an immobilization stress model for 20 minutes. During the immobilization, animals received a session of tDCS (0.5mA) or sham-tCDS procedure. Furthermore, animals were divided into groups according to the period for behavioral retesting and euthanasia (30, 60, 120min or 24hours). Control animals did not receive any intervention. The spinal cord was removed to assess BDNF levels by ELISA. BDNF levels were diminished in stressed animals (60min, 120min, and 24h). This reduction in neurotrophic level was reversed by tDCS. A single tDCS session was able to reduce mechanical sensitivity up to 60 minutes. The present data indicate that a single session of tDCS induces analgesia and may prevent the effect of stress. The modulation of spinal cord BDNF levels by cortical stimulation suggests that topdown effects are involved.
Key word: **BDNF; Stress, Physiological; Immobilization; tDCS**

Significance statement

Stress can cause health problems that may be underlined by different factors such as sensory (e.g., pain) and neurochemical (e.g., BDNF levels) changes. tDCS has been used to treat pain conditions and some components of stress. The present data suggest that stress induces the decrease of BDNF levels at the spinal cord, and a single session of tDCS can reverse this effect. Furthermore, pain threshold was increased up to 60 minutes after the tDCS application. The influence of tDCS far from the application site indicates that this technique activates descending systems to induce topdown effects. In summary, a single tDCS session can be an alternative to treat patients in stressful situations or to produce analgesia in clinical and surgical procedures.

1 - Introduction

Different environmental stimuli, such as stressful situations, can cause disturbance in people's lives (EPEL; CROSSWELL; MAYER; PRATHER *et al.*, 2018). These events may contribute to generate negative psychological and biological effects (MAUSS; LEVENSON; MCCARTER; WILHELM *et al.*, 2005). Furthermore, stressful conditions can increase sensitivity to pain (CRETTAZ; MARZINIAK; WILLEKE; YOUNG *et al.*, 2013) and disrupt cognitive activities (GAMARO; MICHALOWSKI; CATELLI; XAVIER *et al.*, 1999). There are well-established animal models of stress (e.g. movement restriction stress) for understanding mechanisms and testing new therapies (HEBERT; SEROVA; SABBAN, 2005).

Movement restriction is one of the most used ways to study stress because this is a painless technique and does not generate significant changes in animal's behavior (BUYNITSKY; MOSTOFSKY, 2009). However, this animal model might affect psychological, physiological, and biochemical parameters (GRANDIN; DEESING, 2002; NAGARAJA; JEGANATHAN, 1999). These changes may include alterations in molecular factors related to neuroplasticity, such as neurotrophins. In addition, it has been shown that stress-induced by movement restriction alters the nociceptive threshold (ADACHI; DE OLIVEIRA; VERCELINO; DE MACEDO *et al.*, 2017).

Neurotrophins are essential to regulate the development, survival, and maintenance of the dendritic arborization. One important neurotrophin affected by stress is the Brain-derived Neurotrophic Factor (BDNF). BDNF causes autophosphorylation of TrkB receptors and triggers intracellular cascades resulting in increased neuronal excitation in the spinal cord (BARDE; EDGAR; THOENEN, 1982). This protein can be used as a biomarker to assess a given intervention's effects on a brain region (GHANAVATINEJAD; TABRIZI; OMIDGHAEMI; SHARIFI *et al.*, 2019). For example, BDNF has been used to evaluate the effect of Transcranial Direct Current Stimulation (tDCS) at the spinal cord (ADACHI;

QUEVEDO; DE SOUZA; SCARABELOT *et al.*, 2015). Furthermore, tDCS can be an alternative to reverse the effects caused by stress, both in hyperalgesia and in reducing BDNF (ADACHI; QUEVEDO; DE SOUZA; SCARABELOT *et al.*, 2015).

This neurostimulation technique has been used as an adjunct in treating neurological and psychiatric disorders (BERLIM; DIAS NETO; TURECKI, 2009a; FREGNI; GIMENES; VALLE; FERREIRA *et al.*, 2006). For example, different painful syndromes have been treated by applying tDCS on the primary motor cortex (M1) (the cortical area adjacent to the region responsible for nociceptive processing) (FREGNI; GIMENES; VALLE; FERREIRA *et al.*, 2006). The majority of tDCS models may be explained by those cortico-cortical or cortico-subcortical interactions (FREGNI; FREEDMAN; PASCUAL-LEONE, 2007). However, some studies suggest that the action of tDCS can involve descending systems (i.e., topdown mechanisms) (ADACHI; QUEVEDO; DE SOUZA; SCARABELOT *et al.*, 2015; DIMOV; FRANCIOSI; CAMPOS; BRUNONI *et al.*, 2016). Therefore, neuronal activity starts in cortical areas (i.e., M1) and may modulate neuronal activity at primary afferents, second-order neurons, or other descending pathways (MILLAN, 2002).

Currently, tDCS animal models use several sessions (e.g., eight consecutive days) to treat different pathologies or reverse stress-induced allodynia (ADACHI; QUEVEDO; DE SOUZA; SCARABELOT *et al.*, 2015). However, there are no detailed data to clarify the acute effects of a single session of tDCS, which could be important for clinical application in different situations (e.g., preemptive analgesia). The current model of tDCS in rats (REGNER; TORRES; DE OLIVEIRA; PFLÜGER *et al.*, 2020) allows for testing of stress effects, as the animals must be immobilized for the treatment to be carried out. Therefore, the present study aimed to systematically evaluate the effects of a single session of tDCS in rats submitted to an immobilization stress model on pain behavior and the descending nervous system by assessing neurotrophic factor levels in the spinal cord.

2 - Materials and methods

The present study was approved by the Institutional Committee for Animal Care and Use (CEUA) of Hospital de Clinicas de Porto Alegre (HCPA) (number 2019-0126). A series of steps were taken to control possible measurement bias. Behavioral and biochemical testing was performed by a blinded researcher regarding the group of animals.

Behavior experiments were carried out in the Animal Experimentation Unit (UEA-HCPA). The biochemical analyses were performed in the Laboratory Protein Calcium Binders, Department of Biochemistry - Institute of Biological Sciences and Health – ICBS; Universidade Federal do Rio Grande do Sul - UFRGS.

2.1 Animals

Sixty-day-old male Wistar rats (n =100) weighing 180–230g were used in this study. They were housed in the Animal Experimentation Unit of the Hospital de Clínicas de Porto Alegre in polypropylene home cages (49 x 34 x 17.8 cm). The animals were kept under standard 12-hour light-dark cycle conditions, in a controlled room temperature (22 ° C ± 2 ° C) and humidity (40-60%) and had *ad libitum* access to chow and water. The procedures of scientific animal use were realized in accordance with guide for the care and use of laboratory animals (LEI). The experimental protocol complied with the ethical and methodological standards of the ARRIVE guidelines (KILKENNY; BROWNE; CUTHILL; EMERSON *et al.*, 2012).

2.2 Experimental design

Currently, one of the tDCS rat models requires immobilization of the animal, which also can be used to induce stress. Therefore, the protocols for immobilization stress and tDCS models were arranged to investigate the tDCS effects on acute stress. The rats were divided into

group of three or four animals per cage and habitude to the maintenance room for one week before the start of the experiment. Affter the habituation period, the rats were randomily selected for different groups. The experiments were carried out according to the sequence presented in the timeline described in figure 1.

Insert figure 1

Baseline behavioral measurements (von Frey test) were initially performed (D1). In the following days, a five-minute habituation period was applied daily to adapt the animal to the treatment of tDCS. On day 8 (D8), the immobilization model was employed for twenty minutes. During this period, half of the animals received a tDCS session (ST group), and the other half had a sham-tDCS procedure (S groups).

The animals were euthanized after intervention (stress + tDCS or stress + sham-tDCS) according to the group (30, 60, 120 min, or 24 hours). There were two control groups: 1) Behavioral Control (BhC): animals performed the behavioral tests without receiving any intervention (Stress + tDCS or Stress + sham-tDCS); 2) Biochemical Control (BcC): animals only had baseline behavioral tests (D1) and were euthanized on D8. Therefore, the animals were divided into 10 groups: S30, S60, S120, S24, ST30, ST60, ST120, ST24, BhC, and BcC. Before euthanasia, the mechanical threshold was re-evaluated to assess the effect of stress and tDCS on pain sensitivity. After euthanasia (by decapitation), the spinal cord was removed for biochemical analysis.

2.3 The immobilization stress model

During the immobilization, animals remained gently wrapped in a towel, maintaining the movements of the limbs restricted (CASTAGLIUOLO; LEEMAN; BARTOLAK-SUKI;

NIKULASSON *et al.*, 1996; HEBERT; SEROVA; SABBAN, 2005) for 20 minutes (Fig. 2). This stress model has been used by other authors (HEBERT; SEROVA; SABBAN, 2005).

Insert figure 2

2.4 tDCS session.

The tDCS model requires an adaptation period (i.e., six consecutive daily immobilization periods / 5 minutes) before tCDS application. All ST and S groups were exposed to similar experimental procedures to keep the conditions constant between groups. The application of tDCS consisted of positioning and fixing (adhesive tape, Micropore®) electrocardiogram electrodes (that were cut for proper adaptation, with a final size of 11mm in diameter) with conductive adhesive and applying a current of 500μA for 20 minutes (FREGNI; GIMENES; VALLE; FERREIRA *et al.*, 2006). According to Figure 3 (NITSCHE; COHEN; WASSERMANN; PRIORI *et al.*, 2008), the electrodes were positioned on the head. Animals were trichotomized in the region to guarantee the adherence of the electrocardiogram electrodes. After positioning, the electrodes were fixed with adhesive tape (Micropore®). The current leaves a battery and passes through the electrodes. In the ST (stress + tDCS) groups, the electrodes were kept on during the 20 minutes of stimulation. In contrast, animals in S (stress) groups received similar procedures; however, electrodes were maintained at off position. The animals remained immobilized throughout the intervention with real tDCS or immobilization.

Insert figure 3

2.5 Von Frey test.

The electronic von Frey test was used to assess mechanical sensitivity by measuring the threshold of withdrawal responses. An experimenter who did not participate in the behavioral data collection randomly coded animals. Twenty-four hours before the test, animals were acclimated to the apparatus for ten minutes. During the test, animals were placed individually inside a cage with a metal grid floor. A single stimulus (polypropylene cone) was applied perpendicularly to the medial plantar surface of the hind paw. The force steadily increased until paw withdrawal occurs.

The data were collected twice (Fig. 1). The first test (baseline) was performed at the beginning of the experiment (D0). According to the groups (30, 60, 120 min or 24 h), the second test was taken after the intervention (tDCS+stress or sham-tDCS + stress).

2.6 Euthanasia and samples collection.

After euthanasia by decapitation, the spinal cord was removed by an experienced experimenter and stored in a freezer at -80ºC. In addition, blood was collected to assess corticosterone levels. Decapitation is a restricted model of euthanasia. However, this method was necessary because the action of anesthetic drugs could alter the biochemical data.

2.7 Biochemical assays

Phosphate-buffered saline (0.01M, pH=7.4) was utilized for homogenizing the structures. The amount added corresponding to 5 times the sample mass. To disrupt the tissue, a mechanical homogenizer was used. The homogenate was centrifuged for 5min at 5000xg and the supernatant was applied in all biochemical assays performed in this study. BDNF and corticosterone were measured by sandwich. Procedures were performed as stated by the manufacturer's protocol. Optical density was determined by spectrophotometer at a wavelength

of 450 nm. All data are presented as pg/mg of protein. Total protein was estimated byBradford's method with bovine serum albumin (BSA) as standard.

2.8 Quantification of corticosterone.

The evaluation of serum corticosterone was performed with the Enzyme-Linked Immunosorbent Assay (ELISA) Cayman kit. The manufacturer's instructions were followed for quantification in serum.

2.9 Quantification of BDNF

The ELISA kit FineTest® was used to quantify BDNF levels at the spinal cord. The manufacturer's instructions were followed for quantification in homogenized nervous tissue. The results were expressed in pg/g tissue.

2.10 Statistical analysis

All analyzes were performed using the IBM SPSS® Statistics program. Results are presented as means ± standard error and the difference of $P < 0.05$ was considered significant. Animals were divided into different groups according to the analysis (n = 8–10/group for the behavioral test; n = 6/group for biochemical assays).

First, the normality of the data was assessed using the Kolmogorov-Smirnov test. For the analysis of the data of the levels of corticosterone in the animals of the control groups and of the groups that were immobilized, the Mann-Whitney test was used. For the analysis of the mechanical pain threshold, Repeated measures ANOVA followed by Fisher's LSD was used and data were expressed as a percentage of the baseline data. The analysis of BDNF levels in the spinal cord was performed with One-way ANOVA followed by Fisher's LSD and normalized data were used in relation to the biochemical control group. Pearson's correlation was used to analyze the relationship between mechanical pain threshold and BDNF levels in the spinal cord and normalized BDNF quantification data were tested with absolute and normalized data from the von Frey test. To assess the correlation between analgesic effects of

tDCS/stress, data post-intervention were normalized by using the following equation: [(T – B)/B * 100], where T = mechanical threshold after tDCS/stress intervention; B = mechanical threshold at baseline tests. Therefore, to investigate the relationship between BDNF levels and the analgesic effects of tDCS, a Person Correlation Test was performed using the normalized mechanical pain threshold and the BDNF levels at spinal cord level.

3 - Results

3.1 Effect of stress induced by immobilization

There was an increase in corticosterone levels in animals submitted to stress model (control group vs. stress group; P=0.047) (Fig 3). Therefore, the present data suggest that immobilization model was efficient to induce stress in rats.

Insert figure 4

3.2 Effects of tDCS on the mechanical sensivity threshold

Stressed animals showed increases in the mechanical pain threshold at 30 and 60 minutes after a single tDCS session (p=0.002 and p=0.015, respectively) (Fig. 4). There were no differences in pain sensitivity between baseline and post- tDCS session other time points (p>0.05). This indicates that tDCS was able to increase the mechanical pain threshold up to 60 minutes after the treatment.

However, stressed animals that received a sham-tDCS procedure did not have variations on the pain threshold at any time point after the intervention (p>0.05). These data suggest that immobilization stress did not affect the mechanical pain threshold of rats at the present experimental conditions.

Insert figure 5 here

3.4 Effects of stress induced on the spinal cord BDNF levels is reversed by a single tDCS session

Compared to biochemical control group, stress decreased spinal cord BDNF levels at 60 (BcC vs. S60; p=0.032), 120 (BcC vs. S120; p=0.044) minutes, and 24 hours (BcC vs. S24; p=0.001) after immobilization stress.

The results indicated that treatment with tDCS was able to fully reverse the effects of stress induced by movement restraint. There were no differences between control group and rats treated with tDCS at 30 (BcC vs. S60; p=0.1), 60 (BcC vs. S60; p=0.1), 120 (BcC vs. S60; p=0.7) minutes, and 24 hours (BcC vs. S60; p=0.5) after intervention.

Insert figure 6 here

3.5 Correlation between BDNF levels and mechanical perception

To test the relationship between BDNF levels in the spinal cord and mechanical pain perception, correlation tests were used. Initially the results of the von Frey test (absolute values) were used. There was no correlation between DBNF levels and mechanical pain during baseline ($r = 0.145$, $P = 0.45$). Likewise, no relationship was found after stress ($r = 0.08$, $P = 0.68$) or stress+tDCS session ($r = -0.057$, $P = 0.77$).

To access the analgesia induced by the intervention (stress or stress+tDCS), the post-intervention results were expressed by the percentage of baseline (table 1).

Insert table 1 here

Using these normalized data, a negative correlation was found between the spinal cord BDNF levels and the mechanical pain threshold in stressed animals treated by tDCS (r=-0.253;

P=0.047) (Fig7). However, there was no correlation between BDNF levels and mechanical sensitivity in the group that received only the stress model (r=-0.214; P=0.27).

Insert figure 7 here

4 - Discussion

The present data demonstrate that stress modifies BDNF levels at the central nervous system (CNS). These alterations may cause physiological and perceptual changes (CRETTAZ; MARZINIAK; WILLEKE; YOUNG *et al.*, 2013; MAUSS; LEVENSON; MCCARTER; WILHELM *et al.*, 2005). Furthermore, stressful situations may induce cognitive dysfunctions (GAMARO; MICHALOWSKI; CATELLI; XAVIER *et al.*, 1999). BDNF is one of the molecular factors that may be involved in cellular adaptations. Therefore, it could be essential to make efforts (e.g., new therapies) to avoid BDNF variations during stressful situations. The present study shows that tDCS may prevent changes in BDNF levels in animals exposed to immobilization stress model. Because there was modulation of spinal cord BDNF levels, and electrotherapy was applied to the cerebral cortex, there is a strong indication that the neurotrophic modulation was induced by the descending system (e.g., topdown effect). Moreover, a single tDCS session was able to produce analgesia in a short-term period (up to 60 minutes). Furthermore, the increase in the pain threshold was negatively correlated to the decrease of BDNF levels.

Immobilization model caused stress

An increase in serum corticosterone levels was observed in the group of stressed animals (Fig. 4). This result corroborates studies that observed increases in corticosterone levels after a single session of 10 minutes of immobilization (CLEMENT; KIRSCH; HASSE; OPPER *et al.*, 1998). Likewise, five daily immobilization sessions were able to increase corticosterone levels (CAVALETTI; TREDICI; BRAGA; TAZZARI, 1995); (CLEMENT; KIRSCH;

HASSE; OPPER *et al.*, 1998). The present results indicate that stress was well established in the present study. The application of tDCS protocol used herein, where the animal is detained for treatment, benefited the experimental design to test the current hypothesis.

Daily stress requires physiological adaptations, and the CNS is the main control center for physiological and behavioral responses (GOMES; GARDIM; BERNARDO; PEFFARDINI *et al.*, 2013). When the body is exposed to higher intensity stress and can not adapt, its responses can be harmful (EPEL; CROSSWELL; MAYER; PRATHER *et al.*, 2018) involving neurodegenerative diseases and cognitive impairments (GAMARO; MICHALOWSKI; CATELLI; XAVIER *et al.*, 1999).

Immobilization stress reduces BDNF levels in the spinal cord but did not alter pain sensitivity

The present study showed that stress caused a reduction in BDNF levels in the spinal cord. After the first 30 minutes post-stress, although there was a trend to reduction, it was not different from the control. Stressed rats had BDNF reduction in the spinal cord after 60 minutes up to 24 hours after immobilization. Furthermore, the highest decrease was found at the last time measured (i.e., 24hs) (Fig. 6). The present findings are in accordance with a previous study that demonstrated the diminishing of BDNF at different areas of CNS after an acute immobilization model in other CNS structures (SMITH; MAKINO; KVETNANSKY; POST, 1995).

According to other studies, the acute effects of immobilization start early by reducing the levels of BDNF mRNA in the hippocampus and the dentate gyrus of rats. This decrease in neurotrophins levels is maintained for up to 24 hours (SMITH; MAKINO; KVETNANSKY; POST, 1995). The basal levels of BDNF mRNA are kept in adrenalectomized animals subjected to acute stress. These data suggest that while corticosterone levels contribute to the reduction

of BDNF mRNA during immobilization, and alternative mechanisms should be proposed (SMITH; MAKINO; KVETNANSKY; POST, 1995).

Previous studies showed that repeated-stress models might induce hyperalgesia (ADACHI; QUEVEDO; DE SOUZA; SCARABELOT *et al.*, 2015). However, the present study did not find an alteration of pain sensitivity caused by acute stress. This lack of pain alteration can involve the stress model used or its single application. Perhaps, a more intense stress situation could be required to alter the pain threshold.

The present study demonstrated that BDNF levels in the CNS could be changed more quickly through shorter immobilization sessions and a much smaller time frame.

A single session of tDCS reverses the effect of stress on the CNS

Animals subjected to stress had spinal BDNF levels decreased. However, a single application of tDCS was sufficient to reverse these stress effects, allowing similar neurotrophic levels between the treated and control groups. The application of the tDCS is directed to the primary motor cortex. However, this electrotherapy has effects that go beyond cortico-circuitries and are able to modulate corticospinal excitability (NITSCHE; PAULUS, 2000). Using these mechanisms, tDCS may alter incoming information by descending pathways (ADACHI; QUEVEDO; DE SOUZA; SCARABELOT *et al.*, 2015). One way to modulate ascending neuronal activity is changing synapses connectivity, and BDNF is a critical mediator in the plasticity of synapses induced by tDCS (FRITSCH; REIS; MARTINOWICH; SCHAMBRA *et al.*, 2010). In vitro studies suggest that tDCS participates in the co-release of pro-BDNF and proteases, which modulates synaptic strength (FRITSCH; REIS; MARTINOWICH; SCHAMBRA *et al.*, 2010).

One of the most relevant findings of this study is related to the capacity of a single application of tDCS in the cerebral cortex can modulate activity in remote regions of the CNS. Additionally, electrotherapy can alter neurotrophic levels in the spinal cord (ADACHI;

QUEVEDO; DE SOUZA; SCARABELOT *et al.*, 2015). A similar phenomenon can be observed when cognitive activities, such as attention, cause neurons' firing in higher-order brain areas, and activate descending modulatory systems. Similarly, the same process may occur in the nociceptive system, where spatial attention may modulate the receptor fields of second-order neurons or even primary afferents (QUEVEDO; COGHILL, 2007). This topdown effect may justify the reversal of the decrease in BDNF levels in the spinal cord by tDCS found here.

Future studies are required to confirm whether the tDCS effects found in the present experiments occurred because it may modulate the synthesis of BDNF, or if tDCS prevents the degradation of neurotrophins present in the spinal cord.

A single session of tDCS increases mechanical pain threshold

A single session of tDCS was able to increase the mechanical pain threshold at 30 and 60 minutes after application. Different mechanisms may be related to the present findings, such as the change in the resting membrane potential of neurons (ZAGHI; ACAR; HULTGREN; BOGGIO *et al.*, 2010). Therefore, this change in membrane potential may increase the threshold for firing action potentials during mechanical stimulation (ZAGHI; ACAR; HULTGREN; BOGGIO *et al.*, 2010). Alterations of pain sensitivity have been found 24 hours after chronic stress, and tDCS was able to reverse its hyperalgesia and allodynia (ADACHI; CAUMO; LASTE; MEDEIROS *et al.*, 2012).

These findings corroborate studies showing that the repeated-tDCS is able to reverse biochemical stress effects and increase pain threshold (ADACHI; QUEVEDO; DE SOUZA; SCARABELOT *et al.*, 2015). However, the present study indicates that this effect can be done by the acute effect of a single tDCS session, and some components can be sustained by 24 hours after the electrotherapy. These effects could be due to the ability of the tDCS to modulate regions that process pain, as well as the release of neurotransmitters, causing a global effect of

activation of the descending pain pathways (DIMOV; FRANCIOSI; CAMPOS; BRUNONI *et al.*, 2016).

The BDNF levels in the spinal cord are correlated with tDCS-induced analgesia

To test the hypothesis that the mechanical sensitivity of the animals was correlated with spinal BDNF levels, absolute threshold values were used. No significant correlations were found between spinal neurotrophic levels and mechanical sensitivity in any of the studied groups. However, changes in the sensitivity between baseline and post-intervention (percentage from baseline ratings) might be helpful to assess the relationship between possible analgesia/hyperalgesia induced by the intervention (stress or stress-tDCS) and BDNF levels in the spinal cord. Using normalized data, a negative correlation was found between spinal cord BDNF levels and the increase of mechanical pain threshold in animals treated by tDCS (Fig. 7). There was no such correlation with animals in the stress group, suggesting that this result is due to the treatment effect.

The relationship between mechanical sensitivity and alteration of the BDNF level in CNS regions has been reported (SCARABELOT; DE OLIVEIRA; MEDEIROS; DE MACEDO *et al.*, 2019). However, in the present study, brief immobilization was sufficient to cause a reduction in BDNF. Interestingly in a model of orofacial inflammatory pain, there was an increase in BDNF in the brainstem, which was reversed by treatment with tDCS. This normalization of the neurotrophin levels was accompanied by analgesia (SCARABELOT; DE OLIVEIRA; MEDEIROS; DE MACEDO *et al.*, 2019).

Final considerations

The present data indicate that the neurophysiological changes induced by stress may be present in short periods after the animal is exposed. Changes in the nervous system can have several consequences, such as alterations on mood, emotions, cognition, and somatosensory

sensitivity. New therapies must be investigated, so changes induced by stress can be mitigated. Accordingly, tDCS may be an alternative to prevent the effects of stress, as well as being easy to apply, and an inexpensive method. This brain stimulation is able to induce local (e.g., cortico-cortical) and topdown (by descending systems) effects. Future studies are needed to investigate methods and therapies to alleviate the deleterious effects of stress induced by the modern lifestyle.

6 – References

Adachi LNS, Caumo W, Laste G, Medeiros LF, Rozisky JR, de Souza A, Fregni F, Torres IL (2012) Reversal of chronic stress-induced pain by transcranial direct current stimulation (tDCS) in an animal model. Brain research 1489:17-26.

Adachi LNS, de Oliveira C, Vercelino R, de Macedo IC, Laste G, Quevedo AS, Scarabelot VL, Caumo W, da Silva Torres IL (2017) Evaluation of different procedure involved in the Transcranial Direct Current Stimulation (tDCS) technique experimental application. Clinical & Biomedical Research 37.

Adachi LNS, Quevedo AS, de Souza A, Scarabelot VL, Rozisky JR, de Oliveira C, Marques Filho PR, Medeiros LF, Fregni F, Caumo W (2015) Exogenously induced brain activation regulates neuronal activity by topdown modulation: conceptualized model for electrical brain stimulation. Experimental brain research 233:1377-1389.

Albus U (2012) Guide for the Care and Use of Laboratory Animals (8th edn). In: SAGE Publications Sage UK: London, England.

Barde Y-A, Edgar D, Thoenen H (1982) Purification of a new neurotrophic factor from mammalian brain. The EMBO journal 1:549-553.

Berlim MT, Dias Neto V, Turecki G (2009) Estimulação transcraniana por corrente direta: uma alternativa promissora para o tratamento da depressão maior? Brazilian Journal of Psychiatry 31:S34-S38.

Buynitsky T, Mostofsky DI (2009) Restraint stress in biobehavioral research: recent developments. Neuroscience & Biobehavioral Reviews 33:1089-1098.

Castagliuolo I, Leeman SE, Bartolak-Suki E, Nikulasson S, Qiu B, Carraway RE, Pothoulakis C (1996) A neurotensin antagonist, SR 48692, inhibits colonic responses to immobilization stress in rats. Proceedings of the National Academy of Sciences 93:12611-12615.

Cavaletti G, Tredici G, Braga M, Tazzari S (1995) Experimental peripheral neuropathy induced in adult rats by repeated intraperitoneal administration of taxol. Experimental neurology 133:64-72.

Clement H-W, Kirsch M, Hasse C, Opper C, Gemsa D, Wesemann W (1998) Effect of repeated immobilization on serotonin metabolism in different rat brain areas and on serum corticosterone. Journal of neural transmission 105:1155-1170.

Crettaz B, Marziniak M, Willeke P, Young P, Hellhammer D, Stumpf A, Burgmer M (2013) Stress-induced allodynia–evidence of increased pain sensitivity in healthy humans and patients with chronic pain after experimentally induced psychosocial stress. PloS one 8:): e69460. https://doi.org/10.1371/journal.pone.0069460

Dimov LF, Franciosi AC, Campos ACP, Brunoni AR, Pagano RL (2016) Topdown effect of direct current stimulation on the nociceptive response of rats. PloS one 11.

Epel ES, Crosswell AD, Mayer SE, Prather AA, Slavich GM, Puterman E, Mendes WB (2018) More than a feeling: A unified view of stress measurement for population science. Frontiers in neuroendocrinology 49:146-169.

Fregni F, Gimenes R, Valle AC, Ferreira MJ, Rocha RR, Natalle L, Bravo R, Rigonatti SP, Freedman SD, Nitsche MA (2006) A randomized, sham-controlled, proof of principle study of

transcranial direct current stimulation for the treatment of pain in fibromyalgia. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology 54:3988-3998.

Fritsch B, Reis J, Martinowich K, Schambra HM, Ji Y, Cohen LG, Lu B (2010) Direct current stimulation promotes BDNF-dependent synaptic plasticity: potential implications for motor learning. Neuron 66:198-204.

Gamaro GD, Michalowski MB, Catelli DH, Xavier MH, Dalmaz C (1999) Effect of repeated restraint stress on memory in different tasks. Brazilian Journal of Medical and Biological Research 32:341-347.

Ghanavatinejad F, Tabrizi ZPF, Omidghaemi S, Sharifi E, Møller SG, Jami M-S (2019) Protein biomarkers of neural system. Journal of otology 14:77.

Gomes RL, Gardim CB, Bernardo AFB, Peffardini C, Lorençoni R (2013) Associação entre o nível de estresse e o nível educacional de funcionários de uma universidade pública. In: Colloq Vitae, pp 104-111.

Grandin T, Deesing M (2002) Distress in animals: is it fear, pain or physical stress?

Hebert MA, Serova LI, Sabban EL (2005) Single and repeated immobilization stress differentially trigger induction and phosphorylation of several transcription factors and mitogen-activated protein kinases in the rat locus coeruleus. Journal of neurochemistry 95:484-498.

Huang N, Yang C, Hua D, Li S, Zhan G, Yang N, Luo A, Xu H (2019) Alterations in the BDNF–mTOR Signaling Pathway in the Spinal Cord Contribute to Hyperalgesia in a Rodent Model of Chronic Restraint Stress. Neuroscience 409:142-151.

Mauss IB, Levenson RW, McCarter L, Wilhelm FH, Gross JJ (2005) The tie that binds? Coherence among emotion experience, behavior, and physiology. Emotion 5:175.

Nagaraja H, Jeganathan P (1999) Effect of short & long term restraint stress on some physiological & biochemical parameters in rats. Indian Journal of Medical Research 109:76.

Nitsche MA, Paulus W (2000) Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. The Journal of physiology 527:633-639.

Nitsche MA, Cohen LG, Wassermann EM, Priori A, Lang N, Antal A, Paulus W, Hummel F, Boggio PS, Fregni F (2008) Transcranial direct current stimulation: state of the art 2008. Brain stimulation 1:206-223.

Regner GG, Torres IL, de Oliveira C, Pflüger P, da Silva LS, Scarabelot VL, Ströher R, de Souza A, Fregni F, Pereira P (2020) Transcranial direct current stimulation (tDCS) affects neuroinflammation parameters and behavioral seizure activity in pentylenetetrazole-induced kindling in rats. Neuroscience Letters:135162.

Scarabelot VL, de Oliveira C, Medeiros LF, de Macedo IC, Cioato SG, Adachi LNS, Paz AH, de Souza A, Caumo W, Torres IL (2019) Transcranial direct-current stimulation reduces nociceptive behaviour in an orofacial pain model. Journal of oral rehabilitation 46:40-50.

Smith MA, Makino S, Kvetnansky R, Post RM (1995) Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. Journal of Neuroscience 15:1768-1777.

Ueyama T, Kawai Y, Nemoto K, Sekimoto M, Toné S, Senba E (1997) Immobilization stress reduced the expression of neurotrophins and their receptors in the rat brain. Neuroscience research 28:103-110.

Zaghi S, Heine N, Fregni F (2009) Brain stimulation for the treatment of pain: a review of costs, clinical effects, and mechanisms of treatment for three different central neuromodulatory approaches. Journal of pain management 2:339.

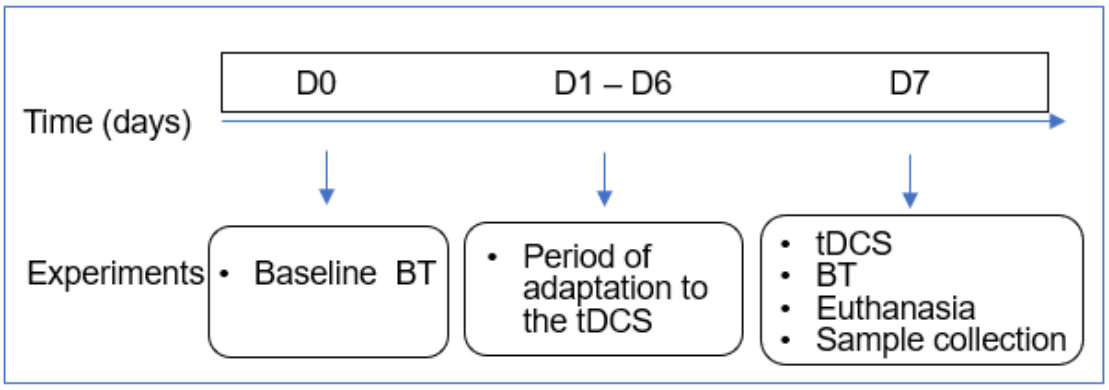
Table 1

Group	von Frey test (% of Baseline)	SD

ST30	152,88	48,99
ST60	130,82	29,35
ST120	129,40	46,17
ST24	106,22	20,58

Mechanical sensitivity in stressed animals treated with tDCS: Pain sensitivity (normalized by baseline values) was increased over 50% and 30% thirty and sixty minutes, respectively, after the tDCS session.

Figure 1

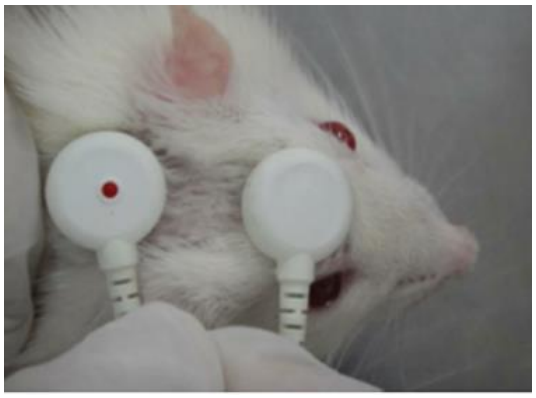


Experimental design. Baseline behavioral tests were performed on day zero (D0). From D1 to D6, animals were handle to prepare to the tDCS or tDCS-sham intervention. On D7, animals were subjected to stress using an immobilization model. The animals in the ST group received a session of tDCS simultaneously with immobilization. Afterwards, the behavior tests were done and euthanasia was performed according to the groups (30, 60 or 120 minutes). Groups S24 and ST24 were tested and euthanized twenty-four hours after the stress / tDCS session. **D** = Day. **BT** = Behavior tests. **S** = Stress group. **ST** = Stress + tDCS group.

Figure 2

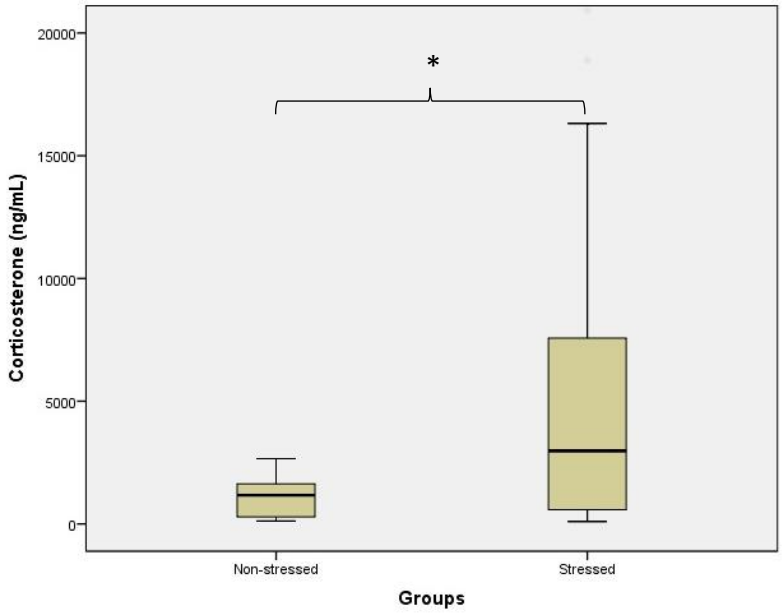
Immobilization stress model: The animals had restricted limb movements for 20 minutes (ADACHI; QUEVEDO; DE SOUZA; SCARABELOT *et al.*, 2015).

Figure 3



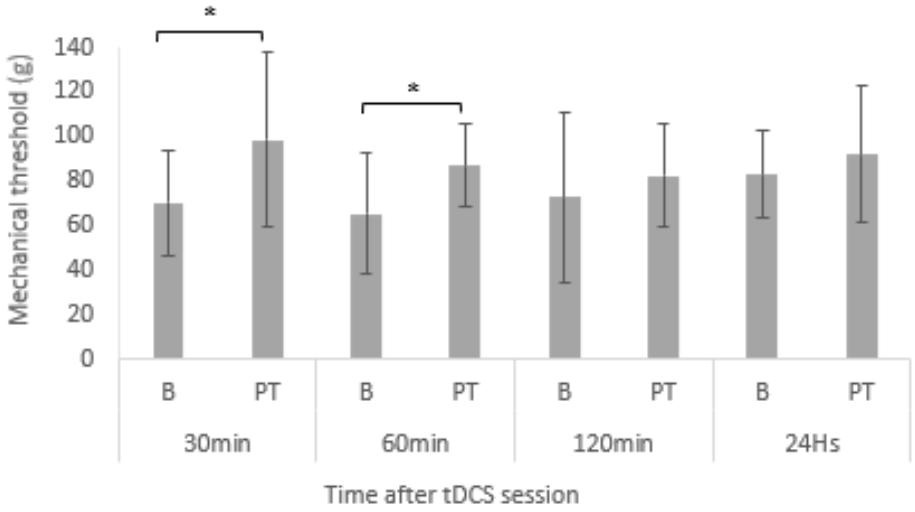
tDCS treatment in rats: Location of electrodes on the rat head during tDCS application. The cathodal electrode was placed between the lateral angles of the eyes. The anodal electrode was positioned on the midline of the parietal areas of the scalp (ADACHI; QUEVEDO; DE SOUZA; SCARABELOT *et al.*, 2015).

Figure 4



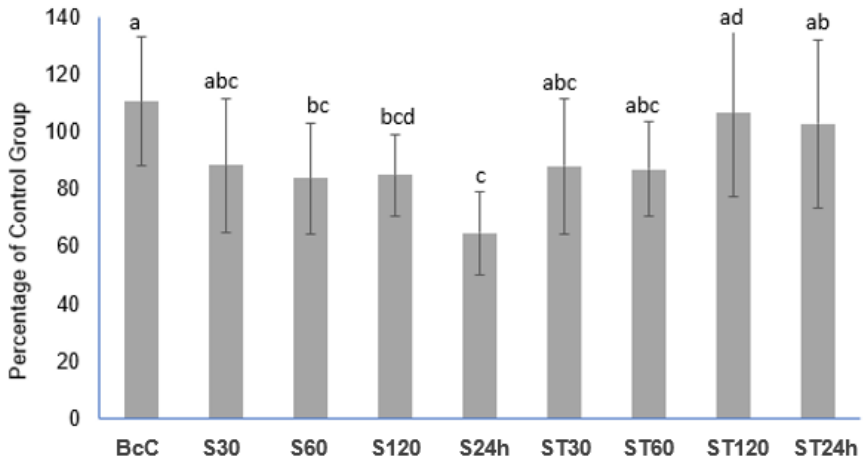
Stress induced by immobilization model. Immobilization stress model caused an significantly increase in serum corticosterone. The asterisk signs (*) indicate a statistically significant difference of $P<0.05$ between the groups.

Figure 5



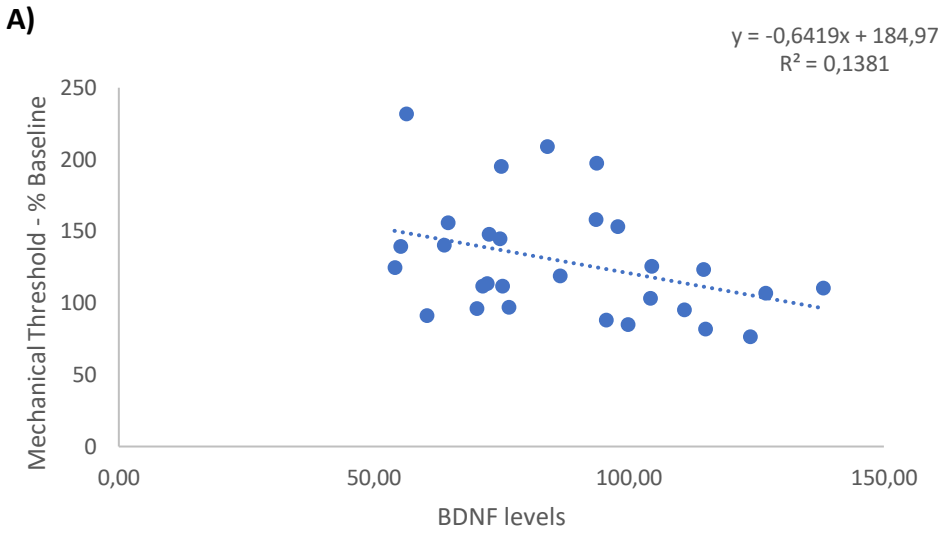
Effect of tDCS on the mechanical pain threshold. Treatment with tDCS increased the mechanical pain threshold at 30 minutes and 60 minutes after the session. The data collected in the baseline (**B**) and post-treatment (**PT**) tests were compared using Two-way repeated measures ANOVA followed by Fisher's LSD. The asterisk signs (*) indicate a statistically significant difference of $P<0.05$ between the groups.

Figure 6



Effect of tDCS on spinal cord BDNF levels.The data were represented as mean ± SD. Stress due to immobilization significantly reduced BDNF levels in the spinal cord at 60, 120 minutes an5 24h (S60xC; S120xC and S24hxC). The tDCS reversed this reduction, as the values returned to the same level as the control group (ST60xC; ST120xC and ST24hxC). Twenty-four hours after stress, the levels were completely reversed (S24hxST24h). **C**=Control group; **S**= Stress groups; **ST**=Stress+tDCS groups. Different letters above the bars mean a difference of $P<0.05$ between the groups.

Figure 7



Correlation between BDNF levels and mechanical pain threshold in tDCS groups. The spinal cord BDNF levels was negatively correlated with increased mechanical pain threshold in animals treated by tDCS (r=-0.253; P=0.047).

Discussão

4 - DISCUSSÃO

Neste estudo, a ETCC reverteu a diminuição de BDNF na medula espinal de ratos submetidos a estresse repetido. Nos primeiros 30 minutos após a estimulação já houve tendência de restauração do nível dessa neurotrofina, e após uma hora, já não houve mais diferença entre os grupos tratados e grupo controle. Este efeito se manteve por 24 horas. Também foi demonstrado o aumento do limiar nociceptivo após 30 minutos da realização do tratamento, sendo este efeito também observado após 60 minutos. A restauração dos níveis de BDNF mostrou-se negativamente relacionada com o limiar de sensibilidade mecânica nos ratos que receberam a neuroestimulação verdadeira.

Testaram-se os efeitos de uma única sessão de neuroestimulação com ETCC nos níveis de BDNF na medula espinal e no limiar de sensibilidade a estímulos mecânicos em ratos submetidos a um modelo de estresse repetido. Os achados desse estudo podem impactar futuramente nos protocolos atuais de aplicação de ETCC, bem como sobre o potencial uso da neuroestimulação para reverter efeitos deletérios causados pelo estresse e diminuir a sensibilidade à dor. O protocolo de estimulação empregado foi 20 minutos de ETCC transcutânea bimodal (500μA) (FITZGERALD; MCQUEEN; DASKALAKIS; HOY, 2014; REGNER; TORRES; DE OLIVEIRA; PFLÜGER *et al.*, 2020). Os animais permaneceram imobilizados e conscientes durante o período de estimulação, logo, a contenção dos movimentos dos animais se fez necessária para garantir a permanência dos eletrodos em posição durante a estimulação (ADACHI; DE OLIVEIRA; VERCELINO; DE MACEDO *et al.*, 2017). A imobilização foi realizada enrolando-se o animal em um pedaço de tecido com o auxílio de fita adesiva (REGNER; TORRES; DE OLIVEIRA; PFLÜGER *et al.*, 2020). Previamente ao dia da estimulação, os animais foram imobilizados durante 5 minutos por 5 dias e por 20 minutos durante a sessão de ETCC totalizando 6 dias de contenção (HEBERT; SEROVA; SABBAN, 2005).

No presente trabalho, o estresse foi avaliado pela dosagem dos níveis séricos de corticosterona (SRINIVASAN; LOGANATHAN; WANKHAR; RATHINASAMY *et al.*, 2016). Constatou-se elevação dos níveis de corticosterona nos grupos que foram imobilizados, ou seja, os animais dos grupos Estresse (*Stress*) e Estresse – ETCC (*Stress-tDCS*), diferente dos animais dos grupos controles (Controle Bioquímico e Controle Comportamental) que não tiveram os níveis alterados (Fig. 4). O aumento de corticosterona é uma das formas de se mensurar o estresse, pois estímulos estressantes levam a liberação de hormônios adrenais como adrenalina, cortisol (em humanos) e corticosterona (em animais) (SRINIVASAN; LOGANATHAN; WANKHAR; RATHINASAMY *et al.*, 2016). Apesar da secreção destes hormônios ser controlada pela alça de retroalimentação negativa do HPA, estímulos intensos, prolongados ou repetidos levam à perda desta regulação (KRISHNAN; HAN; GRAHAM; BERTON *et al.*, 2007). Com isso, ficou demonstrado que, tanto os ratos que receberam a estimulação verdadeira quanto a falsa, apresentaram estresse devido à imobilização.

Com relação aos efeitos da ETCC, os animais que receberam o tratamento ativo tiveram seu limiar de dor mecânica aumentado em 30 e 60 minutos após a estimulação, ou seja, os animais ficaram menos sensíveis frente a estímulo mecânico nesse período (Fig. 5). Nessa análise, cada rato foi comparado com ele mesmo quanto aos valores coletados no teste basal. O fato de não ter sido observado esse aumento nos animais submetidos à falsa aplicação, sugere que este efeito está relacionado com a corrente elétrica. Sugere-se que esses efeitos foram observados devido à capacidade do tDCS modular regiões que processam a dor, bem como à regulação de neurotransmissores como o glutamato, GABA e opioides endógenos, causando um efeito global de ativação das vias descendentes da dor (DIMOV; FRANCIOSI; CAMPOS; BRUNONI *et al.*, 2016).

Outros estudos também mostram que a neuroestimulação é capaz de reverter os efeitos hiperalgésicos causados por estresse. O aumento do limiar mecânico pela ETCC também foi

observado em ratos submetidos ao modelo de constrição do nervo ciático. Quatorze dias após a cirurgia, os ratos foram submetidos a uma sessão de 20 minutos diária de ETCC bimodal de 0.5mA, durante oito dias consecutivos. Semelhante ao nosso modelo, os animais também foram enrolados em uma toalha e permaneceram imobilizados durante o tempo de estimulação para evitar a remoção dos eletrodos (REGNER; TORRES; DE OLIVEIRA; PFLÜGER *et al.*, 2020). Essa diferença no tempo necessário para aumentar limiar nociceptivo reafirma que os efeitos da ETCC dependem da dose e da duração do tratamento (BATSIKADZE; MOLIADZE; PAULUS; KUO *et al.*, 2013; STAGG; NITSCHE, 2011).

A hiperalgesia relacionada ao estresse crônico por restrição de movimento pode ser resultado de alteração na atividade do sistema opioide nos ratos. A neuroestimulação aguda com ETCC induz aumento da liberação endógena de opioides endógenos, que agem em seus respectivos receptores causando efeito analgésico (ADACHI; CAUMO; LASTE; MEDEIROS *et al.*, 2012; DOS SANTOS; LOVE; MARTIKAINEN; NASCIMENTO *et al.*, 2012; TORRES; VASCONCELLOS; SILVEIRA CUCCO; DALMAZ, 2001). Outro mecanismo sugerido para essa mudança na sensibilidade envolve aumento do limiar necessário para desencadear o potencial de ação nos neurônios diante de estímulo mecânico (PURPURA; MCMURTRY, 1965). O glutamato é o principal neurotransmissor excitatório do sistema nervoso, e seu aumento na medula espinal está relacionado com maior condutividade ao íon-cálcio no neurônios, devido à remoção do bloqueio pelo íon-magnésio dos receptores NMDA, levando ao aumento da resposta à dor (LI; SIMONE; LARSON, 1999). A estimulação com ETCC catódica reduz os níveis de glutamato por diminuição da taxa de síntese desse neurotransmissor a partir de glutamina por enzimas (STAGG; BEST; STEPHENSON; O'SHEA *et al.*, 2009). Como a síntese de GABA, principal neurotransmissor inibitório, também depende de glutamato (SONNEWALD; WESTERGAARD; SCHOUSBOE; SVENDSEN *et al.*, 1993), ocorre também a redução de sua concentração (STAGG; BEST; STEPHENSON; O'SHEA *et al.*,

2009). Considerando que os efeitos pós estimulação com ETCC dependem da modulação de sinapses glutamatérgicas (STAGG; NITSCHE, 2011), este é outro mecanismo que pode explicar os efeitos antiálgicos encontrados no presente estudo.

O presente modelo de estresse repetido causou redução nos níveis de BDNF da medula espinal (Fig. 6). Nos 30 minutos após a aplicação da falsa estimulação com os animais imobilizados, já havia tendência a redução dos níveis teciduais dessa neurotrofina, tornando-se significativa após 60 minutos até 24 horas, que foi o último período de tempo em que foi mensurada. Em 24 horas, a redução mostrou-se mais acentuada que nos outros tempos medidos. Sabe-se que acontece a redução do BDNF no hipocampo após estresse agudo e estresse repetido. E esse efeito tende a se estender por 24 horas após a última sessão, e contribui para atrofia neuronal dessa estrutura, visto que essa neurotrofina está relacionada com a manutenção e sobrevivência dos neurônios (MURAKAMI; IMBE; MORIKAWA; KUBO *et al.*, 2005) . São necessários mais estudos para definir por quanto tempo o estresse repetido por imobilização afetou a concentração BDNF medular. Ainda mais que o tempo de exposição ao estresse parece interferir no tempo necessário para início do decaimento dos níveis de mRNA e no ponto que ele começa a retornar aos níveis da linha de base, e a variedade de protocolos de imobilização encontrados na literatura dificulta a comparação dos dados (SMITH; MAKINO; KVETNANSKY; POST, 1995); (HUANG; YANG; HUA; LI *et al.*, 2019; UEYAMA; KAWAI; NEMOTO; SEKIMOTO *et al.*, 1997).

Um dos principais achados da presente pesquisa foi a capacidade da ETCC em reverter/prevenir os efeitos do estresse repetido por imobilização sobre os níveis de BDNF na medula espinal. Os grupos que receberam a estimulação verdadeira não apresentaram diferença em relação ao grupo controle. Duas hipóteses concorrem para explicar o efeito da ETCC. Essa reversão pode ter ocorrido pelo aumento da produção ou da liberação de pró-BDNF, BDNF ou aumento das enzimas responsáveis pela transformação do pró-BDNF na sua forma madura.

Outra sugestão é de que a ETCC apenas impede a degradação da quantidade de BDNF que já existia na medula no momento do experimento. Em um estudo em ratos, 30 minutos de estimulação anódica no lobo parietal de ratos, observou-se aumento de glutamato nessa região (CLARK; COFFMAN; TRUMBO; GASPAROVIC, 2011), e sabe-se que o glutamato estimula a produção de BDNF (GULYAEVA, 2017). Caso este mecanismo tenha ocorrido, podemos sugerir que esse efeito não ficou restrito à área de estimulação, agindo por meio das vias descendente. Os animais que receberam a estimulação verdadeira tiveram seus resultados do teste de sensibilidade mecânica correlacionados de forma inversa em relação aos dados de BDNF, a analgesia se correlacionou inversamente ao BDNF (Fig. 7). Ou seja, os animais tratados com ETCC que mostraram aumento do limiar de sensibilidade mecânica, tiveram redução nos níveis de BDNF na medula espinal. Essa redução de sensibilidade condiz com achados na literatura que mostram que o BDNF tem efeito de atenuação na dor em ratos machos, e o contrário acontece nas fêmeas, pois age facilitados da dor (LI; ZHANG; WEI; LUO *et al.*, 2010). Quando feita a injeção intratecal de BDNF, houve redução da hipersensibilidade térmica e mecânica em modelo de dor por ligação de nervo espinal, mostrando a importância dessa neurotrofina para a regulação da neurotransmissão GABAérgica (MERIGHI; SALIO; GHIRRI; LOSSI *et al.*, 2008; MEYER-TUVE; MALCANGIO; EBERSBERGER; MAZARIO *et al.*, 2001).

A ideia inicial do projeto era fazer uma análise sistemática dos efeitos comportamentais e neurotróficos da ETCC nas vias descendentes de ratos *Wistar*. Contudo, a mudança de perspectiva ocorreu notar-se que no grupo que recebeu falsa neuroestimulação houve alteração nos níveis de BDNF, sendo que o tratamento com a sessão única de ETCC reverteu esse efeito. Enfim, chegamos à conclusão de que a imobilização causou estresse nos animais, dado suportado pela posterior análise de corticosterona, que se mostrou aumentada nos grupos que sofreram restrição de movimento. Além disso, essa reversão nos níveis do fator neurotrófico

em estudo está de acordo com o aumento do limiar de sensibilidade mecânica encontrado nos dados do teste de von Frey, e amparado por estudos anteriores (LI; ZHANG; WEI; LUO *et al.*, 2010).

Como perspectivas futuras, faz-se necessário analisar os efeitos do estresse repetido por imobilização além de 24 horas, para definir por quanto tempo persisitem seus efeitos sobre o BDNF. Além disso, reforça-se a necessidade de investigar por quais mecanismos a ETCC reverteu os efeitos do modelo de estresse apresentado sobre neurotrofinas.

Conclusão

5 - CONCLUSÃO

O estresse repetido por imobilização inerente à técnica de aplicação de ETCC em ratos utilizada neste trabalho (HEBERT; SEROVA; SABBAN, 2005; REGNER; TORRES; DE OLIVEIRA; PFLÜGER *et al.*, 2020) causou redução da atividade neurotrófica na medula espinal de ratos Wistar e foi revertida pelo ETCC. Além disso, a ETCC foi capaz de aumentar o limiar de dor mecânica por até 60 minutos. Também se observou que as alterações na atividade neurotrófica se relacionam inversamente com a sensibilidade mecânica. Efeito que também se observa em humanos após exercício físico, no qual o aumento do BDNF está relacionado com a redução da dor em pacientes com fibromialgia (ISERHARDT, 2016).

Os transtornos de humor podem levar ao aumento da sensação de dor (BERBER; KUPEK; BERBER, 2005). Por exemplo, a prevalência da depressão pode chegar a 80% em pacientes com fibromialgia, em que a dor e o estresse são agravantes (HOMANN; STEFANELLO; GÓES; BREDA *et al.*, 2012). A ETCC, contribui para a redução da sensação de dor em pacientes com fibromialgia (NUNES, 2015), sugerindo um potencial uso na prática clínica.

Além disso, o fato de uma aplicação de ETCC ter reduzido a sensibilidade à dor por até uma hora, sugere o emprego desta técnica para analgesia preemptiva em cirurgias ambulatoriais, como as odontológicas, reduzindo a quantidade de anestésicos locais e analgésicos utilizados (SILVA; GALDINO; ANDRADE; LUCENA *et al.*, 2019). A modulação exercida pela ETCC em regiões que processam a dor e a liberação de neurotransmissores podem ter contribuído para esses efeitos globais no SNC (DIMOV; FRANCIOSI; CAMPOS; BRUNONI *et al.*, 2016).

A diminuição dos níveis de BDNF têm sido correlacionada com distúrbios psiquiátricos em humanos, como a depressão (CASTRÉN; KOJIMA, 2017). Sabe-se que o estresse está

associado ao aumento do risco de transtornos de humor (SHEA; WALSH; MACMILLAN; STEINER, 2005). Um dos efeitos dos antidepressivos é justamente o aumento dessa proteína (COYLE; DUMAN, 2003). Isso torna muito promissora a perspectiva de emprego de ETCC para reversão dos efeitos negativos do estresse diário sobre as neurotrofinas.

Com isso, nosso estudo mostra que a ETCC pode tornar-se uma importante aliada para evitar os efeitos negativos do estresse advindos da redução de BDNF. A restauração dos níveis dessa neurotrofina, relacionada à redução da sensibilidade mecânica, surge como uma aliada na redução da dor, levando ao aumento da qualidade de vida das pessoas em determinadas doenças que têm como sintomas a dor aguda ou crônica (HOMANN; STEFANELLO; GÓES; BREDA *et al.*, 2012).

Como perspectivas, é necessário investigar os mecanismos pelos quais houve a reversão nos níveis de BDNF, e por quanto tempo os efeitos de uma única sessão perduram no organismo além de 24 horas, tempo máximo de investigação nesse estudo.

Referências

6 - REFERÊNCIAS

ADACHI, L. N. S.; CAUMO, W.; LASTE, G.; MEDEIROS, L. F. *et al.* Reversal of chronic stress-induced pain by transcranial direct current stimulation (tDCS) in an animal model. **Brain research**, 1489, p. 17-26, 2012.

ADACHI, L. N. S.; DE OLIVEIRA, C.; VERCELINO, R.; DE MACEDO, I. C. *et al.* Evaluation of different procedure involved in the Transcranial Direct Current Stimulation (tDCS) technique experimental application. **Clinical & Biomedical Research**, 37, n. 2, 2017.

ADACHI, L. N. S.; QUEVEDO, A. S.; DE SOUZA, A.; SCARABELOT, V. L. *et al.* Exogenously induced brain activation regulates neuronal activity by top-down modulation: conceptualized model for electrical brain stimulation. **Experimental brain research**, 233, n. 5, p. 1377-1389, 2015.

ALMEIDA, T. F.; ROIZENBLATT, S.; TUFIK, S. Afferent pain pathways: a neuroanatomical review. **Brain research**, 1000, n. 1-2, p. 40-56, 2004.

ANTAL, A.; FISCHER, T.; SAIOTE, C.; MILLER, R. *et al.* Transcranial electrical stimulation modifies the neuronal response to psychosocial stress exposure. **Human brain mapping**, 35, n. 8, p. 3750-3759, 2014.

BARDE, Y.-A.; EDGAR, D.; THOENEN, H. Purification of a new neurotrophic factor from mammalian brain. **The EMBO journal**, 1, n. 5, p. 549-553, 1982.

BATSIKADZE, G.; MOLIADZE, V.; PAULUS, W.; KUO, M. F. *et al.* Partially non-linear stimulation intensity-dependent effects of direct current stimulation on motor cortex excitability in humans. **The Journal of physiology**, 591, n. 7, p. 1987-2000, 2013.

BERBER, J. D. S. S.; KUPEK, E.; BERBER, S. C. Prevalence of depression and its relationship with quality of life in patients with fibromyalgia syndrome. **Revista Brasileira de Reumatologia**, 45, n. 2, p. 47-54, 2005.

BERLIM, M. T.; DIAS NETO, V.; TURECKI, G. Estimulação transcraniana por corrente direta: uma alternativa promissora para o tratamento da depressão maior? **Brazilian Journal of Psychiatry**, 31, p. S34-S38, 2009a.

BERLIM, M. T.; DIAS NETO, V.; TURECKI, G. Transcranial direct current stimulation: a promising alternative for the treatment of major depression? **Revista Brasileira de Psiquiatria**, 31, p. S34-S38, 2009b.

BRUNETTO, A. L.; PAGNUSSATO, F.; GALIA, C. R. Expression of neurotrophins and their receptors in primary osteosarcoma. **Rev Col Bras Cir**, 46, n. 2, p. e2094, 2019.

BUYNITSKY, T.; MOSTOFSKY, D. I. Restraint stress in biobehavioral research: recent developments. **Neuroscience & Biobehavioral Reviews**, 33, n. 7, p. 1089-1098, 2009.

CASTAGLIUOLO, I.; LEEMAN, S. E.; BARTOLAK-SUKI, E.; NIKULASSON, S. *et al.* A neurotensin antagonist, SR 48692, inhibits colonic responses to immobilization stress in rats. **Proceedings of the National Academy of Sciences**, 93, n. 22, p. 12611-12615, 1996.

CASTRÉN, E.; KOJIMA, M. Brain-derived neurotrophic factor in mood disorders and antidepressant treatments. **Neurobiology of disease**, 97, p. 119-126, 2017.

CAVALETTI, G.; TREDICI, G.; BRAGA, M.; TAZZARI, S. Experimental peripheral neuropathy induced in adult rats by repeated intraperitoneal administration of taxol. **Experimental neurology**, 133, n. 1, p. 64-72, 1995.

CLARK, V. P.; COFFMAN, B. A.; TRUMBO, M. C.; GASPAROVIC, C. Transcranial direct current stimulation (tDCS) produces localized and specific alterations in neurochemistry: a 1H magnetic resonance spectroscopy study. **Neuroscience letters**, 500, n. 1, p. 67-71, 2011.

CLEMENT, H.-W.; KIRSCH, M.; HASSE, C.; OPPER, C. *et al.* Effect of repeated immobilization on serotonin metabolism in different rat brain areas and on serum corticosterone. **Journal of neural transmission**, 105, n. 10-12, p. 1155-1170, 1998.

COYLE, J. T.; DUMAN, R. S. Finding the intracellular signaling pathways affected by mood disorder treatments. **Neuron**, 38, n. 2, p. 157-160, 2003.

CRETTAZ, B.; MARZINIAK, M.; WILLEKE, P.; YOUNG, P. *et al.* Stress-induced allodynia–evidence of increased pain sensitivity in healthy humans and patients with chronic pain after experimentally induced psychosocial stress. **PloS one**, 8, n. 8, 2013.

DAVIS, S. E.; SMITH, G. A. Transcranial Direct Current Stimulation Use in Warfighting: Benefits, Risks, and Future Prospects. **Frontiers in human neuroscience**, 13, p. 114, 2019.

DE CAMARGO, M. G.; FURLAN, M. M. D. P. < b> Resposta Fisiológica do Corpo às Temperaturas Elevadas: Exercício, Extremos de Temperatura e Doenças Térmicas. **Saúde e Pesquisa**, 4, n. 2, 2011.

DE SANTI, L.; ANNUNZIATA, P.; SESSA, E.; BRAMANTI, P. Brain-derived neurotrophic factor and TrkB receptor in experimental autoimmune encephalomyelitis and multiple sclerosis. **Journal of the neurological sciences**, 287, n. 1-2, p. 17-26, 2009.

DIMOV, L. F.; FRANCIOSI, A. C.; CAMPOS, A. C. P.; BRUNONI, A. R. *et al.* Top-down effect of direct current stimulation on the nociceptive response of rats. **PloS one**, 11, n. 4, 2016.

DOS SANTOS, M. F.; LOVE, T. M.; MARTIKAINEN, I. K.; NASCIMENTO, T. D. *et al.* Immediate effects of tDCS on the μ-opioid system of a chronic pain patient. **Frontiers in psychiatry**, 3, p. 93, 2012.

EPEL, E. S.; CROSSWELL, A. D.; MAYER, S. E.; PRATHER, A. A. *et al.* More than a feeling: A unified view of stress measurement for population science. **Frontiers in neuroendocrinology**, 49, p. 146-169, 2018.

FINK, G. **Encyclopedia of stress**. Academic Press, 2007. 0120885077.

FITZGERALD, P. B.; MCQUEEN, S.; DASKALAKIS, Z. J.; HOY, K. E. A negative pilot study of daily bimodal transcranial direct current stimulation in schizophrenia. **Brain stimulation**, 7, n. 6, p. 813-816, 2014.

FREGNI, F.; FREEDMAN, S.; PASCUAL-LEONE, A. Recent advances in the treatment of chronic pain with non-invasive brain stimulation techniques. **The Lancet Neurology**, 6, n. 2, p. 188-191, 2007.

FREGNI, F.; GIMENES, R.; VALLE, A. C.; FERREIRA, M. J. *et al.* A randomized, sham-controlled, proof of principle study of transcranial direct current stimulation for the treatment of pain in fibromyalgia. **Arthritis & Rheumatism: Official Journal of the American College of Rheumatology**, 54, n. 12, p. 3988-3998, 2006.

FRITSCH, B.; REIS, J.; MARTINOWICH, K.; SCHAMBRA, H. M. *et al.* Direct current stimulation promotes BDNF-dependent synaptic plasticity: potential implications for motor learning. **Neuron**, 66, n. 2, p. 198-204, 2010.

GAMARO, G. D.; MICHALOWSKI, M. B.; CATELLI, D. H.; XAVIER, M. H. *et al.* Effect of repeated restraint stress on memory in different tasks. **Brazilian Journal of Medical and Biological Research**, 32, n. 3, p. 341-347, 1999.

GARLAND, E. L. Pain processing in the human nervous system: a selective review of nociceptive and biobehavioral pathways. **Primary Care: Clinics in Office Practice**, 39, n. 3, p. 561-571, 2012.

GHANAVATINEJAD, F.; TABRIZI, Z. P. F.; OMIDGHAEMI, S.; SHARIFI, E. *et al.* Protein biomarkers of neural system. **Journal of otology**, 14, n. 3, p. 77-88, 2019.

GOMES, R. L.; GARDIM, C. B.; BERNARDO, A. F. B.; PEFFARDINI, C. *et al.*, 2013, **Associação entre o nível de estresse e o nível educacional de funcionários de uma universidade pública**. 104-111.

GRANDIN, T.; DEESING, M. Distress in animals: is it fear, pain or physical stress? **Journal of neurochemistry** 95:484-498, 2002.

GULYAEVA, N. Interplay between brain BDNF and glutamatergic systems: a brief state of the evidence and association with the pathogenesis of depression. **Biochemistry (Moscow)**, 82, n. 3, p. 301-307, 2017.

HEAD, B. P.; PATEL, H. H.; NIESMAN, I. R.; DRUMMOND, J. C. *et al.* Inhibition of p75 neurotrophin receptor attenuates isoflurane-mediated neuronal apoptosis in the neonatal central nervous system. **Anesthesiology: The Journal of the American Society of Anesthesiologists**, 110, n. 4, p. 813-825, 2009.

HEBERT, M. A.; SEROVA, L. I.; SABBAN, E. L. Single and repeated immobilization stress differentially trigger induction and phosphorylation of several transcription factors and mitogen-activated protein kinases in the rat locus coeruleus. **Journal of neurochemistry**, 95, n. 2, p. 484-498, 2005.

HIRSCHLE, A. L. T.; GONDIM, S. M. G. Estresse e bem-estar no trabalho: Uma revisão de literatura. **Ciência & Saúde Coletiva**, 25, p. 2721-2736, 2020.

HOMANN, D.; STEFANELLO, J. M. F.; GÓES, S. M.; BREDA, C. A. *et al.* Percepção de estresse e sintomas depressivos: funcionalidade e impacto na qualidade de vida em mulheres com fibromialgia. **Revista Brasileira de Reumatologia**, 52, n. 3, p. 324-330, 2012.

HUANG, N.; YANG, C.; HUA, D.; LI, S. *et al.* Alterations in the BDNF–mTOR Signaling Pathway in the Spinal Cord Contribute to Hyperalgesia in a Rodent Model of Chronic Restraint Stress. **Neuroscience**, 409, p. 142-151, 2019.

ISERHARDT, D. G. M. Avaliação dos níveis séricos do fator neurotrófico cerebral (BNDF) em pacientes com fibromialgia antes e após a conduta na fisioterapia. 2016.

KANDRATAVICIUS, L.; MONTEIRO, M. R.; SILVA, R. A. D. V.-D.; LEITE, J. P. Neurotrofinas na epilepsia do lobo temporal. **Journal of Epilepsy and Clinical Neurophysiology**, 16, n. 1, p. 7-12, 2010.

KEKIC, M.; BOYSEN, E.; CAMPBELL, I. C.; SCHMIDT, U. A systematic review of the clinical efficacy of transcranial direct current stimulation (tDCS) in psychiatric disorders. **Journal of Psychiatric Research**, 74, p. 70-86, 2016.

KILKENNY, C.; BROWNE, W. J.; CUTHILL, I. C.; EMERSON, M. *et al.* Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. **Osteoarthritis Cartilage**, 20, n. 4, p. 256-260, Apr 2012.

KOMAKI, Y.; HIKISHIMA, K.; SHIBATA, S.; KONOMI, T. *et al.* Functional brain mapping using specific sensory-circuit stimulation and a theoretical graph network analysis in mice with neuropathic allodynia. **Scientific reports**, 6, p. 37802, 2016.

KOPTEV, M.; VYNNYK, N. Morphological substantiation for acute immobilization stress-related disorders of adaptation mechanisms. **Wiadomosci Lekarskie (Warsaw, Poland: 1960)**, 70, n. 4, p. 767-770, 2017.

KRAUS, N.; ANDERSON, S. The ear-brain connection: the role of cognition in neural speech processing. **Hear Res**, 229, n. 1-2, p. 186-203, 2007.

KRISHNAN, V.; HAN, M.-H.; GRAHAM, D. L.; BERTON, O. *et al.* Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. **Cell**, 131, n. 2, p. 391-404, 2007.

LEI, N. 11.794, de 8 de outubro de 2008. **Regulamenta o inciso VII do § 1o do art**, 225.

LEVI-MONTALCINI, R. The nerve growth factor: thirty-five years later. **The EMBO journal**, 6, n. 5, p. 1145-1154, 1987.

LI, F.; ZHANG, J.-W.; WEI, R.; LUO, X.-G. *et al.* Sex-differential modulation of visceral pain by brain derived neurotrophic factor (BDNF) in rats. **Neuroscience letters**, 478, n. 3, p. 184-187, 2010.

LI, J.; SIMONE, D. A.; LARSON, A. A. Windup leads to characteristics of central sensitization. **Pain**, 79, n. 1, p. 75-82, 1999.

LIEBETANZ, D.; KLINKER, F.; HERING, D.; KOCH, R. *et al.* Anticonvulsant effects of transcranial direct-current stimulation (tDCS) in the rat cortical ramp model of focal epilepsy. **Epilepsia**, 47, n. 7, p. 1216-1224, 2006.

LOMMATZSCH, M.; ZINGLER, D.; SCHUHBAECK, K.; SCHLOETCKE, K. *et al.* The impact of age, weight and gender on BDNF levels in human platelets and plasma. **Neurobiology of aging**, 26, n. 1, p. 115-123, 2005.

MAIA, B. R.; DIAS, P. C. Ansiedade, depressão e estresse em estudantes universitários: o impacto da COVID-19. **Estudos de Psicologia (Campinas)**, 37, 2020.

MARGIS, R.; PICON, P.; COSNER, A. F.; SILVEIRA, R. D. O. Relação entre estressores, estresse e ansiedade. **Revista de Psiquiatria do Rio Grande do Sul**, 25, p. 65-74, 2003.

MAUSS, I. B.; LEVENSON, R. W.; MCCARTER, L.; WILHELM, F. H. *et al.* The tie that binds? Coherence among emotion experience, behavior, and physiology. **Emotion**, 5, n. 2, p. 175, 2005.

MERIGHI, A.; SALIO, C.; GHIRRI, A.; LOSSI, L. *et al.* BDNF as a pain modulator. **Progress in neurobiology**, 85, n. 3, p. 297-317, 2008.

MEYER-TUVE, A.; MALCANGIO, M.; EBERSBERGER, A.; MAZARIO, J. *et al.* Effect of brain-derived neurotrophic factor on the release of substance P from rat spinal cord. **Neuroreport**, 12, n. 1, p. 21-24, 2001.

MILLAN, M. J. Descending control of pain. **Progress in neurobiology**, 66, n. 6, p. 355-474, 2002.

MO, C.; RENOIR, T.; HANNAN, A. J. Stress and Glucocorticoids as Experience-Dependent Modulators of Huntington's Disease. *In*: **Stress: Physiology, Biochemistry, and Pathology**: Elsevier, 2019. p. 243-278.

MOFFA, A. H.; BRUNONI, A. R.; NIKOLIN, S.; LOO, C. K. Transcranial direct current stimulation in psychiatric disorders: a comprehensive review. **Psychiatric Clinics**, 41, n. 3, p. 447-463, 2018.

MONTEGGIA, L. M.; BARROT, M.; POWELL, C. M.; BERTON, O. *et al.* Essential role of brain-derived neurotrophic factor in adult hippocampal function. **Proceedings of the National Academy of Sciences**, 101, n. 29, p. 10827-10832, 2004.

MURAKAMI, S.; IMBE, H.; MORIKAWA, Y.; KUBO, C. *et al.* Chronic stress, as well as acute stress, reduces BDNF mRNA expression in the rat hippocampus but less robustly. **Neuroscience research**, 53, n. 2, p. 129-139, 2005.

NAGARAJA, H.; JEGANATHAN, P. Effect of short & long term restraint stress on some physiological & biochemical parameters in rats. **Indian Journal of Medical Research**, 109, p. 76, 1999.

NITSCHE, M. A.; COHEN, L. G.; WASSERMANN, E. M.; PRIORI, A. *et al.* Transcranial direct current stimulation: state of the art 2008. **Brain stimulation**, 1, n. 3, p. 206-223, 2008.

NITSCHE, M. A.; PAULUS, W. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. **The Journal of physiology**, 527, n. 3, p. 633-639, 2000.

NUNES, V. B. D. S. Efeito da estimulação transcraniana de corrente contínua combinado a tarefa neurocognitiva inibitória na dor e capacidade atencional de pacientes com fibromialgia. 2015.

OKANO, A. H.; FONTES, E. B.; MONTENEGRO, R. A.; FARINATTI, P. D. T. V. *et al.* Brain stimulation modulates the autonomic nervous system, rating of perceived exertion and

performance during maximal exercise. **British journal of sports medicine**, 49, n. 18, p. 1213-1218, 2015.

PACAK, K.; MCCARTY, R. Acute stress response: experimental. **Encyclopedia of stress**, 1, p. 8-17, 2000.

PACHECO, P. V. A influência do fator neurotrófico derivado do cérebro e dos exercícios físicos sobre a neuroplasticidade após acidente vascular encefálico. 2009.

PARKER, K. J.; SCHATZBERG, A. F.; LYONS, D. M. Neuroendocrine aspects of hypercortisolism in major depression. **Hormones and behavior**, 43, n. 1, p. 60-66, 2003.

PLOW, E. B.; PASCUAL-LEONE, A.; MACHADO, A. Brain stimulation in the treatment of chronic neuropathic and non-cancerous pain. **The Journal of Pain**, 13, n. 5, p. 411-424, 2012.

PURPURA, D. P.; MCMURTRY, J. G. Intracellular activities and evoked potential changes during polarization of motor cortex. **Journal of neurophysiology**, 28, n. 1, p. 166-185, 1965.

QUEVEDO, A. S.; COGHILL, R. C. Attentional modulation of spatial integration of pain: evidence for dynamic spatial tuning. **Journal of Neuroscience**, 27, n. 43, p. 11635-11640, 2007.

REGNER, G. G.; TORRES, I. L.; DE OLIVEIRA, C.; PFLÜGER, P. *et al.* Transcranial direct current stimulation (tDCS) affects neuroinflammation parameters and behavioral seizure activity in pentylenetetrazole-induced kindling in rats. **Neuroscience Letters**, p. 135162, 2020.

ROTHMAN, S.; MATTSON, M. Activity-dependent, stress-responsive BDNF signaling and the quest for optimal brain health and resilience throughout the lifespan. **Neuroscience**, 239, p. 228-240, 2013.

SCARABELOT, V. L.; DE OLIVEIRA, C.; MEDEIROS, L. F.; DE MACEDO, I. C. *et al.* Transcranial direct-current stimulation reduces nociceptive behaviour in an orofacial pain model. **Journal of oral rehabilitation**, 46, n. 1, p. 40-50, 2019.

SCHOENROCK, S. A.; TARANTINO, L. M. Animal models of environmental manipulations resulting in epigenetic modifications that increase risk for affective disorders. *In*: **Epigenetics in Psychiatry**: Elsevier, 2014. p. 181-205.

SEBBEN, A. D.; COCOLICHIO, F.; SCHMITT, A. P.; CURRA, M. D. *et al.* Effect of neurotrophic factors on peripheral nerve repair [Abstract in English]. **Scientia Medica**, 21, n. 2, p. 81-89, 2011.

SHEA, A.; WALSH, C.; MACMILLAN, H.; STEINER, M. Child maltreatment and HPA axis dysregulation: relationship to major depressive disorder and post traumatic stress disorder in females. **Psychoneuroendocrinology**, 30, n. 2, p. 162-178, 2005.

SILVA, T. D. S. F.; GALDINO, M. K. C.; ANDRADE, S. M. M. D. S.; LUCENA, L. B. S. D. *et al.* Use of non-invasive neuromodulation in the treatment of pain in temporomandibular dysfunction: preliminary study. **BrJP**, 2, n. 2, p. 147-154, 2019.

SMITH, M. A.; MAKINO, S.; KVETNANSKY, R.; POST, R. M. Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. **Journal of Neuroscience**, 15, n. 3, p. 1768-1777, 1995.

SON, H.; YANG, J. H.; KIM, H. J.; LEE, D. K. A chronic immobilization stress protocol for inducing depression-like behavior in mice. **JoVE (Journal of Visualized Experiments)**, n. 147, p. e59546, 2019.

SONNEWALD, U.; WESTERGAARD, N.; SCHOUSBOE, A.; SVENDSEN, J. *et al.* Direct demonstration by [13C] NMR spectroscopy that glutamine from astrocytes is a precursor for GABA synthesis in neurons. **Neurochemistry international**, 22, n. 1, p. 19-29, 1993.

SRINIVASAN, S.; LOGANATHAN, S.; WANKHAR, W.; RATHINASAMY, S. *et al.* Stress effect on humoral and cell mediated immune response: indispensable part of corticosterone and cytokine in neutrophil function. **Trials in vaccinology**, 5, p. 61-70, 2016.

STAGG, C. J.; BEST, J. G.; STEPHENSON, M. C.; O'SHEA, J. *et al.* Polarity-sensitive modulation of cortical neurotransmitters by transcranial stimulation. **Journal of Neuroscience**, 29, n. 16, p. 5202-5206, 2009.

STAGG, C. J.; NITSCHE, M. A. Physiological basis of transcranial direct current stimulation. **The Neuroscientist**, 17, n. 1, p. 37-53, 2011.

SUBHANI, A. R.; KAMEL, N.; SAAD, M. N. M.; NANDAGOPAL, N. *et al.* Mitigation of stress: new treatment alternatives. **Cognitive neurodynamics**, 12, n. 1, p. 1-20, 2018.

TANNO, A. P.; MARCONDES, F. K. Estresse, ciclo reprodutivo e sensibilidade cardíaca às catecolaminas. **Revista Brasileira de Ciências Farmacêuticas**, 38, n. 3, p. 273-289, 2002.

TORRES, I. L. D. S.; VASCONCELLOS, A.; SILVEIRA CUCCO, S.; DALMAZ, C. Effect of repeated stress on novelty-induced antinociception in rats. **Brazilian Journal of Medical and Biological Research**, 34, n. 2, p. 241-244, 2001.

UEYAMA, T.; KAWAI, Y.; NEMOTO, K.; SEKIMOTO, M. *et al.* Immobilization stress reduced the expression of neurotrophins and their receptors in the rat brain. **Neuroscience research**, 28, n. 2, p. 103-110, 1997.

ZAGHI, S.; ACAR, M.; HULTGREN, B.; BOGGIO, P. S. *et al.* Noninvasive brain stimulation with low-intensity electrical currents: putative mechanisms of action for direct and alternating current stimulation. **The Neuroscientist**, 16, n. 3, p. 285-307, 2010.

ZHANG, Z.; WANG, X.; WANG, W.; LU, Y.-G. *et al.* Brain-Derived Neurotrophic Factor–Mediated Downregulation of Brainstem K+–Cl–Cotransporter and Cell-Type–Specific GABA Impairment for Activation of Descending Pain Facilitation. **Molecular pharmacology**, 84, n. 4, p. 511-520, 2013.

HOSPITAL DE CLÍNICAS
PORTO ALEGRE RS

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GRUPO DE PESQUISA E PÓS GRADUAÇÃO
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

Certificamos que o projeto abaixo, que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica, encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) e pelas áreas de apoio indicadas pelo pesquisador.

Projeto: 160408 **Data de Aprovação do Projeto:** 14/10/2016

Título: Avaliação dos efeitos agudos da estimulação transcraniana com corrente continua (ETCC) na ativação de vias neurais descendentes e de mecanismos sistêmicos relacionados ao controle da dor em ratos naive

Pesquisador Responsável: IRACI LUCENA DA SILVA TORRES

Equipe de pesquisa:

ALEXANDRE SILVA DE QUEVEDO, DEISE PONZONI, ETIANE MICHELI MEYER CALLAI, FELIPE ERNESTO ARTUZI, ISABEL CRISTINA DE MACEDO, VANESSA LEAL SCARABELOT

Submissão	Documento	Espécie/Linhagem	Sexo/Idade	Qtd.	Data Reunião	Situação
10/08/2016	APROVAÇÃO	RATO - WISTAR	M/60dias	100	11/10/2016	APROVADO

Total de Animais: 100

Coordenador
Comissão de Ética no Uso de Animais

- Os membros da CEUA/HCPA não participaram do processo de avaliação onde constam como pesquisadores.
- Toda e qualquer alteração do Projeto deverá ser comunicada à CEUA/HCPA.
- O pesquisador deverá apresentar relatórios semestrais de acompanhamento e relatório final ao CEUA/HCPA.