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PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS:

FARMACOLOGIA E TERAPÊUTICA

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AVALIAÇÃO DOS EFEITOS DA ASSOCIAÇÃO DE PREGABALINA E ESTIMULAÇÃO TRANSCRANIANA POR CORRENTE CONTÍNUA (ETCC) EM UM MODELO ANIMAL DE FIBROMIALGIA

PORTO ALEGRE

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Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas: Farmacologia e Terapêutica do Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul, como requisito parcial para a obtenção do título de Doutora em Farmacologia e Terapêutica.

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RESUMO

Síndrome Fibromiálgica caracteriza-se por dor generalizada, fadiga, sono não reparador e sintomas cognitivos. Os grupos farmacológicos mais utilizados para o tratamento desta condição são antidepressivos e anticonvulsivantes. Dentre as terapêuticas disponíveis, a pregabalina tem se mostrado uma importante opção farmacológica na redução da dor e melhora da qualidade de vida em pacientes. Por outro lado, estudos pré-clínicos e clínicos têm demonstrado que técnicas neuromodulatórias são promissoras no tratamento de quadros de dor crônica, podendo ser uma opção para o tratamento da fibromialgia. Este estudo objetiva avaliar o possível efeito sinérgico antinociceptivo entre estimulação transcraniana por corrente contínua (ETCC) e pregabalina em modelo de fibromialgia em ratos. Quarenta ratos Wistar machos adultos foram inicialmente divididos em dois: controle (CT) e fibromialgia (FM) e, após a submissão ao modelo de fibromialgia, foram subdivididos em: grupo controle (CT), reserpina + pregabalina + ETCC-sham (RPTs), reserpina + pregabalina + ETCC-ativo (RPT), reserpina + veículo pregabalina + ETCC-sham (RVTs) e reserpina + veículo pregabalina + ETCC-ativo (RVT). O modelo de fibromialgia foi induzido pela aplicação de reserpina (3 dias, 1 mg/Kg, s.c.). Os comportamentos avaliados foram: resposta nociceptiva (von Frey e placa quente), comportamento do tipo depressivo (nado forçado) e comportamento do tipo ansioso (labirinto em cruz elevado). As intervenções utilizadas foram 8 dias de aplicação de ETCC (20 min/0,5mA) e/ou de administração oral de pregabalina (30mg/kg, gavagem). Os ratos foram mortos por decapitação para coleta das amostras biológicas. Os marcadores bioquímicos fator neurotrófico derivado do encéfalo (BDNF), interleucinas 1β e 10, e fator de necrose tumoral alfa (TNF-α) foram mensurados por ELISA. Este estudo foi aprovado pela CEUA/-HCPA #2015-0272. Os ratos submetidos ao modelo de fibromialgia apresentaram hiperalgesia térmica (placa-quente) e mecânica (von Frey), indexadas por um menor limiar nociceptivo (teste t, $P<0,0001$ e $P=0,01$ respectivamente), aumento do tempo de imobilidade no teste de nado forçado (teste t, $P=0,004$), aumento no comportamento de *grooming*, redução nos comportamentos de *non-protected head-dipping* (NPHD) e *rearing*

(ANOVA de uma via, P= 0,03, P=0,03 e P< 0,01 respectivamente) com redução de níveis séricos de BDNF (teste t, P<0.05). Adicionalmente, ratos com fibromialgia tratados com ETCC (associado ou não a pregabalina) apresentaram uma reversão parcial do comportamento hipernociceptivo (ANOVA de uma via, P<0.05), reversão do aumento do *grooming* e da diminuição dos níveis séricos de BDNF induzido pelo modelo após tratamento com ETCC. A administração de pregabalina reverteu a diminuição do NPHD induzida pelo modelo. O presente estudo demonstra que a ETCC é uma importante ferramenta para aliviar comportamento nociceptivo em quadros de dor crônica, assim como reverter comportamentos do tipo ansioso relacionados ao modelo de fibromialgia. Além de reverter a diminuição dos níveis séricos de BDNF. No entanto, não foi observado efeito sinérgico na associação de ETCC e pregabalina nos efeitos avaliados no modelo de fibromialgia induzido pela reserpina.

Palavras-chave: fibromialgia, ETCC, pregabalina, ratos, dor.

ABSTRACT

Fibromyalgic syndrome is characterized by generalized pain, fatigue, non-restorative sleep, and cognitive symptoms. The pharmacological groups most used for the treatment of this condition are antidepressants and anticonvulsants. Among the available therapies, pregabalin has been shown to be an important pharmacological option in reducing pain and improving quality of life in patients. On the other hand, preclinical and clinical studies have shown that neuromodulatory techniques are promising in the treatment of chronic pain, and may be an option in the treatment of fibromyalgia.This study aims to evaluate the possible synergistic antinociceptive effect between transcranial direct current stimulation (tDCS) and pregabalin in a rat model of fibromyalgia.Forty adult male Wistar rats were initially divided into two: control (CT) and fibromyalgia (FM) and, after submission of the fibromyalgia model, they were subdivided into: control group (CT), reserpine + pregabalin + tDCS-sham (RPTs), reserpine + pregabalin + tDCS-active (RPT), reserpine + pregabalin vehicle + tDCS-sham (RVTs) and reserpine + pregabalin vehicle + active tDCS (RVT). The fibromyalgia model was induced by the application of reserpine (3 days, 1 mg/kg, s.c.). The behaviors evaluated were: nociceptive response (von Frey and hot plate), depressive-like behavior (forced swimming) and anxious-like behavior (elevated plus maze). The interventions used were 8 days of tDCS application (20 min/0.5mA) and/or oral administration of pregabalin (30mg/Kg, gavage). Rats were killed by decapitation to collect biological samples. The biochemical markers brain-derived neurotrophic factor (BDNF), interleukins 1β and 10, and tumor necrosis factor alpha (TNF-α)] were measured by ELISA. This study was approved by CEUA/-HCPA #2015-0272. Rats submitted to the fibromyalgia model showed thermal (hot plate) and mechanical (von Frey) hyperalgesia, indexed by a lower nociceptive threshold (t test, $P<0.0001$ and $P=0.01$ respectively), increased immobility in the forced swimming test (t test, $P=0.004$), increase in grooming behavior, reduction in non- protected head-dipping (NPHD) and rearing behaviors (one-way ANOVA, $P= 0.03$, $P= 0.03$ and $P<0.01$ respectively) with reduced serum BDNF levels (t-test, $P<0.05$). Additionally, rats with fibromyalgia treated with tDCS (with or without pregabalin) showed a partial reversal of hypernociceptive behavior (one-way ANOVA, $P<0.05$), reversal of increased grooming and decreased serum BDNF levels induced by the model. after tDCS treatment. Pregabalin administration reversed the model-induced decrease in NPHD. The present study demonstrates that tDCS is an important tool

for alleviating nociceptive behavior in chronic pain conditions, as well as reversing anxious-like and anxious-like behaviors related to the fibromyalgia model. In addition to reversing the decrease in serum BDNF levels. However, no synergistic effect was observed in the association of tDCS and pregabalin in the effects evaluated in the reserpine-induced fibromyalgia model.

Keywords: fibromyalgia, tDCS, pregabalin, rats, pain

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- **Artigo 2**

LISTA DE ABREVIATURAS

ANOVA - análise de variância (do inglês, *analysis of variance*)

BDNF - fator neurotrófico derivado do encéfalo

DA – dopamine

EA – Efeitos adversos

ELISA - ensaio de imunoabsorção enzimática (do inglês, *enzyme-linked immunosorbent assay)*

ETCC - estimulação transcraniana por corrente contínua (do inglês, *transcranial Direct Current Stimulation)*

EMT - estimulação magnética transcraniana (do inglês, *transcranial magnetic stimulation*).

FDA - *Food and Drug Administration*

GABA - ácido gama-aminobutírico

HCPA- Hospital de Clínicas de Porto Alegre

IL-1β - interleucina 1β

IL-10 – interleucina 10

LCE – labirinto em cruz elevado

LCR –Liquido Cefalorraquidiano

*f*MRI – ressonância magnética funcional

NE – norepinefrina

PG - Pregabalina

PQ – Placa Quente

SNC - Sistema Nervoso Central

SNP - Sistema Nervoso Periférico

VF – Von Frey

TNF-α - fator de necrose tumoral alfa

5-HT - serotonina

APRESENTAÇÃO

Esta tese está estruturada em 3 partes:

- **Parte I** - Introdução, Revisão da literatura, Justificativa, Objetivos e Referências da parte I
- **Parte II** - Materiais e métodos, resultados, discussão e conclusão na forma de dois artigos científicos. O primeiro caracterizando o modelo de fibromialgia em ratos Wistar; e o segundo avaliando os efeitos do tratamento com pregabalina e estimulação transcraniana por corrente contínua em ratos submetidos ao modelo de fibromialgia e os níveis de BDNF, IL1-β, IL-10 e TNF–α em estruturas do sistema nervoso central e soro.

  - **Artigo 1**: *Fibromyalgia-like animal model decreases serum BDNF levels of male Wistar rats.*

  - **Artigo 2:** *No synergic effect between pregabalin and transcranial direct current stimulation (tDCS) upon hyper nociceptive behavior in rats with fibromyalgia model.*

- **Parte III** - Discussão Geral, Conclusões, Referências da parte III, Aprovação da Comissão de Ética, Perspectivas e Produção Acadêmica Durante o Período de Doutorado.

Observação: Detalhes técnicos mais precisos sobre a metodologia empregada em cada um dos trabalhos apresentados podem ser encontrados nos artigos científicos.

SUMÁRIO

PARTE I

1 INTRODUÇÃO

Fibromialgia é uma síndrome musculoesquelética caracterizada por dor difusa crônica generalizada, fadiga, sono não reparador e sintomas cognitivos. Acomete principalmente mulheres, atingindo cerca de 2% da população mundial (Urfaloglu e Berk, 2022). O fenômeno fisiopatológico principal da fibromialgia é a sensibilização central, caracterizada pela limitação das vias descendentes de inibição da dor e pelo aprimoramento das vias ascendentes de sinalização da dor (Poluha e Grossmann, 2018). Alguns modelos pré-clínicos de fibromialgia têm sido propostos com o objetivo de melhor esclarecer a fisiopatologia desta síndrome e de buscar novos tratamentos. Vários modelos fisiopatogênicos têm sido propostos para elucidar esta síndrome, sendo que o mais aceito considera que há um desequilíbrio entre o processo nociceptivo e a modulação endógena da dor (Yunus, 2007). Segundo este modelo, há uma diminuição global das vias inibitórias relacionadas ao processo nociceptivo. Desta forma, estímulos de baixa intensidade ou não-nociceptivos são processados em estruturas pré-corticais e corticais envolvidas no processo afetivo e cognitivo da dor aumentando a resposta nociceptiva (Burgmer, et al., 2009). Portanto, as estratégias terapêuticas objetivam modular processos de neuroplasticidade em múltiplas áreas cerebrais implicadas nas respostas afetivas, cognitivas e perceptivas da dor que compõem a neuromatriz da dor. O caráter sindrômico da fibromialgia, manifestações clínicas com causa desconhecida alterando diferentes fatores em sua fisiopatologia resultam na ausência de diagnóstico e de tratamento específico (Oliveira e Almeida, 2018).

O tratamento da fibromialgia geralmente é multidisciplinar, objetivando o alívio sintomático, o que se traduz em modificação da plasticidade mal adaptativa da neuromatriz da dor. As intervenções farmacológicas incluem analgésicos clássicos, antidepressivos, anticonvulsivantes e relaxantes musculares, objetivando controle sintomático, assim como promover modificação na neuromatriz da dor, no entanto, além dos efeitos adversos, os resultados podem não ser satisfatórios (Crofford, 2004; Goldenberg, 2007). Entre os anticonvulsivantes, a pregabalina foi aprovada pela *Food and Drug Administration* (FDA - EUA) para uso no tratamento de fibromialgia em 2007.

A pregabalina é estruturalmente semelhante ao aminoácido inibitório ácido gama-aminobutírico (GABA), liga-se com elevada afinidade na subunidade alfa-2-delta dos canais de cálcio dependentes de voltagem no sistema nervoso central (Lang et al., 2005). No entanto, não está claro de que forma promove alívio nos sintomas da fibromialgia. A hipótese mais aceita sugere que há hiperatividade glutamatérgica na síndrome fibromiálgica, e que a pregabalina promove bloqueio do influxo de cálcio, estabilizando a membrana em terminais pré-sinápticos (Oliveira e Almeida, 2018). *In vitro*, a pregabalina demonstrou ser capaz de reduzir a liberação de diversos neurotransmissores cálcio-dependentes, incluindo glutamato, norepinefrina, calcitonina e substância P (Lang et al., 2005). Estudo em voluntários saudáveis usando 600 mg de pregabalina e estimulação magnética transcraniana demonstrou efeitos em diferentes circuitos inibitórios no córtex motor, sugerindo um efeito ativador de receptores GABA-B (Lang et al., 2005). Ensaios clínicos randomizados têm demonstrado o efeito neuromodulador da pregabalina ao longo do tempo (Arnold et al., 2012; Hauser et al., 2009). No entanto, poucos estudos abordam o mecanismo de ação deste fármaco (Burgmer et al., 2009). Um estudo comparou o efeito da pregabalina na ativação cortical ao estímulo doloroso usando ressonância magnética funcional (*f*MRI) em grupos de mulheres fibromiálgicas e saudáveis sem tratamento. Este estudo demonstrou efeito no giro supramarginal, giro frontal superior e inferior, giro temporal médio, tálamo, cerebelo e córtex visual primário (Kim et al., 2013). Outro estudo experimental *crossover* avaliou, com técnicas de neuroimagem combinadas (*f*MRI, espectroscopia de prótons) e análise de conectividade funcional, o efeito de 450 mg de pregabalina diários, durante 14 dias em pacientes fibromiálgicas (Harris et al., 2014). O estudo demonstrou que a pregabalina diminui os níveis de neurotransmissores excitatórios (glutamato e glutamina) na ínsula posterior e reduz a conectividade funcional em áreas do cérebro em condições de dor crônica (Harris et al., 2014). Estes estudos sugerem que, além das características farmacodinâmicas da pregabalina, devem ser considerados os efeitos neuromodulatórios que dependem das características basais dos circuitos neurais do paciente.

Associadas às terapias farmacológicas têm sido propostas intervenções não

farmacológicas, incluindo terapia cognitivo comportamental, técnicas de relaxamento, estimulações periféricas como eletroacupuntura e estimulação transcraniana por corrente contínua (ETCC, ou tDCS do inglês "*transcranial Direct Current Stimulation*") e magnética (TMS – *Transcranial Magnetic Stimulation*) (Hassett e Gevirtz, 2009). A ETCC é uma técnica não invasiva e segura, podendo ser combinada com outras estratégias terapêuticas (Zaminotto et al., 2019). Na ETCC, uma corrente contínua de baixa intensidade direcionada ao escalpo é aplicada usando eletrodos (cátodo e ânodo). A corrente atinge o córtex cerebral, produzindo hiperpolarização ou despolarização do potencial de repouso de membrana axonal (conforme montagem dos eletrodos). Ensaios clínicos têm suportado estimativas matemáticas (Mendoça et al., 2011) demonstrando que a ETCC induz correntes significativas em áreas corticais, alterando a excitabilidade cortical (Antal et al., 2010). Este efeito tem sido associado à indução de analgesia na fibromialgia quando aplicada conforme esquemas terapêuticos apropriados (Fagerlund et al., 2015; Villamar et al., 2013). Os efeitos induzidos pela ETCC dependem da montagem dos eletrodos, do local e da duração de estimulação e podem ser de longa duração (Medeiros et al., 2012). Os efeitos de longa duração na excitabilidade se assemelham aos processos de potenciação e depressão em longa duração (da sigla em inglês, LTP e LTD, respectivamente), como alterações na intensidade das sinapses glutamatérgicas (Boros et al., 2008). No entanto, estas alterações podem envolver outros sistemas neurobiológicos tais como o GABAérgico, serotoninérgico, e colinérgico, conforme tem sido demonstrado em estudo utilizando fármacos em sujeitos saudáveis (Medeiros et al., 2012). Porém, os mecanismos neurobiológicos pelos quais esta técnica induz o seu efeito terapêutico em pacientes com fibromialgia ainda não estão esclarecidos. Esta dúvida ganha relevância considerando os mecanismos fisiopatológicos desta doença, como alterações neurofisiológicas significativas envolvendo os sistemas catecolaminérgico, serotoninérgico e dopaminérgico (Dadabhoy et al., 2008), os quais podem ser modulados por fármacos e/ou outras técnicas de estimulação não invasivas.

Considerando que a dor é entendida como um processo de neuroplasticidade

mal adaptativa, que envolve a síntese de mediadores pela neuroglia, os quais apresentam potencial adjuvante a este processo, dentre estes destacam-se BDNF e S100B. Os níveis de BDNF estão associados a medidas psicofísicas da dor tanto em sujeitos hígidos (Stefani et al., 2012) quanto em pacientes fibromiálgicas (Zanette et al., 2014). O efeito desta neurotrofina é complexo, podendo aumentar a excitabilidade ou diminuir a inibição das vias da dor. Este efeito pode estar relacionado à sua concentração, estado neuroplástico do sistema e níveis de hormônios gonadais (Stefani et al., 2012). Em mulheres saudáveis, o incremento dos níveis séricos está associado à elevação no limiar de dor, enquanto em homens esta resposta é reversa. Em fibromiálgicas menopáusicas a elevação do BDNF foi associada a efeito similar ao observado em homens saudáveis. Enquanto a proteína S100B é fixadora de cálcio, que em circunstâncias normais, e no contexto do estresse, pode ativar vias sensibilizadoras da nocicepção. Grupo de pesquisa demonstrou que os níveis séricos de S100B estão correlacionados com redução no limiar de dor em pacientes fibromiálgicas (Zanette et al., 2014).

Estudos pré-clínicos utilizando modelos animais de fibromialgia permitem mimetizar a doença e avaliar hipóteses por meio de experimentos que em humanos não poderiam ser conduzidos. Os modelos animais de fibromialgia mais utilizados são os que envolvem a indução de dor generalizada, como por exemplo, injeções de salina acidificada, fadiga associada a aplicação de injeções de salina acidificada, *priming* hiperalgésico (indução de processo inflamatório agudo e posterior administração de prostaglandina 2), depleção de aminas biogênicas no sistema nervoso central e indução de estresse, todos conhecidos como indutores de sintomas semelhantes aos que ocorrem em pacientes de fibromialgia (Nagakura et al., 2009).

Entre os modelos animais que promovem depleção de aminas biogênicas está o modelo induzido por reserpina. Este modelo foi proposto considerando as alterações descritas em circuitos serotoninérgicos, dopaminérgicos e catecolaminérgicos em pacientes fibromiálgicas (Nagakura et al., 2009). A injeção repetida de reserpina é capaz de induzir depleção das aminas biogênicas em ratos, induzindo hiperalgesia generalizada, achado que é característico da doença. Desta

maneira, este modelo oferece um substrato neurobiológico plausível, com correlação comportamental consistente com a doença, possibilitando o estudo da combinação de intervenções farmacológicas e não farmacológicas buscando avaliar efeitos comportamentais, bioquímicos e moleculares nos animais submetidos ao modelo e aos tratamentos propostos.

2. REVISÃO DE LITERATURA

2.1 ESTRATÉGIAS PARA SELECIONAR E LOCALIZAR INFORMAÇÕES

Para realização desta revisão, buscou-se ressaltar os principais aspectos relacionados à fibromialgia, pregabalina e estimulação transcraniana por corrente contínua (ETCC). A estratégia de busca envolveu as seguintes bases de dados: LILACS, PubMed e Embase, sem restrição de data de publicação. Para a busca foram utilizadas as seguintes palavras-chave: *Fibromyalgia, pregabalin etranscranial direct current stimulation.* Refinando-se a busca por meio de cruzamentoentre as palavras-chave acima, foi encontrado um número reduzido de publicações,conforme demonstrado na figura 1.

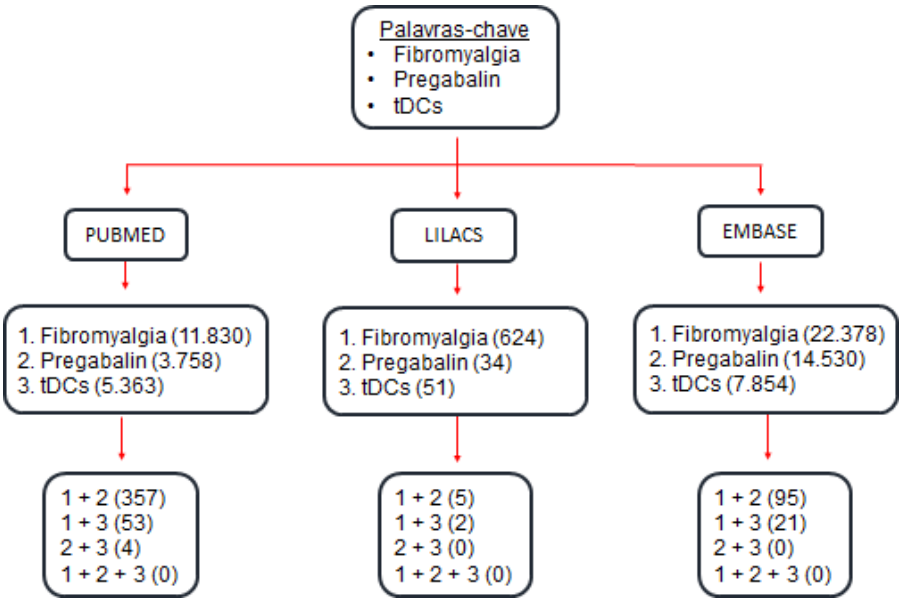

Figura 1. Fluxograma da busca na literatura. Abreviatura: *tDCS* - Estimulação transcraniana por corrente contínua (tradução livre do inglês *transcranial direct current stimulation*).

2.2 FIBROMIALGIA

A fibromialgia é uma síndrome de etiologia desconhecida, caracterizada por dor crônica generalizada, sono não reparador, fadiga e sintomas cognitivos, tais como, lacunas de memória, dificuldades de concentração e alterações de linguagem, acometendo 3% da população mundial, e mais prevalente em mulheres (Bazzichi et al., 2020). Frequentemente, estes pacientes apresentam aumento da prevalência de diagnóstico de depressão, ansiedade e enxaqueca (Monteiro et al., 2021).

A etiologia da síndrome fibromiálgica não está elucidada; no entanto, ela tem sido relacionada à sensibilização central, caracterizada por hiperalgesia e alodinia (Cordeiro et al., 2020). Atualmente, não existem testes clínicos objetivos para o diagnóstico da fibromialgia. A avaliação é baseada em sintomas clínicos e exames de diagnóstico que afastem outras doenças que cursam com dor crônica (Heyman et al., 2010).

Nesse contexto, apesar de poucos achados, tem sido sugerido o envolvimento de citocinas inflamatórias que podem potencializar a neuroinflamação e a sensibilização central e periférica (Galvez-Sánchez e Reyes, 2020). No entanto, têm sido descritas diferentes funções e níveis de citocinas pró-inflamatórias na fibromialgia. Alguns estudos demonstraram uma associação positiva entre os níveis de citocinas no soro e no líquido cefalorraquidiano e a intensidade da dor, enquanto outros mostraram pouca ou nenhuma evidência na correlação entre eles (Galvez-Sánchez e Reyes, 2020; Peck et al., 2020). Tem sido evidenciada o papel das citocinas na geração de dor em múltiplas condições associadas à dor crônica (Galvez-Sánchez e Reyes, 2020; Peck et al., 2020). Por outro lado, além do envolvimento do Sistema imunológico, investigam-se possíveis associações genéticas e variantes de polimorfismos específicos relacionados com a fibromialgia, sugerindo que indivíduos com tais alterações são mais suscetíveis a desenvolver a doença (Peck et al., 2020).

Por conseguinte, estudos clínicos descrevem que na fibromialgia ocorre um aumento do *input* sensorial mediado pelo sistema nervoso central (SNC) (Üçeyler et

al., 2013), assim como pelo sistema nervoso periférico (SNP). Achados demonstram que pacientes fibromiálgicos, quando comparados a indivíduos saudáveis, demonstram maior limiar térmico e menor limiar mecânico, caracterizando alteração funcional de fibras A delta e fibras C. Essa alteração foi confirmada por meio da biópsia de pele que evidenciou menor quantidade de fibras C nociceptivas desmielinizadas nesses pacientes quando comparados com indivíduos saudáveis (Üçeyler et al., 2013).

Evidências demonstram hiperativação global do SNC, com desequilíbrio entre níveis de neurotransmissores excitatórios e inibitórios em pacientes fibromiálgicos comparados com indivíduos saudáveis. Níveis elevados do neurotransmissor excitatório glutamato e glutamina foram encontrados em áreas corticais e subcorticais, incluindo o cingulado posterior, ínsula posterior e amígdala nessa população, quando avaliados por meio de espectroscopia de prótons por ressonância magnéticam (Fayed et al., 2010; Harris et al., 2009). Tal fato pode explicar a indicação do uso de pregabalina como tratamento farmacológico na FM pela *Food and Drug Administration* (FDA), pois o mecanismo de ação desse fármaco envolve a inibição da liberação pré-sináptica de glutamato (Harris et al., 2009). Níveis elevados de glutamato foram correlacionados positivamente com a redução do limiar a baixa pressão e altas pontuações no questionário de impacto da fibromialgia (FIQ, do inglês *Fibromyalgia Impact Questionnaire*) (Harris et al., 2009). Além disso, foram encontrados níveis três vezes mais elevados de substância P no líquido cefalorraquidiano (LCR) destes pacientes, quando comparados com indivíduos saudáveis (Russell et al., 1994; Mountz et al., 1995). Este neuropeptídeo coexiste com o glutamato nas vias aferentes nociceptivas e geram sensibilização dos neurônios do corno dorsal da medulla espinhal (Becker e Schweinhardt, 2012).

Por outro lado, alguns estudos relataram que a dopamina e a norepinefrina estão em níveis metabólitos reduzidos no LCR quando comparados com indivíduos saudáveis (Russell et al., 1994; Becker e Schweinhardt, 2012). Além disso, foram identificados baixos níveis metabólitos de serotonina no LCR e no sangue, indicando

forte envolvimento desse neurotransmissor em transtornos de sono e de humor nesses pacientes (Becker e Schweinhardt, 2012). O ácido gama-aminobutírico (GABA) é um neurotransmissor que desempenha papel importante na inibição da transmissão de dor, principalmente na ínsula anterior direita, área envolvida na percepção de dor (Harris et al., 2009). Em relação às neurotrofinas, os níveis de BDNF estão associados às medidas psicofísicas da dor em sujeitos saudáveis e em pacientes fibromiálgicas (Zanette et al., 2014). Os efeitos do BDNF são complexos, podendo aumentar ou diminuir a excitabilidade neuronal, dependendo da sua concentração, capacidade neuroplástica do sistema e níveis de hormônios gonadais (Stefani et al., 2012).

Considerando o exposto acima, a complexidade da Fibromialgia e sua refratariedade aos tratamentos tradicionais é necessária uma abordagem multidisciplinar, o qual inclui ativamente o paciente e baseia-se nas modalidades farmacológicas e não farmacológicas (Figueiredo-Dourado et al., 2020). Porém, o tratamento farmacológico disponível não é significativamente resolutivo, sendo insatisfatório principalmente no controle dos sintomas dolorosos (Heymann et al., 2010). As intervenções farmacológicas incluem analgésicos simples, sedativos, relaxantes musculares antidepressivos e os anticonvulsivantes, sendo a pregabalina o medicamento mais utilizado para tratar essa síndrome (Poluha e Grossman, 2018).

2.3 PREGABALINA (PG)

A pregabalina tem sido a primeira escolha para tratamento da fibromialgia, possui um perfil conveniente de efeitos adversos e demonstrou uma redução significativa na intensidade da dor quando utilizada pelo período de 12 a 26 semanas na dose de 300 a 600mg (Alles et al., 2020; Oliveira e Almeida., 2018). PG é estruturalmente semelhante ao GABA, sendo sua estrutura química o S-enantiômero do ácido 3-aminometil-5-metilhexanóico (Barata et al, 2019; Gerardi, 2016).

PG possui elevada afinidade à subnudade alfa-2-delta dos canais de cálcio

dependentes de voltagem no SNC (Barata et al., 2019). Alfa-2-delta é uma proteína auxiliar associada aos canais de cálcio dependente de voltagens, que são subdivididos em quatro subtipos, mas a pregabalina, assim como a gabapentina, somente se liga com alta afinidade aos subtipos 1 e 2 (Gerardi et al., 2016; Maitra et al., 2017). A ligação da pregabalina à proteína alfa-2 delta é o que confere o efeito analgésico, ansiolítico e anticonvulsivante (Gerardi et al., 2016), uma vez que modula o influxo de cálcio nos terminais nervosos, inibindo a liberação de neurotransmissores excitatórios como glutamato, serotonina, noradrenalina, dopamina e substância P (Illez et al., 2022). A pregabalina é um dos fármacos de primeira linha para o tratamento da fibromialgia, com favorável perfil de efeitos adversos. O tratamento deve ser iniciado com doses baixas e aumentar gradualmente (Girardi et al., 2016).

Os efeitos adversos (EA) da pregabalina tem sido descritos em vários ensaios clínicos randomizados (Crofford et al., 2004; Arnold et al., 2008; Mease et al., 2008; Ohta et al., 2012; Dogan et al., 2011; Arnold et al., 2012; Ohta et al., 2013), eles são frequentes, mas a taxa de EA graves são semelhantes ao placebo. A taxa de descontinuação do uso do fármaco também é baixa e está relacionada com a dose. Dentre os EA mais frequentes estão tontura, sonolência, ganho de peso e edema (Gerardi et al., 2016).

Estudo em voluntários saudáveis usando 600 mg de pregabalina e estimulação magnética demonstrou que o fármaco pode agir em diferentes circuitos inibitórios no córtex motor humano, o que sugere um efeito ativador de receptores GABA-B (Lang et al., 2005). Vários ensaios clínicos randomizados têm demonstrado o efeito modulador da pregabalina ao longo do tempo (Arnold et al., 2012; Hauser et al., 2009), no entanto, são escassos estudos sobre o mecanismo de ação deste fármaco *in vivo* (Burgmer et al., 2009).

Dentre os poucos estudos existentes, um deles comparou o efeito da pregabalina na ativação cortical ao estímulo doloroso usando ressonância magnética funcional (fMRI) em mulheres fibromiálgias e mulheres saudáveis sem tratamento. O estudo demonstrou efeito no giro supramarginal, giro frontal superior e inferior, giro temporal médio, tálamo, cerebelo e córtex calcarino (Kim et al., 2013). Outro estudo

experimental, crossover, com pacientes fibromiálgicas avaliou o efeito de 450 mg de pregabalina diários, durante 14 dias. O efeito foi avaliado com técnicas de neuroimagem combinadas (fMRI, espectroscopia de prótons, e análise de conectividade funcional), onde foi demonstrado que a pregabalina diminui os níveis de neurotransmissores excitatórios (glutamato e glutamina) na ínsula posterior, e redução da conectividade funcional nas áreas de funcionamento em repouso do encéfalo (Harris et al., 2013). Usando estes métodos de avaliação é possívelobservar *in vivo* os mecanismos de ação de um fármaco nos sistemas modificados pela doença.

Os efeitos adversos do tratamento com pregabalina geralmente são leves e moderados e bem tolerados pelo paciente em longo prazo, tornando favorável o uso quando avaliado o risco/benefício e a sua eficácia. Estudos mostram que a pregabalina para tratamento da fibromialgia é promissora, pois um número significativo de pacientes alcançou benefícios no controle da dor, melhora da qualidade do sono e funcionalidade, impactando diretamente na qualidade de vida do indivíduo (Bhusal et al., 2016).

2.4 ESTIMULAÇÃO TRANSCRANIANA POR CORRENTE CONTÍNUA

Associado às terapias farmacológicas, tem sido propostas intervenções não farmacológicas com potencial efeito analgésico, como as técnicas neuromoduladoras, e mais especificamente a estimulação transcraniana por corrente contínua (ETCC) (Silva et al., 2020).

Há cerca de 60 anos atrás, foi mostrado em um estudo pré-clínicos, utilizando ratos anestesiados, que a atividade neuronal e a excitabilidade cortical poderiam ser modificadas por uma estimulação de corrente contínua no córtex sensoriomotor (Bindmann et al., 1964). Anos depois, a Eletroestimulação Transcraniana de Corrente Contínua (ETCC), mostrando que a excitabilidade do córtex motor ao ser estimulado por uma corrente contínua, modula a atividade neuronal e psicológica (Nitsche e Paulus, 2000). A estimulação ocorre por um deslocamento subliminar dos potenciais de membrana em repouso, causando hiperpolarização ou despolarização,

dependendo da direção da corrente. Estimulações de poucos segundos são suficientes para induzir alterações neuronais, entretanto não duram muito tempo após a aplicação. Já estimulações de minutos podem durar mais de uma hora (Coêlho et al., 2021). No geral, sabe-se que a plasticidade sináptica é dependente de cálcio de neurônios glutamatérgicos, sendo uma das vias de ação da ETCC, uma vez que o bloqueio de receptores do tipo N-metil-D-aspartato (NMDA) diminui os efeitos da estimulação (Nitsche et al., 2003). Além disso, a ETCC pode reduzir localmente a neurotransmissão do GABA, independentemente da polaridade da estimulação e isso pode impactar na neuroplasticidade devido a relação entre os dois neurotransmissores, além disso, pode envolver o sistema serotoninérgico e colinérgico.

A ETCC, por modular os potenciais da membrana em repouso no nível sináptico, pode promover efeitos não sinápticos e, portanto, apresentar efeitos prolongados de duração (Ardolino et al., 2005). A ETCC utiliza uma corrente contínua de baixa intensidade aplicada no escalpo, por meio de eletrodos (cátodo e ânodo). A polaridade da estimulação pode ser anodal ou catodal, aumentando ou diminuindoa excitabilidade cortical, respectivamente, podendo atingir áreas corticais e subcorticais do encéfalo (Esteves et al., 2019). Os efeitos dessa terapia, entre outros fatores, dependem do tempo de estimulação, posicionamento dos eletrodos, intensidade da corrente aplicada e número de sessões realizadas, permanecendo por horas ou até por dias (Stagg e Nitsche, 2011; Medeiros et al., 2012). Seu mecanismo de ação pode estar relacionado à modulação de diferentes neurotransmissores (Medeiros et al., 2012, Souza et al., 2018), destacando os serotoninérgicos, glutamatérgicos, adenosinérgicos, opioidérgicos, gabaérgicos e outros. Esse estímulo pode alterar a excitabilidade neural, excitando ou inibindo as regiões corticais e subcorticais, promovendo, por exemplo, a liberação de opióides endógenos que auxiliam a via descendente da dor (Souza et al, 2021).

3 JUSTIFICATIVA

Fibromialgia é uma síndrome caracterizada por dor generalizada, fadiga, transtornos do sono, alterações de humor e rigidez musculoesquelética, associada à plasticidade mal-adaptativa nos circuitos neuronais centrais. O tratamento farmacológico da fibromialgia demonstra eficácia limitada, não oferecendo alívio satisfatório da dor (Matias et al., 2022), evidenciando a necessidade de estudos com tratamentos não farmacológicos associados, objetivando resultados mais efetivos. Embora haja disponibilidade de fármacos como a pregabalina para tratamento desta condição, pela sua capacidade de reduzir a liberação de neurotransmissores associados à sensibilização central, os pacientes não apresentam alívio completo dos sintomas. A ETCC tem sido descrita como uma técnica segura e eficaz, sendo aplicada no tratamento de diversas doenças. Além disso, existem poucos estudos pré-clínicos que investigam os mecanismos de ação relacionados a este tratamento, os efeitos no SNC, no processo nociceptivo e comportamental em ratos. Desta forma, este estudo se propõe a contribuir para um melhor entendimento dos mecanismos de ação da ETCC na fibromialgia, viabilizando o uso para o tratamento desta condição.

4 OBJETIVOS

4.1 Objetivo geral

Avaliar os efeitos do tratamento combinado de Pregabalina e ETCC sobre parâmetros comportamentais e neuroquímicos em ratos submetidos à um modelo de fibromialgia.

4.2 Objetivos específicos

Refinar o modelo animal de fibromialgia induzido por reserpina avaliando;

- comportamento hipernociceptivo, hiperalgesia mecânica e térmica;
- comportamento do tipo depressivo;

- os níveis do fator neurotrófico derivado do encéfalo (BDNF) em tronco encefálico, medula espinhal, córtex cerebral, hipocampo e soro;
- os níveis de TNF-α em tronco encefálico, córtex cerebral, hipocampo e soro.

Avaliar o efeito do tratamento combinado de pregabalina e ETCC em ratos submetidos a um modelo de fibromialgia sobre:

- comportamento hipernociceptivo, hiperalgesia mecânica;
- comportamento do tipo ansioso de ratos;
- os níveis do fator neurotrófico derivado do encéfalo (BDNF) em tronco encefálico, córtex cerebral, hipocampo e soro;
- os níveis de citocinas (IL-1beta, TNF-α e IL-10) em estruturas do sistema nervoso central (tronco encefálico, córtex cerebral, hipocampo) e soro.

PARTE II

ARTIGOS CIENTÍFICOS

5 MANUSCRITO 1

Submetido para a revista Anais da Academia Brasileira de Ciências

FIBROMYALGIA-LIKE ANIMAL MODEL DECREASES SERUM BDNF LEVELS OF MALE WISTAR RATS

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Running title: Decreased serum BDNF in fibromyalgia rats

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ABSTRACT

Fibromyalgia syndrome is characterized by central sensitization, with imbalance between the descending pain inhibition pathways and the ascending pain signaling pathways, including changes in the serotonergic, dopaminergic and catecholaminergic circuits. The aim was to refine a fibromyalgia-like model induced by reserpine in Wistar rats, assessing nociceptive response, depression-like behavior, and central and peripheral biomarkers levels (BDNF and TNF-α). Forty male adult Wistar rats were allocated by weight in control and fibromyalgia groups. Mechanical and thermal hyperalgesia were evaluated by Von Frey test and Hot Plate test respectively, and depression-like behavior was evaluated by Forced Swim test. Tests were accomplished before the administration and 5 days after the last administration of the reserpine (8th day). Rats in the fibromyalgia group presented a lower thermal and mechanical threshold (Student t test, $P<0.001$ and $P=0.01$, respectively), and an increased immobility time (Student t test, $P=0.004$). also reduced serum BDNF levels (Student t test, $P=0.05$), without changes in TNF-α levels. In summary, it is possible to conclude that the fibromyalgia-like model induced by reserpine was able to mimic fibromyalgia symptoms, at least in the nociceptive response, which is related to BDNF levels reduction.

Keywords: Fibromyalgia, Rats, BDNF, TNF-α

INTRODUCTION

Fibromyalgia syndrome is characterized by chronic widespread pain, fatigue, non-restorative sleep, and cognitive symptoms (Ceca et al. 2020), afflicting mainly women and affecting nearly 2% of the world population. The main pathophysiological phenomenon of fibromyalgia is the central sensitization, characterized by a limitation of descending pain inhibition pathways and by the improvement of the ascending pain signaling pathways (Poluha and Grossmann 2018). Several pathophysiological hypotheses have been proposed to elucidate this syndrome, which the most accepted considers that there is an imbalance between nociception and physiological pain control (Yunus 2007). According to this hypothesis, there is a global decrease in inhibitory pathways related to the pain, thereby allowing low-intensity or non-nociceptive stimuli to be processed in pre-cortical and cortical structures involved in the affective and cognitive pain process, resulting in an increase of painful perception (Burgmer et al. 2009). Therefore, therapeutic strategies aim to modulate neuroplasticity processes in the pain neuromatrix (multiple brain areas implied in affective, cognitive, and evaluative responses to pain).

Animal models of disease allow us to evaluate hypotheses through experiments that cannot be tested on humans. Due to the serotonergic, dopaminergic and catecholaminergic described circuit changes, in fibromyalgia patients, a model of this disease has been proposed in rats, using the drug reserpine (Nagakura et al. 2009). The repeated injection of reserpine can induce depletion of biogenic amines in rats, inducing generalized allodynia, a finding that is characteristic of this disease. In this way, this model of the disease offers a plausible neurobiological substrate, with a

consistent disease behavioral correlation which thus offers subsidies to study the combination of pharmacological and non-pharmacological intervention, their behavioral, neuroanatomical, and neurobiological effects.

In face of this, the goal of this study was to refine the fibromyalgia-like model induced by reserpine, assessing nociceptive response, depression-like behavior, and central and peripheral neurotrophy and inflammatory biomarkers levels in adult Wistar rats.

MATERIALS AND METHODS

Animals

A total of 40 male Wistar rats [55 - 65 days old (≃ 250g) at the beginning of treatment were used, which was provided by the Animal Reproduction and Experimentation Center (CREAL) through the Institute of Basic Health Sciences(ICBS) of Federal University of Rio Grande do Sul (UFRGS). Rats were assigned or weight-matched (in grams) and housed in groups of 2 to 3 rats per polypropylene cage(49 cm × 34 cm × 16 cm). Rats were kept at a 12-hour light/dark standard cycle (lightson at 7am and lights off at 7pm), in a controlled temperature environment (22±2°C) having free access to water and to Nuvital® feed.

All experiments and procedures were approved by the Institutional Committee for Animal Care and Use (GPPG-HCPA protocol Nº 2015-0272) and according to the Guide for the Care and Use of Laboratory Animals 8th ed. 2011 and law 11.794 (Brazil). The experimental protocol complied with the ethical and methodological standards of the ARRIVE guidelines (Kilkenny et al. 2013).

Experimental Design

Rats were assigned or weight-matched and divided into 2 experimental groups: Control group (reserpine vehicle) and fibromyalgia groups (reserpine). The mechanical hyperalgesia threshold was evaluated by the electronic von Frey test before the administration of reserpine and five days after the last dose of the drug. Also, forced swim and hot plate tests were performed five days after the last dose of the drug.

---------- Insert Figure 1 ----------

Fibromyalgia Model

To induce a fibromyalgia-like model, rats received vehicle or reserpine subcutaneously (1mg/kg) diluted in 0.5% acid acetic glacial for three consecutive days. This drug can induce depletion of biogenic amines in rats, inducing generalized hyperalgesia, a finding that is characteristic of the disease (Nagakura et al. 2012). Five days after the last reserpine administration (8th day), the rats were assessed for behavioral tests (von Frey, hot plate and forced swim). For weight maintenance, it was needed to add hypercaloric food and sunflower seed, which were placed on the floor of the housing box, due to the difficulty of movement presented by the animals.

Von Frey Test

Mechanical hyperalgesia was assessed by the electronic von Frey test (Cioato et al. 2016). First, rats were allowed to acclimate in acrylic boxes on a wire metal grid for 15 minutes 24h before testing to avoid stress-induced novelty. Then, in the next

day rats were allowed to the boxes again, and after 5 minutes a plastic tip was perpendicularly probed under the animal's right rear paw. Flick, biting or shaking responses were taken as nociceptive measures and recorded in grams (g) throughout the mean of 3 trials.

Hot Plate Test

For thermal hyperalgesia, the rats were also acclimated 24h before testing for allowing them to explore the apparatus for 5 minutes in the switched off mode. On day testing the temperature was set up to 55°C. After that, rats were lowered on a warmed surface circumvented by a transparent polypropylene funnel. The time between the placement, and the first aversive response to thermal noxious stimulus understood as paw shaking or biting was timed in a single trial and expressed in seconds (s). A 20 second cutoff was established to avoid tissue damage (Woolfe and Macdonald 1944).

Forced Swim Test

This test was performed to assess depressive-like behavior. The animals were placed into a round glass tank (dimensions 40 cm x 40 cm x 52 cm), the tank was filled with water (22-25°C) until a depth of 35 cm, in such a way that the tail of the rats could not touch the bottom of the tank. The animals were placed into water for five minutes, the immobility time of the rat was recorded in seconds, considering the total of immobility and/or movements to keep the head out of the water with no intention of escaping. After the test, the rats were dried with cotton pads and a hairdryer (Moreira et al. 2016). The tests were filmed and analyzed by two trained researchers, with the

goal of subsequently performing a double check of obtained results to guarantee their reliability and was carried out five days after the last dose of reserpine.

Tissue collection

Rats were killed by decapitation 24 hours after the last behavioral evaluation; and the cerebral cortex, spinal cord, hippocampus, and brainstem were harvested and immediately stored in the -80°C freezer. Also, the serum was collected and stored in the -80°C freezer for further analysis.

Biochemical assays

TNF-α and BDNF levels were determined by enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies for each cytokine and neurotrophin (R&D Systems, Minneapolis, United States) using the manufacturer's protocol. Optical density was measured using an ELISA reader at a wavelength of 450 nm. Total protein was measured by the Bradford method using bovine serum albumin as the standard (Bradford 1976). Data were expressed in picograms per milligram (pg/mg) of protein.

Statistical analysis

For behavioral and biochemical data, a t test for independent samples (Student t test) was used by comparing the control group and fibromyalgia-like model induced by reserpine injection. A $P<0.05$ was taken as significant. All data are expressed as mean ± standard error of the mean (S.E.M.) and were run in the SPSS 26.0 statistical program.

RESULTS

Behavioral measures

There was no difference between groups upon the mechanical hyperalgesia at baseline (P>0.05; Panel A). After the reserpine-induced fibromyalgia-like model there was difference between groups, rats that received reserpine displayed decrease in the mechanical nociceptive threshold compared to those that received vehicle, five days after the last injection (Student t test P<0.05; Figure 1, Panel A).

Regarding thermal sensitivity, we found differences between groups by showing that reserpine-induced fibromyalgia-like model group displayed a marked thermal hyperalgesia compared to the vehicle group five days after the last injection (Student t test, P<0.05; Figure 1, Panel B; P<0.05).

Concerning the forced swimming test, we also noted that a reserpine-induced fibromyalgia model triggered an increased immobility time compared to vehicle groups (Student t test, P=0.004, Figure 1, Panel C).

---------- Insert Figure 2 ----------

Biochemical Analysis

Regarding cerebral cortex, brainstem, and spinal cord BDNF levels, we found no statistical difference between groups (t test for independent samples, P>0.05). However, the serum BDNF levels were decreased in rats subjected to reserpine-

induced fibromyalgia rat model in relation to the control group (t test for independent samples; $P<0.05$; Table 1).

Regarding TNF-α no significant differences were found in all evaluated structures (hippocampus, brainstem, cerebral cortex, and serum) (t test for independent samples, $P>0.05$; Table 1).

---------- Insert Table 1 ----------

DISCUSSION

The results of the current study demonstrated that rats submitted to the fibromyalgia model presented mechanical and thermal hyperalgesia, an increase in immobility time. In addition, there was a reduction in serum BDNF levels in those rats. Altogether, these results outline that reserpine-induced fibromyalgia-like models can unleash behavioral phenotypes related to chronic pain, probably with participation of the peripheral BDNF levels.

It is important to note that on the third day of reserpine, the rats began to reveal eye compression, vocalization at the manipulation moment, arched posture, weight loss and prostration behaviors, that indicate the presence of pain (Neves et al. 2013). According to the four behavior units of the Grimace scale for rats (orbital tightening, nose/cheek flattening, ear changes and whisker change) (Sotocina et al. 2011), in the current study, the rats presented an indication of severe pain, and also loss of weight. Altogether, our findings showed that rats in the fibromyalgia model displayed a hyper

nociceptive behavior, such as thermal and mechanical hyperalgesia, both linked to central sensitization (Price et al. 2022; Staud and Rodriguez, 2006; Yuto et al. 2022).

It is important to highlight that in the hot plate evaluation, the lower thermal threshold was characterized by presenting only ocular compression and vocalization for thermal sensitivity signaling, and not presenting the expected response which was the “tap dance” movement and the licking of the feet (Woolfe and Macdonald 1944). Another previous study showed that monoamines (serotonin, norepinephrine, and dopamine) are reduced in the spinal cord in rats subjected to an animal model of fibromyalgia (Nagakura et al. 2009). So, considering this changed answer in the test, it is possible to suggest that the fibromyalgia rats present alterations in the supraspinal pain process, since the hot plate test involves complex supraspinal sensory integration (Kiso et al. 2018).

In addition, depressive-like behavior was assessed, the fibromyalgia rats presented a significant increase in immobility time, indicating the depressive-like behavior. Previous studies have used this model also to evaluate antidepressant drugs (Gao et al. 2016; Kim et al, 2021). Thus, the depletion of monoamines resulting from the administration of reserpine may be reproducing, in addition to fibromyalgia, comorbidities attributed to this condition, such as depression. It is known that the pathophysiology of depression is complex, but there is an involvement with deficiency of monoaminergic neurotransmitters, specifically norepinephrine (NE) and serotonin (5-HT) (Miri et al. 2017).

A clinical diagnosis from the patient's complaints related to several complicated trouble spots throughout the body, such as pain, aches, palpitations, and often psychopathological changes, such as anxiety and depression (Alves et al., 2022). Also,

evidence suggests that patients diagnosed with fibromyalgia have reduced serum levels of BDNF, corroborating the worsening of the psychopathologies involved in this condition (Merino and Simon 2022).

In relation to BDNF levels in the model like fibromyalgia, there was a reduction in serum BDNF levels in those rats. It is possible to infer that the depression-like behavior also observed in the forced swimming test to be related to the decreased peripheral BDNF levels. This result corroborated another study that showed a reduction in this neurotrophin induced by a fibromyalgia-like model conducted by intermittent cold stress with slight modification (Lee et al. 2018), however, we did not observe change in the central BDNF levels, at least in the structures evaluated.

In conclusion, this study was effective in proving the establishment of an animal model of fibromyalgia induced by reserpine, contributing to a better understanding of pathophysiology of this disease. In this way, it will also contribute to future studies that can evaluate pharmacological and non-pharmacological therapies.

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Authors contributions

VAS, LFM, CLO, HRM, JADS, AS and ILST have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data. VAS, LFM and ILST have been involved in drafting the manuscript or revising it

critically for important intellectual content. All authors have given final approval of the version to be published.

REFERENCES

BRADFORD MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248-54.

BURGMER M, POGATZKI-ZAHN E, GAUBITZ M, WESSOLECK E, HEUFT G, PFLEIDERER B. 2009. Altered brain activity during pain processing in fibromyalgia. Neuroimage 44(2):502-8.

CECA D, PABLOS A, ELVIRA L, LÓPES-HERNÁNDEZ L, ORTEGA AL. 2020. Effectiveness of a self-myofascial conditioning programme on pain, depression, anxiety and sleep quality in people with Fibromyalgia. CPD 20(1):147-165.

CIOATO SG et al. 2016. Long-Lasting Effect of Transcranial Direct Current Stimulation in the Reversal of Hyperalgesia and Cytokine Alterations Induced by the Neuropathic Pain Model. Brain Stimul 9(2):209-17.

EJIRI Y, UTA D, OTA H, MIZUMURA K, TAGUCHI T. 2022. Nociceptive chemical hypersensitivity in the spinal cord of a rat reserpine-induced fibromyalgia model. Neurosci Res 181;87-94.

GAO ZY, YANG P, HUANG QJ, XU HY. 2016. The influence of dizocilpine on the reserpine-induced behavioral and neurobiological changes in rats. Neurosci Lett 614:89-94.

KISO T, MORIYAMA A, FURUTAMI N, MATSUDA R, FUNATSU Y. 2018. Effects of pregabalin and duloxetine on neurotransmitters in the dorsal horn of the spinal cord in a rat model of fibromyalgia. Eur J Pharmacol 827:117-124.

KILKENNY C, BROWNE WJ, CUTHILL IC, EMERSON M, ALTMAN DG. 2013. Improving Bioscience Research Reporting: The Arrive Guidelines for Reporting Animal Research. Animals 4(1): 35–44.

KIM YR, PARK BK, SEO CS, KIM NS, LEE MY. 2021. Antidepressant and Anxiolytic-Like Effects of the Stem Bark Extract of Fraxinus rhynchophylla Hance and Its Components in a Mouse Model of Depressive-Like Disorder Induced by Reserpine Administration. Front Behav Neurosc 15:650833.

LEE H et al. 2018. Antinociceptive effect of Valeriana fauriei regulates BDNF signaling in an animal model of fibromyalgia. Int J Mol Med 41(1):485-492.

MERINO AS, SIMON D. 2022. Fatores genéticos associados à fibromialgia: uma revisão narrativa. Research, Society and Development 11(3):e11211326421.

MIRI AL, HOSNI AP, GOMES JC, KERPPERS II, PEREIRA MCS. Estudo do L-triptofano em um modelo experimental de depressão causado pela Doença de Alzheimer. J Phys Educ 28;e2839.

MOREIRA SFS ET AL. Hypoestrogenism alters mood: Ketamine reverses depressive-like behavior induced by ovariectomy in rats. Pharmacol Rep 68(1):109-115.

NAGAKURA Y, OE T, AOKI T, MATSUOKA N. 2009. Biogenic amine depletion causes chronic muscular pain and tactile allodynia accompanied by depression: A putative animal model of fibromyalgia. Pain 146(1-2):26-33.

NAGAKURA Y, TAKAHASHI M, NOTO T, SEKIZAWA T, OE T, YOSHIMI E, TAMAKI K, SHIMIZU Y. 2021. Different pathophysiology underlying animal models of fibromyalgia and neuropathic pain: comparison of reserpine-induced myalgia and chronic constriction injury rats. Behav Brain Res 226(1):242-9.

NEVES SM, ONG SMP, RODRIGUES LD, SANTOS RA, FONTES RS, SANTANA RO. 2013. Manual de cuidados e procedimentos com animais de laboratório do biotério de produção e experimentação da FCF-IQ/USP. São Paulo,

POLUHA RL, GROSSMANN E. 2018. A pregabalina melhora os distúrbios do sono na fibromialgia ?. BrJP 1(2):163-166.

PRICE DD, STAUD R, ROBINSON ME, MAUDERLI AP, CANNON R, VIERCK CJ. 2002. Enhanced temporal summation of second pain and its central modulation in fibromyalgia patients. Pain 99: 49–59.

SOTOCINAL SG et al. The Rat Grimace Scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. Mol Pain. 2011 Jul 29;7:55. doi: 10.1186/1744-8069-7-55. PMID: 21801409; PMCID: PMC3163602.

STAUD R, RODRIGUEZ ME. 2006. Mechanisms of disease: Pain in fibromyalgia syndrome. Nat Clin Pract Rheumatol 2:90–98.

WOOLFE G, MACDONALD AD. 1944. The evaluation of the analgesic action of pethidine hydrochloride. J Pharmacol Exp Ther 80:300-307.

YUNUS MB. 2007. Fibromyalgia and overlapping disorders: the unifying concept of central sensitivity syndromes. Semin Arthritis Rheum 36(6):339-56.

FIGURE CAPTIONS

Figure 1. Experimental design

Figure 2. Behavioral assessments (N = 40).

Panel A. Mechanical hyperalgesia threshold assessed in the Von Frey test. *Significant difference from control group (Student t test, P = 0.01). The vertical lines indicate the mean ± S.E.M of paw withdrawal threshold in grams (g).

Panel B. Thermal hyperalgesia assessed by the Hot Plate test. The vertical lines indicate the mean ± S.E.M of paw withdrawal threshold in seconds (s) *Significant difference from control group (Student t test, P < 0.001).

Panel C. Immobility time assessed by the Forced Swim test. The vertical lines indicate the mean ± S.E.M of immobility time in seconds (s). *Significant difference from control group (Student t test, P = 0.004).

Table 1. Biomarkers profile after reserpine-induced fibromyalgia rat model in serum and central nervous system structures (brainstem, cerebral cortex, spinal cord and hippocampus).

FIGURES

FIGURE 1

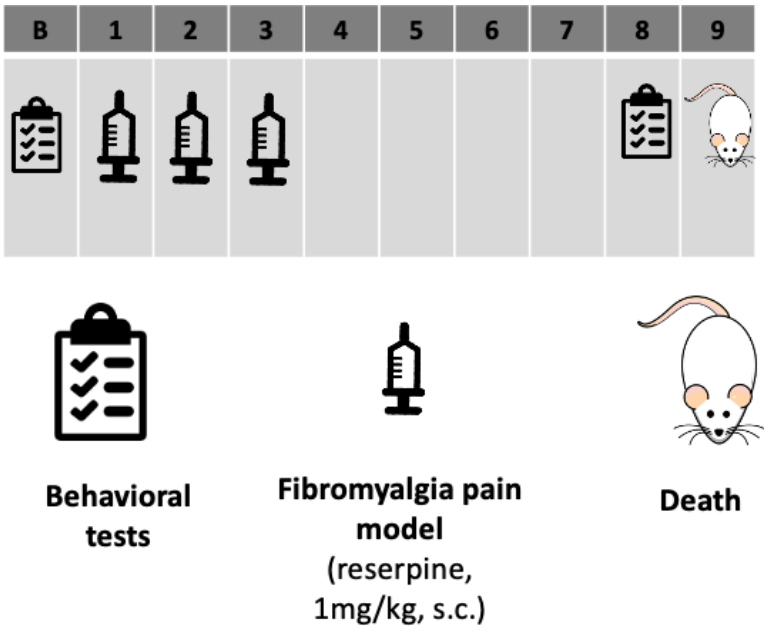

FIGURE 2

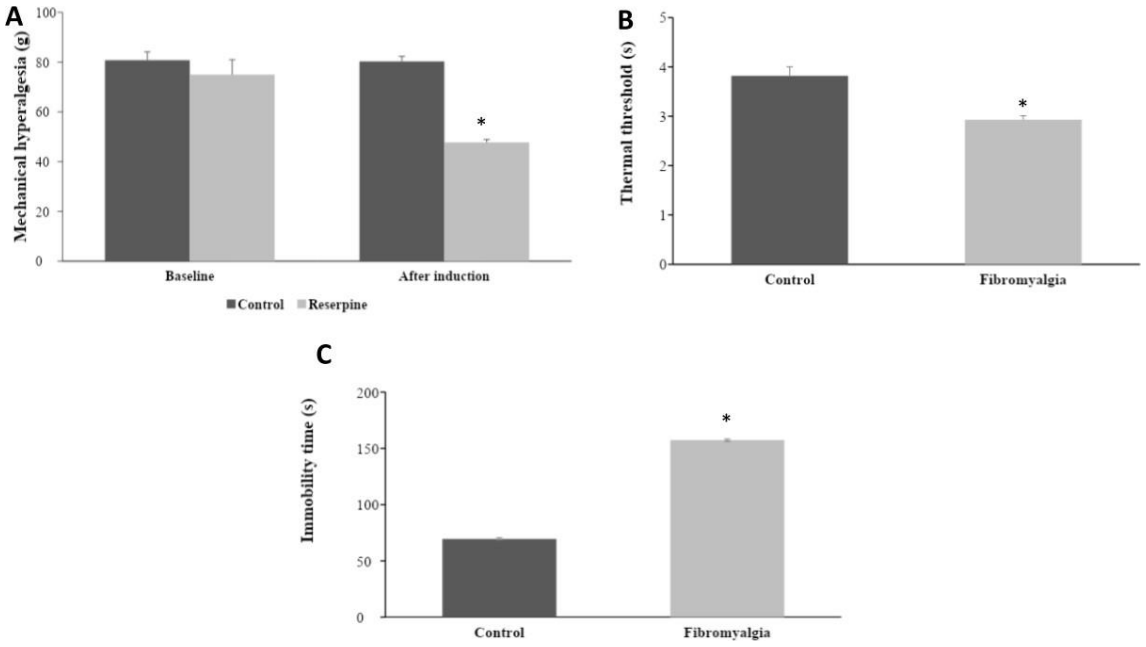
A
Mechanical hyperalgesia (g)
100
80
60
40
20
0
*
Baseline
After induction
Control
Reserpine
B
Thermal threshold (s)
5
4
3
2
1
0
*
Control
Fibromyalgia
C
Immobility time (s)
200
150
100
50
0
*
Control
Fibromyalgia

Table 1. Biomarkers profile after reserpine-induced fibromyalgia rat model in serum and central nervous system structures (brainstem, cerebral cortex, spinal cord and hippocampus).

Biomarker	Structure	Control group	Fibromyalgia group	P value
BDNF (pg/mg of protein)	Brainstem	3.23±0.21	3.44±0.21	0.507
	Cerebral Cortex	0.61±0.15	0.46±0.12	0.453
	Spinal Cord	3.11±0.44	2.59±0.75	0.576
	Serum	0.60±0.01	0.45±0.02*	**<0.01**
TNF-a (pg/mg of protein)	Brainstem	2.15±0.44	3.29±0.84	0.262
	Cerebral Cortex	1.87±0.49	0.88±0.29	0.120
	Hippocampus	2.17±1.05	0.71±0.13	0.213
	Serum	10.86±2.59	13.29±1.79	0.459

5.2 MANUSCRITO 2

Submetido para Neuroscience Letters

No synergic effect between pregabalin and transcranial direct current stimulation (tDCS) upon hyper nociceptive behavior in rats with fibromyalgia model

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ABSTRACT

Fibromyalgia is a widespread musculoskeletal disease, in which the etiology and the pathophysiology are not well elucidated. As a complex disease and refractoriness, there is no specific and single treatment. The pregabalin, as a pharmacological tool, has shown effective benefits; however, its side effects may decrease the treatment adherence. In this context, neuromodulatory techniques, like transcranial direct current stimulation (tDCS), may be used as a complementary tool to relieve pain and increase life quality in fibromyalgia. Thus, the objective of this study was to evaluate the effects of association of pregabalin and tDCS in a rat model of fibromyalgia, in behavioral parameters and biomarkers levels. Forty male adult Wistar rats were randomized in five experimental groups: control group (CT), reserpine + pregabalin + tDCS-sham (RPTs), reserpine + pregabalin + tDCS-active (RPT), reserpine + pregabalin vehicle + tDCS-sham (RVTs) and reserpine + pregabalin vehicle + active tDCS (RVT). This study was approved by the Institutional Ethics Committee (GPPG-HCPA #2015-0272). The fibromyalgia model was induced by administration of one dose of reserpine (1 mg/kg) for 3 consecutive days. The behavioral parameters evaluated were mechanical allodynia (von Frey test); anxiety-like behaviors (elevated plus-maze and forced swim) The nociceptive response was evaluated five days after the last administration of reserpine and 24 hours after the end of treatment. The animals submitted to the reserpine-induced fibromyalgia model exhibited a reduced mechanical threshold, which was partially reversed by tDCS (isolated or combined with pregabalin) (one way ANOVA/SNK, $P<0.001$). There was no difference between the groups in the percentage of arm entries ($P>0.05$) and percentage of time spent in the open arms ($P>0.05$). There was an increase in grooming time ($P=0.03$), and a decrease in NPHD ($P=0.03$) and rearing ($P<0.01$); while the grooming increase was reversed by tDCS, the NPHD decrease was reversed by pregabalin. There was a decrease in serum BDNF levels, which was reversed by tDCS (one-way ANOVA/SNK, $P<0.01$). In summary, considering the translational aspect, our findings, in a rat fibromyalgia-like model in rats, suggest that tDCS can be a potential non-pharmacologic treatment to fibromyalgia. However, in this study, no synergic effect between tDCS and pregabalin was observed.

Keywords: fibromyalgia model, pregabalin, tDCS, rats, biomarkers

INTRODUCTION

Fibromyalgia is a disease of unknown etiology, characterized by generalized chronic pain, non-restorative sleep, fatigue, mood disorders and cognitive symptoms. Fibromyalgia patients have biological, psychological, and social changes, negatively influencing the individual's quality of life [1]. Its prevalence is estimated at 2.5% in Brazil, affecting 2 to 10% of the adult population [2].

Despite this condition having no specific treatment, patients have obtained improvement after pharmacological approach. Pregabalin is a pharmacological option for the management of fibromyalgia in humans, being structurally like the neurotransmitter gamma-aminobutyric acid, but it binds to the alpha2-delta subunits of the voltage-dependent calcium channels [3]. However, the mechanisms of pregabalin promoting clinical benefits in fibromyalgia patients remains unknown [3]. It is considered an “anchor drug” for the treatment of fibromyalgia, since it has significant effects in terms of pain control, improvement of sleep disorders and individual functionality, positively impacting the quality of life of patients [4].

On the other hand, non-invasive neuromodulatory techniques have been proposed, and can be used in combination with pharmacological or isolated treatment [5]. Among them, the Transcranial Direct Current Stimulation (tDCS) has been used to treat different conditions, since depression, chronic pain, anxiety, compulsive disorders, among others. It is a non-invasive technique that modulates neuronal thresholds through weak, anodal and cathodic electrical currents that can increase and decrease the cortical excitability [6]. The application of the electric current is through electrodes placed on the scalp and its effects are related to the applied brain region and the polarity of the electrodes, which can persist for hours or even days [7].

The tDCS action mechanisms are involved with different neurotransmitter signaling, such as serotonergic, glutamatergic, adenosinergic, opioidergic, gabaergic, among others [8]. In the same sense, our previous studies have described that the antinociceptive effect of tDCS in different chronic pain animal models may be linked to central levels of neurotrophins and cytokines [9–11].

Clinical and preclinical research aimed to search for biomarkers that can facilitate the diagnosis of fibromyalgia. A recent study has found no difference in the

brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) plasma levels between fibromyalgia patients and pain-free controls [12]. On the other hand, Jablochkova and colleagues [13] have shown that fibromyalgia patients display higher BDNF and lower NGF levels than healthy controls [14]. In the preclinical setting, a previous study has shown a reduction in the BDNF and phosphorylated cAMP response element-binding protein (p-CREB) expression in central nervous system structures, like medial prefrontal cortex and hippocampus, of mice in a fibromyalgia-like model, using chronic restraint stress and intermittent cold stress paradigm [15]. Considering these findings, no successful biomarkers were found until nowadays once controversial results have been found in the literature.

Thus, our objective was to evaluate the synergic effect of pregabalin combined with tDCS upon nociceptive response, depressive-like and anxiety-like behaviors in rats subjected to fibromyalgia pain model. Also, we have investigated the effect of this treatment combination in the central levels of biomarkers (BDNF, IL-1β, TNF-α and IL- 10) in the central nervous structures.

METHODS

Animals

Forty male Wistar rats (55 - 65 days old, ≃250g) were provided by the Animal Reproduction and Experimentation Center (CREAL) of Federal University of Rio Grande do Sul (UFRGS). Rats were randomized by weight (in grams) and housed in groups of 2 to 3 rats per polypropylene cage (49 × 34 × 16 cm). Rats were kept at a 12-hour light/dark standard cycle (lights on at 7am), in a controlled temperature environment (22 ± 2 °C) having free access to water and Nuvital® chow. All experiments and procedures were approved by the Institutional Committee for Animal Care and Use (GPPG-HCPA protocol Nº 2015-0272). The experimental protocol complied with the ethical and methodological standards of the ARRIVE guidelines [16].

Experimental Design

The experimental design was performed according to Figure 1, briefly after 14 days of adaptation, the animals were randomized by weight and von Frey into 5 experimental groups: total control (CT), which received only the vehicle reserpine (acetic acid glacial 0.5% in distilled water); reserpine + pregabalin + tDCS-sham (RPTs), the animals received reserpine, were treated with pregabalin and subjected to sham stimulation; reserpine + pregabalin + tDCS- active (RPT), the animals received reserpine, treated with pregabalin and submitted to active stimulation; reserpine + pregabalin vehicle + tDCS-sham (RVTs), animals received reserpine, treated with pregabalin vehicle and subjected to sham stimulation; reserpine + pregabalin vehicle + active tDCS (RVT), the animals received reserpine, treated with pregabalin vehicle and submitted to active stimulation. The mechanical hyperalgesia test was performed at the beginning of the study, after induction of the model and after treatment, the evaluation of thermal hyperalgesia was carried out after the induction of the model and after treatment, and the elevated plus maze test was performed after the end of the treatment. The fibromyalgia model was induced by administering reserpine 1 mg/Kg, subcutaneously (s.c.) for three consecutive days, after an interval of five days, to establish the model, behavioral tests were performed (von Frey), 24 hours after the end of the treatment, behavioral tests (von frey and elevated plus maze), 48 hours after the end of treatment, the animals were decapitated to collect the structures.

Insert Figure 1

Fibromyalgia Model

To induce a fibromyalgia-like model, rats received vehicle or reserpine subcutaneously (1mg/kg) diluted in 0.5% acetic glacial acetic acid for three consecutive days. This drug can induce depletion of biogenic amines in rats, inducing generalized hyperalgesia, a finding that is characteristic of the disease [17].

Von Frey Test

Mechanical hyperalgesia was assessed by the electronic von Frey test [9,10]. First, rats were allowed to acclimate in acrylic boxes on a wire metal grid for 15 minutes

24h before testing to avoid stress-induced novelty. Then, in the next day rats were allowed to the boxes again, and after 5 minutes a plastic tip was perpendicularly probed under the animal's right rear paw. Flick, biting or shaking responses were taken as nociceptive measures and recorded in grams (g) throughout the mean of 3 trials.

Elevated plus-maze test (EPM)

The anxious-like behavior was assessed in the EPM test. This apparatus consists of two enclosed and two open arms (50 x 40 x 10 cm) in an orthogonal fashion positioned 50 cm from the floor level. The animals were placed in the central area of the equipment, facing one of the closed arms, then it was allowed to explore for 5 min. The following measures were evaluated: number of protected head-dipping (PHD); number of unprotected head-dipping (NPHD); number of entries in the open arms (EOA); number of entries in closed arms (ECA); time spent in the open arms (TOA); tim spent in closed arms (TCA); grooming time and breeding number (Moreira et al, 2015). The percent of open arm entries (100 x open/total entries) and of time spent in the open arms (100 open/open + enclosed arms) was calculated for each rat [18]. The experimental tests were recorded using a video camera placed 150cm above the apparatus. The videos were subsequently analyzed by a blinded experimenter.

Pregabalin treatment

After the establishment of fibromyalgia model, pregabalin (Lyrica, Pfizer®) was dissolved in saline to a final concentration of 30 mg/kg and administered by gavage [19] for 8 consecutive days 30 minutes before tDCS session. Treatments were carried out between 9 and 11 am.

tDCS treatment

Initially, rats had their heads shaved and were gently restrained in a soft and clean towel. Then, EEG electrodes containing conductive gel were trimmed to 3.5mm and connected to a battery driven stimulator with an intensity of 0.5mA and current density of 0.33mA/cm^2. Next, the rat's head was soaked with saline 0.9% and the

anode and cathode electrodes were placed on the supraorbital and parietal areas, respectively [9,10].

Tissue collection

Rats were killed by decapitation 24 hours after the last behavioral evaluation; and brainstem, cerebral cortex, hippocampus, were harvested and stored immediately in the -80°C freezer. Also, the serum was collected and stored in the -80°C freezer for further analysis.

Biochemical assays

TNF-α, IL-1β, IL-10 and BDNF levels were determined by enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies for each cytokine and neurotrophin (R&D Systems, Minneapolis, United States) using manufacturer's protocol. Optical density was measured using an ELISA reader at a wavelength of 450 nm. Total protein was measured by the Bradford method using bovine serum albumin as the standard [20]. Data were expressed in picograms per milligram (pg/mg) of protein.

Statistical analysis

The data were analyzed in the SPSS 26.0 statistical program and expressed as mean ± standard error of the mean (S.E.M.). It was considered a significant difference when $P<0.05$. One-way ANOVA followed by Student Newman Keuls (SNK) was performed to compare differences between groups in each time point assessed (before or after treatment).

RESULTS

Mechanical Hyperalgesia

Regarding mechanical hyperalgesia, there was an increased hypersensitivity in the reserpine-induced fibromyalgia-like model compared to the control group (one-way ANOVA/SNK, $F_{(4,33)}=17.793$, $P<0.001$, Figure 2), comparison made at time point

before treatment. After treatment, it is possible to observe tDCS associated or not (RVT and RPT) partially reversed the mechanical hypersensitivity when compared to the control group (one-way ANOVA/SNK, $F_{(4,24)}$=9.690, P<0.001, Figure 2). In addition, pregabalin alone (RPTs) was not efficient to reverse the mechanical hypersensitivity, once it was not different from reserpine plus vehicle plus sham-tDCS (RVTs)(one way ANOVA, P>0.05). Also, no synergic effect was found between the combination between tDCS and pregabalin, as we have hypothesized.

---------- Insert Figure 2 -------

Anxious-like behavior

In the elevated plus maze test, no significant differences were found in the percentage of open arm entries (one-way ANOVA/ SNK, P = 0.265) and percentage of time spent in the open arms (one-way ANOVA/ SNK, P = 0.419) among the groups assessed.

---------- Insert Figure 3 -------

Also, it is possible to observe that there was a statistically significant increase in the grooming time in rats subjected to reserpine (RPTs, RPT and RVTs groups), which was reversed by tDCS alone (one-way ANOVA/ SNK, P=0.03, Table 1). In addition, we found a decrease in the number of NPHD (one-way ANOVA/ SNK, P = 0.03, Table 1) and in the number of rearing induced by reserpine (one-way ANOVA/ SNK, P<0.01, Table 1), however pregabalin alone was able to reverse NPHD behavior. We did not observe any significant difference in the number of fecal boluses and PHD (one-way ANOVA/ SNK, P = 0.55 and P = 0.21, respectively, Table 1).

---------- Insert Table 1 -------

Biomarkers

Regarding biomarkers, we showed a statistically significant decrease in serum BDNF levels in reserpine-induced fibromyalgia rat models (one way ANOVA/SNK,

P=0.003, Table 2), which was completely reversed by tDCS. However, no significant differences were found regarding the other biomarkers assessed (IL-10, TNF-α, IL-1β, and BDNF) in all structures analyzed (brainstem, cerebral cortex, spinal cord or serum)(one-way ANOVA/ SNK, P>0.05, Table 2).

---------- Insert Table 2 -------

DISCUSSION

Our findings highlight those rats subjected to a reserpine-induced fibromyalgia model displayed reduced mechanical threshold. In addition, the tDCS (combined or not with pregabalin) probed antinociceptive effects as shown by a partial reversal of mechanical hyperalgesia. On the other hand, pregabalin alone was not effective in showing reduction in the mechanical hypersensitivity in the current rat fibromyalgia model used. Here, we also showed that reserpine induced increased grooming behavior which was reversed only by tDCS. Also, it induced decreased NPHD and rearing behaviors, and NPHD was reversed by pregabalin treatment. Additionally, reserpine decreased serum BDNF levels that were restored after tDCS treatment.

Altogether, these effects might be attributed to the depletion of important neurotransmitters involved in the pain descending control such as monoaminergic systems as well as through an imbalance between excitatory (glutamatergic) and inhibitory (GABAergic) mechanisms. Corroborating our results, previous studies of our group have demonstrated that tDCS mediate analgesia by means of all these pathways [21]. As shown before, norepinephrine, dopamine, and serotonin from the dorsal horn were significantly lower after reserpine-induced myalgia in rats [22]. And tDCS can modulate all these neurotransmitters, while pregabalin increases only norepinephrine [22]. Thus, it can contribute to explain the partial reversion of the hyper nociceptive behavior found in our data. Furthermore, it is important to stress that complementary methods targeting the combination between therapies to provide more powerful and consistent outcomes (pain relief) should be pursued.

Pregabalin is considered a gold standard treatment for fibromyalgia patients

[23]. However, in preclinical settings it has been used to alleviate hyper nociceptive behavior in different chronic pain conditions [19,24]. The binding is believed to be to the alpha-2 delta subunit responsible for its antinociceptive effect, since with a binding, an excessive reduction of multiple excitatory neurotransmitters (noradrenaline, serotonin, dopamine, glutamate, and substance P) occurs [25]. In the same sense, a previous study has shown no improvement in acute or chronic muscle hyperalgesia after pregabalin-treated groups [26]. Also, it is interesting to note that pregabalin (10 and 30 mg/kg) significantly increased muscle pressure threshold in reserpine-induced myalgia rats [22]. However, in the current study using a similar dose (30 mg/kg) we did not observe a decrease in the hyper nociceptive behavior, however, the nociceptive tests in this study were performed 24 hours after the last dose of reserpine. A study demonstrated the reversal of mechanical hyperalgesia in a rat neuropathic model, with when evaluated 1 hour after pregabalin administration [27]. Altogether these findings highlight the complexity of the action mechanism of pregabalin in front of different chronic conditions, including in the fibromyalgia model.

We also observed that reserpine increased grooming behavior, decreased NPHD and rearing behaviors. The grooming behavior, which is a rodent self-cleaning movement related to anxiety [28], was reverted by tDCS. Previous studies from our research group showed the anxiolytic effect of tDCS, reversing the anxiety-like behavior in rats submitted to a neuropathic pain model [29,30]. On the other hand, our results showed that the pregabalin reversed only NPHD. The involvement of the GABAergic system in the imbalance between excitatory and inhibitory pathways may be related to this behavior anxious profile presented by the animals, and the anxiolytic effect of pregabalin reversed this behavior [31].

In the current study, the reserpine-induced fibromyalgia model induced a reduction in the BDNF serum levels, which was reverted by tDCS. There was no reserpine effect in the BDNF levels in the central nervous structures. It is important to note that a previous study using a different fibromyalgia model showed a reduction in the BDNF expression in central nervous system structures (prefrontal cortex and hippocampus) of mice [15]. It is important to highlight that the tDCS effects upon peripheral and central biomarkers need to be elucidated, once no consensus was achieved yet [32–34].

Unfortunately, any effect of the pain model was observed, neither treatment in the cytokine's levels, such as TNF-α, IL-1β, and IL-10, in central and peripheral samples regarding the pain model and after-effects of pregabalin or tDCS action and structures assessed. On the other hand, we have showed that bimodal tDCS treatment induced analgesia in rats submitted to different pain models, and this was associated with alterations in neurotrophin and cytokines levels [9,10,35,36]. It is important to highlight that fibromyalgia syndrome is still not well understood in the clinical or preclinical settings; however more studies are necessary to increase knowledge about this condition.

In summary, our findings highlight no synergic effect between tDCS and pregabalin treatments in reserpine-induced fibromyalgia rat model. However, there were complementary effects in the anxiety-like behavior. It is known that fibromyalgia is a complex syndrome, and its mechanisms are not well elucidated, further studies can contribute with a better understanding of the pathophysiology of this disease and the treatment options to relieve pain and improve quality of life.

REFERENCES

[1] A. Chica, F. Guirval, R. Garrido, G. Chaves, A. Mendo, Efectos de un programa de danza española en mujeres con fibromialgia, Cuad. Psicol. Del Deport. 19 (2019). https://doi.org/10.6018/cpd.343401.

[2] M.R. De Assis, E. Dos Santos Paiva, M. Helfenstein, R.E. Heymann, D.F. Pollak, J.R. Provenza, A. Ranzolin, M.C. Rezende, L.S. Ribeiro, E.J.R. Souza, J.E. Martinez, Treatment data from the Brazilian fibromyalgia registry (EpiFibro), Adv. Rheumatol. 60 (2020). https://doi.org/10.1186/s42358-019-0108-2.

[3] J.O. de Oliveira Júnior, M.B. de Almeida, The current treatment of fibromyalgia TT - O tratamento atual da fibromialgia, BrJP. 1 (2018).

[4] S. Bhusal, S. Diomampo, M.N. Magrey, Clinical utility, safety, and efficacy of pregabalin in the treatment of fibromyalgia, Drug. Healthc. Patient Saf. 8 (2016). https://doi.org/10.2147/DHPS.S95535.

[5] A.A. da Silva, E. Kakuta, E. Henrique Loreti, Estimulação transcraniana por corrente contínua em fibromialgia: uma revisão sistemática, Rev. Neurociências. 28 (2020). https://doi.org/10.34024/rnc.2020.v28.10884.

[6] M.G.L. Matias, A.F.L. Cavalcante, K.A. Mescouto, E.M. Silva-Filho, A.F. Baptista, A.H. Okano, R. Pegad, A estimulação transcraniana por corrente contínua anódica sobre o córtex motor esquerdo apresenta diferença na dor entre os hemicorpos na fibromialgia?, Brazilian J. Pain. 5 (2022). https://doi.org/10.5935/2595-0118.20220020-pt.

[7] M.L.S. Coêlho, P.M. Cabral, L.Y.N. Morello, G.G. do Rêgo, P.S. Boggio, Estimulações cerebrais não invasivas: as aplicações de Estimulação Transcraniana por Corrente Contínua (ETCC) e Estimulação Magnética Transcraniana (EMT) na aprendizagem e na clínica, in: Tecnol. Apl. Em Educ. e Saúde, 2021. https://doi.org/10.29327/558730.1-21.

[8] L.F. Medeiros, I.C.C. de Souza, L.P. Vidor, A. de Souza, A. Deitos, M.S. Volz, F. Fregni, W. Caumo, I.L.S. Torres, Neurobiological effects of transcranial direct current stimulation: A review, Front. Psychiatry. 3 (2012). https://doi.org/10.3389/fpsyt.2012.00110.

[9] S.G. Cioato, L.F. Medeiros, P.R. Marques Filho, R. Vercelino, A. de Souza, V.L. Scarabelot, C. de Oliveira, L.N.S. Adachi, F. Fregni, W. Caumo, I.L.S. Torres, Long-Lasting Effect of Transcranial Direct Current Stimulation in the Reversal of Hyperalgesia and Cytokine Alterations Induced by the Neuropathic Pain Model, Brain Stimul. (2016) 209–17. https://doi.org/10.1016/j.brs.2015.12.001.

[10] B.C. Lopes, L.F. Medeiros, V. Silva de Souza, S.G. Cioato, H.R. Medeiros, G.G. Regner, C. Lino de Oliveira, F. Fregni, W. Caumo, I.L.S. Torres, Transcranial direct current stimulation combined with exercise modulates the inflammatory profile and hyperalgesic response in rats subjected to a neuropathic pain model: Long-term effects, Brain Stimul. (2020). https://doi.org/10.1016/j.brs.2020.02.025.

[11] D.S. Santos, B.C. Lopes, L.F. Medeiros, J.A.F. Assumpção, A. de Souza, A.A. Salvi, L.S. da Silva, F. Fregni, W. Caumo, I.L.S. Torres, Transcranial Direct Current Stimulation (tDCS) Induces Analgesia in Rats with Neuropathic Pain and Alcohol Abstinence, Neurochem. Res. 45 (2020). https://doi.org/10.1007/s11064-020-03116-w.

[12] D. Baumeister, W. Eich, S. Saft, O. Geisel, R. Hellweg, A. Finn, C.I. Svensson, J. Tesarz, No evidence for altered plasma NGF and BDNF levels in fibromyalgia patients, Sci. Rep. 9 (2019). https://doi.org/10.1038/s41598-019-49403-7.

[13] A. Jablochkova, E. Bäckryd, E. Kosek, K. Mannerkorpi, M. Ernberg, B. Gerdle, B. Ghafouri, Unaltered low nerve growth factor and high brain-derived neurotrophic factor levels in plasma from patients with fibromyalgia after a 15-week progressive resistance exercise, J. Rehabil. Med. 51 (2019).

https://doi.org/10.2340/16501977-2593.

[14] L. Haas, L.V.C. Portela, A.E. Böhmer, J.P. Oses, D.R. Lara, Increased plasma levels of brain derived neurotrophic factor (BDNF) in patients with fibromyalgia., Neurochem. Res. 35 (2010) 830–834. https://doi.org/10.1007/s11064-010-0129-z.

[15] H. Lee, J. Im, H. Won, J.Y. Kim, H.K. Kim, J.T. Kwon, Y.O. Kim, S. Lee, I.H. Cho, S.W. Lee, H.J. Kim, Antinociceptive effect of Valeriana fauriei regulates BDNF signaling in an animal model of fibromyalgia, Int. J. Mol. Med. 41 (2018). https://doi.org/10.3892/ijmm.2017.3203.

[16] C. Kilkenny, W.J. Browne, I.C. Cuthill, M. Emerson, D.G. Altman, Improving bioscience research reporting: The arrive guidelines for reporting animal research, Animals. 4 (2013) 35–44. https://doi.org/10.3390/ani4010035.

[17] Y. Nagakura, M. Takahashi, T. Noto, T. Sekizawa, T. Oe, E. Yoshimi, K. Tamaki, Y. Shimizu, Different pathophysiology underlying animal models of fibromyalgia and neuropathic pain: Comparison of reserpine-induced myalgia and chronic constriction injury rats, Behav. Brain Res. (2012). https://doi.org/10.1016/j.bbr.2011.09.023.

[18] C.M. Padovan, F.S. Guimarães, Restraint-induced hypoactivity in an elevated plus-maze, Brazilian J. Med. Biol. Res. 33 (2000). https://doi.org/10.1590/S0100-879X2000000100011.

[19] S. Tanimoto-Mori, E. Nakazato-Imasato, K. Toide, Y. Kita, Pharmacologic investigation of the mechanism underlying cold allodynia using a new cold plate procedure in rats with chronic constriction injuries, Behav. Pharmacol. (2008). https://doi.org/10.1097/FBP.0b013e3282f3d0a3.

[20] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein dye binding, Anal. Biochem. 72 (1976) 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

[21] A. Souza, D.F. Martins, L.F. Medeiros, C. Nucci-Martins, T.C. Martins, A. Siteneski, W. Caumo, A.R.S. dos Santos, I.L.S. Torres, Neurobiological mechanisms of antiallodynic effect of transcranial direct current stimulation (tDCS) in a mice model of neuropathic pain, Brain Res. 1682 (2018). https://doi.org/10.1016/j.brainres.2017.12.005.

[22] T. Kiso, A. Moriyama, M. Furutani, R. Matsuda, Y. Funatsu, Effects of pregabalin and duloxetine on neurotransmitters in the dorsal horn of the spinal cord in a rat model of fibromyalgia, Eur. J. Pharmacol. 827 (2018). https://doi.org/10.1016/j.ejphar.2018.03.011.

[23] S. Derry, M. Cording, P.J. Wiffen, S. Law, T. Phillips, R.A. Moore, Pregabalin for pain in fibromyalgia in adults, Cochrane Database Syst. Rev. 2016 (2016). https://doi.org/10.1002/14651858.CD011790.pub2.

[24] O.C. Pires, M.L. Dalcim, A.L.M. Pigozzi, F.M.S. de L.A. Caldeira, M.H.R. Pires, L. de Almeida Neto, I. de P. Posso, Effects of duloxetine, fluoxetine and pregabalin on fentanyl-induced hyperalgesia in rattus novergicus, Brazilian J. Pain. 3 (2020). https://doi.org/10.5935/2595-0118.20200004.

[25] C. Toth, Pregabalin: Latest safety evidence and clinical implications for the management of neuropathic pain, Ther. Adv. Drug Saf. 5 (2014). https://doi.org/10.1177/2042098613505614.

[26] Y. Ohmichi, M. Ohmichi, N. Murai, M. Yasui, N. Takeshita, H. Oshibuchi, M. Naito, T. Nakano, J. Sato, Therapeutic effects of diclofenac, pregabalin, and duloxetine on disuse-induced chronic musculoskeletal pain in rats, Sci. Rep. 8 (2018). https://doi.org/10.1038/s41598-018-21429-3.

[27] R.G. Ungard, Y.F. Zhu, S. Yang, P. Nakhla, N. Parzei, K.L. Zhu, G. Singh, Response to pregabalin and progesterone differs in male and female rat models of neuropathic and cancer pain, Can. J. Pain. 4 (2020). https://doi.org/10.1080/24740527.2020.1724776.

[28] C. Estanislau, A.W.N. Veloso, G.B. Filgueiras, T.P. Maio, M.L.C. Dal-Cól, D.C.

Cunha, R. Klein, L.F. Carmona, A. Fernández-Teruel, Rat self-grooming and its relationships with anxiety, dearousal and perseveration: Evidence for a self-grooming trait, Physiol. Behav. 209 (2019). https://doi.org/10.1016/j.physbeh.2019.112585.

[29] D.S. Santos, L.F. Medeiros, D.J. Stein, I.C. De Macedo, D.E. Da Silva Rios, C. De Oliveira, R.S. Toledo, F. Fregni, W. Caumo, I.L.S. Torres, Bimodal transcranial direct current stimulation reduces alcohol consumption and induces long-term neurochemical changes in rats with neuropathic pain, Neurosci. Lett. 759 (2021). https://doi.org/10.1016/j.neulet.2021.136014.

[30] B.C. Lopes, L.F. Medeiros, D.J. Stein, S.G. Cioato, V.S. de Souza, H.R. Medeiros, P.R.S. Sanches, F. Fregni, W. Caumo, I.L.S. Torres, tDCS and exercise improve anxiety-like behavior and locomotion in chronic pain rats via modulation of neurotrophins and inflammatory mediators, Behav. Brain Res. 404 (2021). https://doi.org/10.1016/j.bbr.2021.113173.

[31] D.S. Baldwin, K. Ajel, Role of pregabalin in the treatment of generalized anxiety disorder, Neuropsychiatr. Dis. Treat. 3 (2007). https://doi.org/10.2147/nedt.2007.3.2.185.

[32] S.F. Da Silva Moreira, L.F. Medeiros, A. De Souza, C. De Oliveira, V.L. Scarabelot, F. Fregni, W. Caumo, I.L.S. Torres, Transcranial direct current stimulation (tDCS) neuromodulatory effects on mechanical hyperalgesia and cortical BDNF levels in ovariectomized rats, Life Sci. (2016). https://doi.org/10.1016/j.lfs.2015.10.011.

[33] H. Hadoush, S.A. Banihani, H. Khalil, Y. Al-Qaisi, A. Al-Sharman, M. Al-Jarrah, Dopamine, BDNF and motor function postbilateral anodal transcranial direct current stimulation in Parkinson’s disease, Neurodegener. Dis. Manag. 8 (2018). https://doi.org/10.2217/nmt-2017-0048.

[34] A.R. Brunoni, F. Padberg, E.L.M. Vieira, A.L. Teixeira, A.F. Carvalho, P.A. Lotufo, W.F. Gattaz, I.M. Benseñor, Plasma biomarkers in a placebo-controlled trial comparing tDCS and escitalopram efficacy in major depression, Prog.

Neuro-Psychopharmacology Biol. Psychiatry. 86 (2018). https://doi.org/10.1016/j.pnpbp.2018.06.003.

[35] L.N. Spezia Adachi, A.S. Quevedo, A. de Souza, V.L. Scarabelot, J.R. Rozisky, C. de Oliveira, P.R. Marques Filho, L.F. Medeiros, F. Fregni, W. Caumo, I.L.S. Torres, Exogenously induced brain activation regulates neuronal activity by top-down modulation: conceptualized model for electrical brain stimulation, Exp. Brain Res. 233 (2015) 1377–1389. https://doi.org/10.1007/s00221-015-4212-1.

[36] G. Laste, W. Caumo, L.N.S. Adachi, J.R. Rozisky, I.C. de Macedo, P.R.M. Filho, W.A. Partata, F. Fregni, I.L.S. Torres, After-effects of consecutive sessions of transcranial direct current stimulation (tDCS) in a rat model of chronic inflammation., Exp. Brain Res. (2012) 75–83. https://doi.org/10.1007/s00221-012-3149-x.

FIGURE CAPTIONS

FIGURE 1: Experimental Design. tDCS: transcranial direct current stimulation.

FIGURE 2: Threshold of mechanical hyperalgesia in an animal model of fibromyalgia, assessed by the von Frey test before and after treatment. The data are presented as mean ± S.E.M of paw withdrawal threshold in grams (g) (n=40). Control (CT); Reserpine + pregabalin + Sham tDCs (RPTs); Reserpine + pregabalin + tDCs (RPT); Reserpine + vehicle pregabalin + Sham tDCs (RVTs); Reserpine + vehicle pregabalin + tDCs (RVT).
*Significant difference from CT group at time point before treatment (one way ANOVA/SNK, $P<0.001$).
Different letters mean significant statistical difference at time point after treatment (one way ANOVA/SNK, $P<0.001$).

FIGURE 3: Elevated plus maze test. Data expressed as mean ± SEM of percentage of control (N = 40). Control (CT); Reserpine + pregabalin + Sham tDCs (RPTs); Reserpine + pregabalin + tDCs (RPT); Reserpine + pregabalin vehicle + sham tDCs (RVTs); Reserpine + pregabalin vehicle + tDCs (RVT).

PAINEL A: Percentage of the animals' stay in the open arms assessed by the elevated plus-maze test. There was no difference between groups (one Way ANOVA/ SNK, $P = 0.265$).

PAINEL B: Percentage of time spent in open arms assessed by the elevated plus-maze test. There was no difference between (one Way/ANOVA/ SNK, $P= 0.419$).

FIGURE 1

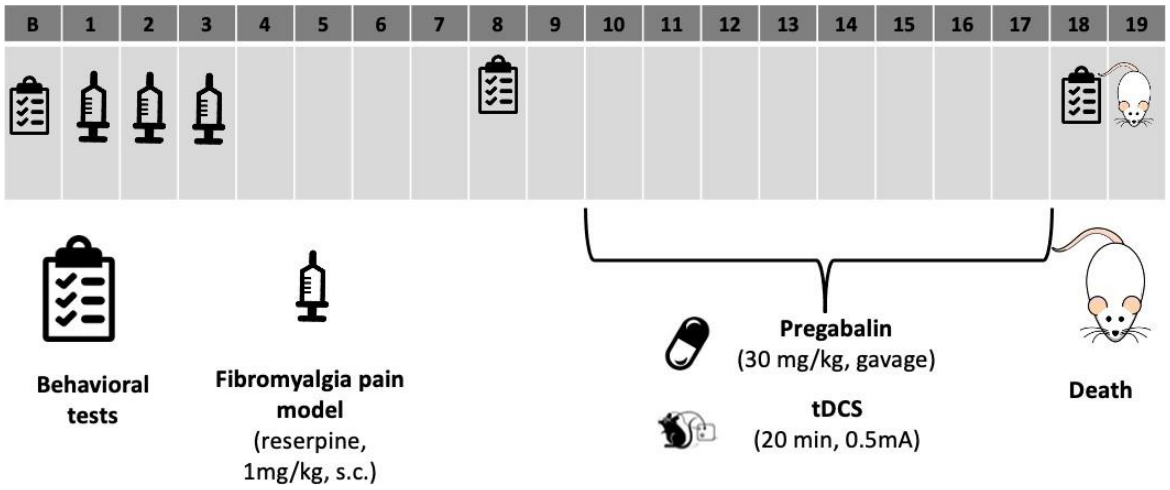

FIGURE 2

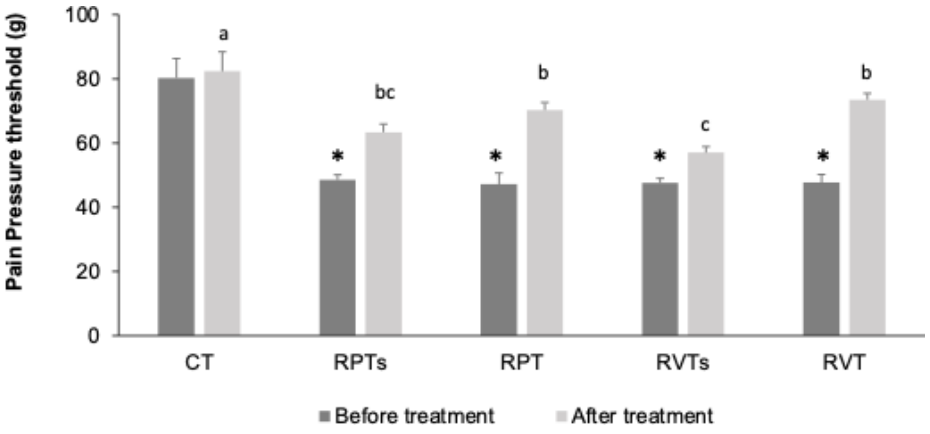

FIGURE 3

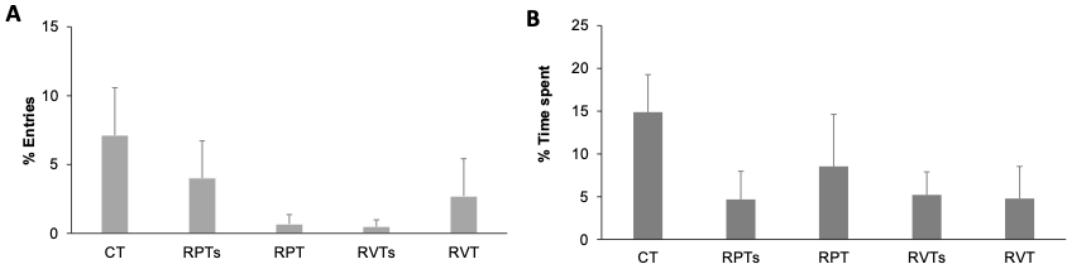

Table 1. Behavioral parameters assessed in the Elevated Plus Maze. Data expressed as mean ± SEM (N = 40) of number of fecal boluses, protected head-dipping (PHD), unprotected head-dipping (NPHD) and rearing, and time of grooming (seconds). Control (CT); Reserpine + pregabalin + Sham tDCs (RPTs); Reserpine + pregabalin + tDCs (RPT); Reserpine + pregabalin vehicle + sham tDCs (RVTs); Reserpine + pregabalin vehicle + tDCs (RVT). [a] significant difference from the CT and RVT groups (one way ANOVA/ SNK, P<0.05); [b] significant difference from the CT and RPTs groups (one way ANOVA/ SNK, P<0.05); [c] significant difference from the CT group (one way ANOVA/ SNK, P<0.05).

Group	*Grooming (s)*	*Fecal boluses (n)*	*PHD (n)*	*NPHD (n)*	*Rearing (n)*
CT	4.41 ± 2.51	0.25 ± 0.25	3.00 ± 0.78	2.57 ± 0.86	16.29 ± 0.71
RPTs	34.25 ± 13.62[a]	0.50 ± 0.50	1.13 ± 0.47	1.25 ± 0.77	9.38 ± 0.86[c]
RPT	10.02 ± 2.76[a]	0.00 ± 0.00	1.75 ± 0.62	0.25 ± 0.25[b]	9.63 ± 1.67[c]
RVTs	17.88 ± 7.94[a]	0.00 ± 0.00	2.75 ± 0.70	0.25 ± 0.16[b]	12.50 ± 1.30[c]
RVT	3.42 ± 1.88	0.00 ± 0.00	1.63 ± 0.56	0.50 ± 0.50[b]	8.25 ± 0.81[c]
P	**0.03**	0.55	**0.21**	0.03	**< 0.01**

Table 2. **Biomarkers analyzed in the central and peripheral samples.** Data expressed as mean ± SEM (N = 40). Control (CT); Reserpine + pregabalin + Sham tDCs (RPTs); Reserpine + pregabalin + tDCs (RPT); Reserpine + pregabalin vehicle + sham tDCs (RVTs); Reserpine + pregabalin vehicle + tDCs (RVT). * significant difference from the CT and RVT groups (one way ANOVA/ SNK).

Biomarker	Structure	CT group	RPTs group	RPT group	RVTs group	RVT group	P value
BDNF (pg/mg of protein)	Brainstem	3.18±0.24	3.64±0.40	3.58±0.65	3.43±0.08	3.80±0.34	0.852
	Cerebral Cortex	0.53±0.10	0.63±0.06	0.65±0.05	0.35±0.06	1.09±0.49	0.207
	Spinal Cord	2.93±0.51	2.56±0.36	4.21±0.89	2.59±0.75	2.76±0.30	0.828
	Serum	**0.59±0.02**	**0.45±0.03***	**0.51±0.04***	**0.45±0.03***	**0.61±0.04**	**0.003**
TNF-α (pg/mg of protein)	Brainstem	1.34±0.93	4.65±1.76	1.85±0.68	3.02±1.01	3.44±1.21	0.514
	Cerebral Cortex	1.54±0.005	1.00±0.56	2.29±0.97	1.20±0.31	0.78±0.10	0.339
	Hippocampus	1.01±0.18	1.58±0.71	0.92±0.21	0.75±0.19	0.62±0.12	0.610
	Serum	9.20±8.76	6.91±4.46	9.63±1.72	14.20±2.42	12.51±2.88	0.337
IL-1β (pg/mg of protein)	Hippocampus	1.47±0.18	2.82±0.72	1.45±0.34	0.69±0.19	1.25±0.37	0.315
IL-10 (pg/mg of protein)	Brainstem	19.71±6.50	24.96±2.56	16.22±3.76	16.87±2.79	24.93±3.67	0.267
	Cerebral Cortex	43.77±27.76	8.30±1.77	11.86±2.55	12.97±1.48	23.12±10.32	0.039

PARTE III

DISCUSSÃO GERAL

6 DISCUSSÃO GERAL

Os resultados desta tese demonstram que os ratos submetidos ao modelo de fibromialgia induzido por reserpina apresentaram hiperalgesia mecânica e térmica, comportamento do tipo ansioso, e redução nos níveis séricos de BDNF. Desta forma, é possível sugerir que este modelo reproduziu características da síndrome fibromiálgica, demonstrando comportamentos relacionados à dor crônica, com provavelmente a participação do BDNF. É importante destacar que no terceiro dia de indução do modelo de fibromialgia, os ratos já começaram a apresentar fenótipos indicativos de dor, como, compressão ocular, vocalização no momento de manipulação, postura arqueada, e comportamentos de prostração (Neves et al., 2013). Essa sintomatologia reproduzida pelos animais, são condizentes com a doença, que caracteriza-se por dor intensa difusa, fadiga e por muitas vezes está associada a comorbidades como ansiedade e depressão (Alves et al., 2022).

Nesta tese, a ETCC apresentou efeitos antinociceptivos demonstrados pela reversão parcial da hiperalgesia mecânica, já a pregabalina isolada não foi eficaz em mostrar redução da hipersensibilidade mecânica no modelo utilizado. Considerando que o modelo de fibromialgia utilizado induz a depleção de dopamina, serotonina e norepinefrina provocando um desequilíbrio entre os mecanismos excitatórios (glutamatérgicos) e inibitórios (GABAérgicos), é possível sugerir que a modulação destas vias de neurotransmissão pela ETCC esteja relacionada ao seu efeito analgésico (Souza et al., 2018).

Por outro lado, a pregabalina, considerada um tratamento padrão ouro em pacientes fibromiálgicos (Allen et al., 2020), têm sido utilizada em estudos pré-clínicos, no alívio do comportamento hipernociceptivo em diferentes modelos de dor (Tanimoto-Morri et al., 2008; Pires et al., 2020). Sua ligação à subunidade alfa-2 delta pode ser responsável por seu efeito antinociceptivo, promovendo redução de neurotransmissores excitatórios (noradrenalina, serotonina, dopamina, glutamato e substância P) (Toth, 2014). É interessante ressaltar que a pregabalina (10 e 30 mg/kg) aumentou significativamente o limiar de pressão muscular em ratos com mialgia induzida por reserpina (Kiso et al., 2018). Por outro lado, um estudo não mostrou melhora na hiperalgesia muscular aguda ou crônica após tratamento com pregabalina

(Ohmichi et al., 2018). Este último estudo corrobora os dados desta tese utilizando dose semelhante (30 mg/kg) em que não houve diminuição no comportamento hipernociceptivo. No entanto, é importante salientar que, nesta tese, os testes nociceptivos foram realizados 24 horas após a última dose de pregabalina. Um recente estudo avaliando 1 hora após a administração de pregabalina mostrou que esta foi capaz de reverter a hiperalgesia mecânica em um modelo neuropático em ratos (Ungard et al., 2020). Em suma, esses achados destacam a complexidade da pregabalina, considerando propriedades farmacocinéticas e farmacodinâmicas, frente a diferentes condições crônicas, inclusive no modelo de fibromialgia.

O modelo de fibromialgia induzido pela reserpina promoveu aumento no comportamento de autolimpeza (*grooming*), induziu diminuição de *NPHD* e números de *rearing*, comportamentos esses relacionados à ansiedade (Estanislau et al., 2019). O tratamento com a pregabalina reverteu a *NPHD*, demonstrando seu efeito ansiolítico (Baldwin and Ajel, 2007), enquanto o comportamento de autolimpeza foi restaurado pela ETCC, efeito corroborado por estudos prévios do nosso grupo de pesquisa que mostraram o efeito ansiolítico da ETCC, revertendo o comportamento do tipo ansioso em ratos submetidos a um modelo de dor neuropática (Santos et al., 2021; Lopes et al., 2021).

O modelo de fibromialgia induzida por reserpina induziu uma redução nos níveis séricos de BDNF, que foi revertida pela ETCC. E, não houve efeito da reserpina sobre os níveis de BDNF nas estruturas cerebrais centrais. Prévio estudo usando um outro modelo de fibromialgia mostrou uma redução na expressão de BDNF em córtex pré-frontal e hipocampo de camundongos (Lee et al., 2017). Por outro lado, associado a estes dados comportamentais observados nesta tese, a reserpina diminuiu os níveis séricos de BDNF, e estes foram restaurados pela ETCC. É importante salientar que o BDNF é um neurotrofina envolvida na neuroplasticidade, sendo um componente crítico de mecanismos moleculares, principalmente na presença de processos mal adaptativos como a fibromialgia (Bidari, et al; 2022). Não foi observado efeito do ETCC nos níveis periféricos de BDNF, corroborando a não existência de consenso na literatura neste tópico (Brunoni et al., 2018; Moreira et al., 2016).

Não foram observados efeitos do modelo de dor ou dos tratamentos nos níveis de citocinas, como TNF-α, IL-1β e IL-10 tanto em nível periférico quanto central. Prévios

achados do grupo de pesquisa que demonstram que o tratamento com ETCC bimodal induz analgesia associada a alterações nos níveis centrais de neurotrofinas e citocinas em ratos submetidos a diferentes modelos de dor (Cioato et al., 2016; Lopes et al., 2020; Adachi et al. 2015; Laste et al., 2012), no entanto no modelo de fibromialgia não foi observado este efeito. É importante destacar que a síndrome da fibromialgia ainda não é bem compreendida no cenário clínico ou pré-clínico; porém mais estudos são necessários para aumentar o conhecimento sobre esta condição.

Sumarizando os dados desta tese não demonstram efeito sinérgico entre os tratamentos com ETCC e pregabalina em um modelo animal de fibromialgia induzida por reserpina dentre os parâmetros avaliados. No entanto, houve efeitos complementares no comportamento de ansiedade. Sabe-se que a fibromialgia é uma síndrome complexa, e seus mecanismos não estão bem elucidados, portanto mais estudos podem contribuir para um melhor entendimento da fisiopatologia desta doença, e a busca de métodos complementares, visando a combinação de terapias para fornecer resultados mais consistentes no alívio da dor, proporcionando melhor qualidade de vida.

CONCLUSÕES

7 CONCLUSÕES

Com base nos resultados desta tese, foi possível demonstrar que o modelo animal de fibromialgia induzido por reserpina é capaz de reproduzir características da síndrome fibromiálgica., sendo desta forma uma ferramenta adequada para a busca de um melhor entendimento da fisiopatologia da doença. No entanto, não foi observado efeito sinérgico entre os tratamentos pregabalina e ETCC em relação aos parâmetros analisados. Houve uma complementação dos tratamentos em relação a reversão de comportamentos do tipo ansioso nos ratos com dor. No entanto, os resultados demonstram que a ETCC pode ser uma potencial terapêutica não farmacológica adjuvante no tratamento da síndrome fibromiálgica e de outras doenças de difícil manejo terapêutico, trazendo benefícios de impacto positivo na qualidade de vida dos indivíduos.

PERSPECTIVAS

8 PERSPECTIVAS

A partir deste trabalho, novas pesquisas serão desenvolvidas buscando esclarecer os mecanismos de ação envolvidos nos efeitos antinociceptivos da ETCC, bem como desenvolvimento de protocolos que possibilitem a avaliação de tratamentos mais longos, duração dos seus efeitos e possíveis efeitos sinérgicos.

Portanto, pretende-se avaliar em novos estudos:

- Protocolos de tratamento de ETCC mais prolongados;
- Associação da ETCC com outros fármacos utilizados para o manejo da fibromialgia e possíveis efeitos sinérgicos;
- Avaliar diferentes vias de sinalização que podem estar relacionados com o efeito da ETCC neste modelo de fibromialgia, como vias serotonérgicas, dopaminérgicas e noradrenérgicas.

REFERÊNCIAS

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Alles, S., Cain, S. M., & Snutch, T. P. (2020). Pregabalin as a Pain Therapeutic: Beyond Calcium Channels. Frontiers in cellular neuroscience, 14, 83. https://doi.org/10.3389/fncel.2020.00083

Antal, A., Terney, D., Kühnl, S., & Paulus, W. (2010). Anodal transcranial direct current stimulation of the motor cortex ameliorates chronic pain and reduces short intracortical inhibition. Journal of pain and symptom management, 39(5), 890–903. https://doi.org/10.1016/j.jpainsymman.2009.09.023

Ardolino, G., Bossi, B., Barbieri, S., & Priori, A. (2005). Non-synaptic mechanisms underlie the after-effects of cathodal transcutaneous direct current stimulation of the human brain. The Journal of physiology, 568(Pt 2), 653–663. https://doi.org/10.1113/jphysiol.2005.088310

Arnold, L. M., Emir, B., Murphy, T. K., Zeiher, B. G., Pauer, L., Scott, G., & Petersel, D. (2012). Safety profile and tolerability of up to 1 year of pregabalin treatment in 3 open-label extension studies in patients with fibromyalgia. Clinical therapeutics, 34(5), 1092–1102. https://doi.10.1016/j.clinthera.2012.03.003

Arnold, L. M., Russell, I. J., Diri, E. W., Duan, W. R., Young, J. P., Jr, Sharma, U., Martin, S. A., Barrett, J. A., & Haig, G. (2008). A 14-week, randomized, double-blinded, placebo-controlled monotherapy trial of pregabalin in patients with fibromyalgia. The journal ofpain, 9(9), 792–805.. https://doi.org/10.1016/j.jpain.2008.03.013

Barata, P. C., Santos, M. J., & Maia, T. (2019). Treating akathisia with pregabalin–A case report. Archives of Clinical Psychiatry (São Paulo), 46, 81-82. https://doi.org/10.1590/0101-60830000000199

Bazzichi, L., Giacomelli, C., Consensi, A., Giorgi, V., Batticciotto, A., Di Franco, M., & Sarzi-Puttini, P. (2020). One year in review 2020: fibromyalgia. Clinical and experimental rheumatology, 38 Suppl 123(1), 3–8.

Becker, S., & Schweinhardt, P. (2012). Dysfunctional neurotransmitter systems in fibromyalgia, their role in central stress circuitry and pharmacological actions on these systems. Pain research and treatment, 2012, 741746. https://doi.org/10.1155/2012/741746

Bindman, LJ, Lippold, OC, e Redfearn, JWT (1964). A ação de correntes polarizadoras breves no córtex cerebral do rato (i) durante o fluxo de corrente e (2) na produção de efeitos posteriores de longa duração. J. Fisiol. 172, 369-382.

Boros, K., Poreisz, C., Münchau, A., Paulus, W., & Nitsche, M. A. (2008). Premotor transcranial direct current stimulation (tDCS) affects primary motor excitability in humans. European Journal of Neuroscience, 27(5), 1292-1300.https://doi.org/10.1111/j.1460-9568.2008.06090.x

Burgmer, M., Pogatzki-Zahn, E., Gaubitz, M., Wessoleck, E., Heuft, G., & Pfleiderer, B. (2009). Altered brain activity during pain processing in fibromyalgia. Neuroimage, 44(2), 502-508.https://doi.10.1016/j.neuroimage.2008.09.008

Bhusal, S., Diomampo, S., & Magrey, M. N. (2016). Clinical utility, safety, and efficacy of pregabalin in the treatment of fibromyalgia. Drug, Healthcare and Patient Safety, 8, 13. https://doi.10.2147/DHPS.S95535.

Coêlho, M. L. S., Cabral, P. M., Morello, L. Y. N., do Rêgo, G. G., & Boggio, P. S. (2021). Estimulações cerebrais não invasivas: as aplicações de Estimulação Transcraniana por Corrente Contínua (ETCC) e Estimulação Magnética Transcraniana (EMT) na aprendizagem e na clínica. Tecnologias Aplicadas em Educação e Saúde, 341.

Cordeiro, B. L. B., Fortunato, I. H., Lima, F. F., Santos, R. S., Costa, M. D. C., & Brito, A. F. (2020). Influence of the Pilates method on quality of life and pain of individuals with fibromyalgia: integrative review. BrJP, 3, 258-262. https://doi.org/10.5935/2595-0118.20200049.

Crofford L. J. (2004). Pharmaceutical treatment options for fibromyalgia. Current rheumatology reports, 6(4), 274–280. https://doi.org/10.1007/s11926-004-0035-y

Dadabhoy, D., Crofford, L. J., Spaeth, M., Russell, I. J., & Clauw, D. J. (2008). Biology and therapy of fibromyalgia. Evidence-based biomarkers for fibromyalgia syndrome. Arthritis Research & Therapy, 10(4), 1-18.https://doi.org/10.1186/ar2443.

Doğan, S., Ozberk, S., & Yurci, A. (2011). Pregabalin-induced hepatotoxicity. European journal of gastroenterology & hepatology, 23(7),628. https://doi.org/10.1097/MEG.0b013e328346df7a

Esteves, G. J., Motoyama, Y. L., Pereira, P. E. D. A., Elcadi, G. H., Pereira, R., & Azevedo, P. H. S. M. D. (2019). Effect of transcranial direct current stimulation on supramaximal intermittent exercise performance. Motriz: Revista de Educação Física, 25. https://doi.org/10.1590/s1980-6574201900040215

Fagerlund, A. J., Hansen, O. A., & Aslaksen, P. M. (2015). Transcranial direct current stimulation as a treatment for patients with fibromyalgia: a randomized controlled trial. Pain, 156(1), 62-71.https://doi.org/10.1016/j.pain.0000000000000006

Fayed, N., Garcia-Campayo, J., Magallón, R., Andrés-Bergareche, H., Luciano, J. V., Andres, E., & Beltrán, J. (2010). Localized 1H-NMR spectroscopy in patients with fibromyalgia: a controlled study of changes in cerebral glutamate/glutamine, inositol, choline, and N-acetylaspartate. Arthritis research & therapy, 12(4), 1-12. https://doi.org/10.1186/ar3072

Figueiredo-Dourado, S. S., Lima, L. V., Givigi, R., & DeSantana, J. M. (2020). Effect of environmental enrichment associated or not with physical activity on nociceptive and motor functions in an animal model of fibromyalgia. BrJP, 3, 99-104. https://doi.org/10.5935/2595-0118.20200022

Galvez-Sánchez, Carmen M., and Gustavo A. Reyes del Paso. (2020). "Diagnostic Criteria for Fibromyalgia: Critical Review and Future Perspectives" *Journal of Clinical Medicine* 9, no. 4: 1219.https://doi.org/10.3390/jcm9041219

Gerardi, M. C., Atzeni, F., Batticciotto, A., Di Franco, M., Rizzi, M., & Sarzi-Puttini, P. (2016). The safety of pregabalin in the treatment of fibromyalgia. Expert opinion on drug safety, 15(11), 1541–1548.https://doi.org/10.1080/14740338.2016.1242575

Goldenberg D. L. (2007). Pharmacological treatment of fibromyalgia and other chronic musculoskeletal pain. Best practice & research. Clinical rheumatology, 21(3), 499–511. https://doi.org/10.1016/j.berh.2007.02.012

Harris, R. E., Napadow, V., Huggins, J. P., Pauer, L., Kim, J., Hampson, J., Sundgren, P. C., Foerster, B., Petrou, M., Schmidt-Wilcke, T., & Clauw, D. J. (2013). Pregabalin rectifies aberrant brain chemistry, connectivity, and functional response in chronic pain patients. Anesthesiology, 119(6), 1453–1464. https://doi.org/10.1097/ALN.0000000000000017

Hassett, A. L., & Gevirtz, R. N. (2009). Nonpharmacologic treatment for fibromyalgia: patient education, cognitive-behavioral therapy, relaxation techniques, and complementary and alternative medicine. Rheumatic diseases clinics of North America, 35(2), 393–407. https://doi.org/10.1016/j.rdc.2009.05.003

Häuser, W., Bernardy, K., Uçeyler, N., & Sommer, C. (2009). Treatment of fibromyalgia syndrome with gabapentin and pregabalin--a meta-analysis of randomized controlled trials. Pain, 145(1-2), 69–81. https://doi.org/1016/j.pain.2009.05.014

Heymann, R. E., Paiva, E., Helfenstein, M., Jr, Pollak, D. F., Martinez, J. E., Provenza, J. R., Paula, A. P., Althoff, A. C., Souza, E. J., Neubarth, F., Lage, L. V., Rezende, M. C., de Assis, M. R., Lopes, M. L., Jennings, F., Araújo, R. L., Cristo, V. V., Costa, E. D., Kaziyama, H. H., Yeng, L. T., … Fonseca, M. C. (2010). Brazilian consensus on the treatment of fibromyalgia. Revista brasileira de reumatologia, 50(1), 56–66. https://doi.org/10.1590/S0482-50042010000100006

Illeez, O. G., Oktay, K., Aktas, I., Ozkan, F. U., Nazligül, T., Begoglu, F. A., Kaysin, M. Y., Atici, A., & Akpinar, P. (2022). Comparison of the effects of duloxetine and pregabalin on pain and associated factors in patients with knee osteoarthritis. Revista da Associacao Medica Brasileira (1992), 68(3), 377–383. https://doi.org/10.1590/1806-9282.20211047.

Kim, S. H., Lee, Y., Lee, S., & Mun, C. W. (2013). Evaluation of the effectiveness of pregabalin in alleviating pain associated with fibromyalgia: using functional magnetic resonance imaging study. PloS one, 8(9), e74099. https://doi.org/10.1371/journal.pone.0074099

Lang, N., Siebner, H. R., Ward, N. S., Lee, L., Nitsche, M. A., Paulus, W., Rothwell, J. C., Lemon, R. N., & Frackowiak, R. S. (2005). How does transcranial DC stimulation of the primary motor cortex alter regional neuronal activity in the human brain?. The European journal of neuroscience, 22(2), 495–504. https://doi.org/10.1111/j.1460-9568.2005.04233.x

Matias, M. G. L., Cavalcante, A. F. L., Mescouto, K. A., Silva Filho, E. M., Baptista, A. F., Okano, A. H., & Pegado, R. (2022). Does anodal transcranial direct current stimulation over left motor cortex show body side pain-related difference in fibromyalgia?. BrJP, (AHEAD). *https://doi.org/10.5935/2595-0118.20220020-pt*

Mease, P. J., Russell, I. J., Arnold, L. M., Florian, H., Young, J. P., Jr, Martin, S. A., & Sharma, U. (2008). A randomized, double-blind, placebo-controlled, phase III trial of pregabalin in the treatment of patients with fibromyalgia. The Journal of rheumatology, 35(3), 502–514. PMID: 18278830.

Medeiros, L. F., de Souza, I. C., Vidor, L. P., de Souza, A., Deitos, A., Volz, M. S., Fregni, F., Caumo, W., & Torres, I. L. (2012). Neurobiological effects of transcranial direct current stimulation: a review. Frontiers in psychiatry, 3, 110. https://doi.org/10.2289/fpsyt.2012.00110.

Mendonca, M. E., Santana, M. B., Baptista, A. F., Datta, A., Bikson, M., Fregni, F., & Araujo, C. P. (2011). Transcranial DC stimulation in fibromyalgia: optimized cortical target supported by high-resolution computational models. The journal of pain, 12(5), 610–617. https://doi.org/10.1016/j.jpain.2010.12.015

Maitra, S., Baidya, D. K., Bhattacharjee, S., & Som, A. (2017). Gabapentina e pregabalina no período perioperatório em cirurgia cardíaca: uma revisão sistemática e metanálise [Perioperative gabapentin and pregabalin in cardiac surgery: a systematic review and meta-analysis]. Revista brasileira de anestesiologia, 67(3), 294–304. https://doi.org/10.1016/j.bjane.2016.07.014

Monteiro, É. A. B., Oliveira, L. D., & Oliveira, W. L. (2021). Aspectos psicológicos da fibromialgia-revisão integrativa. Mudanças, 29(1), 65-76.

Mountz, J.M., Bradley, L.A., Modell, J.G., Alexander, R.W., Triana-Alexander, M., Aaron, L.A., Stewart, K.E., AlarcóN, G.S. and Mountz, J.D. (1995), Fibromyalgia in women. Arthritis & Rheumatism, 38: 926-938. https://doi.org/10.1002/art.1780380708

Nagakura, Y., Oe, T., Aoki, T., & Matsuoka, N. (2009). Biogenic amine depletion causes chronic muscular pain and tactile allodynia accompanied by depression: A putative animal model of fibromyalgia. Pain, 146(1-2), 26-33. https://doi.org/10.16/j.pain.2009.05.024

Nitsche, M. A., Fricke, K., Henschke, U., Schlitterlau, A., Liebetanz, D., Lang, N., & Paulus, W. (2003). Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. The Journal of physiology, 553(1), 293-301. https://doi.org/10.1113/jphysiol.2003.049916

Nitsche, M.A. and Paulus, W. (2000), Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. The Journal of Physiology, 527: 633-639. https://doi.org/10.1111/j.1469-7793.2000.t01-1-00633.x

Oliveira Júnior, J. O. D., & Almeida, M. B. D. (2018). The current treatment of fibromyalgia. BrJP, 1, 255-262.https://doi.org/10.5935/2595-0118.20180049

Ohta, H., Oka, H., Usui, C., Ohkura, M., Suzuki, M., & Nishioka, K. (2012). A randomized, double-blind, multicenter, placebo-controlled phase III trial to evaluate the efficacy and safety of pregabalin in Japanese patients with fibromyalgia. Arthritis research & therapy, 14(5), R217. https://doi.org/10.1186/ar4056

Ohta, H., Oka, H., Usui, C., Ohkura, M., Suzuki, M., & Nishioka, K. (2013). An open-label long-term phase III extension trial to evaluate the safety and efficacy of pregabalin in Japanese patients with fibromyalgia. Modern rheumatology, 23(6), 1108–1115. https://doi.org/10.1007/s10165-012-0803-x

Peck, M. M., Maram, R., Mohamed, A., Ochoa Crespo, D., Kaur, G., Ashraf, I., & Malik, B. H. (2020). The Influence of Pro-inflammatory Cytokines and Genetic Variants in the Development of Fibromyalgia: A Traditional Review. Cureus, 12(9), e10276. https://doi.org/10.7759/cureus.10276

Poluha, R. L., & Grossmann, E. (2018). Does pregabalin improve sleep disorders in fibromyalgia?. BrJP, 1, 163-166. https://doi.org./10.5935/2595-0118.20180031

Russell, I.J., Orr, M.D., Littman, B., Vipraio, G.A., Alboukrek, D., Michalek, J.E., Lopez, Y. and Mackillip, F. (1994), Elevated cerebrospinal fluid levels of substance p in patients with the fibromyalgia syndrome. Arthritis & Rheumatism, 37: 1593-1601. https://doi.org/10.1002/art.1780371106

da Silva, A. A., Kakuta, E., & Loreti, E. H. (2020). Estimulação transcraniana por corrente contínua em fibromialgia: uma revisão sistemática. Revista Neurociências, 28, 1-18. https://doi.org/10.5935/2595-0118.20180049

Souza, A., Martins, D. F., Medeiros, L. F., Nucci-Martins, C., Martins, T. C., Siteneski, A., Caumo, W., Dos Santos, A., & Torres, I. (2018). Neurobiological mechanisms of antiallodynic effect of transcranial direct current stimulation (tDCS) in a mice model of neuropathic pain. Brain research, 1682, 14–23. https://doi.org/10.1016/j.brainres.2017.12.005

Souza, R. V. S., Maciel, D. G., & Cerqueira, M. S. (2021). Effects of transcranial direct current stimulation associated or combined with exercise on musculoskeletal pain: systematicreview. BrJP.https://doi.org/10.5935/2595-0118.20210029

Stagg, C. J., & Nitsche, M. A. (2011). Physiological basis of transcranial direct current stimulation. The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry, 17(1), 37–53. https://doi.org/10.2277/107385841038661

Stefani, L. C., Torres, I. L., de Souza, I. C., Rozisky, J. R., Fregni, F., & Caumo, W. (2012). BDNF as an effect modifier for gender effects on pain thresholds in healthy subjects. Neuroscience letters, 514(1), 62–66. https://doi.org/10.1016/j.neulet.2012.02.057

Üçeyler, N., Zeller, D., Kahn, A. K., Kewenig, S., Kittel-Schneider, S., Schmid, A., Casanova-Molla, J., Reiners, K., & Sommer, C. (2013). Small fibre pathology in patients with fibromyalgia syndrome. Brain : a journal of neurology, 136(Pt 6), 1857–1867. https://doi.org/10.1093/brain/awt053

Urfalıoglu, S., & Berk, E. (2022). Assessment of retinal nerve fiber thickness and optic nerve head blood flow in female patients diagnosed with fibromyalgia syndrome. Arquivos brasileiros de oftalmologia, 85(1), 30–36. https://doi.org/10.5935/0004-2749.20220005

Villamar, M. F., Wivatvongvana, P., Patumanond, J., Bikson, M., Truong, D. Q., Datta, A., & Fregni, F. (2013). Focal modulation of the primary motor cortex in fibromyalgia using 4×1-ring high-definition transcranial direct current stimulation (HD-tDCS): immediate and delayed analgesic effects of cathodal and anodal stimulation. The journal of pain, 14(4), 371–383. https://doi.org/10.1016/j.pain.2012.12.007

Yunus M. B. (2007). Fibromyalgia and overlapping disorders: the unifying concept of central sensitivity syndromes. Seminars in arthritis and rheumatism, 36(6), 339–356. https://doi:10.1016/j.semarthrit.2006.12.009

Zanette, S. A., Dussan-Sarria, J. A., Souza, A., Deitos, A., Torres, I. L., & Caumo, W. (2014). Higher serum S100B and BDNF levels are correlated with a lower pressure-pain threshold in fibromyalgia. Molecular pain, 10, 46. https://doi.org/10.1186/1744-8069-10-46

Zaninotto, A. L., El-Hagrassy, M. M., Green, J. R., Babo, M., Paglioni, V. M., Benute, G. G., & Paiva, W. S. (2019). Transcranial direct current stimulation (tDCS) effects on traumatic brain injury (TBI) recovery: A systematic review. Dementia & neuropsychologia, 13(2), 172–179. https://doi.org/10.1590/1980-57642018dn13-020005

Spezia Adachi, L. N., Caumo, W., Laste, G., Fernandes Medeiros, L., Ripoll Rozisky, J., de Souza, A., Fregni, F., & Torres, I. L. (2012). Reversal of chronic stress-induced pain by transcranial direct current stimulation (tDCS) in an animal model. Brain research, 1489, 17–26.https://doi.org/10.1016/j.brainres.2012.10.009

Alles, S., Cain, S. M., & Snutch, T. P. (2020). Pregabalin as a Pain Therapeutic: Beyond Calcium Channels. Frontiers in cellular neuroscience, 14, 3. https://doi.org/10.3389/fncel.2020.00083

Baldwin, D. S., & Ajel, K. (2007). Role of pregabalin in the treatment of generalized anxiety disorder. Neuropsychiatric Disease and Treatment, 3(2), 185. https://doi.org/10.2147/nedt.2007.3.2.185

Bidari, A., Ghavidel-Parsa, B. & Gharibpoor, F. (2022). Comparação do fator neurotrófico derivado do cérebro sérico (BDNF) entre fibromialgia e grupos de dor nociceptiva; e efeito da duloxetina no nível de BDNF. Distúrbio Musculoesquelético BMC 23, 411. https://doi.org/10.1186/s12891-022-05369-8.

Brunoni, A. R., Padberg, F., Vieira, E. L. M., Teixeira, A. L., Carvalho, A. F., Lotufo, P. A., & Benseñor, I. M. (2018). Plasma biomarkers in a placebo-controlled trial comparing tDCS and escitalopram efficacy in major depression. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 86, 211-217.https://doi.org/10.16/j.pnpbp.2018.06.003

Cioato, S. G., Medeiros, L. F., Marques Filho, P. R., Vercelino, R., de Souza, A., Scarabelot, V. L., ... & Torres, I. L. (2016). Long-lasting effect of transcranial direct current stimulation in the reversal of hyperalgesia and cytokine alterations induced by the neuropathic pain model. Brain stimulation, 9(2), 209-217.https://doi.org/10.1016/j.brs.2015.12.001

da Silva Moreira, S. F., Medeiros, L. F., de Souza, A., de Oliveira, C., Scarabelot, V. L., Fregni, F., ... & Torres, I. L. (2016). Transcranial direct current stimulation (tDCS) neuromodulatory effects on mechanical hyperalgesia and cortical BDNF levels in ovariectomized rats. Life Sciences, 145, 233-239. https://doi.org/10.1016/jlfs.2015.10011

de Carvalho Alves, R., Nepomuceno, V. R., Marson, P. G., Neto, J. B., Silveira, J. M., Rodrigues, E. S. R., ... & da Silva, K. C. C. (2022). Aspectos Epidemiológicos e Diagnóstico da Fibromialgia na Região Norte do Brasil. Research, Society and Development, 11(4), e53511427704-e53511427704.https://doi.org/10.33448/rsd-v11i4.27704

Estanislau, C., Veloso, A. W., Filgueiras, G. B., Maio, T. P., Dal-Cól, M. L., Cunha, D. C., ... & Fernández-Teruel, A. (2019). Rat self-grooming and its relationships with anxiety, dearousal and perseveration: Evidence for a self-grooming trait. Physiology & behavior, 209, 112585. https://doi.org/10.1016/j.physbeh.2019.112585

Kiso, T., Moriyama, A., Furutani, M., Matsuda, R., & Funatsu, Y. (2018). Effects of pregabalin and duloxetine on neurotransmitters in the dorsal horn of the spinal cord in a rat model of fibromyalgia. European Journal of Pharmacology, 827, 117-124. https://doi.org/10.1016/j.ejphar.2018.03.011

Lee, H., Im, J., Won, H., Kim, J. Y., Kim, H. K., Kwon, J. T., ... & Kim, H. J. (2018). Antinociceptive effect of Valeriana fauriei regulates BDNF signaling in an animal model of

fibromyalgia. International Journal of Molecular Medicine, 41(1), 485-492. https://doi.org/10.3892/ijmm.2017.3203

Laste, G., Caumo, W., Adachi, L. N. S., Rozisky, J. R., de Macedo, I. C., Partata, W. A., ... & Torres, I. L. (2012). After-effects of consecutive sessions of transcranial direct current stimulation (tDCS) in a rat model of chronic inflammation. Experimental brain research, 221(1), 75-83.https://doi.org/10.1007/s00221-012-3149-x

Lopes, B. C., Medeiros, L. F., Silva de Souza, V., Cioato, S. G., Medeiros, H. R., Regner, G. G., Lino de Oliveira, C., Fregni, F., Caumo, W., & Torres, I. (2020). Transcranial direct current stimulation combined with exercise modulates the inflammatory profile and hyperalgesic response in rats subjected to a neuropathic pain model: Long-term effects. Brainstimulation, 13(3), 774–782. https://doi.org/10.1016/j.brs.2020.02.025

Neves, S.M, Ong, S. M. P, Rodrigues, L.D, Santos, R.A, Fontes, R.S, Santana, R.O. (2013). Manual de cuidados e procedimentos com animais de laboratório do biotério de produção e experimentação da FCF-IQ/USP. São Paulo

Ohmichi, Y., Ohmichi, M., Murai, N., Yasui, M., Takeshita, N., Oshibuchi, H., ... & Sato, J. (2018). Therapeutic effects of diclofenac, pregabalin, and duloxetine on disuse-inducedchronic musculoskeletal pain in rats. Scientific reports, 8(1), 1-11. https://doi.org/10.1038/s41598-018-21429-3

Pires, O. C., Dalcim, M. L., Pigozzi, A. L. M., Caldeira, F. M. S. D. L. A., Pires, M. H. R., Almeida Neto, L. D., & Posso, I. D. P. (2020). Effects of duloxetine, fluoxetine and pregabalin on fentanyl-induced hyperalgesia in rattus novergicus. BrJP, 3, 14-18. https://doi.org/10.5935/2595-0118.20200004

Santos, D. S., Medeiros, L. F., Stein, D. J., De Macedo, I. C., Rios, D. E. D. S., De Oliveira, C., ... & Torres, I. L. (2021). Bimodal transcranial direct current stimulation reduces alcohol consumption and induces long-term neurochemical changes in rats with neuropathic pain. Neuroscience Letters, 759, 136014. https://doi.org/10.1016/j.neulet.2021.136014

Souza, A., Martins, D. F., Medeiros, L. F., Nucci-Martins, C., Martins, T. C., Siteneski, A., & Torres, I. L. (2018). Neurobiological mechanisms of antiallodynic effect of transcranial direct current stimulation (tDCS) in a mice model of neuropathic pain. Brain research, 1682, 14-23.https://doi.org/10.1016/j.brainres.2017.12.005

Tanimoto-Mori, S., Nakazato-Imasato, E., Toide, K., & Kita, Y. (2008). Pharmacologic investigation of the mechanism underlying cold allodynia using a new cold plate procedure in rats with chronic constriction injuries. Behavioural pharmacology, 19(1), 85-90. https://doi.org/10.1097FBP.0b013e3282f3d0a3

Toth C. (2014). Pregabalin: latest safety evidence and clinical implications for the management of neuropathic pain. Therapeutic advances in drug safety, 5(1), 38–56. https://doi.org/10.1177/2042098613505614

Ungard, R. G., Zhu, Y. F., Yang, S., Nakhla, P., Parzei, N., Zhu, K. L., & Singh, G. (2020). Response to pregabalin and progesterone differs in male and female rat models of neuropathic and cancer pain. Canadian Journal of Pain, 4(1), 39-58. https://doi:10.1080/24740527.2020.1724776.

APÊNDICE

10 APÊNDICE

12.1 Artigos publicados

- SOUZA, VANESSA SILVA; RIBEIRO, HUGO DANIEL WELTER; MEDEIROS, LICIANE FERNANDES; CASTRO, MARIANE SCHAFFER; SOUZA, ANDRESSA. Nociceptive profile and analgesic use of chronic pain patients submitted to rotator cuff repair surgery: a prospective cohort. Revista Brasileira de Ortopedia, 2020.
- LOPES, BETTEGA COSTA; MEDEIROS, LICIANE FERNANDES; CIOATO, STEFANIA GIOTTI; MEDEIROS, HELOISE RICHARDT; REGNER, GABRIELA GREGORY; OLIVEIRA, CAMILA LINO; FREGNI, FELIPE; CAUMO, WOLNEI; TORRES, IRACI L.S. Transcranial direct current stimulation combined with exercise modulates the inflammatory profile and hyperalgesic response in rats subjected to a neuropathic pain model: Long-term effects. Brain Stimulation, 2020.
- OLIVEIRA, CAMILA LINO; MEDEIROS, LICIANE FERNANDES; SOUZA, VANESSA SILVA; LOPES, BETTEGA COSTA; OLIVEIRA, FABRÍCIO FERNANDES; MARQUES, LUANA XAVIER; TORRES, IRACO L.S; SOUZA, ANDRESSA. Low-dose naltrexone reverses facial mechanical allodynia in a rat model of trigeminal neuralgia. Neuroscience Letters, 2020.

12.2 Resumos publicados em anais de congresso

- SOUZA, V.S.; OLIVEIRA, C.L.; DUSSAN-SARRIA, J.A.; CAUMO. W.; TORRES, I.L.S.; SOUZA, A. Fibromialgia através da reserpina: um modelo animal. In: II Simpósio Gaúcho de Farmacologia, 2018, Porto Alegre.
- SOUZA, V.S.; CAUMO, W.; SOUZA, A. Perfil Nociceptivo em pacientes portadores de dor crônica submetidos a cirurgia de reparo do manguito rotador. In: 38º Semana Científica do Hospital de Clínicas de Porto Alegre, 2018, Porto Alegre.
- SOUZA, V.S.; OLIVEIRA, C.L.; LOPES, B.C.; CASTRO, J.M.; MEDEIROS, H.R.; MARQUES, L.X.; DUSSAN-SARRIA, J.A.; MEDEIROS, L.F.; TORRES, I.L.; SOUZA, A. Avaliação dos efeitos da combinação de eletroestimulação transcraniana por corrente contínua em um modelo animal de fibromialgia: resultados preliminares. In: 39° Semana Científica do Hospital de Clínicas de

Porto Alegre, 2019, Porto Alegre.

- OLIVEIRA, C.L.; SOUZA, V.S.; DUSSAN-SARRIA, J.A.; CAUMO, W.;TORRES, I.L.S.; SOUZA, A. Modelo animal de fibromialgia: resultados parciais. In: V Congresso Sul-Brasileiro de Dor e I Congresso Gaúcho de Cuidados Paliativos, 2018, Porto Alegre.
- OLIVEIRA, C.L.; SOUZA, V.S.; DUSSAN-SARRIA, J.A.; CAUMO, W.;TORRES, I.L.S.; SOUZA, A. Modelo animal de fibromialgia através da reserpina. In: 38º Semana Científica do Hospital de Clínicas de Porto Alegre, 2018, Porto Alegre.
- LOPES, B.C.; CIAOTO, S.G.; SOUZA, V.S.; OLIVEIRA, C.L.; MARQUES, L.X.; MEDEIROS, L.F.; FREGNI, F.; CAUMO, W.; TORRES, I.L.S. Long-term effects of combined treatment of physical exercise and transcranial direct current stimulation (tDCS) upon mechanical hyperalgesia in rats. In: X Simpósio Internacional em Neuromodulação, 2018, São Paulo-SP. X Simpósio Internacional em Neuromodulação, 2018.
- ROCHA, R. A. P. L.; ROCHA, E. M.; SOUZA, V.S.; LOPES, B.C.; MEDEIROS, L.F.; SOUZA, A. Efeito Analgésico da Associação de Baixas Doses de Naltrexona e Exercício Físico em Modelo de Fibromialgia. Anais do SEFIC 2019.
- PALUDO, R.H.; ROCHA, R. A. P. L.; ROCHA, E.M.; SOUZA, V.S.; LOPES, B.C.; CESTRO, M.S.; MEDEIROS, L.F.; CAUMO, W.; TORRES, I.L.S.; SOUZA, A. A. Low-Dose Naltrexone effects in pain modulation in Fibromyalgia Rats. Annals of XI International Symposium on Neuromodulation 2019.

APROVAÇÃO DA COMISSÃO DE ÉTICA NO USO DE ANIMAIS

HOSPITAL DE CLÍNICAS DE PORTO ALEGRE

Grupo de Pesquisa e Pós Graduação

Carta de Aprovação

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: 2015/0272

Título: Avaliação dos efeitos da combinação de pregabalina e Eletroestimulação Transcraniana de Corrente Contínua (ETCC) em um modelo animal de fibromialgia

Pesquisador Responsável: **IRACI LUCENA DA SILVA TORRES**

Equipe de Pesquisa:

CAMILA LINO DE OLIVEIRA	JAIRO ALBERTO DUSSÁN SARRIA	LICIANE FERNANDES MEDEIROS
CARLA DE OLIVEIRA	ROBERTA STRÖHER	VANESSA SILVA DE SOUZA
ANDRESSA DE SOUZA		

Data de Aprovação: 23/04/2018

Data de Término: 31/08/2019

Espécie/Linhagem	Sexo/Idade	Quantidade
RATO HETEROGÊNICO	M/65 Dia(s)	50

- 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.

COMPROVANTES DE SUBMISSÃO

Neuroscience Letters

No synergic effect between pregabalin and transcranial direct current stimulation (tDCS) upon hyper nociceptive behavior in rats with fibromyalgia model

--Manuscript Draft--

Manuscript Number:	
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Abstract:	Fibromyalgia is a widespread musculoskeletal disease, in which the etiology and the pathophysiology are not well elucidated. As a complex disease and refractoriness, there is no specific and single treatment. The pregabalin, as a pharmacological tool, has shown effective benefits; however, its side effects may decrease the treatment adherence. In this context, neuromodulatory techniques, like transcranial direct current stimulation (tDCS), may be used as a complementary tool to relieve pain and increase life quality in fibromyalgia. Thus, the objective of this study was to evaluate the effects of association of pregabalin and tDCS in a rat model of fibromyalgia, in behavioral parameters and biomarkers levels. Forty male adult Wistar rats were randomized in five experimental groups: control group (CT), reserpine + pregabalin + tDCS-sham (RPTs), reserpine + pregabalin + tDCS-active (RPT), reserpine + pregabalin vehicle + tDCS-sham (RVTs) and reserpine + pregabalin vehicle + active tDCS (RVT). This study was approved by the Institutional Ethics Committee (GPPG-HCPA #2015-0272). The fibromyalgia model was induced by administration of one dose of reserpine (1 mg/kg) for 3 consecutive days. The behavioral parameters evaluated were mechanical allodynia (von Frey test); anxiety-like behaviors (elevated plus-maze and forced swim) The nociceptive response was evaluated five days after the last administration of reserpine and 24 hours after the end of treatment. The animals submitted to the reserpine-induced fibromyalgia model exhibited a reduced mechanical threshold, which was partially reversed by tDCS (isolated or combined with pregabalin) (one way ANOVA/SNK, $P<0.001$). There was no difference between the groups in the percentage of arm entries ($P>0.05$) and percentage of time spent in the open arms ($P>0.05$). There was an increase in grooming time ($P=0.03$), and a decrease in NPHD ($P=0.03$) and rearing ($P<0.01$); while the grooming increase was reversed by tDCS, the NPHD decrease was reversed by pregabalin. There was a decrease in serum BDNF levels, which was reversed by tDCS (one-way ANOVA/SNK, $P<0.01$). In summary, considering the translational aspect, our findings, in a rat fibromyalgia-like model in rats, suggest that tDCS can be a potential non-pharmacologic treatment to fibromyalgia. However, in this study, no synergic effect between tDCS and pregabalin was observed.
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	Tetsuo Kiso

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FIBROMYALGIA-LIKE ANIMAL MODEL DECREASES SERUM BDNF LEVELS OF MALE WISTAR RATS

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