UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS:
FARMACOLOGIA E TERAPÊUTICA

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ESTIMULAÇÃO MAGNÉTICA TRANSCRANIANA REPETITIVA EM MODELO ANIMAL DE DOR NEUROPÁTICA

PORTO ALEGRE
2021

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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 Doutor em Farmacologia e Terapêutica.

Orientadora: Prof. Dra. Iraci LS Torres
Coorientador: Dr. Dirson J Stein

PORTO ALEGRE
2021

CIP - Catalogação na Publicação

Toledo, Roberta Ströher
ESTIMULAÇÃO MAGNÉTICA TRANSCRANIANA REPETITIVA EM MODELO ANIMAL DE DOR NEUROPÁTICA / Roberta Ströher Toledo. -- 2021.
86 f.
Orientadora: Iraci Lucena da Silva Torres.

Coorientador: Dirson João Stein.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Instituto de Ciências Básicas da Saúde, Programa de Pós-Graduação em Ciências Biológicas: Farmacologia e Terapêutica, Porto Alegre, BR-RS, 2021.

1. Dor neuropática. 2. Estimulação magnética transcraniana. 3. Ratos. I. Torres, Iraci Lucena da Silva, orient. II. Stein, Dirson João, coorient. III. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

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"Se não puder voar, corra.
Se não puder correr, ande.
Se não puder andar, rasteje,
mas continue em frente de qualquer jeito."

Martin Luther King Jr

Aos meus pais,

Ao meu grande amor William,

Ao meu filho Tomás,

À minha filha Antonella que está a caminho.

AGRADECIMENTOS

Agradeço à minha professora orientadora Dra. Iraci Lucena da Silva Torres, pela paciência, confiança e dedicação por toda essa jornada, estando ao meu lado desde o início na minha caminhada pela pós-graduação.

Ao meu coorientador e amigo Dirson J Stein, cruzando nossos caminhos mais uma vez, agora na pós-graduação! Obrigada pelas contribuições nesse trabalho e pelo conhecimento compartilhado.

À professora Dra. Vera Maria Vieira Paniz pelos ensinamentos e confiança depositados no início de minha formação profissional, da qual serei eternamente grata.

Aos professores do Programa de Pós-Graduação em Ciências Biológicas: Farmacologia e Terapêutica/UFRGS pelos ensinamentos compartilhados durante toda minha formação na pós-graduação.

Aos queridos colegas, amigos e parceiros Dra. Helouise R Medeiros, Ms. Josimar M de Castro e Dr. José Antônio Fagundes Assumpção que foram fundamentais para a realização desse trabalho.

Agradecimento especial às amigas Dra. Carla de Oliveira, Dra. Vanessa Leal Scarabelot, Dra. Gabriela Gregory Regner e Dra. Fernanda dos Santos Pereira, amizades que a pós-graduação me presentou e que hoje são essenciais em minha vida. Agradeço imensamente minha amiga Ms. Lisiane Santos Silva pelo todo suporte, companhia e dedicação nessa trajetória. O melhor "Robin" que poderia ter estado comigo neste caminho. Esse trabalho é nosso!

Agradeço também as queridas alunas de Iniciação Científica Mayra A. de Souza Antunes e Thais Moraes por todo suporte, interesse e dedicação neste trabalho.

Aos demais colegas e amigos do Laboratório de Farmacologia da Dor e Neuromodulação: Investigações Pré-clínicas pelo convívio e contribuições ao longo de todo o período da minha pós-graduação.

Ao Grupo de Pesquisa e Pós-Graduação do Hospital de Clínicas de Porto Alegre – GPPG/HCPA, pelo apoio financeiro para o desenvolvimento do projeto (2017-0438) e à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela concessão de bolsa de doutorado durante minha formação.

À Unidade de Experimentação Animal (UEA), Unidade de Análises Moleculares e Proteína (UAMP), Laboratório de Hepatologia do Hospital de Clínicas de Porto Alegre

onde foram desenvolvidas as diversas etapas desse trabalho. Um agradecimento particular a toda equipe do Serviço de Pesquisa e Desenvolvimento em Engenharia Biomédica/HCPA, especialmente ao Dr. Paulo Roberto Stefani Sanches por toda dedicação, proatividade e paciência depositados neste estudo.

À minha família, meus irmãos Rafael e Christiano Ströher e especialmente aos meus pais, Roberto Fischer Ströher e Maria Silvia Ströher, pelo amor, incentivo, investimento e apoio incondicional nesta longa jornada.

Ao meu grande amor William de Aguiar Toledo pelo companheirismo, incentivo e compreensão ao decorrer de todos esses anos.

Aos meus filhos Tomás Ströher Toledo e Antonella Ströher Toledo, minhas inspirações! Tomás chegou às nossas vidas durante este doutorado e em meio a pandemia. Antonella está a caminho neste momento histórico e conturbado. Eles são hoje o maior motivo de todas nossas alegrias e responsáveis por passarmos por este momento tão difícil de forma um pouco mais leve.

Por fim, agradeço a todos que, diretamente ou indiretamente, fizeram parte de minha formação e que me incentivaram durante toda pós-graduação. Muito obrigada!

RESUMO

Dor neuropática (DN) está relacionada à presença de hiperalgesia, alodinia e dor espontânea, afetando de 7% a 10% da população geral. É uma dor de difícil tratamento, sendo necessário a busca de novas opções terapêuticas. Neste contexto, a estimulação magnética transcraniana repetitiva (EMTr) vem sendo aplicada para o alívio da DN, especialmente em pacientes com dor refratária. Considerando a relevância do tema, o objetivo desta tese foi avaliar o efeito do tratamento com EMTr sobre a resposta nociceptiva e a memória de longo prazo de ratos submetidos a um modelo de DN, além de avaliar seus efeitos nos níveis centrais de neurotrofinas e citocinas pró e anti-inflamatórias. Para tanto, foram utilizados 106 ratos Wistar machos adultos (60 dias de idade), divididos em 9 grupos experimentais: controle (C), controle + sham EMTr (C+s.EMTr), controle + EMTr ativo (C+EMTr), sham dor neuropática (s.DN), sham dor neuropática + sham EMTr (s.DN+s. EMTr), sham dor neuropática + EMTr ativo (s.DN+EMTr), dor neuropática (DN), dor neuropática + sham EMTr (DN+s. EMTr) e dor neuropática + EMTr ativo (DN+EMTr). O estabelecimento do modelo de DN ocorreu 14 dias após a cirurgia de constrição crônica do nervo isquiático (CCI). A partir disso, os ratos foram tratados com sessões diárias de 5 minutos de EMTr por 8 dias consecutivos. As respostas de hiperalgesia térmica e alodinia mecânica foram avaliadas antes do procedimento cirúrgico, após o estabelecimento da DN e 24h após o término do tratamento, por meio dos testes da Placa Quente (PQ) e von Frey (VF). A memória de longo prazo foi avaliada pelo teste de reconhecimento de objetos, 48h após o término do tratamento. Em seguida, os animais foram eutanasiados e estruturas foram coletadas para análises bioquímicas. Os ensaios bioquímicos (níveis de BDNF, TNF-α e IL-10) foram realizados em homogenatos de córtex pré-frontal (CPF), medula espinhal e hipocampo. O tratamento com EMTr promoveu reversão total da hiperalgesia térmica (Placa Quente) induzida pelo modelo de DN e reversão parcial da alodinia mecânica (Von-Frey). Além disso, a EMTr aumentou os níveis de BDNF e TNF-α no CPF e reverteu a diminuição dos níveis hipocampais de IL-10 nos animais com DN. Complementarmente, o tratamento com EMTr reverteu o déficit na memória de longo prazo induzido pela DN. Desta forma, a EMTr apresenta-se como uma ferramenta promissora para o manejo da DN, e as alterações induzidas pela estimulação parecem ser estado-dependente, ou seja, EMTr induz alterações em parâmetros neuroquímicos, modulando a neuroplasticidade, apenas nos animais com um estado mal adaptativo como a DN, aumentando o limiar

nociceptivo e recuperando a memória de longo prazo desses animais.

Palavras-chave: ratos, dor crônica, neuromodulação, memória, nocicepção, interleucina, neurotrofina.

ABSTRACT

Neuropathic pain (NP) is related to the presence of hyperalgesia, allodynia, and spontaneous pain, affecting 7% to 10% of the general population. The management of NP is difficult, demanding new therapeutic options. In this context, repetitive transcranial magnetic stimulation (rTMS) has been applied for NP relief, especially in patients with refractory pain. Considering the relevance of this theme, the aim of the present thesis was to evaluate the effects of rTMS treatment on the nociceptive response and long-term memory of rats in a NP model, and to evaluate its effects on central levels of neurotrophins and pro and anti-inflammatory cytokines. For this purpose, 106 adult male Wistar rats (60 days old) were used, divided into 9 experimental groups: control(C), control + sham rTMS (C+s.rTMS), control + active rTMS (C+ rTMS), sham neuropathic pain (s.NP), sham neuropathic pain + sham rTMS (s.NP+s.rTMS), sham neuropathic pain + active rTMS (s.NP+rTMS), neuropathic pain (NP), neuropathic pain + sham rTMS (NP+s.rTMS) and neuropathic pain + active rTMS (NP+rTMS). The establishment of the NP model occurred 14 days after surgery for chronic constriction injury (CCI) of the sciatic nerve. Thereafter, the rats were submitted to daily 5-minute rTMS sections during 8 consecutive days. The responses of thermal hyperalgesia and mechanical allodynia were evaluated before the surgical procedure, after the establishment of NP and after treatment, respectively, by the hot plate and von-Frey tests, 24h after the end of the treatment. Long-term memory was assessed by the object recognition test, 48 hours after the end of treatment. After, animals were killed, and the structures collected for biochemical assays. BDNF, TNF-α and IL-10 levels were performed in prefrontal cortex (PFC), spinal cord and hippocampus homogenates. Treatment with rTMS promoted total reversal of thermal hyperalgesia (hot plate) and partial reversal of mechanical allodynia (von-Frey) induced by the NP model. Furthermore, rTMS increased BDNF and TNF-α levels in the PFC and reversed the decrease in hippocampal IL-10 levels in animals with NP. In addition, rTMS treatment reversed the long-term memory deficit induced by NP. Thus, rTMS presents itself as a promising tool for the management of NP; changes induced by the stimulation seem to be state-dependent, that is, rTMS induces changes in neurochemical parameters, modulating neuroplasticity, only in animals with a maladaptive state (i.e. NP), increasing nociceptive thresholds and long-term memory restoration in these animals.

Keywords: rats, chronic pain, neuromodulation, memory, nociception, interleukin, neurotrophin.

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LISTA DE ABREVIATURAS

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

AMPA - amino-3-hidroxi-5-metil-4 isoxazolproprionato

ANVISA- Agência Nacional de Vigilância Sanitária

AVC – acidente vascular cerebral

AVE- acidente vascular encefálico

BDNF – fator neurotrófico derivado do encéfalo (do inglês, *brain-derived neurotrophic factor*)

CCI – constrição crônica do isquiático (nervo)

CPF – córtex pré-frontal

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

EMT- estimulação magnética transcraniana

EMTr- estimulação magnética transcraniana repetitiva

ETCC- estimulação transcraniana por corrente contínua

GABA- ácido gama-aminobutírico

GPPG – Grupo de Pesquisa e Pós-Graduação

HCPA – Hospital de Clínicas de Porto Alegre

IASP - associação internacional para estudos da dor (do inglês, *International Association for the Study of Pain*)

IFN-γ – interferon gama

IL1β – interleucina 1 beta

IL-10 – Interleucina 10

LTM- memória de longo prazo (do inglês, *long-term memory*)

NGF– fator de crescimento neuronal (do inglês, *nerve growth factor*)

NMDA- N-metil-D-aspartato (receptor ionotrópico)

ORT- teste de reconhecimento de objetos (do inglês *object recognition test*)

PFC- córtex pré-frontal (do inglês, *prefrontal cortex*)

PQ- placa quente

rTMS- estimulação magnética transcraniana repetitiva (do inglês, *repetitive transcranial magnetic stimulation*)

SNC – sistema nervoso central

SNK- Student-Newman-Keuls (teste estatístico)

tDCS- estimulação transcraniana por corrente contínua (do inglês, *transcranial direct current stimulation*)

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

TNF-α – fator de necrose tumoral alfa (do inglês, *tumor necrosis factor alpha*)

TRO – teste do reconhecimento de objetos

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 e Discussão na forma de dois artigos científicos: o primeiro demonstrando os efeitos da estimulação magnética transcraniana repetitiva (EMTr) sobre a nocicepção e parâmetros neuroquímicos (BDNF, TNF-α e IL-10) em ratos submetidos ao modelo de dor neuropática (publicado na revista *Brain Research*); e o segundo avaliando os efeitos da EMTr sobre o comportamento de reconhecimento de objetos e níveis de BDNF e IL-10 hipocampais de ratos submetidos ao modelo de dor neuropática (publicado na revista *Neuroscience*).

  - **Artigo 1:** *rTMS induces analgesia and modulates neuroinflammation and neuroplasticity in neuropathic pain model rats.*

  - **Artigo 2**: *Repetitive transcranial magnetic stimulation (rTMS) reverses the long-term memory impairment and the decrease of hippocampal interleukin-10 levels, both induced by neuropathic pain in rats.*

- **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.

PARTE I

INTRODUÇÃO

1 INTRODUÇÃO

Dor neuropática (DN) se refere à disfunção do sistema nervoso somatossensorial e é caracterizada pela presença de hiperalgesia, alodinia e dor espontânea (Nishikawa e Nomoto 2017), acometendo cerca de 7 a 10% da população em geral. Sua incidência tem aumentado principalmente devido ao envelhecimento da população global, a maior incidência de doenças como diabetes mellitus e a maior sobrevida ao câncer após a quimioterapia (Colloca et al. 2017).

Apesar do controle da dor ser uma prioridade em pacientes com DN, ainda existem limitações quanto ao seu manejo, como dificuldades de avaliação, relato de dor subestimado, uso inadequado de analgésicos e anti-inflamatórios e efeitos adversos dos tratamentos farmacológicos (Bruguerolle e Labrecque 2007; Nishikawa e Nomoto 2017). Entre as principais opções de tratamento farmacológico da DN estão a pregabalina (análogo GABA), a gabapentina (alta afinidade pela subunidade alfa-2-delta dos canais de cálcio voltagem-dependentes), a duloxetina (inibidor da recaptação da serotonina-noradrenalina) e antidepressivos tricíclicos como recomendação de primeira linha no tratamento da DN periférica e central (Collaca et al. 2017). A abordagem pode incluir também o uso de adesivos de capsaicina e de lidocaína (Kraychete et al. 2016). Fármacos opioides não são primeira escolha no manejo da DN, devido principalmente a baixa eficácia e risco de dependência (Rosenblum et al. 2008). No entanto, em torno de 50% dos pacientes apresentam melhora significativa com estes tratamentos, além da ocorrência de efeitos adversos, que limitam a adesão ao tratamento (Finnerup et al. 2015).

Considerando essas limitações no tratamento da DN tem sido investigado, tanto em estudos pré-clínicos quanto clínicos, novas terapias não farmacológicas que possam complementar ou substituir o tratamento farmacológico. Entre os métodos alternativos para o tratamento de quadros dolorosos estão as técnicas de neuromodulação não-invasivas. A estimulação transcraniana por corrente contínua (ETCC) e a estimulação magnética transcraniana repetitiva (EMTr) têm demonstrado bons resultados no tratamento de doenças relacionadas à plasticidade cerebral mal adaptativa como a DN (Ngernyam et al. 2013; 2015; Leung et al. 2009; S. Yang e Chang 2020), incluindo trabalhos realizados em nosso grupo de pesquisa, que tem avaliado os benefícios e efeitos da ETCC na DN em estudos pré-clínicos (Laste et al. 2012; Cioato et al. 2016; Santos et al. 2020; Lopes et al. 2021). Porém, este trabalho representa o primeiro estudo utilizando a EMTr no manejo da DN em nosso grupo. Salientamos que o equipamento de

estimulação magnética foi desenvolvido em parceria com a equipe de engenharia biomédica do Hospital de Clínicas de Porto Alegre (HCPA), o que permitiu o emprego desta ferramenta não-farmacológica em animais (ratos Wistar).

Os mecanismos pelos quais as técnicas neuromodulatórias atuam ainda não foram totalmente elucidados, especialmente quando são empregadas no tratamento da DN. Por este motivo, marcadores neuroquímicos tem sido alvo de estudo, tais como o fator neurotrófico derivado do encéfalo (BDNF) e citocinas pró e anti-inflamatórias, como o fator de necrose tumoral (TNF-α) e interleucina 10 (IL-10).

O BDNF possui um papel importante na plasticidade sináptica: no corno dorsal da medula espinhal, sinaliza a informação nociceptiva (Miletic e Miletic 2002). Ele atua como um modulador da resposta nociceptiva após lesão de nervo, desempenhando papel importante no desenvolvimento da DN (Fukuoka et al. 2001). Além disso, os níveis de BDNF aparecem alterados após tratamentos com EMT e ETCC (Dall'Agnol et al. 2014; Ganguly e Poo 2013) e por essa razão, esta neurotrofina tem sido utilizada como um biomarcador de excitabilidade cortical e de plasticidade neuronal (Soltész et al. 2014).

As citocinas também apresentam atividade no processo nociceptivo (Ren e Torres 2009; Uçeyler, Schäfers, e Sommer 2009). Dentre elas, a IL-1β e o TNF-α, liberados pela glia, regulam o desenvolvimento e a plasticidade de circuitos neuronais e vias nociceptivas (Deverman e Patterson 2009; Schäfers, Sorkin, e Sommer 2003; McMahon e Malcangio 2009; Ren e Torres 2009). As evidências indicam que a hiperalgesia induzida pelo TNF-α pode estar relacionada à indução de outros mediadores inflamatórios (Schäfers, Sorkin, e Sommer 2003; Woolf et al. 1997; Claudia Sommer e Kress 2004). Além disso, a IL-10 tem um importante papel antinociceptivo na DN, reduzindo a hipersensibilidade da dor induzida por lesão nervosa (Austin e Moalem-Taylor 2010). Estudos pré-clínicos têm demonstrado que o sucesso do tratamento com EMTr em outras patologias, como na esclerose múltipla, acidente vascular encefálico e doença de Parkinson, pode ter contribuição do aumento dos níveis de IL-10 nos animais ativamente estimulados (L. Yang et al. 2020; Hong et al. 2020; J. Y. Lee et al. 2020). Por outro lado, em um estudo clínico com pacientes idosos com depressão refratária e tratados com EMTr apresentaram uma redução de marcadores inflamatórios, como IL-1β e TNF-α, melhorando os sintomas da doença (Zhao et al. 2019).

Dores crônicas, como a DN, podem afetar a função cognitiva, alterando a atenção, dificultando a execução de tarefas cognitivas e de memória (Berryman et al. 2013;

Moriarty, McGuire, e Finn 2011). Estudos têm demonstrado declínio tanto na memória de trabalho quanto no desempenho da memória de longo prazo em pacientes com dores crônicas, incluindo a DN (Mazza, Frot, e Rey 2018; Jacobsen et al. 2021). Tem sido sugerida dor crônica como uma consequência mal adaptativa de mecanismos de memória como por exemplo a LTP (do inglês, *long term potentiation*) (Mazza, Frot, e Rey 2018). Outros estudos sugerem que o declínio da memória em pacientes com dor crônica está associado à intensidade da dor (Hart, Martelli, e Zasler 2000). Porém, foi demonstrado que o alívio da dor nestes pacientes não melhorou a função cognitiva (Dick e Rashiq 2007). Portanto, os mecanismos pelos quais esses os pacientes apresentam declínio na memória ainda estão sendo investigados, incluindo a possível contribuição de mediadores neuroquímicos envolvidos na dor crônica (Hart, Martelli, e Zasler 2000).

Apesar disso, existem poucos estudos utilizando EMTr em animais, especialmente envolvendo o manejo da DN. Isso se deve principalmente ao fato da dificuldade de aplicação da técnica, especialmente em animais não anestesiados. Embora existam evidências dos efeitos terapêuticos da EMTr, os seus mecanismos de ação não estão totalmente elucidados, o que justifica a necessidade de estudos pré-clínicos utilizando essa técnica. Sendo assim, o objetivo desta tese foi avaliar os efeitos da EMTr sobre a alodinia, hiperalgesia e alterações na memória de longo prazo em ratos submetidos ao modelo de DN, assim como, verificar as possíveis alterações de níveis de marcadores neuroquímicos nesses animais.

REVISÃO DA LITERATURA

2 REVISÃO DA LITERATURA

2.1. DOR NEUROPÁTICA (DN)

Dor é uma experiência multidimensional de caráter subjetivo e sua percepção sofre influência de fatores internos e externos (Sá et al. 2009), e é classificada temporalmente em dor aguda e crônica. A primeira é evolutivamente vantajosa, uma vez que é um alerta de dano promovendo a evitação de estímulos danosos. Já a dor crônica, está dissociada das ameaças reais, permanece por mais de 3 meses e ela própria é a doença. Pacientes com dor crônica moderada a intensa tem redução na qualidade de vida, apresentando dificuldade em realizar atividades profissionais e sociais, frequentemente associadas a quadros depressivos (Treister et al. 2013). Um exemplo de dor crônica é a DN, causada por doenças ou lesões que danificam as vias neurais sensíveis à dor periférica ou central, promovendo disparos sinápticos inadequados desencadeando a dor sem causa definida (Treister et al. 2013). Esse tipo de dor pode ser sentida distante da lesão ou doença do sistema nervoso (ex.: na perna e/ou pé, em pacientes com compressão radicular, ou dor fantasma em pessoas com membro amputado) (DeSantana et al. 2020).

De acordo com a última atualização da Associação Internacional para Estudos da Dor (IASP, da sigla em inglês), dor e nocicepção são fenômenos diferentes. O processo nociceptivo refere-se à resposta do sistema nervoso a um estímulo nocivo, enquanto a dor é uma resposta subjetiva e modificada pelas experiências de vida do indivíduo (DeSantana et al. 2020). Ainda de acordo com a IASP, a fisiopatologia da dor pode ser nociceptiva, neuropática e nociplástica. Esta última desempenha um papel nas condições de dor crônica como fibromialgia, dor lombar, e dor de cabeça (Kosek et al. 2016; DeSantana et al. 2020).

Alodinia e hiperalgesia são sintomas característicos da DN. A primeira refere-se a resposta dolorosa a um estímulo não nocivo, enquanto a hiperalgesia se caracteriza por resposta nociceptiva exacerbada a um estímulo nocivo. A sensibilização periférica e central mal adaptativas contribuem para a geração e manutenção destes sintomas (Jensen e Finnerup 2014). Esta tese, um estudo pré-clínico, utilizou testes comportamentais nociceptivos a fim de avaliar esses sintomas: teste do Von-Frey para alodinia mecânica, e Placa Quente para hiperalgesia térmica. Alguns estudos na literatura referem-se ao teste do Von-Frey também para avaliação da hiperalgesia mecânica (Lambert, Mallos, e Zagami 2009; Ameyaw et al. 2014). Porém, por acreditarmos que o estímulo deste teste

não é nocivo, neste trabalho iremos utilizar o termo "alodinia" para o teste do Von-Frey (Pitcher e Henry 2000; Chung 2013; Li et al. 2020). Ademais, maiores informações sobre os testes serão abordadas no item 2.4 desta revisão da literatura.

2.2 MODELO DE DN EM ROEDORES

Em estudos pré-clínicos, especialmente em roedores, os modelos animais de DN envolvem em sua maioria a lesão de nervos periféricos ou centrais, além de modelos citotóxicos. Os modelos de lesão de nervos periféricos incluem axotomia, ligadura parcial ou constrição crônica do nervo isquiático, ligadura das raízes espinhais e lesão poupadora de nervo. Enquanto isso, modelos centrais envolvem contusões compressivas e modelos citotóxicos incluem a neuropatia diabética induzida por estreptozocina, quimioterápicos e álcool (Sousa et al. 2016; Burma et al. 2017).

Em 1979, Wall et al. descreveram o primeiro modelo animal de neuropatia dolorosa. Neste procedimento, foram seccionados os nervos safeno e isquiático do rato e o coto proximal foi amarrado, deixando a pata traseira completamente desenervada, ficando conhecido como o modelo de neuroma. Este procedimento mimetiza as síndromes humanas vistas após uma amputação (dor fantasma) ou após a transecção do nervo em um membro intacto (anestesia dolorosa). É importante salientar que após alguns dias da indução deste modelo, os animais passam a apresentar comportamento de automutilação da pata onde é realizada a secção do nervo (comportamento que os autores chamaram de "autotomia" em resposta a dor espontânea ou "disestesia"), sendo a principal limitação desta técnica (Bennett 1993).

Em 1988 foi proposto por Bennet e Xie um modelo onde os animais apresentavam, além da dor espontânea, a presença de alodinia e hiperalgesia. Neste modelo, o nervo isquiático do animal é exposto e nele são feitas amarraduras que induzem a formação de um edema intraneural, conhecido como modelo de constrição crônica do nervo isquiático (CCI) (Bennett e Xie 1988; Bennett 1993) **(Figura 1)**. Nesta técnica os animais não apresentam o comportamento de autotomia, e devido a presença de hiperalgesia e alodinia, foi o modelo animal de DN utilizado nesta tese.

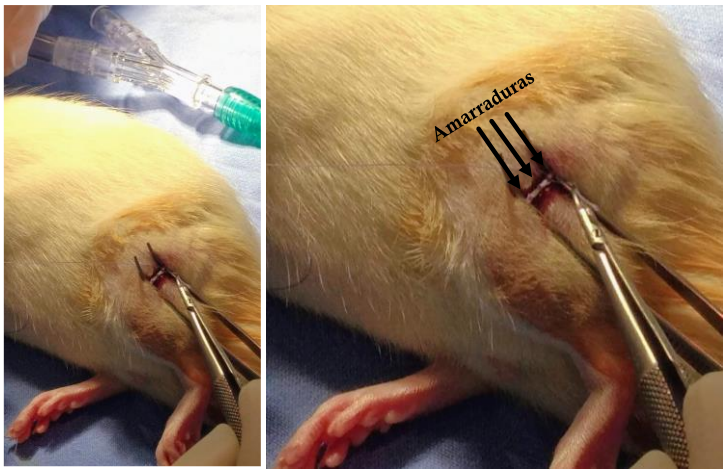

Figura 1. Cirurgia de constrição crônica do nervo isquiático (CCI). Imagem demonstrando três amarraduras ao redor do nervo para estabelecimento da DN. Créditos da imagem: Roberta S Toledo.

2.3 DN E ÁREAS DE INTERESSE

A informação de percepção dolorosa está comprometida em pacientes com DN, especialmente na transdução, etapa onde ocorre a conversão de um estímulo químico em um impulso elétrico. Neurônios de segunda ordem que se originam na medula espinhal transmitem estímulos nociceptivos ao tálamo por meio de vias ascendentes, onde o trato espinotalâmico retransmite a informação para os centros corticais superiores, incluindo o córtex cingulado anterior (envolvido na ansiedade, antecipação da dor, atenção à dor e respostas motoras), o córtex insular (papel no sistema sensorial aspectos discriminativos e afetivos da dor), o córtex pré-frontal (importante para os sentidos de integração, tomada de decisão, recuperação de memória e processamento da atenção em relação à dor), o núcleo accumbens - relacionado ao efeito placebo em alguns estudos-, a amígdala e o hipocampo e outras partes do sistema límbico (envolvidos na formação e armazenamento de memórias associadas a eventos emocionais, afeto, excitação e atenção à dor e ao aprendizado) (Cohen e Mao 2014).

Mecanismos periféricos também podem estar presentes na DN. A sensibilização periférica pode ser causada por diversos fatores, incluindo mediadores inflamatórios, como a calcitonina e a substância P, que aumentam a permeabilidade vascular, induzindo edema localizado, recrutamento de prostaglandinas, bradicinina, fatores de crescimento e citocinas, levando a sensibilização dos nociceptores (Cohen e Mao 2014).

A maioria dos estudos utilizando técnicas neuromodulatórias para manejo da DN tem como alvo de estimulação, regiões do córtex pré-frontal dorsolateral e córtex motor primário (M1) (Graff-Guerrero et al. 2005; Fregni et al 2007). O córtex pré-frontal dorsolateral é uma região funcional e estruturalmente heterogênea, implicado no processamento cognitivo, afetivo e sensorial (Seminowicz e Moayedi 2017). Na dor crônica, sua função parece estar alterada, apresentando redução da sua massa cinzenta quando comparada a indivíduos saudáveis (Davis e Moayedi 2013). Já o córtex M1 apresenta conexões com alguns dos núcleos de retransmissão sensorial no tálamo e com fibras eferentes e aferentes na medula espinhal responsáveis pela transmissão de estímulos dolorosos e modulação da resposta motora ao contato nocivo. Na dor crônica, pacientes apresentam uma plasticidade mal adaptativa desta região, e a modulação desta área induz analgesia (Saavedra et al 2014).

2.4 ESTIMULAÇÃO MAGNÉTICA TRANSCRANIANA REPETITIVA (EMTr)

EMT se refere a uma técnica neuromodulatória não invasiva que utiliza um campo magnético transiente gerado por uma bobina sobre o escalpo, que é capaz de gerar uma corrente elétrica e estimular regiões do encéfalo (Ni e Chen 2008) (**Figura 2**). Dependendo do tipo de estimulação a ser utilizado (pulsos únicos ou repetitivos) a EMT pode ter finalidades diferentes: diagnóstico ou terapêutico (Müller et al. 2013). EMT com pulso único ou com pares de pulsos, são métodos utilizados predominantemente para diagnóstico, principalmente para doenças neurodegenerativas (ex, doença do neurônio motor), acidente vascular encefálico, mielopatia compressiva e esclerose múltipla, por meio da representação do potencial evocado motor e do período silente (Groppa et al. 2012). Já com finalidades terapêuticas, a EMT com pulsos repetitivos vem ganhando cada vez mais destaque. Nela, são emitidos vários pulsos seguidamente de acordo com a frequência determinada: baixa frequência ($\leq$ 1 Hz) leva à diminuição da excitabilidade neuronal inibindo a atividade cortical; alta frequência (> 1 Hz, podendo chegar até 60 Hz), produz um efeito contrário, aumentando a excitabilidade neuronal e a estimulação da atividade cortical (Müller et al. 2013). Atualmente, outras modalidades de estímulo vêm sendo aplicadas, como *theta burst* contínua ou intermitente, que apresentam como principal vantagem, menor tempo de sessão em relação a EMTr convencional. A variação de estímulo intermitente está relacionada a estimulação excitatória, enquanto a contínua, a estimulação inibitória (Sanna et al. 2019). Há também a estimulação “quadripulsada”,

que dependendo do intervalo entre os estímulos, apresenta efeitos diferentes: intervalos curtos estão relacionados ao aumento na excitabilidade cortical enquanto intervalos longos com a diminuição na excitabilidade corticoespinhal (Jung et al. 2016).

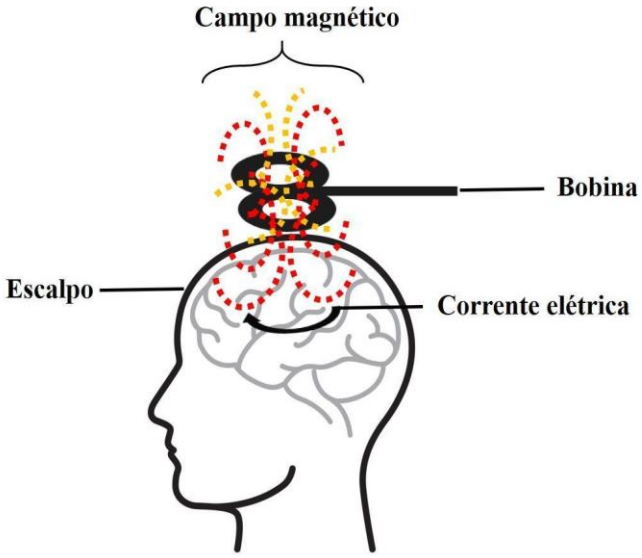

Figura 2. Princípios da EMTr. Desenho esquemático demonstrando a indução da corrente elétrica por meio de um campo magnético gerado pela bobina (Adaptado de (Ridding e Rothwell 2007).

Além do tipo de frequência a ser utilizada, o local de estimulação também é um ponto importante a ser observado na EMTr com finalidades terapêuticas. Estimular o córtex motor primário (M1) geralmente fornece o melhor alívio da dor, enquanto para a depressão, por exemplo, a região mais adequada é o córtex pré-frontal dorsolateral (Treister et al. 2013). A estimulação desta área em pacientes com DN tem demonstrado resultados promissores (Cruccu et al. 2007; Leo e Latif 2007; Leung et al. 2009). Recentemente, foi publicada uma diretriz baseada em evidência, onde a EMTr de alta frequência sob o córtex motor primário (M1) contralateral ao lado doloroso, apresenta nível A de eficácia para o tratamento da DN (Lefaucheur et al. 2020).

Em 1985, a EMT foi primeiramente descrita para uso médico no Reino Unido por Barker e colaboradores (Barker, Jalinous, e Freeston 1985). A *Food and Drug Administration* dos Estados Unidos concedeu aprovação a este método em 2008 para o tratamento da depressão (Noohi e Amirsalari 2016). Já no Brasil, a Agência Nacional de Vigilância Sanitária (ANVISA) regulamentou o uso do aparelho de Estimulação Magnética Transcraniana em março de 2006 e o uso clínico da EMTr foi reconhecido pelo Conselho Federal de Medicina para o tratamento da depressão e alucinações

auditivas relacionadas a esquizofrenia somente em 2011 (CFM n° 37/11).

Para este estudo pré-clínico, o aparelho de EMTr e as bobinas no formato de "borboleta" foram desenvolvidos em parceria com a Equipe de Engenharia Biomédica do Hospital de Clínicas de Porto Alegre (**Figura 3**). O dispositivo gera pulsos com ciclo de trabalho de 1ms na frequência de 1 Hz e com intensidade do campo magnético de 200 mT (militesla).

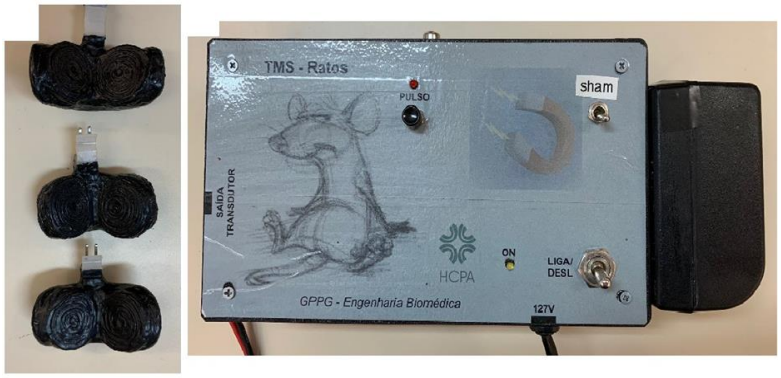

Figura 3. Aparelho de EMTr para ratos. Imagem demonstrando as bobinas no formato de "borboletas" utilizadas no experimento (lado esquerdo) e o equipamento de EMTr (lado direito). Créditos da imagem: Paulo RS Sanches.

2.5 TESTES NOCICEPTIVOS E AVALIAÇÃO DE MEMÓRIA DE LONGO-PRAZO EM RATOS

Animais, especialmente roedores, são utilizados em experimentos para estudo da fisiopatologia da dor devido às limitações éticas de experimentos em humanos (Deuis, Dvorakova, e Vetter 2017). A dor não pode ser diretamente medida em animais: por este motivo, é dito que se avalia o "comportamento do tipo dor" ou a nocicepção. Estes testes utilizam estímulos elétricos, térmicos, mecânicos ou até mesmo químicos (Le Bars, Gozariu, e Cadden 2001). Também, os métodos de avaliação comportamental podem ser evocados ou não por estímulo (espontâneo), dependendo se há ou não a aplicação de um estímulo externo para provocar uma resposta reflexa ao estímulo nocivo. Dentre as metodologias que utilizam estímulos evocados, pode-se citar os testes de Von-Frey, Placa Quente, Hargreaves e Randall-Sellito. Por outro lado, a análise de marcha, comportamento de escavar, e o teste de incapacitação se enquadram em metodologias de avaliação da nocicepção espontânea (Deuis, Dvorakova, e Vetter 2017).

Nesta tese, a avaliação nociceptiva foi realizada por meio de dois testes, ambos evocados por estímulo nocivo externo: Placa Quente e Von-Frey eletrônico. No primeiro

teste, o animal é colocado em uma superfície de metal **(Figura 4)** mantida a uma temperatura constante (±55°C) e avalia-se o tempo necessário (em segundos) para o comportamento de retirada da pata (sapateado), sons agudos elevados e/ou lamber as patas.

Figura 4. Aparato onde é realizado o teste da Placa Quente. A superfície metálica mantém a temperatura constante de ±55°C. Créditos da imagem: Roberta S Toledo.

Este teste de estímulo térmico avalia a hiperalgesia e envolve dor tônica, sendo que os estímulos de longa duração desencadeiam uma resposta nociceptiva envolvendo principalmente fibras do tipo C (Le Bars, Gozariu, e Cadden 2001). Além disso, há integração de vias supra espinhais, uma vez que ratos com transecção espinhal não apresentam reflexo de sapateado ou retirada das patas posteriores quando submetidos ao teste (Giglio et al. 2006).

Para o teste de Von-Frey, os animais são colocados em uma pequena gaiola com piso gradeado **(Figura 5 – A e B).** O estímulo mecânico externo ocorre por meio de um filamento que exerce uma força (em gramas) sobre a pata do animal **(Figura 5 - C)**. Essa força é aumentada gradativamente, até que ocorra a retirada da pata. O registro da força exercida até o momento do reflexo de retirada é detectado pelo aparelho (Deuis, Dvorakova, e Vetter 2017).

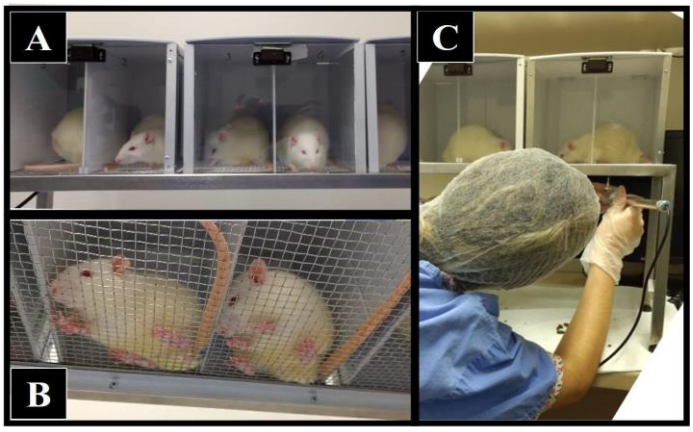

Figura 5. Teste do Von-Frey. A- Gaiola onde os animais são dispostos durante o teste. **B-** Visualização da base gradeada da caixa. **C-** Estímulo na pata do animal sendo realizado por meio do Von-Frey eletrônico. Créditos da imagem: Roberta S. Toledo.

Neste teste avalia-se a alodinia mecânica decorrente do envolvimento de fibras do tipo Aβ (Millan 1999). Nesta tese, o teste do Von-Frey foi realizado em três momentos: no basal (antes da cirurgia para indução da DN), para randomização dos animais, 14 dias após a cirurgia, para confirmação do estabelecimento da DN e 24h após o final do tratamento com EMTr. Os animais são submetidos a uma avaliação 14 dias após a cirurgia, uma vez que estudos prévios do nosso grupo demonstraram que após este período os animais que foram submetidos à cirurgia *sham* apresentam os mesmos limiares de retirada da pata dos animais controle (sem cirurgia), enquanto os animais submetidos ao modelo cirúrgico efetivo, apresentam limiares nociceptivos diminuídos, demonstrando a alodinia mecânica característica do modelo (Laste et al. 2012; Cioato et al. 2016).

Quanto aos métodos de avaliação de memória utilizados em roedores, um dos mais conhecidos é o teste de reconhecimento de objetos (TRO). Nele avalia-se a memória declarativa dos animais, com a participação de algumas regiões cerebrais que parecem estar envolvidas, tais como o lobo temporal, o lobo frontal e o hipocampo, frequentemente avaliado em pesquisas envolvendo as doenças de Alzheimer e de Parkinson (Magen et al. 2012; Grayson et al. 2015; Bengoetxea, Rodriguez-Perdigon, e Ramirez 2015; Pavia-Collado et al. 2018). É importante salientar que, dependendo do tempo após a fase de aprendizado em que a análise for feita, é possível diferenciar a memória de curto prazo da memória de longo prazo. Este teste tem por vantagem sobre os demais, o fato de que a tarefa é baseada no comportamento espontâneo do animal, sem necessidade de recompensa ou punição, além de ser realizado com uma única fase de aprendizado (Ennaceur e Delacour 1988). Quando roedores são expostos a um objeto familiar ao lado

de um objeto novo, passam naturalmente a explorar por mais tempo o objeto desconhecido. Essa aparente "preferência não condicionada" por um objeto novo indica que o objeto familiar existe na memória do animal, o que fundamenta esse teste para estudo de memória em roedores (Bengoetxea, Rodriguez-Perdigon, e Ramirez 2015).

O teste é dividido em três fases: habituação, familiarização e teste. Na primeira fase (habituação) os animais são colocados no aparato de *open field* e permanecem livres para explorar o local, geralmente por um período de 5 minutos; na próxima fase, os animais são colocados novamente no mesmo aparato, porém contendo dois objetos semelhantes em forma, tamanho e textura, dispostos equidistantemente um do outro **(Figura 6)**. A última fase (teste), é semelhante à fase de familiarização, porém um novo objeto substitui um dos objetos antigos **(Figura 6)**. Os objetos devem ser os mais diferentes possíveis em forma (mas semelhantes em tamanho) e altos o suficiente para que o animal não suba neles (podendo ficar sobre eles sem exploração) (Bengoetxea, Rodriguez-Perdigon, e Ramirez 2015). Uma importante observação é que o intervalo entre a fase de familiarização e a fase teste varia de acordo com o objetivo de se avaliar memória de curto ou longo-prazo. Para estudos de memória de curto prazo, a maioria dos estudos utiliza intervalos entre 2 min a 1 h entre a segunda e terceira fase, enquanto para avaliação da memória de longo prazo o intervalo, na maioria dos estudos, passa a ser de 24 h (Moore et al. 2013; Leffa et al. 2018; 2016; Lueptow 2017; Cruz-Sanchez et al. 2020).

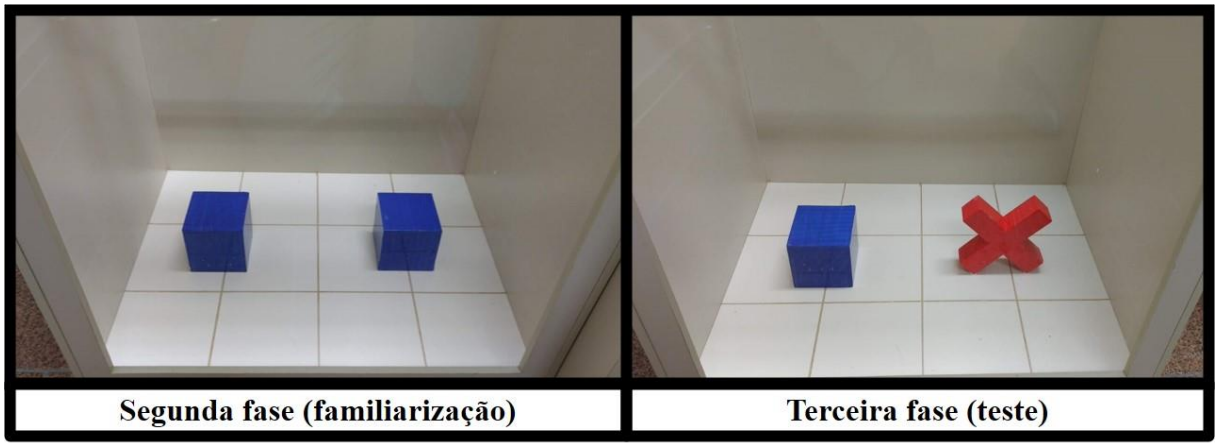

Figura 6. Disposição dos objetos nas fases de familiarização e teste durante o TRO no aparato de *open field*. Créditos da imagem: Roberta S Toledo.

Uma das formas de expressar os resultados deste teste é por meio do índice de discriminação. Ele é definido pela diferença no tempo de exploração entre o objeto novo e o familiar, dividido pelo tempo total de exploração entre os dois objetos. Os resultados

variam entre +1 e -1, onde a pontuação positiva indica mais tempo gasto com o objeto novo, enquanto a pontuação negativa indica mais tempo gasto com o objeto familiar (Bengoetxea, Rodriguez-Perdigon, e Ramirez 2015; Leffa et al. 2016; 2018). Nesta tese utilizamos essa representação para avaliação da memória de longo-prazo (24h de intervalo entre a segunda e terceira fases).

2.6 MARCADORES NEUROQUÍMICOS

Diversos biomarcadores já foram relacionados ao desenvolvimento, manutenção e redução de quadros dolorosos da DN, como neurotrofinas e citocinas pró e anti-inflamatórias.

As neutrofinas, que participam dos processos de sobrevivência e de crescimento neuronal durante o desenvolvimento do sistema nervoso, já demonstraram desempenhar importante papel na transmissão da dor fisiológica e patológica (Obata e Noguchi 2006). Dentre elas, destaca-se o BDNF, sintetizado nos neurônios sensoriais primários, é transportado para os terminais centrais dos aferentes primários no corno dorsal da medula espinhal, envolvendo-se na modulação de estímulos dolorosos (Zhou e Rush 1996; Michael et al. 1997; S. L. Lee et al. 1999). Tem sido hipotetizado que o bloqueio de BDNF nos neurônios sensoriais pode ser uma estratégia no desenvolvimento de analgésicos, uma vez que esta neurotrofina é capaz de contribuir para a hipersensibilidade à dor crônica no gânglio da raiz dorsal e na medula (Obata e Noguchi 2006).

O TNF-α, uma importante citocina pró-inflamatória, é capaz de estimular a produção adicional de outras citocinas, processo associado principalmente à manutenção da DN (Austin e Moalem-Taylor 2010). De fato, diversos estudos demonstram que a utilização de anticorpos ou antagonistas de seus receptores, são capazes de reverter a hiperalgesia em modelos de lesão de nervo periférico (Homma, Brull, e Zhang 2002; Onda, Yabuki, e Kikuchi 2003; C. Sommer, Schmidt, e George 1998). Já existem no mercado medicamentos anti-TNF- α, como o infliximabe e o etanercepte, indicados para doenças inflamatórias, porém são indicados para o tratamento da artrite reumatoide, espondilite anquilosante ativa e psoríase. Apesar desses medicamentos não terem sido ainda validados para o manejo da DN (Austin e Moalem-Taylor 2010), o etanercepte induziu uma redução na hiperalgesia após lesão do nervo em camundongos (C. Sommer et al. 2001).

Dentre as citocinas anti-inflamatórias, a IL-10 possui um importante papel no

processo antinociceptivo. Ela é produzida por células T ativadas, células B, macrófagos e mastócitos, sendo capaz de inibir a liberação de citocinas pró-inflamatórias, como a IL-1β, IL-6 e TNF-α (Kanaan et al. 1998; Poole et al. 1995), o que leva a uma redução no recrutamento e ativação de células gliais no local da lesão e na medula espinhal (Austin e Moalem-Taylor 2010). Por este motivo, a IL-10 humana recombinante já foi utilizada na clínica para tratamento de doenças autoimunes (psoríase e doença de Crohn) (Kimball et al. 2002; Schreiber et al. 2000), e devido a sua atuação como supressor endógeno de inflamação excessiva após lesão nervosa, também pode ser benéfica no tratamento da DN (Austin e Moalem-Taylor 2010).

Tem-se demonstrado que técnicas neuromodulatórias não-invasivas, como a EMTr, podem alterar os níveis de neurotrofinas e interleucinas melhorando o prognóstico de diferentes tipos de doenças. Em pacientes idosos com diagnóstico de depressão refratária, a EMTr aumentou os níveis séricos de BDNF e diminuiu os níveis de IL-1β e TNF-α apenas em pacientes com a doença, não alterando nenhum desses fatores em indivíduos saudáveis (Zhao et al. 2019). Um recente estudo demonstrou sinergismo da EMTr e o transplante de células-tronco mesenquimais humanas na doença de Parkinson, regulando positivamente a expressão de IL-10 e diminuindo a produção de Interferon gama (IFN-γ) e TNF-α (J. Y. Lee et al. 2020). Acredita-se que a inibição da polarização astrocítica neurotóxica seja um mecanismo potencial para a eficácia da EMTr de alta frequência (10 Hz) no AVC isquêmico cerebral, com a colaboração de IL-10 (Hong et al. 2020). Desta forma, nesta tese espera-se que a EMTr seja capaz de induzir alterações neuroquímicas em animais submetidos ao modelo de DN promovendo a melhora da hiperalgesia e alodinia induzidas pela CCI

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JUSTIFICATIVA E OBJETIVOS

3 JUSTIFICATIVA

A DN está relacionada à presença de hiperalgesia, alodinia e dor espontânea, afetando de 7 a 10% da população geral, contribuindo para diminuição na qualidade de vida do paciente. Seu manejo ainda é considerado um desafio, devido à baixa eficácia dos tratamentos, assim como a ocorrência de efeitos adversos com os fármacos atualmente disponíveis (J. B. Lee et al. 2010). Estima-se que apenas 30 a 40% dos pacientes com dor neuropática apresentam alívio satisfatório da dor (Baron, Binder, e Wasner 2010). Assim sendo, investigações de novas alternativas terapêuticas analgésicas que sejam eficazes e que apresentem menores efeitos adversos para o tratamento da dor neuropática são altamente relevantes. A estimulação magnética transcraniana repetitiva (EMTr) tem sido descrita como uma técnica segura e eficaz, sendo aplicada para alívio da DN, especialmente em pacientes com dor refratária. Apesar disso, existem poucos estudos pré-clínicos que investigam os mecanismos de ação envolvidos neste tratamento, e seus efeitos no SNC tanto no processo nociceptivo quanto na memória declarativa em ratos. Desta forma, esperamos que os achados deste estudo possam contribuir para um melhor entendimento dos mecanismos de ação da EMTr na DN, viabilizando sua utilização para casos específicos de dores crônicas.

4 OBJETIVOS

4 .1 Objetivo geral

Esta tese teve como objetivo geral avaliar o efeito do tratamento de EMTr na memória e em parâmetros nociceptivos e neuroquímicos de ratos machos adultos Wistar submetidos a um modelo de DN.

4.2 Objetivos específicos

Verificar os efeitos da EMTr em ratos submetidos ao modelo de DN sobre:

- a resposta nociceptiva (hiperalgesia) por meio da medida da resposta ao estímulo térmico nocivo (placa quente) nos tempos: basal, 14 dias após a cirurgia do modelo de DN, e 24h após o final do tratamento com EMTr;
- a resposta nociceptiva (alodinia) por meio da medida da resposta ao estímulo mecânico (von Frey) nos tempos: basal, 14 dias após a cirurgia do modelo de DN, e 24h após o final do tratamento com EMTr;
- memória declarativa de longo prazo em roedores por meio do teste de

reconhecimento de objetos;

- os níveis dos marcadores neuroquímicos BDNF, citocinas pró-inflamatória (TNF-α) e anti-inflamatória (IL-10) em córtex pré-frontal e medula espinhal; e BDNF e IL-10 em hipocampo, todos após 48h do final do tratamento com EMTr.

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PARTE II

6 ARTIGOS CIENTÍFICOS

6.1 ARTIGO I

"*rTMS induces analgesia and modulates neuroinflammation and neuroplasticity in neuropathic pain model rats*" - DOI 10.1016/j.brainres.2021.147427.

Periódico: *Brain Research*

Situação: Versão aceita para publicação

rTMS induces analgesia and modulates neuroinflammation and neuroplasticity in neuropathic pain rats

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ABSTRACT

Neuropathic pain (NP) is related to the presence of hyperalgesia, allodynia, and spontaneous pain, affecting 7%–10% of the general population. Repetitive transcranial magnetic stimulation (rTMS) is applied for NP relief, especially in patients with refractory pain. As NP response to existing treatments is often insufficient, we aimed to evaluate rTMS treatment on the nociceptive response of rats submitted to an NP model and its effect on pro-and anti-neuroinflammatory cytokine and neurotrophin levels. A total of 106 adult male Wistar rats (60 days old) were divided into nine experimental groups: control, control+sham rTMS, control+rTMS, sham NP, sham neuropathic pain+sham rTMS, sham neuropathic pain+rTMS, NP, neuropathic pain+sham rTMS, and neuropathic pain+rTMS. NP establishment was achieved 14 days after the surgery to establish chronic constriction injury (CCI) of the sciatic nerve. Rats were treated with 5 min daily sessions of rTMS for eight consecutive days. Nociceptive behavior was assessed using von Frey and hot plate tests at baseline, after NP establishment, and post-treatment. Biochemical assays to assess the levels of brain-derived neurotrophic factor (BDNF), tumor necrosis factor-alpha (TNF-α), and interleukin (IL)-10, were performed in the prefrontal cortex (PFC) and spinal cord tissue homogenates. rTMS treatment promoted a partial reversal of mechanical allodynia and total reversal of thermal hyperalgesia induced by CCI. Moreover, rTMS increased the levels of BDNF, TNF-α, and IL-10 in the PFC. rTMS may be a promising tool for the treatment of NP. The alterations induced by rTMS on neurochemical parameters may have contributed to the analgesic effect presented.

Keywords: BDNF; TNF-α; IL-10; neuromodulation; chronic pain; brain stimulation

1. INTRODUCTION

Neuropathic pain (NP) is related to somatosensory nervous system dysfunction and is characterized by the presence of hyperalgesia, allodynia, and spontaneous pain [1]. NP affects 7%–10% of the general population, and its incidence has been increasing mainly due to the aging global population, increased incidence of diabetes mellitus, and the rise of survival from cancer after chemotherapy [2].

Non-invasive therapies, such as repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS), are being applied for NP pain relief [3], especially in patients with refractory pain [4]. tDCS delivers a low electric current to the brain, while rTMS, the technique applied in the current study, employs a magnetic field delivered by a stimulation coil located over the skull [5]. Both techniques promote the modulation of brain function, inducing neuroplasticity in the central nervous system (CNS) by changing the resting membrane potential and modifying neuronal activity [6].

Our research group has demonstrated that tDCS can reverse hyperalgesia induced by chronic stress [7], chronic inflammatory pain [8], NP [9], and chronic trigeminal pain [10] in rats. However, we have not yet tested rTMS in pain conditions. Preclinical studies have shown that rTMS is an effective rehabilitative approach in rat models of spinal cord injury [11] and traumatic brain injury [12], promoting neuroplasticity.

Low-frequency rTMS has been reported to have inhibitory effects on the brain, contributing to its analgesic effect [13–14]. Several brain regions have been targeted in neuromodulatory studies with both invasive and non-invasive approaches. However, the cerebral cortex is the principal area for neuromodulatory therapies for pain [3]. Studies on the use of rTMS for pain have primarily focused on the primary motor cortex (M1) and/or dorsolateral prefrontal cortex (DLPFC). Both are related to decreasing pain perception in humans, where rTMS has been applied to treat NP, fibromyalgia, and regional pain syndrome [15–17]. Analgesia induced by neuromodulatory techniques appears to be associated with the re-establishment of normal cortical excitability and is more dependent on the remodeled endogenous opioid system [18].

Nociceptive signaling may be influenced by neurotrophic and inflammatory factors. Brain-derived neurotrophic factor (BDNF) induces synaptic plasticity, especially in the spinal dorsal horn [19–20]. It has been used as a biomarker for cortical excitability and enhanced neuronal activity [21]. On the other hand, the expression of tumor necrosis factor-alpha (TNF-α) in the spinal cord contributes to the induction and maintenance of NP in rodents [22]. In addition, increased interleukin-10 (IL-10) expression, which plays

a classic anti-inflammatory role [23], could be involved in the development of NP after peripheral nerve injury [24–26].

NP management is a complex issue, and the response to existing treatments is often unsatisfactory. Therefore, the investigation of new tools, which may be useful for the management of NP, is of great interest. This study aimed to evaluate the effects of rTMS treatment on the nociceptive response of rats submitted to an NP model and its effect on pro-and anti-neuroinflammatory cytokine and neurotrophin levels.

2. RESULTS

2.1 Nociceptive tests

2.1.1 Von Frey test

Mechanical allodynia, assessed by the von Frey test, showed an interaction between groups at all three evaluation times (generalized estimating equation (GEE), Wald $\chi2=5121.60$, $P<0.001$). At baseline, no difference was found among groups in the paw withdrawal threshold (GEE/Bonferroni, $P>0.05$). Fourteen days after CCI surgery, animals from the NP groups presented lower paw withdrawal latency, confirming the establishment of NP (GEE/Bonferroni, $P<0.05$). Twenty-four h after the end of the treatment, animals from the NP and NP+Sham.rTMS groups presented a lower paw withdrawal threshold. Active rTMS-treated animals presented an increase in the nociceptive threshold compared to the non-treated or sham rTMS-treated rats but did not reach the nociceptive threshold of the control animals, indicating a partial reversal of the mechanical allodynia induced by CCI (GEE/Bonferroni, $P<0.05$; Figure 1).

INSERT FIGURE 1

2.1.2 Hot plate test

Thermal hyperalgesia showed an interaction between the groups at all three evaluation times, as assessed by the hot plate test (GEE, Wald $\chi2=75.26$, $P<0.001$). At baseline, no difference was found among groups in the thermal latency thresholds (GEE/Bonferroni, $P>0.05$). Fourteen days after CCI surgery, animals from the NP groups presented a lower thermal threshold, confirming the establishment of the NP model (GEE/Bonferroni, $P<0.05$). Twenty-four h after the end of rTMS treatment, animals from the NP and NP+Sham.rTMS groups presented a lower thermal threshold. In contrast, the rTMS-treated animals presented an increased nociceptive threshold that reached the same

threshold as the control animals, demonstrating a total reversal of the thermal hyperalgesia induced by CCI (GEE/Bonferroni, $P<0.05$; Figure 2).

INSERT FIGURE 2

2.2 Biochemical assays

2.2.1 BDNF levels

Differences in the BDNF levels among the groups were found in the PFC and spinal cord (one-way analysis of variance (ANOVA), $F_{8,70}=3.96$, and $F_{8,71}=6.90$, respectively, $P<0.001$ for both). Animals from the NP rTMS-treated group presented higher BDNF levels in the PFC than the other groups (one-way ANOVA/SNK, $P<0.05$). In the spinal cord, all animals that underwent a surgical procedure (sham NP or NP) presented higher BDNF levels (Figure 3).

INSERT FIGURE 3

2.2.3 TNF-α levels

Differences in the TNF-α levels among the groups were found in the PFC and spinal cord (one-way ANOVA, $F_{8,66}= 8.81$, and $F_{8,71}= 16.87$, respectively, $P<0.001$ for both). Animals from the NP rTMS-treated group presented higher levels of this cytokine in the PFC and spinal cord compared to the other groups (one-way ANOVA/SNK, $P<0.05$; Figure 4).

INSERT FIGURE 4

2.2.4 IL-10 levels

Differences in the IL-10 levels among the groups were found in the PFC and spinal cord (one-way ANOVA, $F_{8,70}=4.28$, and $F_{8,71}=24.06$, respectively, $P<0.001$ for both). The NP rTMS-treated group presented higher levels of this IL in the PFC than the other groups (one-way ANOVA/SNK, $P<0.05$). In the spinal cord, the NP rTMS-treated group presented higher IL-10 levels than the other groups (one-way ANOVA/SNK, $P<0.05$).

The NP+Sham.rTMS group also presented higher IL-10 levels in this structure. However, these levels were different from the rTMS-treated group (one-way ANOVA/SNK, $P<0.05$; Figure 5).

INSERT FIGURE 5

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3 DISCUSSION

This is the first study that shows that rTMS treatment reversed thermal hyperalgesia and increased the levels of central BDNF, IL10, and TNF-α in NP model rats. The alterations in the neuroinflammatory cytokines and BDNF levels, especially in the PFC, may contribute to the analgesic effect of rTMS. In this way, we can suggest that rTMS, as proposed in this study, can be an effective tool in the treatment of NP. Similar to other studies from our group, the establishment of NP was reached 14 days after CCI surgery, according to the nociceptive tests [8–9]. Animals from the NP groups presented lower mechanical and thermal thresholds than the other groups. At this time, the proposed rTMS treatment was applied, which could partially reverse mechanical allodynia and reverse thermal hyperalgesia.

In the current study, rTMS was applied at a low-frequency which can have inhibitory effects on the brain [13–14]. TMS stimulation frequencies at or below 1 Hz cause neuronal inhibition, whereas, at higher frequencies, neuronal facilitation [27]. High-frequency TMS has already been shown to reduce the nociceptive threshold in rats, probably by the involvement of N-methyl-D-aspartate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate/kainate receptors [28]. This does not mean that the descending inhibitory pathways would be inhibited. Instead, they can often be enhanced, depending on the stimulation protocol [29]. Sampson and colleagues [14] reported that low-frequency rTMS over the DLPFC in human patients was effective in pain relief. In addition, it has been demonstrated that stimulation of the motor cortex by rTMS modulates the cortical and spinal cord circuits, inducing neuronal plasticity [30–31], despite the distance from the stimulated area. Thus, in our study, the reduction in descending modulation due to the NP process could influence the nociceptive response in remote areas, such as in the paw. It is interesting to note that different circuits at the spinal and supraspinal levels were assessed in the behavioral nociceptive tests. We

assessed local mechanical hyperalgesia using the von Frey test and remote thermal hyperalgesia using the hot plate test. It is important to highlight that the von Frey test involves Aδ-fibers, while the hot plate test assesses tonic pain mainly by the C-fibers [32–33]. In this way, we suggest that the top down rTMS modulation is related to the direction (structures involved) in the process, that is, from the cerebral cortex to other structures below. It has been postulated that rTMS induces alterations in the activity of cortical and subcortical brain structures that are related to pain modulation and processing, including the orbitofrontal cortices, medial thalamus, anterior cingulate, and periaqueductal gray matter [34]. Moreover, rTMS reduces chronic pain by triggering the descending inhibitory neural pathways to act at the dorsal horn level [35].

Rats with chronic NP had increased BDNF levels in the PFC. BDNF levels were also higher in the spinal cord in rats subjected to a surgical procedure (sham NP and NP) than in the control rats. The involvement of the BDNF signaling cascade in the development of chronic pain has already been described [36]. This neurotrophin is first synthesized as proBDNF, which is proteolytically arranged into mature BDNF (mBDNF) [37]. Both forms bind to two different types of receptors: mBDNF preferentially binds to the tyrosine receptor kinase B (TrkB), and proBDNF binds to the pan-neurotrophin receptor p75 (p75NTR) [38]. Activation of TrkB is more related to synaptic potentiation and maturation, whereas the activation of p75NTR suppresses synaptic transmission and axonal retraction [39]. Decreasing BDNF levels in the spinal cord have been associated with a reduction in pain behaviors [40–41]. On the other hand, increasing BDNF levels are related to chronic pain development [42]. In the current study, the NP-rTMS-treated group presented higher levels of this neurotrophin in the PFC. Besides, rTMS has been reported to enhance BDNF and its receptor interaction (TrkB) signaling in the cerebral cortex [43]. In elderly patients with refractory depression, rTMS increased BDNF and decreased IL-1β and TNF-α serum levels [44]. In post-stroke patients, rTMS combined with rehabilitation therapy improved motor function in the affected limb by activating BDNF processing [45]. Moreover, the relationship between BDNF and cannabinoid receptor 1 (CB1R), a pathway that modulates nociception, has already been reported [46–48]. Thus, we can suggest that the enhanced BDNF levels induced by rTMS when a disease has been established induces alterations in neuroplasticity, contributing to its analgesic effects in rats subjected to NP.

Another unexpected finding from our study was that the NP-rTMS-treated animals presented higher levels of TNF-α in the PFC and spinal cord than the other groups. As

mentioned, the opposite effect of rTMS has already been shown in elderly patients with refractory depression [44]. On the other hand, another study showed that in a parkinsonian rat model, low-frequency rTMS inhibited the expression of TNF-α and COX-2 and prevented dopaminergic neuron apoptosis [49]. It is important to note that TNF-α is a key regulator of homeostatic synaptic scaling and synaptic transmission [50–51]. The association between pain and microglial TNF-α has been proposed since it improved long-term potentiation at C-fiber synapses in the spinal horn of an animal model of peripheral nerve injury [52]. Although TNF-α activates p38 mitogen-activated protein kinases in spinal microglia after nerve injury, contributing to NP development and maintenance [53], inflammatory cytokines are also involved in repair [54]. We suggest that the pathways activated by central TNF-α signaling contributed to the observed analgesic effects of rTMS.

While enhanced neuroinflammation can protect the CNS at first, it also leads to severe detrimental consequences if sustained or too extreme [55]. IL-10 plays an important role in this scenario, contributing to limiting or resolving neuroinflammation [56]. Similar to other studies from our group using tDCS [8–9], the current study using rTMS showed an increase in the IL-10 levels in the PFC and spinal cord in animals with NP. It has been shown that the central inflammatory cytokines, IL-6 and TNF-α, induce IL-10 production by microglia in a dose-dependent manner [57]. Moreover, glutamate can enhance IL-10 production [58]. In patients with chronic myofascial pain syndrome, rTMS could not modify peripheral biomarkers, including the level of IL-10 [59]. We suggest that the alteration in the levels of IL-10 seen in NP and rTMS-treated rats was reflected by TNF-α enhancement, altering neuroplasticity, and contributing to a central homeostatic scenario.

The application of non-invasive brain stimulation in small animals, such as rTMS, is always a challenge in preclinical studies. We consider that the inability to restrict the stimulation area is a limitation of the current study, which may compromise the translationality of our results. It has been shown in humans that the DLPFC is a brain region involved in the top down-modulation of pain [60] and that the M1 region is a valuable stimulation target in chronic and NP treatments [3,18,61]. Therefore, restricting stimulation to these regions may be more effective in the treatment of pain. Additionally, our TMS device was designed only for treatment, not to diagnose brain excitability, such as TMS application in humans. Further studies should separately evaluate the two existing forms of BDNF since this may shed light on the possible role of this neurotrophin in pain

management. Altogether, the results of the present study may guide a new treatment option for pain.

In conclusion, rTMS treatment reversed thermal hyperalgesia induced by NP. The alterations induced by rTMS on neurochemical parameters may have contributed to this analgesic effect. The major neurochemical alterations induced by rTMS in our study were related to the PFC, which can be due to the proximity to the stimulation site. In addition, alterations in the neuroinflammatory parameters may contribute to the regeneration process. In this way, we can suggest that low-frequency rTMS is a potential tool for NP treatment, possibly due to the modulation of plasticity involving BDNF and neuroinflammatory cytokines.

4 EXPERIMENTAL PROCEDURE

4.1 Animals

A total of 106 adult male Wistar rats were used in this study. They were 60 days old at the beginning of the experiment and weighed an average of 250 g. Animals were group-housed (3) in polypropylene cages (49 cm × 34 cm × 16 cm) with sawdust-covered floors. The rats were maintained in a controlled environment (22 ± 2 °C) under a standard light-dark cycle (lights on at 7 a.m.). They had *ad libitum* access to water and rodent chow (Nuvital®, Curitiba, Brazil). The experiments in this study were conducted in male rats because the nociceptive responses are altered by modulation of the hormone state. All procedures were approved by the Institutional Committee for Animal Care and Use (GPPG/HCPA protocol # 2017–0438) and conducted in compliance with Brazilian law [62–64]. The animals' husbandry followed Brazilian law #11.794, which regulates the scientific use of animals. We tried to minimize animal suffering as much as possible, decrease external sources of pain and discomfort and use the minimum number of animals required to produce reliable scientific data. This study was performed at Hospital de Clínicas de Porto Alegre (Unidade de Experimentação Animal), Porto Alegre/RS, Brazil.

4.2 Experimental design

After 14 days of adaptation to the vivarium, the animals were randomized by von Frey test and divided into nine experimental groups: control (C), control plus sham rTMS (C+s.rTMS), control plus rTMS (C+rTMS), sham NP (s.NP), sham neuropathic pain plus sham rTMS (s.NP+s.rTMS), sham neuropathic pain plus rTMS (s.NP+rTMS), NP, neuropathic pain + sham rTMS (NP+s.rTMS), and neuropathic pain plus rTMS

(NP+rTMS). After that, rats from the s.NP and NP groups were subjected to sham surgery or chronic constriction injury (CCI) of the sciatic nerve, respectively. Then, 14 days after CCI surgery, the establishment of NP was assessed using von Frey and hot plate tests. From day 15 after CCI surgery, the animals underwent daily rTMS sessions for eight consecutive days. Nociception was evaluated using von Frey and hot plate tests at baseline, 14 days after CCI surgery, and 24 h after the last session of rTMS. The rats were then killed by decapitation 48 h after the end of the proposed treatment, and their cerebral structures were removed for further biochemical assays (Figure 6).

INSERT FIGURE 6

4.3 NP model

NP was induced by CCI of the left sciatic nerve [65]. First, the animals were anesthetized with isoflurane (5% induction / 2.5% maintenance) and laid in the left dorsal position. The paw was trichotomized, and antisepsis was made with 2% iodine-alcohol. An incision was made to expose the biceps femoris muscle and the sciatic nerve, which was exposed to approximately 5 mm. Three ligatures were tied (Vycril 4.0) around the nerve 1 mm apart (Figure 7). This procedure reduces the diameter of the nerve while maintaining normal epineurial blood flow [14]. The same investigator performed all the ligatures. The skin was sutured using mononylon 4.0. Animals from the s.NP groups had the sciatic nerve exposed similarly to the NP groups, but without any nerve ligature. The control group did not undergo any surgery.

INSERT FIGURE 7

4.4 rTMS

Fifteen days after CCI surgery and the establishment of NP, the animals from the treated groups underwent a 5 min daily session of rTMS for eight consecutive days (always starting at 8:30 a.m.). The magnetic stimulator and butterfly coils were developed in the Biomedical Engineering Lab of Hospital de Clínicas de Porto Alegre (Figure 8). The device generated pulses with a 1 ms duty cycle in a 1 Hz frequency, and the magnetic

field intensity was 200 mT (millitesla). The animals were restrained during stimulation and wrapped in a cloth. The coil was fixed to the head using adhesive tape (Micropore™) (Figure 9). For sham stimulation, the animals were also restrained, and the coil was placed and fixed in the same position, similar to the active stimulation. However, the magnetic stimulator remained off throughout the procedure. The animals received sham stimulation at the same time as the active animals in the same room so that they were exposed to the characteristic noise of the active stimulation. We highlight that stress immobilization can also be controlled by a sham stimulation group since animals were immobilized without any active treatment.

INSERT FIGURES 8 AND 9

4.5 Von Frey test

Mechanical hyperalgesia was assessed using an automatic von Frey anesthesiometer (Insight, São Paulo, Brazil). The rats were placed in 12 × 20 × 17 cm polypropylene cages with wire grid flooring and habituated to this environment for 10 min 24 h before the first test to prevent novelty-induced analgesia [66]. Von Frey stimuli were applied to the mid-plantar surface of the operated hind paw through the mesh floor. The application was only performed when the animal's paws were in contact with the floor and were measured three times, with an interval of at least 5 s. The withdrawal threshold of the operated left hind paw was expressed in grams (g) [14]. This test was performed at baseline, 14 days after surgery, and 24 h after the end of rTMS treatment.

4.6 Hot plate test

Thermal hyperalgesia was evaluated using the hot plate test. The rats were exposed to the apparatus for habituation for 5 min, 24 h before the first hot plate test to avoid novelty-induced analgesia [66]. The temperature of the plate was set to 55 ± 0.1 °C. The time (in seconds) between the placement of the rat onto the plate and the first response (foot licking, jumping, or rapid removal of paws) was recorded as the latency of the nociceptive response. A cutoff time of 20 s was used to avoid tissue damage [67]. This test was conducted at baseline, 14 days after CCI surgery, and 24 h after the end of rTMS treatment.

4.7 Tissue collection

The animals were euthanized by decapitation 48 h after the last session of rTMS

treatment. The PFC was collected by an experienced researcher, according to Spijker [68], and the entire spinal cord from the cervical to the lumbar region was removed. The structures were kept frozen at −80 °C until biochemical assays were performed.

4.8 Biochemical assays

The PFC and spinal cord BDNF, TNF-α, and IL-10 levels were determined by sandwich enzyme-linked immunosorbent assay using monoclonal specific antibodies (R&D Systems, Minneapolis, United States). Data are expressed in pg/mg of protein. Total protein was determined by Bradford's method using bovine serum albumin as a standard. The PFC and spinal cord were homogenized with a handheld homogenizer with a 1:10 protease inhibitor cocktail (Sigma® #P8340) and centrifuged for 5 min at 10,000 rpm, using the supernatant for the techniques.

4.9 Statistical analysis

Nociceptive behavioral tests were analyzed using a GEE/Bonferroni. For biochemical assays, a one-way ANOVA/SNK was performed. The data had a normal distribution (Shapiro–Wilk test). Results were considered statistically significant if $P<0.05$ and were expressed as the mean ± standard error of the mean. Outlier animals were excluded. SPSS version 18.0 software was used for all statistical analyses.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Acknowledgments and funding sources

This research was supported by the following Brazilian funding agencies: National Council for Scientific and Technological Development–CNPq (Dr. ILS Torres, Dr. W Caumo); Brazilian Committee for the Development of Higher Education Personnel – CAPES (RS Toledo, LS da Silva, HR Medeiros), and CAPES/PNPD Edital PPGCM 07/2016 (Dr. DJ Stein); Graduate Research Group of Hospital de Clínicas de Porto Alegre - GPPG (ILS Torres – Grant 2017–0438); Research Support Foundation of the State of Rio Grande do Sul (PRS Sanches, Grant FAPERGS-PRONEM 16/2551-0000249-5).

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FIGURE CAPTIONS

Figure 1. Von Frey test. Data expressed as mean ± SEM of paw withdrawal threshold in grams (g). n=11-12 animals/group. *Significant difference from Controls and Sham NP groups 14 days after CCI surgery, demonstrating NP establishment (GEE/Bonferroni, P<0.05). #Significant difference of NP and NP+Sham.rTMS groups after treatment (GEE/Bonferroni, P<0.05). $Significant difference of NP+rTMS group after treatment (GEE/Bonferroni, P<0.05).

Figure 2. Hot plate test. Data expressed as mean ± SEM of time in seconds (s) for nociceptive latency response. n=11-12 animals/group. *Significant difference of NP groups 14 days after CCI, demonstrating NP establishment (GEE/Bonferroni, P<0.05). #Significant difference of NP and NP+ Sham.rTMS groups after treatment (GEE/Bonferroni, P<0.05).

Figure 3. BDNF levels. Data expressed as mean ± SEM (pg/mg of protein). n=8-9 animals/group. Different letters indicate statistically significant differences among groups in the same structure (One-way ANOVA/SNK, P<0.05).

Figure 4. TNF-α levels. Data expressed as mean ± SEM (pg/mg of protein). n=8-9 animals/group. Different letters indicate statistically significant differences among groups in the same structure (One-way ANOVA/SNK, P<0.05).

Figure 5. IL-10 levels. Data expressed as mean ± SEM (pg/mg of protein). n=8-9 animals/group. Different letters indicate statistically significant differences among groups in the same structure (One-way ANOVA/SNK, P<0.05).

Figure 6. Experimental design. CCI: chronic constriction injury surgery. rTMS: repetitive Transcranial Magnetic Stimulation.

Figure 7. Neuropathic Pain model. Three ligatures tied around the left sciatic nerve ±1mm apart.

Figure 8. TMS device: A - Butterfly coils, B - Pulse generator.

Figure 9. rTMS treatment. Rat restrained during the stimulation.

FIGURE 1

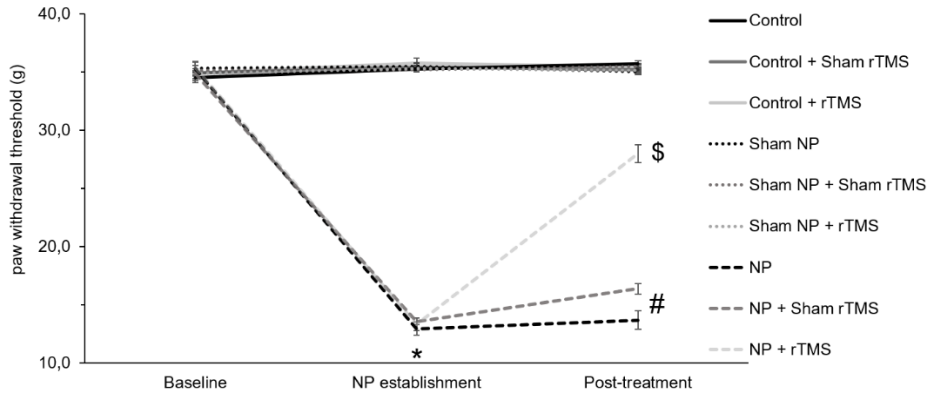
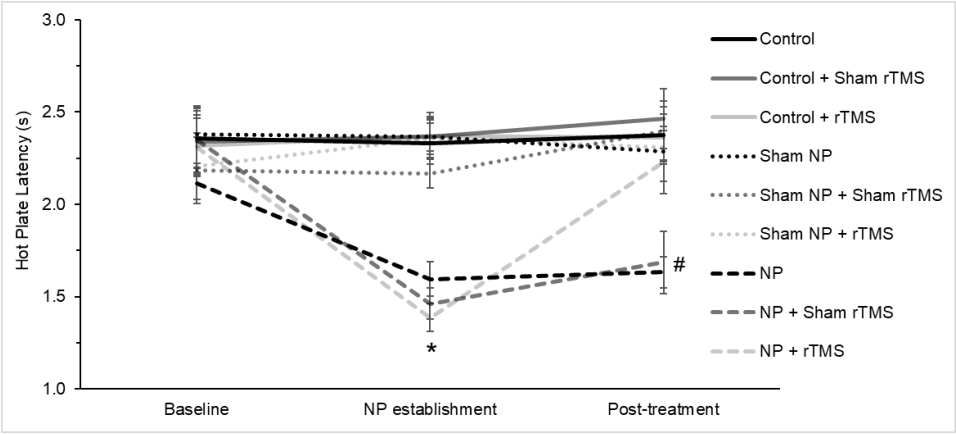


FIGURE 2



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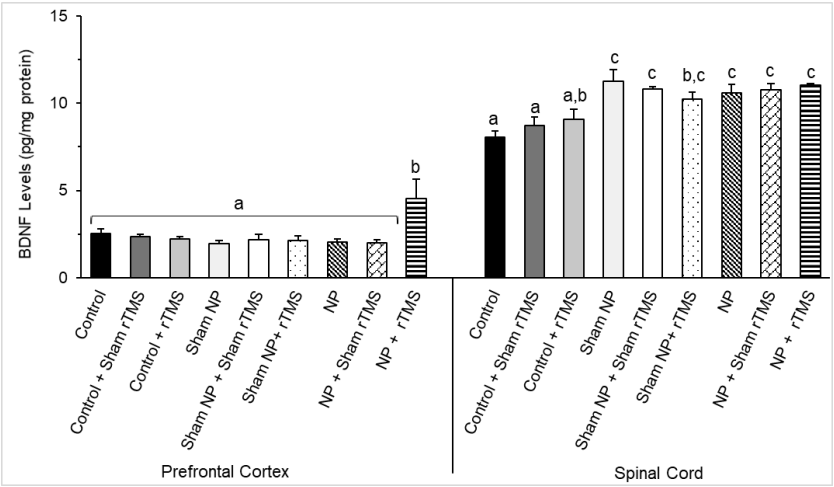

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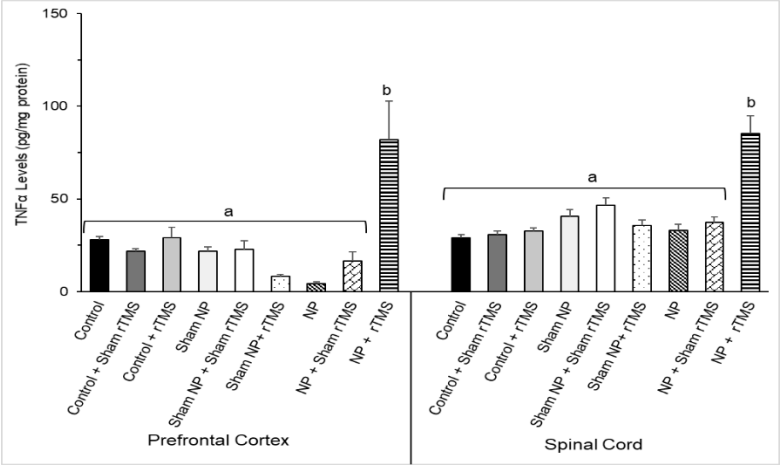

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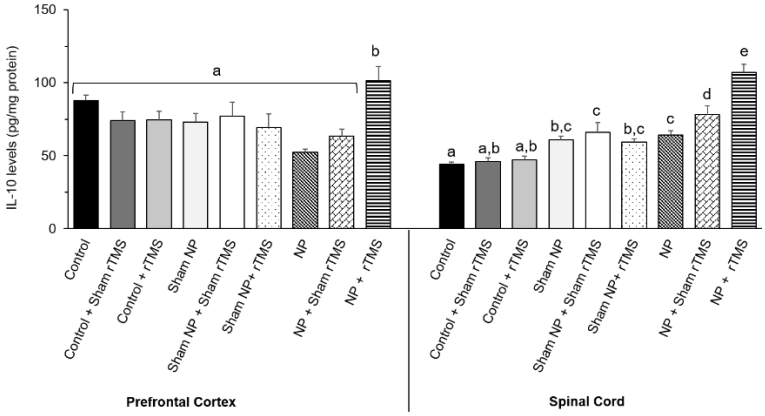

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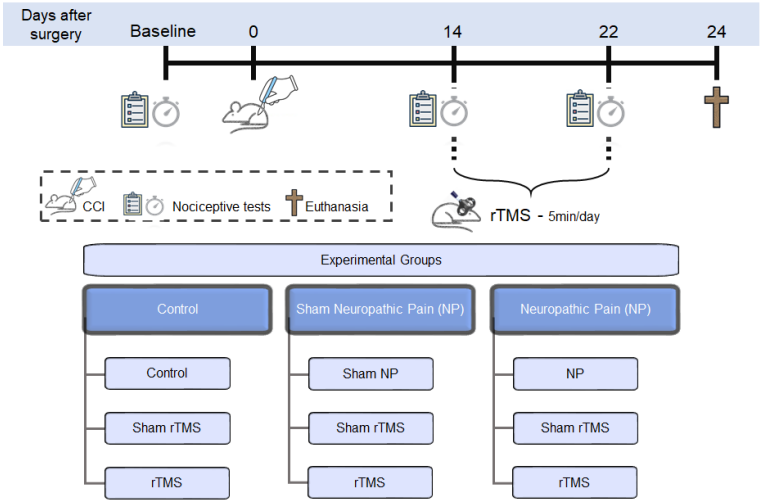


FIGURE 7

FIGURE 8

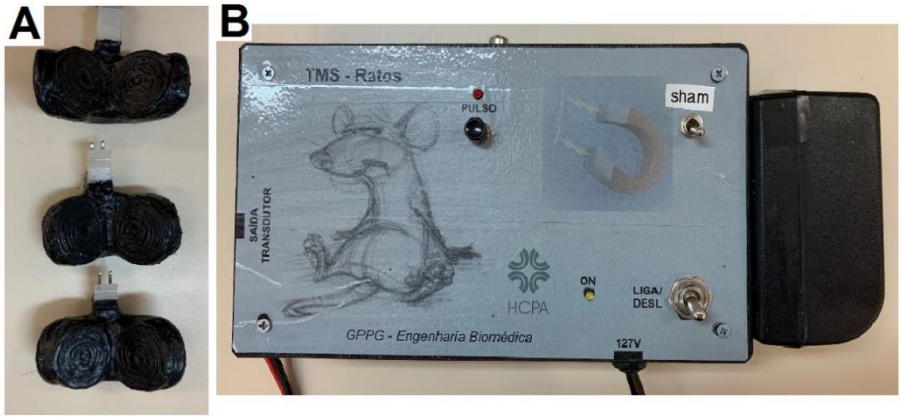
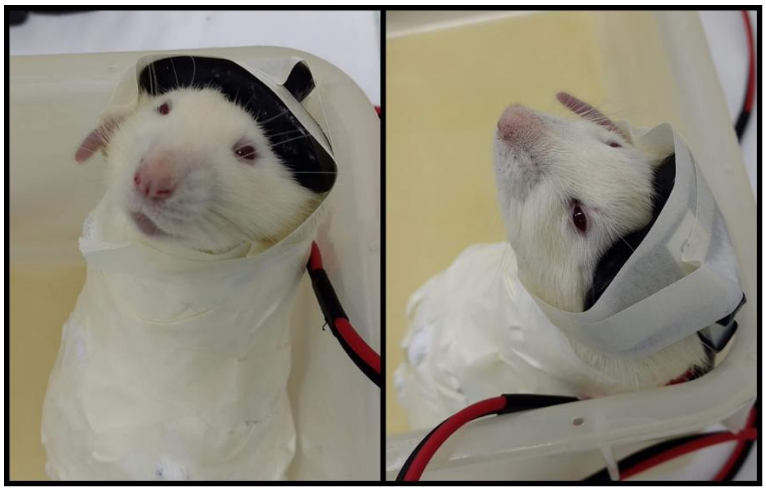


FIGURE 9



6.2 ARTIGO II

"Repetitive transcranial magnetic stimulation (rTMS) reverses the long-term memory impairment and the decrease of hippocampal interleukin-10 levels, both induced by neuropathic pain in rats" - *DOI 10.1016/j.neuroscience.2021.07.030.*

Periódico: *Neuroscience*

Situação: Versão aceita para publicação

Repetitive transcranial magnetic stimulation (rTMS) reverses the long-term memory impairment and the decrease of hippocampal interleukin-10 levels, both induced by neuropathic pain in rats

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ABSTRACT

Neuropathic pain (NP) is characterized by the presence of spontaneous pain, allodynia and hyperalgesia. Repetitive transcranial magnetic stimulation (rTMS) is one of neuromodulatory techniques that induces satisfactory NP relief, including that from refractory pain patients. The objective of this study was to evaluate rTMS treatment over long term memory (LTM) and hippocampal BDNF and IL-10 levels in rats submitted to a NP model. A total of 81 adult (60-days old) male Wistar rats were randomly allocated to one of the following 9 experimental groups: control, control+ sham rTMS, control+rTMS, sham neuropathic pain, sham neuropathic pain+sham rTMS, sham neuropathic pain+rTMS, neuropathic pain (NP), neuropathic pain+sham rTMS and neuropathic pain+rTMS. Fourteen days after the surgery for chronic constriction injury(CCI) of the sciatic nerve, NP establishment was accomplished. Then, rats were treated with daily 5-minute sessions of rTMS for eight consecutive days. LTM was assessed by the object recognition test (ORT) twenty-four hours after the end of rTMS treatment. Biochemical assays (BDNF and IL-10 levels) were performed in hippocampus tissue homogenates. rTMS treatment reversed the reduction of the discrimination index in the ORT and the hippocampal IL-10 levels in NP rats. This result shows that rTMS reverses the impairment LTM and the increase in the hippocampal IL-10 levels, both induced by NP. Moreover, it appears to be a safe non-pharmacological therapeutic tool since it did not alter LTM and neurochemical parameters in naive animals.

Keywords: BDNF; IL-10; neuromodulation; chronic pain; memory; brain stimulation.

INTRODUCTION

Neuropathic pain (NP) is defined by the International Association for the Study of Pain (IASP) as a manifestation resulting from injury or disease of the somatosensory system (Colloca et al. 2017). The main characteristics are described as pain sensation with intermittent or continuous burning, allodynia, and hyperalgesia (Wang et al. 2020). According to the literature, the prevalence of NP is about 6.9 to 10% in the general population (Hecke et al. 2014) and may rise in the future due to increased life expectancy and the incidence of neuropathic-related diseases, such as cancers and diabetes (Colloca et al. 2017). NP is considered a major public health problem worldwide (Ye et al. 2018), resulting in overload of health services, especially associated with difficulty in the management of the disease. It is evident that many affected patients do not receive satisfactory treatment, in view of the lack of accurate diagnosis, refractoriness, ineffective medication, insufficient knowledge of the pathophysiological mechanisms and drug application in clinical practice (Finnerup et al. 2015).

Due to the difficulty of managing NP, new therapies have been tested in the context of refractoriness to treatments, especially repetitive Transcranial Magnetic Stimulation (rTMS). rTMS is a noninvasive neuromodulatory technique designed to modulate the electrical activity of the brain, through the application of recurrent magnetic fields, delivered by a coil, in a brain region generating action potentials in cortical neural circuits (Mylius, Borckardt and Lefaucheur 2012; Zhao et al. 2019; Udupa 2010). rTMS can be associated with the modulation of cortical excitability: while high frequency stimulation (>1Hz) is related to increase of excitability, low frequencies (≤ 1Hz) will transiently depress cortical excitability (Udupa 2010).

Stimulation of the primary motor cortex (M1) is the most prevalent target in experimental studies (Mylius, Borckardt, and Lefaucheur 2012), mainly because this is considered the only validated design for the treatment of refractory pain by neurostimulation at the cortical level, also for the ease of observing motor evoked potentials (MEPs) (Lefaucheur et al. 2004). MEPs can be obtained, for example, by a single TMS pulse, a technique used to explore brain function, while rTMS is able to induce changes in brain activity that can last beyond the stimulation period, being used mostly for therapeutic purposes (Klomjai, Katz and Lackmy-Vallée 2015).

The rTMS has been recently investigated by our research group and we have shown that this technique can be used to reverse the hyperalgesia/allodynia induced by a NP model

in rats, changing biomarkers in prefrontal cortex (Toledo et al. 2021). Previous studies from our research group using tDCS, another neuromodulatory technique, in rats submitted to a neuropathic pain model showed that it induces analgesia, altering neuromodulators such as interleukins and neurotrophins (Cioato et al. 2016; Lopes et al. 2021; Santos et al. 2020), being able to revert the increase of anxiety-like behavior and locomotor activity induced by pain (Lopes et al. 2021).

Long-term memory (LTM) is the ability to recall information long after a learning episode, in periods lasting hours, days, years, or a lifetime (Lee et al. 2016). Clinical and preclinical research highlight the evident role of the hippocampus in LTM. Besides that, it is known that rTMS can affect attention, memory, and other brain functions (Rossi et al. 2009). Wang and colleagues (2014) have previously shown that multiple sessions of stimulation increase the functional connectivity between distributed regions of the cortical network of the hippocampus and, likewise, improve the performance of associative memory in healthy adults (Wang et al. 2014). However, the effects of rTMS on LTM in rodents, especially in those with NP, are yet unknown.

NP may be closely related to brain-derived neurotrophic factor (BDNF) levels, one of those responsible for modulating CNS pain processing (Pezet and McMahon 2006). In addition, increased expressions of BDNF are related to the analgesic effect provided by rTMS in NP models, resulting from a modulatory effect of that stimulation (Zhao et al. 2019; Cao et al. 2020). Previous studies have shown that Interleukin 10 (IL-10), an anti-inflammatory cytokine (Khan et al. 2015), has antinociceptive activity in animal models of NP (Wu et al. 2018). In this sense, another neuromodulatory technique (i.e. low frequency electroacupuncture) is effective in relieving NP by activating the spinal pathway of IL-10 / β-microglial endorphin (Ali et al. 2020). It is well established that synaptic plasticity is a critical component of the neural mechanisms underlying learning and memory (Lynch et al. 2004). In this context, BDNF plays an important role in synaptic transmission and plasticity (Waterhouse and Xu 2009). The hippocampus is an area of the brain that is involved in learning and memory and exhibits various forms of short- and long-term synaptic plasticity (Shang et al. 2016). Thus, evaluating these markers in this structure may be a key factor for understanding the underlying mechanisms.

Given the evidence on the therapeutic use of rTMS in NP (Lefaucheur et al. 2004) and the scarcity of preclinical studies addressing the mechanism and possible underlying

effects of brain stimulation, we investigated the effects of rTMS on LTM, and hippocampal BDNF and IL-10 levels in rats with NP.

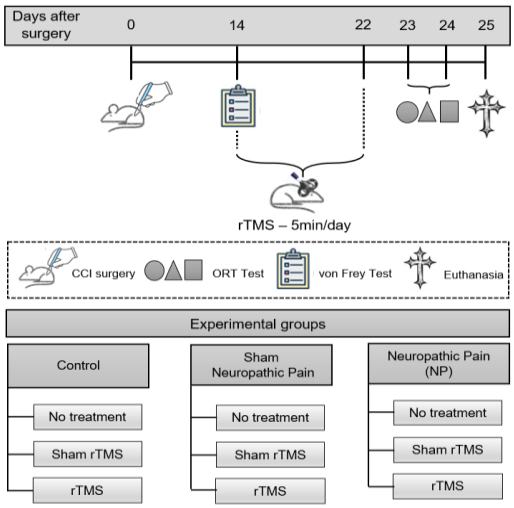
MATERIALS AND METHODS

Animals

A total of 81 adult male Wistar rats (60 days old; weight ≅250g) were group-housed in 3 animals per polypropylene cages (49 × 34 × 16cm) with sawdust-covered floors. They were kept under a standard light-dark cycle (lights on at 7 a.m/off at 7 p.m), in a controlled environment (22 ± 2°C), having *ad libitum* access to rodent chow (Nuvital®, Curitiba, Brazil) and water. We conducted all experiments in male rats since different hormonal states in females may alter the modulatory effect over nociceptive responses (Ribeiro et al. 2005). The procedures were approved by the Institutional Committee for Animal Care and Use (GPPG/HCPA protocol #2017-0438) and conducted in compliance with Brazilian law (Brazil 2008; 2013a; 2013b). Animals' husbandry followed Brazilian Law #11.794 that regulates the scientific use of animals. All procedures were performed at Hospital de Clínicas de Porto Alegre (Unidade de Experimentação Animal), Porto Alegre/RS, Brazil.

Experimental Design

Animals were randomized by baseline nociceptive threshold using the von Frey test, and divided into nine experimental groups: control, control + sham rTMS, control + rTMS, sham neuropathic pain, sham neuropathic pain + sham rTMS, sham neuropathic pain + rTMS, neuropathic pain, neuropathic pain + sham rTMS, and neuropathic pain + rTMS. Afterwards, rats from the groups s.NP and NP were submitted to sham surgery or chronic constriction injury (CCI) of the sciatic nerve, respectively. Then, 14 days after CCI surgery, the establishment of NP was confirmed by the von Frey test. The next day, animals were submitted to daily 5-minutes rTMS sessions for 8 consecutive days. The LTM was assessed by the object recognition test (ORT) 24h after the last session of rTMS. Rats were killed by decapitation 24 hours after the end of the ORT, removing the hippocampus for further biochemical assays (Figure 1).



Neuropathic pain model

Neuropathic pain was induced by CCI of the left sciatic nerve (Bennett e Xie 1988). All the procedures were conducted according to Cioato et al. 2016. Rats from the sham neuropathic pain groups had the sciatic nerve exposed as animals from the neuropathic pain groups, but without any nerve ligature. Control animals did not suffer any manipulation. Fourteen days after CCI surgery, the establishment of neuropathic pain was assured by the von Frey test, and the next day rats were able to start rTMS treatment.

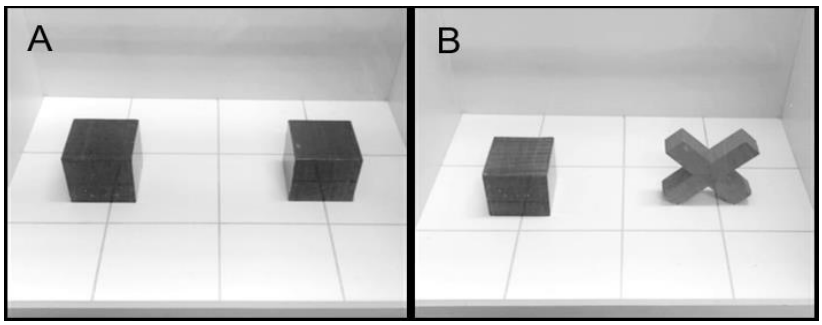
rTMS

Fifteen days after CCI surgery and the establishment of the neuropathic pain, rats from the treated groups underwent one daily 5-minute session of rTMS for eight consecutive days (always starting at 8:30 a.m.). The magnetic stimulator and the butterfly coils were developed in the Biomedical Engineering Laboratory of Hospital de Clínicas de Porto Alegre. All the procedures were conducted according to Toledo et al. 2021. The coil was placed and fixed in a position as same as to the active stimulation, but the magnetic stimulator remained turned off during the procedure for the animals in the sham stimulation groups.

Object recognition test (ORT)

LTM was evaluated by the object recognition test (ORT) conducted 24 hours after the end of rTMS treatment. It was performed on an open field apparatus, in a sound-attenuated room under low-intensity light (375 lx). This test is based on rats' differential exploration of familiar and new objects (Ennaceur et al. 1989) and consists of three phases: habituation, sample, and discrimination. The objects' allocation and shape during

the test is demonstrated in Figure 2. All the procedures were conducted according to Leffa et al. 2018.



Discrimination index was used as a measure of LTM, defined by the difference in exploration time between the novel and the familiar object divided by the total time exploring these two objects in the last phase (Leffa et al. 2018). The objects were constructed using wood and their shapes and colors were distinct to be differentiated by the animals, but with a similar texture and height, with the intention of minimizing the object preference (Ennaceur 2010; Leffa et al. 2016; Lueptow 2017).

Tissue collection

Animals were killed by decapitation 24 hours after the ORT and the hippocampus was collected. It was kept frozen at −80 °C until biochemical assays were performed.

Biochemical assays

Hippocampal BDNF and IL-10 levels were determined by sandwich ELISA using monoclonal specific antibodies (R&D Systems, Minneapolis, United States). Data were expressed in pg/mg of protein. Total protein was detected by Bradford's method (Kruger 1994) with bovine serum albumin (BSA) as standard. The hippocampus was homogenized in a handheld homogenizer with 1:10 Protease Inhibitor Cocktail (Sigma® catalog #P8340) and centrifuged for 5 min at 10.000 rpm, utilizing the supernatant for the assays.

Statistical analysis

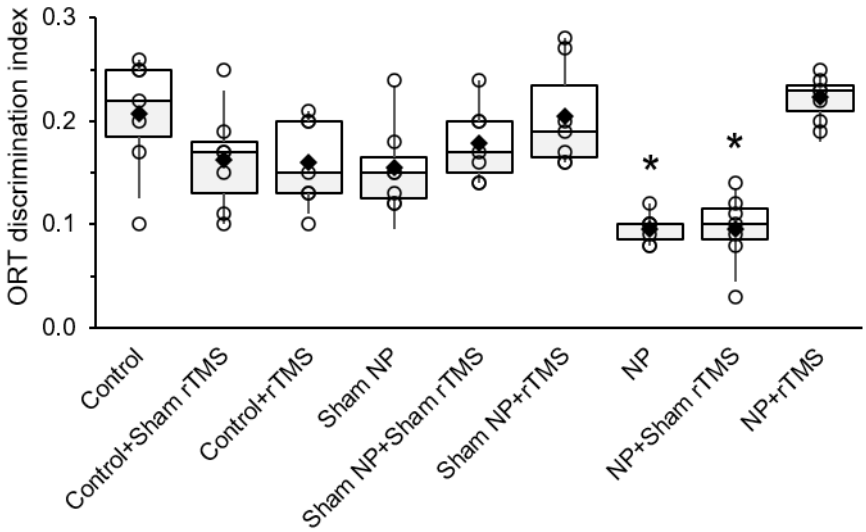
The ORT and biochemical data were analyzed by one-way ANOVA followed by the Student–Newman–Keuls (SNK) *post hoc* test to identify the differences between groups. Results were considered statistically significant if $P<0.05$ and were expressed in box-and-whisker plots showing the median, 25^{th} and 75^{th} percentiles of the groups, using fixed-effects models. We assumed homogeneity of variance and the normality of data

distribution using the Levene and Shapiro Wilk tests. Thus, the null hypothesis of equal population variances was assumed, and we used parametric analyses, since there were no violations of these two assumptions. Seven animals per group were used in the ORT test and nine animals per group for each biochemical analysis. The SPSS 18.0 software was used for all statistical analyses.

RESULTS

Object recognition test (ORT)

Since neuropathic pain can induce LTM impairment (Ren et al. 2011), we decided to evaluate if rTMS might improve this outcome. We found a difference between groups in the ORT discrimination index (one-Way ANOVA/SNK, $F_{8,62}$=8.527, P<0.05). It is possible to observe that the neurophatic pain decrease the discrimination index (NP and NP+ShamrTMS groups), and this effect was reverted by rTMS treatment (NP+rTMS) (Figure 3). This data indicates that the mnemonic effect of rTMS is state dependent since it is observed only in NP animals, with no effects in the control groups (C and Sham-NP groups).

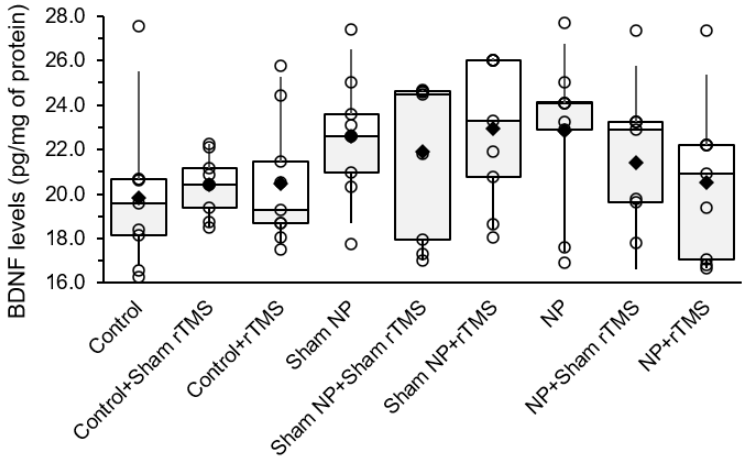


Biochemical Assays

BDNF Levels

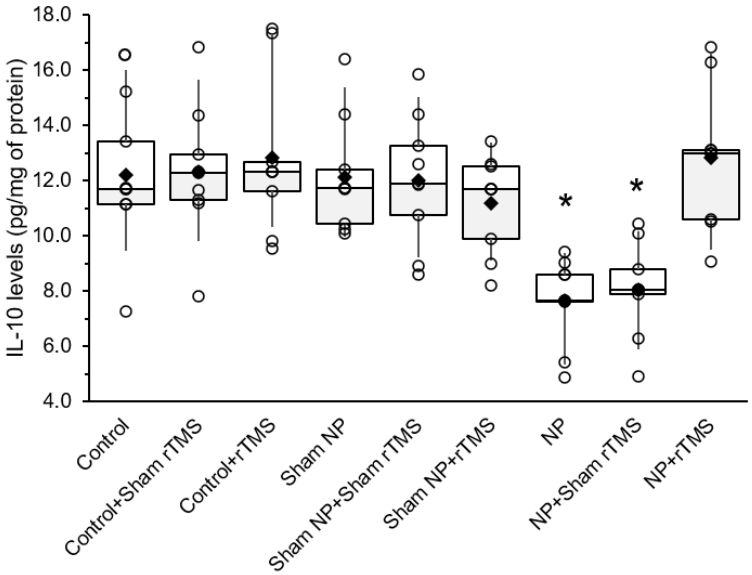
The BDNF, an important neurotrophin involved in neuroplasticity (Chakrapani et al. 2020), might help to reverse the impaired LTM. However, in our study, no difference between groups was found in hippocampal BDNF levels (one-Way ANOVA, P>0.05)

(Figure 4).



IL-10 Levels

The IL-10 also plays an important role in memory improvement (Worthen et al 2020). For this reason, we evaluated its levels in the hippocampus. In fact, a difference between groups was found (one-Way ANOVA/SNK, $F_{8,80}$=6.921, P<0.05). The SNK *post hoc* test showed a decrease in IL-10 levels induced by neuropathic pain, which was reversed by rTMS treatment (Figure 5). And this rTMS effect, such as the mnemonic effect, is state dependent since it is observed only in NP animals, without effect in Control and Sham-NP groups.



DISCUSSION

This is the first preclinical study that shows that rTMS reverses memory impairment and

reductions of hippocampal IL-10 levels, both induced by NP in rats. We highlight that in the current study, rTMS, as a modulatory technique, did not impact the LTM in control nor in Sham-NP groups, that is, animals without pain, only in animals with pain. This is an important result, since it may have clinical applications, demonstrating that rTMS is a safe tool in healthy subjects and, at the same time, effective when there is an unbalance in one or more systems, such as in NP conditions.

To date, there are no studies in rats showing rTMS effects over chronic pain-induced memory impairment. However, in primates it has been shown that rTMS relieves pain induced by stroke and normalizes the inappropriately strengthened functional connectivity between the ipsilesional mediodorsal nucleus of the thalamus and the amygdala, which are regions associated with emotion and memory (Kadono et al. 2021). rTMS has also been applied to improve the impaired memory and cognition in elderly patients (for a review see Gomes-Osman et al. 2018), modulating learning and memory functions (Grafman and Wassermann 1999). Besides, our research group has already observed that tDCS, another neuromodulatory technique, is able to improve short-term memory (STM) (Leffa et al. 2016) and LTM (Leffa et al. 2018) in Spontaneously Hypertensive Rats (SHR), and induced an increase of hippocampal ROS production in SHR and GSH enzyme levels in both strains. Moreover, an anti-inflammatory effect was observed in the brain of Wistar Kyoto (WKY) rats after treatment. These studies demonstrate that noninvasive tools can be used for memory improvement.

Memory can be influenced by several biomarkers, such as inflammatory mediators and neurotrophins (Azizi et al 2015; Miranda et al. 2019). It is important to note that the increase in the production of anti-inflammatory cytokines is generally associated with neurogenesis (Pereira et al. 2015), and IL-10 receptors are presented in the major glial populations promoting survival of both glia and neurons (Strle et al. 2001). IL-10, an anti-inflammatory interleukin, has a critical role in promoting learning and memory in rodents, as demonstrated in the learned helplessness paradigm, where the administration of IL-10 provides pro-cognitive actions, acting in the synapse balance, suggesting a potential therapeutic use (Worthen et al. 2020). IL-10 also plays an important role in NP conditions, as demonstrated in a study in which chronic and partial nerve injury decreased its levels in the dorsal root ganglion and in the sciatic nerve (Khan et al. 2015). IL-10 reverses learning and memory deficits in inflammation-dependent models by blocking the damaging effects of lipopolysaccharide (LPS) or interleukin 1 beta (IL-1β) on long-term

potentiation (LTP) (Kelly et al. 2001; Lynch et al. 2004; Kiyota et al. 2012).

rTMS has the capacity to alter IL-10 levels under many other conditions. In a recent study, using adolescent mice that were exposed to a model of multiple sclerosis, deep rTMS reduced microglial activation and increased the cortical IL-10 levels (Yang et al. 2020), alleviating neurologic anomalies. Another preclinical study, using rats submitted to a Parkinson's disease model, showed a synergism of the rTMS treatment and the human mesenchymal stem cells transplantation, upregulating IL-10 expression and decreasing Interferon gamma (IFN-γ) and Tumor Necrosis Factor alpha (TNF-α) production in the substantia nigra (Lee et al. 2020). Additionally, the inhibition of neurotoxic astrocytic polarization, with an IL-10 role, has been suggested as a potential mechanism in the effectiveness of high-frequency rTMS (10Hz) in cerebral ischemic stroke in rats (Hong et al. 2020). In this way, we can suggest that the increasing levels of IL-10 induced by rTMS can be the biological basis for the improved LTM in rats with NP. Our hypothesis is that changes in neurogenesis are involved in the allodynia (Toledo et al., 2021) and impaired memory performance induced by NP (current study), which are reverted by rTMS.

Besides that, in the current study we evaluated levels of BDNF in the hippocampus, an important brain area for memory mechanisms (Thompson and Kim 1996). However, there were no changes in hippocampal BDNF levels in NP rats, suggesting a CNS region-specific effect, since in the spinal cord it has been observed that BDNF has an important role in NP processes. BDNF is produced by primary sensory neurons located in the dorsal root ganglion (Tsai 2005), and it is transported to the dorsal horn of the spinal cord (Cho et al. 1997), where it activates the tyrosine kinase B (TrkB) receptor (Widenfalk et al. 2001). Previous studies from our group already showed BDNF levels changes in spinal cord in rats submitted to the same NP model (Cioato et al. 2016; Lopes et al. 2021; Santos et al. 2020), corroborating our hypothesis that levels and role of BDNF can be CNS region-specific. Additionally, it is known that aged rats are more susceptible to the development of pain sensitization after nerve injury, due to the reduction in hippocampal BDNF level (Tateiwa et al. 2018). However, we used adult, not aged rats in the current study, and this can also be an explanation for the lack of BDNF alterations in the hippocampus.

As for the effects of rTMS on BDNF, preclinical studies have shown an increase in the BDNF mRNA expression in hippocampal areas such as CA3, granule cell layer of the

dentate gyrus, besides the parietal cortex and the piriform cortex in rats (Müller et al. 2000). In a clinical study in elderly patients diagnosed with refractory depression, rTMS increased BDNF and decreased IL-1β and TNF-α serum levels only in patients with the disease, with no changes in healthy individuals (Zhao et al. 2019). In the current study, the hippocampal BDNF levels were not altered by NP nor by rTMS treatment, demonstrating that the improvement in LTM in NP rats was mediated, at least in part, by hippocampal IL-10 levels, but not hippocampal BDNF levels. However, we cannot rule out that other mediators, not evaluated in this study, are involved in these observed rTMS effects.

It is important to note that rTMS modifies neuronal excitability outside the skull inducing neuroplasticity not only in the target area but also indirectly in other brain regions (Pernia et al. 2020). In humans, it has been demonstrated that the stimulation of the motor cortex by rTMS is able to modulate cortical and spinal cord circuits, inducing neuronal plasticity (Hallett 2007; Yang et al. 2020), despite the distance from the stimulated area. Still in humans, it has already been shown that rTMS can influence targeted hippocampal-cortical networks, producing increased functional MRI connectivity of these networks and concomitant improvements in memory that outlast stimulation by ~24 hours. Also, multiple-day targeted stimulation of hippocampal-cortical networks produces even longer-lasting enhancement (Wang and Voss 2015). In fact, rTMS has been used to modulate abnormal brain activities changing neuronal excitability. High-frequency stimulation (> 5 Hz) is associated with increased cortical excitability, while low-frequency stimulation (< 1 Hz) decreases cortical excitability (Yang and Chang 2020). It does not mean that the inhibitory descending pathways would be inhibited by rTMS application: instead, they can often be enhanced, depending on the stimulation protocol (Lazzaro et al. 2010). A systematic review showed that rTMS can produce clinically significant relief from chronic pain, but the heterogeneity among the studies doesn't enable definitive conclusions about the ideal parameters (Hamid, Malik and Hussain 2018). Despite that, the importance of the stimulation site has been described. It has been postulated that rTMS induces alterations in the activity of cortical and subcortical brain structures, including the orbitofrontal cortices, medial thalamus, anterior cingulate, and periaqueductal gray matter (Lefaucheur 2008; Yang and Chang 2020). Sampson and colleagues (2011) have shown that low-frequency rTMS over the dorsolateral prefrontal cortex in human patients was effective in pain relief. Corroborating this clinical study,

our recent preclinical study, using rTMS at low-frequency, showed a total reversal of the thermal hyperalgesia and partial reversion of the allodynia in NP rats (Toledo et al. 2021). Also, in the same study, we showed that the major neurochemical alterations induced by rTMS were related to the PFC and spinal cord, which had increased the levels of BDNF, IL-10, and TNF-α, suggesting that it can be due to the proximity to the stimulation site. In the current manuscript, due to the size of the rats' head/brain, we could not define the region of rTMS application, and we recognize this as a limitation of the study.

Previous studies have found that rTMS can reduce proinflammatory and increase anti-inflammatory cytokines (Zhao et al. 2019; Lee et al. 2020; Yang et al. 2020), demonstrating an important role in inflammation. However, considering that this is the first preclinical study that assesses the effect of rTMS on memory impairment induced by NP, we cannot infer what is the anti-inflammatory mechanism that is promoting this effect. Moreover, it does not mean that it happens to a lower neuronal activity, since rTMS inhibitory effects can be also related to the stimulation of inhibitory pathways, not only the decrease of excitatory pathways (Damien et al. 2018), as cited above. For this, we could not affirm that a sort of general "lower" neural activity could lead to an anti-inflammatory effect found here. However, this does not mean that IL-10 enhancement by rTMS does not have an important role in the LTM improvement. We thus believe that, although the stimulation in rodents is still not focal, the observed results have translational potency, since the analyzed parameters have similarity with results from clinical investigations.

In conclusion, this pioneer preclinical study showed that low-frequency rTMS, a potential tool for NP treatment, improves NP-impaired LTM, and suggests the increasing hippocampal IL-10 levels as one of the likely biological bases of rTMS mnemonic effects. Furthermore, rTMS appears to be a safe therapeutic tool since it does not alter memory or biochemical parameters in naive animals.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Acknowledgments and funding sources

This research was supported by the following Brazilian funding agencies: National Council for Scientific and Technological Development–CNPq (Dr. ILS Torres, Dr. W

Caumo); Brazilian Committee for the Development of Higher Education Personnel – CAPES (RS Toledo, LS da Silva, HR Medeiros), and CAPES/PNPD Edital PPGCM 07/2016 (Dr. DJ Stein); Graduate Research Group of Hospital de Clínicas de Porto Alegre - GPPG (ILS Torres – Grant 2017-0438); Research Support Foundation of the State of Rio Grande do Sul (PRS Sanches – Grant FAPERGS-PRONEM 16/2551-0000249-5).

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PARTE III

DISCUSSÃO GERAL

7 DISCUSSÃO GERAL

Estudos pré-clínicos que investigam a fisiopatologia e novas abordagens terapêuticas da DN são de suma importância, devido ao crescente número de casos na população e ao grande número de pacientes que são refratários aos tratamentos farmacológicos tradicionais.

Os resultados desta tese demonstram que a EMTr é capaz de reverter a hiperalgesia térmica e o prejuízo da memória de longo-prazo induzidos pelo modelo animal de DN por meio da CCI. Além disso, o tratamento foi capaz de aumentar os níveis centrais de BDNF, IL-10 e TNF-α e reverter a redução dos níveis hipocampais de IL-10, nesses animais. A modulação cortical induzida pela EMTr nos ratos com DN pode ter contribuído para o efeito analgésico encontrado neste estudo, enquanto o aumento dos níveis hipocampais de IL-10 podem ter colaborado para a reversão do prejuízo da memória de longo prazo nos animais com DN.

Observou-se também que os efeitos da EMTr parecem ser "estado-dependentes", ou seja, as alterações centrais (comportamentais e neuroquímicas) induzidas pelo tratamento estão presentes somente nos animais com DN, sem alteração em animais controle e *sham* cirurgia (ambos sem a presença de lesão periférica). Visando a translacionalidade, este é um importante achado, pois demonstra que a EMTr parece ser uma ferramenta segura em indivíduos saudáveis, sendo eficaz quando há um desequilíbrio em um ou mais sistemas, como nos quadros de DN. A capacidade de uma técnica neuromodulatória como a EMTr, de apresentar efeitos apenas em casos em que há uma doença estabelecida, ou seja, em animais com um estado mal adaptativo como a DN, foi também demonstrado por nosso grupo utilizando a ETCC (Cioato et al. 2016; Filho et al. 2016).

O córtex cerebral é a primeira estrutura a receber o estímulo da EMTr. O fato de estar próximo do local da estimulação pode ter contribuído para o aumento nos níveis corticais dos marcadores neuroquímicos estudados (BDNF, IL-10 e TNF- α). Por se tratar de uma estimulação de baixa frequência (1Hz), está associada a uma diminuição da excitabilidade cortical (Yang e Chang 2020), sendo transmitida para outras regiões cerebrais. De fato, já foi demonstrado que a EMTr induz alterações na atividade das estruturas cerebrais corticais e subcorticais relacionadas à modulação e processamento da dor, incluindo os córtices orbito frontais, o tálamo medial, o cíngulo anterior e a substância cinzenta periaquedutal (Lefaucheur 2008). Também, estimula vias neurais inibitórias descendentes

no corno dorsal da medula espinhal, reduzindo a dor (Leung et al. 2009).

Mais especificamente sobre o BDNF, não foi observada alteração dos seus níveis nas regiões cerebrais estudadas nos animais com DN. Este foi um achado não esperado, principalmente em medula espinhal, uma vez que esta neurotrofina está estreitamente relacionada a DN, modulando sinalizações rápidas excitatória (glutamatérgica) e inibitória (gabaérgica), assim como a neurotransmissão peptidérgica na medula espinhal (Merighi et al. 2008). Na verdade, somente o fato de os animais terem passado por um evento cirúrgico, *sham* ou efetivo, foi capaz de aumentar os níveis medulares de BDNF. Já a EMTr induziu um aumento observado nos níveis de BDNF em córtex pré-frontal somente nos animais com DN, o que pode ter contribuído para o efeito antinociceptivo encontrado. É importante salientar que o BDNF atua como um neuromodulador central da dor e induz neuroplasticidade, principalmente na presença de eventos mal adaptativos, como a DN. Alguns estudos têm demonstrado um papel antinociceptivo do BDNF, por meio da ativação de vias serotoninérgicas, opioidérgicas e GABAérgicas (Siuciak et al. 1994; Pezet et al. 2002). Quando foi avaliada a possível contribuição do BDNF hipocampal na melhora do desempenho da memória de longo prazo nos animais com DN e estimulados com EMTr, não foi encontrada diferença entre os grupos. Desta forma, destaca-se o possível papel da IL-10 hipocampal na melhora deste parâmetro, não podendo, porém, ser afirmado que outros mediadores, aqui não avaliados, também não possam estar envolvidos neste efeito.

Prévios estudos têm demonstrado que a dor crônica envolvendo lesões de nervo periférico é frequentemente relacionada a prejuízos de memória (Ren et al. 2011) e potenciação de longa duração (do inglês, *long-term potentiation*, LTP) em sinapses de fibras C em medula espinhal, porém há também inibição LTP em hipocampo (Liu et al. 2017). BDNF (Basbaum et al. 2009) e TNF-α (Liu et al. 2017 são mediadores que têm sido relacionados a processos envolvidos em respostas mal adaptativas decorrentes da dor crônica. Desta forma podemos sugerir que terapêuticas que contraponham as alterações na plasticidade sináptica induzidas pela dor crônica, podem contribuir para o alívio da dor e para a também para a melhora da memória. Apesar disso, não foi encontrado nesta tese alteração nos níveis medulares de TNF-α nos animais com DN. Surpreendentemente, as alterações encontradas nos níveis dessa citocina, tanto no córtex pré-frontal quanto na medula espinhal, foram nos grupos com DN estimulados. Sabe-se que alterações em parâmetros neuroinflamatórios podem não ser apenas prejudiciais como também

benéficas, uma vez que mediadores inflamatórios contribuem para os processos de regeneração e, como no caso do TNF-α, como estimuladores da produção de citocinas anti-inflamatórias como a IL-10 (Sheng et al. 1995). Assim sendo, o aumento, induzido pela EMTr, nos níveis centrais de TNF-α, pode ter contribuído para os seus efeitos antinociceptivos observados nos animais com DN e estimulados.

Uma importante interleucina nos processos de DN é a IL-10, que também participa dos processos de aprendizado e memória em roedores (Worthen et al. 2020). Ratos submetidos ao modelo de DN apresentam níveis reduzidos de IL-10 no nervo isquiático e no gânglio da raiz dorsal (Khan et al. 2015). Nessa tese, observou-se um padrão dos níveis de IL-10 semelhante ao do BDNF, com aumento no córtex pré-frontal somente nos animais com DN estimulados com EMTr, enquanto na medula, o processo cirúrgico (*sham* ou ativo) foi capaz de causar aumento nos níveis desta interleucina. O aumento nos níveis de IL-10, principalmente no CPF, pode ter contribuído para o efeito analgésico induzido pela EMTr. Essa citocina exerce uma função anti-inflamatória, inativando as células apresentadoras de antígeno do sistema imunológico e diminuindo a secreção de citocinas pró-inflamatórias (Iyer e Chen 2012). O uso de terapia genética com IL-10 tem sido indicado para o alívio da dor neuropática periférica crônica (Milligan et al. 2012). Em relação ao hipocampo, foi verificado uma reversão da redução nos níveis de IL-10 induzida pela DN, nos animais tratados com EMTr, o que pode ter contribuído para a melhora do déficit na memória de longo prazo que estes animais apresentaram (Worthen et al. 2020).

Dados clínicos demonstram que estimular distintas regiões cerebrais está relacionado a diferentes efeitos: por exemplo, estimulação do córtex motor primário resulta na redução da dor, enquanto a estimulação do córtex pré-frontal dorsolateral é indicada para tratamento da depressão (Treister et al. 2013). Infelizmente, devido ao reduzido tamanho da cabeça do rato em relação à bobina de estimulação, não foi possível delimitar a região estimulada com EMTr, o que é considerado um limitante deste estudo. Porém, esta limitação não impediu o potencial translacional, uma vez que diversos resultados observados nesta tese são semelhantes ao que é observado na prática clínica. Desta forma, o desenvolvimento de novos estudos buscando possíveis mecanismos de ação, por meio da utilização de fármacos antagonistas/agonistas das vias estudadas, e a associação da técnica neuromodulatória com fármacos tradicionalmente utilizados no manejo da DN, buscando verificar possíveis efeitos sinérgicos, colocam-se como perspectivas.

8 CONCLUSÕES

Com base nos resultados desta tese, é possível sugerir a EMTr de baixa frequência

como uma ferramenta para o tratamento da DN, considerando sua capacidade de modular os níveis centrais de BDNF, TNF-α e a IL-10, com prováveis alterações na neuroplasticidade, contribuindo desta forma para a redução da alodinia e da hiperalgesia, e para a reversão do prejuízo de memória induzidos pela DN. Esse experimento pré-clínico demonstra a segurança da aplicabilidade da EMTr, uma vez que não foi observada nenhuma alteração nos diversos parâmetros avaliados em animais saudáveis (controle total e *sham* cirurgia). Sendo assim, é possível concluir que a EMTr apresenta relevante potencial no tratamento da DN e de outras doenças de difícil manejo terapêutico, trazendo benefícios e melhora da qualidade de vida aos pacientes.

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9 REFERÊNCIAS DA PARTE III

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APROVAÇÃO DA COMISSÃO DE ÉTICA NO USO DE ANIMAIS

10 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: 2017/0438

Título: ESTIMULAÇÃO MAGNÉTICA TRANSCRANIANA EM MODELO ANIMAL DE DOR NEUROPÁTICA

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

Equipe de Pesquisa: BETTEGA COSTA LOPES; CARLA DE OLIVEIRA; ROBERTA STRÔHER; LISIANE SANTOS DA SILVA;

Data de Aprovação: 29/09/2017

Data de Término: 21/08/2019

Espécie/Linhagem	Sexo/Idade	Quantidade
RATO ISOGÊNICO	M/55 Dia(s)	108
RATO ISOGÊNICO	M/55 Dia(s)	12
RATO ISOGÊNICO	M/55 Dia(s)	10

- 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.

Profª Patrícia Ashton Prolla

Coordenadora GPPG/HCPA

Impresso do sistema AGHU-Pesquisa por RAFAEL LEAL ZIMMER em 26/07/2018 15:02:33

PERSPECTIVAS

12 PERSPECTIVAS

A partir deste trabalho, novas pesquisas serão desenvolvidas buscando esclarecer mecanismos de ação envolvidos nos efeitos antinociceptivos da EMTr, assim como verificar se há maiores benefícios na aplicação de protocolos de tratamento mais longos, duração destes efeitos e possíveis efeitos sinérgicos. Também, espera-se aplicar essa ferramenta não-farmacológica em outros modelos animais, como por exemplo, dor aguda e acidente vascular encefálico (AVE).

Portanto, pretende-se avaliar em novos estudos:

- Protocolos de tratamento EMTr mais prolongados,
- Verificar os efeitos a longo prazo deste tratamento,
- Associação da EMTr com fármacos tradicionalmente utilizados no manejo da DN e possíveis efeitos sinérgicos,
- Uso de fármacos agonistas/antagonistas para elucidar possíveis mecanismos de ação da EMTr,
- Aplicar a EMTr isoladamente e/ou em associação a fármacos em outros modelos animais, como dor aguda e AVE.

PRODUÇÃO ACADÊMICA DURANTE O DOUTORADO

12 PRODUÇÃO ACADÊMICA DURANTE O DOUTORADO

12.1 Artigos Publicados

- TOLEDO, ROBERTA STRÖHER; STEIN, DIRSON JOÃO; SANCHES, PAULO ROBERTO STEFANI; DE SOUZA, ANDRESSA; DA SILVA, LISIANE SANTOS; MEDEIROS, HELOUISE RICHARDT; ANTUNES, MAYRA ANGÉLICA DE SOUZA; DE CASTRO, JOSIMAR MACEDO; FREGNI, FELIPE; CAUMO, WOLNEI; TORRES, IRACI L.S. Repetitive Magnetic Stimulation (rTMS) reverses the long-term memory impairment and the drecrease of hippocampal interleukin-10 levels, both induced by neuropathic pain in rats. NEUROSCIENCE, 2021.

- TOLEDO, ROBERTA STRÖHER; STEIN, DIRSON JOÃO; SANCHES, PAULO ROBERTO STEFANI; DA SILVA, LISIANE SANTOS; MEDEIROS, HELOUISE RICHARDT; FREGNI, FELIPE; CAUMO, WOLNEI; TORRES, IRACI L.S. rTMS induces analgesia and modulates neuroinflammation and neuroplasticity in neuropathic pain model rats. BRAIN RESEARCH, 2021.

- STRÖHER, ROBERTA; DE OLIVEIRA, CARLA; STEIN, DIRSON JOÃO; DE MACEDO, ISABEL CRISTINA; GOULARTE, JÉFERSON FERRAZ; DA SILVA, LISIANE SANTOS; REGNER, GABRIELA GREGORY; MEDEIROS, HELOUISE RICHARDT; CAUMO, WOLNEI; TORRES, IRACI L.S. Maternal deprivation and sex alter central levels of neurotrophins and inflammatory cytokines in rats exposed to palatable food in adolescence. NEUROSCIENCE, v. 00, p. 000, 2020.

- OLIVEIRA, C; TOLEDO, RS; SCARABELOT, VL; VERCELINO, R; DA SILVA, LS; REGNER, GG; DE SOUZA, A; SILVEIRA, NP; CAUMO, W; TORRES, ILS. Neonatal morphine exposure and maternal deprivation alter nociceptive response and central biomarkers' levels throughout the life of rats. NEUROSCIENCE LETTERS, v.738, p.135350, 2020.

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- STRÖHER, ROBERTA; DE OLIVEIRA, CARLA; COSTA LOPES, BETTEGA; DA SILVA, LISIANE SANTOS; REGNER, GABRIELA GREGORY; RICHARDT MEDEIROS, HELOUISE; DE MACEDO, ISABEL CRISTINA; CAUMO, WOLNEI; TORRES, IRACI LS. Maternal Deprivation Alters Nociceptive Response in a GENDER-Dependent Manner in Rats. INTERNATIONAL JOURNAL OF DEVELOPMENTAL NEUROSCIENCE, v. 18, p. 30266-1, 2019.

- DE OLIVEIRA, CARLA; DE FREITAS, JOICE SOARES; MACEDO, ISABEL CRISTINA; SCARABELOT, VANESSA LEAL; STRÖHER, ROBERTA; SANTOS, DANIELA SILVA; SOUZA, ANDRESSA; FREGNI, FELIPE; CAUMO, WOLNEI; TORRES, IRACI L.S. Transcranial direct current stimulation (tDCS) modulates biometric and inflammatory parameters and anxiety-like behavior in obese rats. NEUROPEPTIDES, v. 18, p. 30064-30067, 2018.

- *12.2 Resumos Publicados em Anais de Congresso*

- TOLEDO, ROBERTA S; STEIN, D. J. ; SANCHES, P. R. S. ; SILVA, L. S. ; MEDEIROS, HELOUISE RICHARDT ; CAUMO, W. ; TORRES, IRACI LS . Repetitive transcranial magnetic stimulation (rTMS) alters biomarkers in prefrontal cortex of rats submitted to a neuropathic pain model. In: XII SIMPÓSIO INTERNACIONAL EM NEUROMODULAÇÃO, 2020, São Paulo. XII Simpósio de Neuromodulação (International Neuromodulation Symposium), 2020.

- TOLEDO, ROBERTA S; STEIN, D. J. ; SANCHES, P. R. S. ; SILVA, L. S. ; MEDEIROS, HELOUISE RICHARDT ; ANTUNES, M. A. S. ; CAUMO, W. ; TORRES, IRACI L.S. . ESTIMULAÇÃO MAGNÉTICA TRANSCRANIANA REPETITIVA (EMTR) INDUZ ANALGESIA EM RATOS SUBMETIDOS A UM MODELO DE DOR NEUROPÁTICA. In: 40ª Semana Científica do Hospital de clínicas de Porto Alegre, 2020, Formato digital. 40ª Semana Científica do HCPA, 2020.

- ANTUNES, M. A. S. ; TOLEDO, ROBERTA S; STEIN, D. J. ; SANCHES, P. R. S. ; SILVA, L. S. ; MEDEIROS, HELOUISE RICHARDT ; CAUMO, W. ; TORRES, IRACI LS. AVALIAÇÃO DA ESTIMULAÇÃO MAGNÉTICA TRANSCRANIANA REPETITIVA EM ANIMAIS COM DOR NEUROPÁTICA SOBRE A MEMÓRIA DE LONGO PRAZO E NÍVEIS DE BDNF NO CÓRTEX PRÉ-FRONTAL. In: 40ª Semana Científica do Hospital de clínicas de Porto Alegre, 2020, Formato digital. 40ª Semana Científica do HCPA, 2020.

- VICENZI, C. B. ; ZANCANARO, M.; ASSUMPCAO, J. A. F. ; LOPES, B. C. ; SOUZA, A. ; STRÖHER, R ; DE MACEDO, ISABEL CRISTINA ; FREGNI, F. ; CAUMO, W. ; TORRES, I. L. S. . USO PREVENTIVO DA ESTIMULAÇÃO TRANSCRANIANA POR CORRENTE CONTÍNUA REDUZ INFLAMAÇÃO CENTRAL EM RATOS WISTAR. In: 40ª Semana Científica do Hospital de clínicas de Porto Alegre, 2020, Porto Alegre. 40ª Semana Científica do HCPA, 2020.

- STRÖHER, R; SANCHES, P.R.S.; SILVA, L. S.; MEDEIROS, H.; STEIN, D. J.; FREGNI, F.; CAUMO, W.; TORRES, I.L.S. Repetitive Transcranial Magnetic Stimulation (rTMS): development of a preclinical equipament. In: XI Simpósio Internacional em Neuromodulação, 2019, São Paulo. XI Simpósio Internacional em Neuromodulação, 2019.

- SILVA, L. S.; OLIVEIRA, C.; STRÖHER, R ; SALVI, A. A. ; MEDEIROS, H. ; CAUMO, W.; SANCHES, P.R.S. ; VERCELINO, R.; FREGNI, F. ; TORRES, I.L.S. Effect of acupunture and/or Trancranial Direct Stimulation (tDCS) on nociceptive response and central levels of IL-1B and IL-10 in rats submitted to a Neurophatic pain model. In: XI Simpósio Internacional em Neuromodulação, 2019, São Paulo. XI Simpósio Internacional em Neuromodulação, 2019.

- SILVA, L. S.; OLIVEIRA, C.; STRÖHER, R; SALVI, A. A.; MEDEIROS, H.; CASTRO, J. M.; PEREIRA, F. S.; VERCELINO, R.; TORRES, I.L.S. Terapias Up-Down E Bottom-Up Alteram Comportamento Antinociceptivo E Parâmetro Inflamatório Em Modelo Animal De Dor Neuropática. In: 39ª Semana Científica Do Hospital De Clínicas De Porto Alegre, 2019, Porto Alegre. 39ª Semana Científica Do Hospital De Clínicas De Porto Alegre, 2019.

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- STRÖHER, R; OLIVEIRA, C.; SILVA, L. S.; MEDEIROS, H.; MACEDO, I. C.; TORRES, I. L. S. Exposição Ao Alimento Palatável Na Adolêscencia Não Altera Os Níveis Hipotalâmicos E Hipocampais De Citocinas Pró-Inflamatórias Entre Ratos Machos E Fêmeas Submetidos À Deprivação Materna No Periodo Neonatal. In: 39ª Semana Científica Do Hospital De Clínicas De Porto Alegre, 2019, Porto Alegre. 39ª Semana Científica Do Hospital De Clínicas De Porto Alegre, 2019.

- CASTRO, J. M.; SILVA, L. S.; OLIVEIRA, C.; STRÖHER, R; MEDEIROS, H.; CAUMO, W.; VERCELINO, R.; TORRES, I.L.S. Acupunture improves articular function and combined with transcranial direct current stimulation, modulates anti-inflammatory cytokine IL4 in animal model of neuropathic pain.. In: X Congresso Internacional de Atualização em Neurociência, 2019, São Paulo. X Congresso Internacional de Atualização em Neurociência, 2019.

- *12. 3 Orientação de bolsista de Iniciação Científica*

- Orientação da aluna de graduação do curso de Medicina/UFRGS Mayra Angélica de Souza Antunes a partir de Jan/2020 até o presente momento.