Faculdade de Medicina

Programa de Pós-Graduação em Medicina: Ciências Médicas

DANIELA SILVA SANTOS

Impacto da Estimulação Transcraniana por Corrente Contínua (ETCC) em ratos Wistar machos submetidos a um modelo de dor crônica e / ou exposição ao álcool

Porto Alegre

2021

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

Faculdade de Medicina

Programa de Pós-Graduação em Medicina: Ciências Médicas

TESE DE DOUTORADO

Impacto da Estimulação Transcraniana por Corrente Contínua (ETCC) em ratos Wistar machos submetidos a um modelo de dor crônica e / ou exposição ao álcool

Autora: Daniela Silva Santos

Orientadora: Prof.ª Dr.ª Iraci Lucena da Silva Torres

Tese de Doutorado apresentada ao Programa de Pós-Graduação em Medicina: Ciências Médicas, como requisito parcial para obtenção do título de Doutora em Ciências Médicas, da Universidade Federal do Rio Grande do Sul.

Porto Alegre

2021

Impacto da Estimulação Transcraniana por Corrente Contínua (ETCC) em ratos Wistar machos submetidos a um modelo de dor crônica e/ou à exposição ao álcool.

CIP - Catalogação na Publicação

SANTOS, DANIELA SILVA
Impacto da Estimulação Transcraniana por Corrente Contínua (ETCC) em ratos Wistar machos submetidos a um modelo de dor crônica e / ou exposição ao álcool / DANIELA SILVA SANTOS. -- 2021.
156 f.
Orientadora: Iraci Lucena da Silva Torres.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Medicina, Programa de Pós-Graduação em Medicina: Ciências Médicas, Porto Alegre, BR-RS, 2021.

1. Dor neuropática. 2. Etanol. 3. Wistar. 4. Biomarcadores. 5. NPY. I. Torres, Iraci Lucena da Silva, orient. II. Título.

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

BANCA EXAMINADORA

Dr.ª Luciana Cadore Stefani

(PPGCM-UFRGS)

Dr. Ricardo Xavier

(PPGCM-UFRGS)

Dr.ª Mirna Leal

(PPGFT-UFRGS)

Dr.ª Izabel Cristina Custodio de Souza

(UFPEL)

Ao meu pai (*in memorian*).

AGRADECIMENTOS

Esta tese foi resultado de muitos. Foi uma jornada de desafios, construção e amadurecimento. Não foi fácil nem sem esforço, mas em meio às dificuldades sempre tive a convicção de não desistir jamais. Afinal somos sempre autores, desde o nascimento... Autor e ator de nossa vida e de capítulos de vidas alheias próximas a nossa. Por isso, agradeço muito à minha Orientadora, Professora Dr.ª Iraci Torres, pela decisão em me permitir o privilégio de tê-la em meu livro da vida: pessoal e profissional. A Professora Iraci sempre me abriu os braços, mas não foi única. Agradeço sincera e profundamente a todas as pessoas que muito me encorajaram e me ajudaram: não poderia aqui citar todas, pois são muitas...

Reforço o agradecimento ao meu grande exemplo, a professora Iraci. Tenho imenso orgulho de tê-la como orientadora desde os primeiros semestres da graduação. Jamais esquecerei seus ensinamentos e inestimável confiança. Muito obrigada!

Manifesto aqui minha gratidão aos eternos amigos: o Prof. Diogo Onofre Souza, o Prof. Rafael Roesler, a Prof.ª Lavínia Faccini, a Prof.ª Isabel Macedo e a Prof.ª Dr.ª Carla de Oliveira. Muito obrigada!

Meus respeitosos agradecimentos ainda aos membros da banca examinadora e ao imprescindível apoio da CAPES (Coordenação de Aperfeiçoamento e Pessoal de Nível Superior). Ao Hospital de Clínicas de Porto Alegre, em especial nas pessoas da Enfermeira Marta e do Jeferson responsáveis, respectivamente pelo UEA (Unidade de Experimentação Animal) e UAMP (Unidade de Análises Moleculares e de Proteínas); à minha instituição mãe, a UFRGS; e ao Programa de Pós-graduação em Medicina: Ciências Médicas.

Não posso deixar de agradecer ao essencial suporte da Prof.ª Liciane, do Dr. Dirson, e do colega de Doutorado, Bettega. Agradeço ainda a cada um dos demais colegas do grupo de pesquisa, Laboratório de Farmacologia da Dor e Neuromodulação: Investigações Pré-Clínicas, que foram fundamentais para o resultado desse trabalho.

Meu muito obrigada a todos os meus amigos de vida, tão importantes e que me encorajam sempre, em especial à Pamela.

Por fim, agradeço às pessoas mais importantes da minha vida e que me apoiam incondicionalmente: minha amada família. Em especial às minhas irmãs, Drica e Val, à minha mãe Lucia e ao Paulinho.

"Aqueles que passam por nós, não vão sós, não nos deixam sós.

Deixam um pouco de si, levam um pouco de nós. "

Antoine de Saint-Exupéry

RESUMO

Dor crônica e o uso nocivo do álcool representam um problema de saúde pública mundial, com altas prevalências, redução da qualidade de vida e elevados custos sociais e econômicos. Ambas as doenças resultam de processos mal adaptativos no sistema nervoso e as inter-relações entre a dor e o consumo de álcool são consideradas de natureza bidirecional. Mecanismos complexos envolvendo processos neuroplásticos são intrinsicamente ligados e a ineficácia das terapias atuais para o tratamento de dor crônica e do uso nocivo do álcool, pelo menos em parte, resulta da pouca compreensão científica para os processos envolvidos em cada uma dessas condições, assim como na associação delas. Por outro lado, as técnicas neuromodulatórias, como a estimulação transcraniana por corrente contínua (ETCC, ou tDCS, da sigla em inglês), atuam por múltiplos mecanismos de ação e apresentam poucos efeitos adversos, sendo promissoras abordagens terapêuticas. Considerando a relevância do tema, esta tese, composta por 3 manuscritos científicos (artigo 1 – publicado, artigo 2 – submetido, artigo 3 – submetido), teve como objetivo avaliar o efeito do tratamento repetido com a ETCC sobre parâmetros comportamentais e neurais em ratos machos adultos submetidos ao modelo animal de dor neuropática crônica e / ou exposição involuntária ou voluntária ao álcool (dependendo do experimento). Foram desenvolvidos 2 experimentos, sendo em ambos, utilizado o modelo de dor neuropática pela constrição do nervo isquiático (CCI, da sigla em inglês). No primeiro experimento (artigos 1 e 2), os animais dos grupos que receberam álcool, foram tratados 1 vez ao dia, durante 15 dias por gavagem e foram divididos em 5 grupos: controle (CT), dor neuropática (NP), dor neuropática + ETCC (NPtDCS), dor neuropática + álcool (NPAL), dor neuropática + álcool + ETCC (NPALtDCS). No segundo experimento (artigo 3), os animais foram expostos ao consumo de álcool de forma voluntaria junto à água de beber, podendo optar por uma de duas garrafas, e foram divididos em 6 grupos: controle (CT), dor neuropática + shamETCC (NP), dor neuropática + álcool (NPAL), álcool + shamETCC (AL), álcool + ETCC

(ALtDCS) , dor neuropática + álcool + ETCC (NPALtDCS). No artigo 1 foram avaliados a hiperalgesia térmica e os níveis de NGF, IL-10, IL-1 α e IL-1 β em córtex e tronco encefálico; no artigo 2 foram avaliados o comportamento do tipo ansioso e os níveis centrais de IL-1 α e IL-1 β (hipocampo, estriado, cerebelo e medula espinhal), RNA mensageiro da *Il1a* e *IL1b* em cerebelo, hipocampo e medula espinhal; e imunorreatividade de neuropeptídeo Y (NPY) em hipotálamo; e o artigo 3 avaliou consumo de álcool, imunorreatividade de NPY em córtex pré-frontal (CPF), amígdala e estriado, e BDNF, NGF, IL-10,e IL-6 em cerebelo e estriado. Os ratos com CCI apresentaram hiperalgesia térmica que foi revertida pelo tratamento com ETCC, e a ação da ETCC foi retardada pela abstinência ao álcool. Além disso, a CCI induziu um progressivo aumento no consumo de álcool que foi reduzido ao longo do tempo pelo tratamento com a ETCC. Tanto o CCI quanto a exposição ao álcool induziram a comportamentos do tipo ansioso, que foram revertidos pela ETCC. Dor neuropática crônica, exposição ativa ou passiva ao álcool e o tratamento com ETCC modularam a imunorreatividade central do NPY, os níveis centrais de IL-1α, IL-1ß e IL-10 e de NGF; aumentaram expressão de *Il1a* e *Il1b* em medula espinhal e de *Il1b* em cerebelo. CCI e / ou ETCC reduziram os níveis de BDNF no estriado. ETCC aumentou os níveis cerebelares de IL-6 e IL-10. CCI e / ou exposição ao tDCS reduziram os níveis de IL-1α no estriado e ratos expostos a associação de CCI, álcool e ETCC apresentaram um aumento nos níveis de IL-1α em hipocampo. Concluindo, nossos resultados sugerem a ETCC como uma ferramenta promissora no tratamento de quadros de dor crônica e do uso nocivo do álcool. Neste sentido esta tese aumenta a compreensão neurobiológica relacionada ao paradigma da associação das duas doenças e dos mecanismos envolvidos no tratamento com a ETCC. É importante salientar o aspecto translacional, pois tratamentos não farmacológicos como a ETCC são importantes ferramentas na terapia de transtornos que envolvam neurocircuitos complexos, como processos de dor crônica e abuso de álcool.

Palavras-Chave: Dor neuropática; Etanol; Wistar; Biomarcadores; NPY; interleucinas.

ABSTRACT

Chronic pain and harmful use of alcohol represent a worldwide public health problem, with high prevalence, reduced quality of life and high social and economic costs. Both diseases result from maladaptive processes in the nervous system and the interrelationships between pain and alcohol consumption are considered bidirectional in nature. Complex mechanisms involving neuroplastic processes are intricately linked and the ineffectiveness of current therapies for the treatment of chronic pain and the harmful use of alcohol, at least in part, results from little scientific understanding of the processes involved in each of these conditions, as well as in their association. On the other hand, neuromodulatory techniques, such as transcranial direct current stimulation (tDCS), act by multiple mechanisms and have few adverse effects, being promising therapeutic approaches. Considering the relevance of the theme, this doctoral dissertation, composed of 3 scientific manuscripts (article 1 - published, article 2 - in preparation, article 3 - submitted), aimed to evaluate the effect of repeated treatment with tDCS on behavioral and neural parameters in adult male rats submitted to the animal model of chronic neuropathic pain and / or forced or voluntary exposure to alcohol (depending on the experiment). Two experiments were developed, and both used the neuropathic pain model due to sciatic nerve constriction (CCI). In the first experiment (articles 1 and 2), the animals in the groups that received alcohol were treated once a day, during 15 days by gavage and were assigned to one of 5 groups: control (CT), neuropathic pain (NP), neuropathic pain + tDCS (NPtDCS), neuropathic pain + alcohol (NPAL), neuropathic pain + alcohol + tDCS (NPALtDCS). In the second experiment (article 3), the animals were voluntarily exposed to alcohol consumption, in which alcohol and water were offered in separate bottles, and rats were assigned to one of 6 groups: control (CT), neuropathic pain + shamtDCS (NP), neuropathic pain + alcohol (NPAL), alcohol + shamtDCS (AL), alcohol + tDCS (ALtDCS), neuropathic pain + alcohol + tDCS (NPALtDCS). In article 1, thermal hyperalgesia and the levels of NGF, IL-10, IL-1α and IL-1β

in the cortex and brain stem were evaluated. In article 2, anxious-like behavior and central levels of IL-1α and IL-1β (hippocampus, striatum, cerebellum and spinal cord), mRNA of *Il1a* and *Il1b* in cerebellum, hippocampus and spinal cord were evaluated, and NPY immunoreactivity in hypothalamus. Finally, in article 3 alcohol consumption, NPY immunoreactivity in PFC, amygdala and striatum, and BDNF, NGF, IL-10, and IL-6 in cerebellum and striatum were assessed. The rats with CCI showed thermal hyperalgesia that was reversed by treatment with tDCS, and the action of tDCS was delayed by alcohol withdrawal. In addition, CCI induced a progressive increase in alcohol consumption that was reduced over time by treatment with tDCS. Both the CCI and exposure to alcohol induced anxiety-like behaviors, which were reversed by the tDCS. Chronic neuropathic pain, active or passive exposure to alcohol and treatment with tDCS modulated the central immunoreactivity of NPY, the central levels of IL-1α, IL-1ß, IL-10 and NGF, and increased the expression of *Il1a* and *Il1b* in the spinal cord and *Il1b* in the cerebellum. CCI and / or tDCS reduced the levels of BDNF in the striatum. tDCS increased the cerebellar levels of IL-6 and IL-10. CCI and / or exposure to tDCS reduced levels of IL-1α in the striatum and rats exposed to the association of CCI, alcohol and tDCS showed an increase in IL-1α levels in the hippocampus. In conclusion, our results suggest tDCS as a promising tool for the treatment of chronic pain and of harmful alcohol use. In this sense, this dissertation increases the neurobiological understanding related to the paradigm of the association of the two diseases and the mechanisms involved in the treatment with tDCS. It is important to highlight the translational aspect, since non-pharmacological treatments such as tDCS are important tools in the treatment of disorders involving complex neurocircuits, such as chronic pain processes and alcohol abuse.

Keywords: Neuropathic pain; Ethanol; Wistar; Biomarkers; NPY; interleukins.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS

AC1 – Adenilil ciclase tipo 1

AINES – Anti-inflamatórios não esteroidais

AMPA – Ácido α-Amino-3-Hidróxi-5-Metil-4-Isoxazolepropiônico

ANOVA – Analysis of Variance (Análise de Variância)

ARBD – Alcohol-related birth defects (defeitos congênitos relacionados ao álcool)

ARND – Alcohol-related neurodevelopmental disorder (transtorno do neurodesenvolvimento relacionado ao álcool)

BDNF – Brain-Derived Neurotrophic Factor (fator neurotrófico derivado do encéfalo)

BRFSS – Behavioral Risk Factor Surveillance System (Sistema de Vigilância de Fatores de Risco Comportamentais)

CCI – Chronic constriction injury (Constrição crônica do nervo isquiático)

CGRP – Peptídeo relacionado ao gene da calcitonina

CPF – Córtex pré-frontal

CRF – Corticotropin-releasing factor

DA – Dopamina

DALYs – Disability-adjusted life years

DLPFC – Dorsolateral prefrontal cortex (Córtex pré-frontal dorsolateral)

DSM – Manual Diagnóstico e Estatístico de Transtornos Mentais

EEG – Eletroencefalograma

ETCC – Estimulação Transcraniana por Corrente Contínua

FAS – Fetal alcohol syndrome (síndrome alcoólica fetal)

FASDs – Fetal alcohol spectrum disorders (Transtorno do espectro do álcool fetal)

GABA – Ácido gama-aminobutírico

GEEs – Generalized estimated equations

H^+ – Hidrogênio

IASP – International Association for the Study of Pain (Associação Internacional para o Estudo da Dor)

IL – Interleucina

K^+ – Potássio

NAc – Núcleo accumbens

ND-PAE – Neurobehavioral disorder associated with prenatal alcohol exposure (distúrbio neurocomportamental associado à exposição pré-natal ao álcool)

NGF – Nerve Growth Factor (fator de crescimento neural)

NMDA – N-metil-d-aspartato

NP – Neuropathic pain (Dor neuropática)

NPY – Neuropeptídio Y

OMS – Organização Mundial da Saúde

PKCε – proteína quinase C-épsilon

RNAm – Ácido ribonucleico mensageiro

SN – Sistema Nervoso

SNC – Sistema Nervoso Central

SNK – Student–Newman–Keuls

tDCS – Transcranial direct current stimulation

UCNs – Urocortinas

APRESENTAÇÃO

Esta Tese está estruturada em 3 partes:

- Parte I - Introdução, Revisão da Literatura, Objetivos e Referências Bibliográficas;

- Parte II - Materiais e Métodos, Resultados e Discussão na forma de 3 manuscritos científicos;

- Parte III – Considerações Finais e anexos.

O item Referências Bibliográficas refere-se somente às referências contidas nos itens Introdução, Revisão da Literatura e Considerações Finais.

Detalhes técnicos mais precisos sobre a metodologia empregada em cada um dos trabalhos apresentados podem ser encontrados nas referências citadas nos manuscritos.

SUMÁRIO

I. INTRODUÇÃO

INTRODUÇÃO

Dor crônica é uma das condições mais complexas na prática de atenção à saúde, com altas prevalências, redução da qualidade de vida e elevados custos sociais e econômicos [1]–[4]. O processamento e interpretação de sinais de dor envolve a ativação de nervos periféricos e de circuitos ascendentes e descendentes deste a medula espinhal até estruturas supraespinhais [5]. Assim como no sistema nociceptivo, as diferentes fases do processo de dependência ao álcool demonstram uma notável capacidade de sofrer alterações neuroplásticas que envolvem processos mal adaptativos [6]. Medir os efeitos do álcool sobre a saúde requer consideração cuidadosa de múltiplos fatores, como volume e padrão de consumo [7], mas sem dúvidas representa outro complexo problema de saúde pública [8].

Evidências têm mostrado que as condições de dor crônica assim como os quadros envolvendo o uso nocivo de álcool desencadeiam uma série de alterações nos mecanismos moleculares e celulares de áreas específicas, tais como a amígdala, o hipotálamo, o hipocampo e o córtex pré-frontal [9], [10], promovendo neuromodulações em sistemas de neurotransmissão como o glutamatérgico, GABAérgico, opioidérgico, e mediadores como CRF / urocortinas (Ucns) e proteína quinase C-épsilon (PKCε) [11]. A desregulação dessas vias decorrentes de lesão nervosa e / ou envolvida no uso nocivo do álcool promove, por exemplo, liberação de interleucinas (ILs), entre elas a IL-1 [12]–[14]; alteração do neuropeptídio Y (NPY) que é um importante promotor da plasticidade neuronal e amplamente distribuído no sistema nervoso [15]–[17].

Neste contexto, a relação entre a dor crônica e o uso nocivo do álcool é estreita e parece bidirecional. Revisão sobre o tema aponta três principais interações entre o uso de álcool e a dor: I. o uso de álcool levando à hiperalgesia, II. o uso de álcool modulando a

hiperalgesia, III. a dor crônica como um fator de risco predispondo à recaída ao álcool [11]. A compreensão dos circuitos de sobreposição mal adaptativa entre ambas as doenças pode fornecer avanços no entendimento do processo de comorbidade entre as mesmas, assim como nas abordagens terapêuticas. Mais especificamente, a estimulação transcraniana por corrente contínua (ETCC) apresenta efeito primário de modulação do potencial de repouso neuronal [18] já sendo usada na prática clínica como alternativa de tratamento para essas doenças [19].

Por outro lado, os estudos experimentais utilizando modelos animais permitem a investigação e detalhamento daqueles neurocircuítos por detrás dessas doenças complexas [20]. Bem como, permitem o estudo dos mecanismos de ação das terapias farmacológicas e não-farmacológicas, avaliando impacto nas respostas nociceptivas, nos efeitos neurais, no perfil de segurança, entre outros. Portanto, considerando o conhecimento ainda insuficiente para a intrínseca ligação entre as condições de dor crônica e uso nocivo do álcool, e a ineficácia das terapias atuais que, pelo menos em parte, também resulta da lacuna de conhecimento na área, essa tese tem importante aspecto translacional. Esta tese utilizou modelos animais de condições com alta relevância clínica, dor neuropática crônica e uso nocivo de álcool, demonstrando o efeito promissor do uso repetido da ETCC no tratamento destas condições e contribuindo para um melhor entendimento dos mecanismos que contribuem e perpetuam essas doenças.

II. REVISÃO DA LITERATURA

2.1 ESTRATÉGIAS PARA LOCALIZAR E SELECIONAR INFORMAÇÕES

Nesta revisão de literatura buscou-se apresentar os principais aspectos da dor, álcool e ETCC, e suas relações com biomarcadores. A estratégia de busca envolveu as seguintes bases de dados: MEDLINE (site PubMed), LILACS e SciELO. Foram selecionados artigos publicados entre 2000 e 2020.

Nos sites PubMed, LILACS e SciELO foram realizadas buscas utilizando os seguintes termos: *pain*, *alcohol*, *tDCS*, *NPY*, *BDNF*, *NGF*, *IL-6*, *IL-10*, *IL-1α, IL-1β*. Em relação ao termo *pain*, foram encontrados 635.230 artigos no PubMed e 21.839 artigos no LILACS, já no SciELO foram encontrados 17.324 artigos. Utilizando-se o termo *alcohol* foram encontrados 548.582 artigos no PubMed, 9.073 artigos no LILACS e 8.261 no SciELO. Com o descritor *tDCS* a busca no PubMed encontrou 6.257 artigos, 47 no LILACS e 39 no SciELO. Para NPY, 6.422 artigos foram encontrados no PubMed, 36 no LILACS e 0 no SciELO. Com o descritor *BDNF*, foram encontrados 24.051 no PubMed, 131 no LILACS e 102 no SciELO. Para *NGF*, 9.866 artigos foram encontrados no PubMed, 89 no LILACS e 33 no SciELO. Para *IL-6*, 128.795 artigos foram encontrados no PubMed, 1.746 no LILACS e 1.531 no SciELO. Ao utilizar o descritor *IL-10* foram encontrados 60.803 artigos no PubMed, 1.391 no LILACS e 6.794 no SciELO. Em relação a *IL-1α,* 6.178 artigos foram encontrados no PubMed, 0 no LILACS e 423 no SciELO. Por último, a busca simples de *IL-1β* revelou 78.088 artigos no PubMed, 0 no LILACS e 407 no SciELO.

Refinando-se a busca, com cruzamentos entre as palavras-chave, foi encontrado um reduzido número de artigos como mostrado na Figura 1.

Palavras-Chave

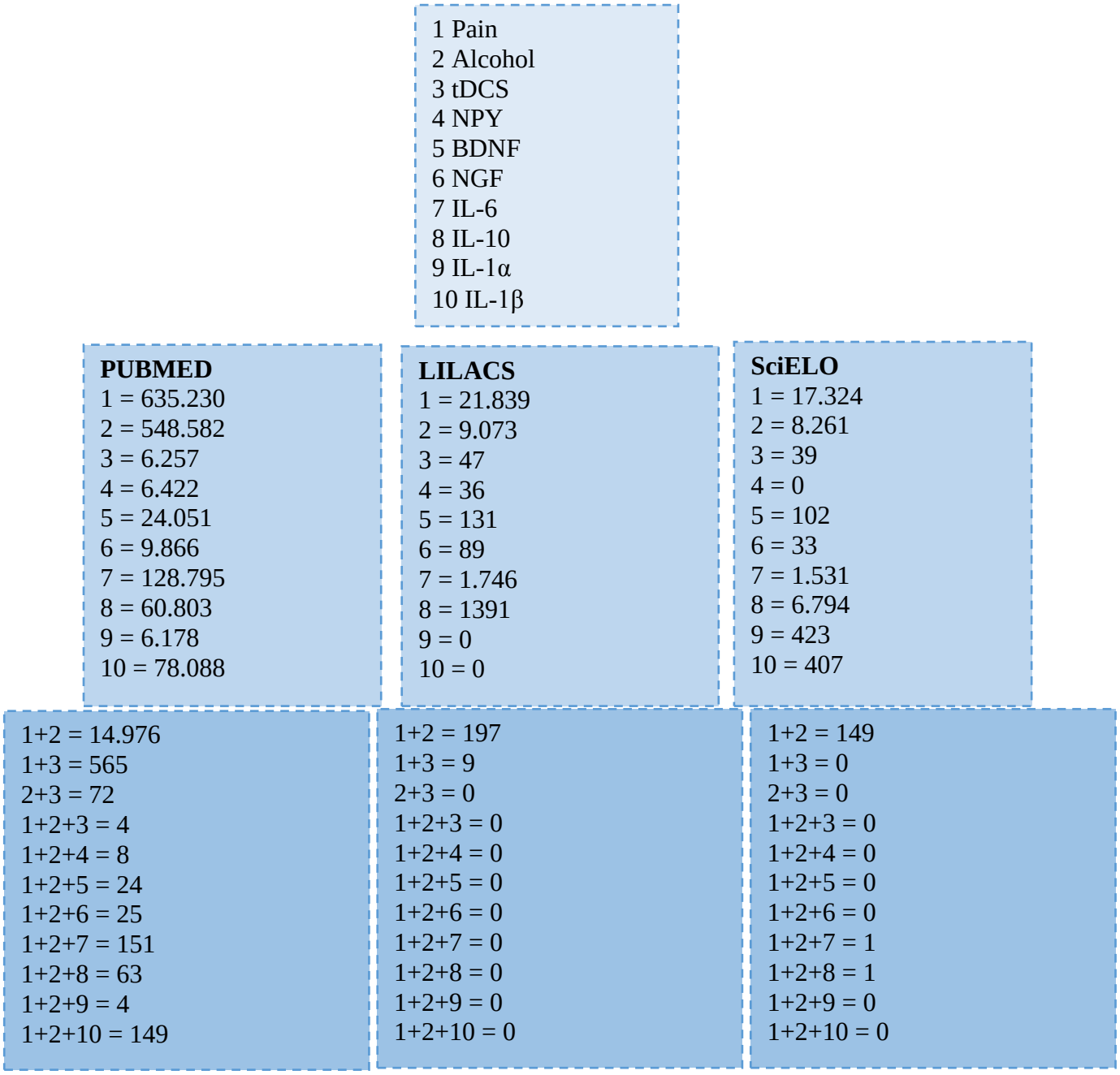

Figura 1. Fluxograma da pesquisa realizada com as palavras-chave Pain, Alcohol, tDCS, NPY, BDNF, NGF, IL-6, IL-10, IL-1α e IL-1β.

2.2 DOR

Registros pré-históricos mostram que antigas civilizações já buscavam entender o porquê da ocorrência de dor e os métodos de como controlá-la [21]. De acordo com definição atualizada pela Associação Internacional para Estudos da Dor (International Association for the Study of Pain – IASP), dor é "uma experiência sensorial e emocional desagradável associada, ou semelhante àquela associada a dano real ou potencial ao tecido" [22]. A dor é considerada um fenômeno subjetivo complexo, na qual cada indivíduo tem uma percepção única, influenciada por fatores biológicos, psicológicos e sociais [23].

2.2.1 Epidemiologia e impacto para a saúde dos quadros de dor

A dor, como um sintoma de lesão ou doença, é experimentada por quase todos os indivíduos em algum momento ao longo da vida [4]. Porém, na maioria das pessoas, a experiência da dor é breve e facilmente resolvida com o tratamento ou procedimento adequado que resolva a sua causa [24]. Atualmente, a dor é avaliada como o quinto sinal vital em ambientes hospitalares [4], [25]. Por outro lado, a dor crônica é uma das condições mais complexa na prática de atenção à saúde, deixando de ser um sintoma de doença para ser tratado como uma doença, sendo responsável por 40% da procura por profissionais da saúde [3], [26]. Apesar da variação nas estimativas de prevalência de dor crônica, mais de 20% da população mundial sofre de dor crônica, segundo dados da Organização Mundial da Saúde (OMS) [1], corroborando a importância epidemiológica da dor [4], [27].

Como é possível observar, dor crônica é um importante problema de saúde [28], onde mais de um terço dos indivíduos com dor crônica definem sua dor como grave e

40% daqueles que sofrem de dor crônica não apresentam analgesia satisfatória [29], [30], gerando altos custos aos sistemas de saúde pública [28].

2.2.2 Fisiopatologia da dor

Fisiologicamente, a dor é um alerta de que algo está errado no organismo, por exemplo, a dor produzida ao tocarmos em uma chapa quente, ao desenvolver uma lesão em músculo, osso ou órgão, a dor no peito decorrente de isquemia, entre outras [24], [31]. Nesses casos, a dor desempenha um papel de alerta, com função protetora, é um sintoma, conhecido como dor aguda [32]. No entanto, quando esta dor persiste por um tempo prolongado, decorrente de um processo mal adaptativo, passa a ser uma dor crônica, tornando-se doença e não mais um sintoma [24], [26].

O processo nociceptivo é o processamento da informação nociceptiva desde a periferia até o sistema nervoso central (SNC). Este processo compreende as etapas de transdução, transmissão e modulação de sinais neurais gerados em resposta a um estímulo nocivo [5]. Os nociceptores são receptores sensoriais de alto limiar que enviam sinais, induzindo a percepção da dor em resposta a um estímulo com potencial de dano. Nociceptores são terminações nervosas livres das fibras dos tipos C e Aδ [33], que possuem diferentes características em relação à constituição de fibras e velocidade de condução, lenta e rápida, respectivamente. Podem ser classificados, de acordo com a modalidade do estímulo ao qual respondem: mecânicos, químicos, térmicos e polimodais [33]. Os nociceptores são estimulados por substâncias, como a serotonina, H^+, K^+, histamina, bradicinina, colecistocinina e substância P [5], [33]. O estímulo nociceptivo detectado na periferia é transmitido ao SNC como estímulo elétrico, por meio de potenciais de ação [34].

A comunicação entre os neurônios de 1ª e 2ª ordem ocorre no corno dorsal da medula espinhal por meio de neurotransmissores, como o glutamato, substância P e

peptídeo relacionado ao gene da calcitonina (CGRP) [5]. Enquanto os dois últimos geram potenciais lentos, o glutamato promove potenciais excitatórios pós-sinápticos rápidos por meio da ativação de receptores ácido α-amino-3-hidróxi-5-metil-4-isoxazolepropiônico (AMPA) [5], [35].

No SNC, a ativação do neurônio de segunda ordem conduz a informação ao tálamo e, via neurônio de terceira ordem, chega ao córtex cerebral, onde a informação é percebida como dor, em especial no córtex somatossensorial e sistema límbico [5]. Dependendo do substrato neural, a percepção da dor pode ser subdivida em dois componentes: 1) componente sensorial, que codifica os aspectos discriminativos da dor como localização, intensidade, duração e caráter; e 2) componente afetivo, que envolve contexto afetivo-cognitivo, emoção, percepção, aprendizado e memória [26], [36].

O processo de transição da dor aguda para crônica é complexo [26]. A IASP adotou parâmetros temporais para classificar a dor crônica, considerando três meses de presença de dor como o ponto mais conveniente de transição da dor aguda à dor crônica [2], [37]. Dor crônica é uma doença debilitante que pode levar, em longo prazo, à incapacidade física, redução da qualidade de vida e aumento dos gastos com assistência médica [28]. Embora o mecanismo exato de seu desenvolvimento seja desconhecido, sabe-se que são desencadeadas alterações plásticas relacionadas aos processos de sensibilização periférica e central [34], [38]. Por exemplo, alterações neuroplásticas podem durar além do processo cicatricial ou da cessação da nocicepção periférica, tornando-se "mal adaptativas", desencadeando um processo de sensibilização central [24], [38]. Além disso, alterações em circuitos neurais nociceptivos induzem alterações nos sistemas sensorial e afetivo-emocional relacionados a quadros de hiperalgesia e alodinia [5], [39], [40]. Evidências mostram que três processos neurais estão envolvidos

nos quadros de dor crônica: (i) sistema corticoespinal motor; (ii) sistema de modulação descendente da dor; e (iii) neuroplasticidade [41].

Dor também é classificada de acordo com o mecanismo fisiopatológico em: a) dor nociceptiva ou inflamatória; b) dor neuropática; c) dor mista [42]. A dor nociceptiva é decorrente de ativação de nociceptores e relacionada à lesão de ossos, músculos ou ligamentos, e órgãos. A transmissão nociceptiva é influenciada por processos centrais e periféricos e pela liberação de substâncias mediadoras (neurotransmissores, peptídeos e neuromoduladores) que podem ativar ou inibir este processo [5]. São exemplos de dores crônicas nociceptivas: osteoartrose, artrite reumatoide, fratura e rigidez muscular na dor lombar inespecífica, entre outras. Geralmente, responde bem ao tratamento com analgésicos, anti-inflamatórios clássicos como os não-esteroidais (AINES) e opioides fracos [43]. Por outro lado, a dor neuropática é induzida por lesão ou disfunção do sistema nervoso central ou periférico [31], [34], [43]. Além disso, caracterizada como em queimação, agulhadas ou dormência, tem uma distribuição anatômica plausível e uma condição de base predisponente, como - diabetes ou tratamento quimioterápico. Não responde bem aos tratamentos clássicos, sendo necessário o uso outras abordagens farmacológicas que atuem nos sistemas opioidérgico, gabaérgico, glutamatérgico e adrenérgico, além de bloqueio de canais de sódio e cálcio. Os fármacos mais frequentemente descritos em dor neuropática são os antidepressivos, os anticonvulsivantes, os beta-bloqueadores e os antagonistas de receptores NMDA. Entretanto, estes fármacos são relacionados a efeitos adversos que muitas vezes limitam o seu uso [22], [44], assim como seus efeitos analgésicos não abrangem todos os grupos de pacientes com a doença. Por fim, a dor mista é relacionada à lesão de nervos e

inflamação em tecidos adjacentes, como ocorre, por exemplo, na dor oncológica e síndrome do túnel do carpo [42].

A dor neuropática, alvo desta tese, é causado por doença, lesão ou disfunção no sistema somatossensorial ou pelo seu envolvimento em outros processos mórbidos, como compressão ou inflamação [31]. Constitui uma síndrome, com mecanismos ainda pouco esclarecidos, como, por exemplo, neuropatia alcoólica e neuralgia do trigêmeo. Há aumento da atividade neuronal e de circuitos nociceptivos (multicelulares, neuronais, e gliais) [45], [46], induzidos por aumento na excitabilidade de membrana, na eficácia sináptica e na plasticidade do SN somatossensitivo e / ou por redução da inibição nociceptiva descendente [31], [33], [34]. Estes fenômenos geralmente ocorrem depois de um estímulo nociceptivo intenso ou repetitivo, podendo ser desencadeados em resposta a inflamação periférica, atividade neuronal anormal ou lesão nervosa, levando a cronificação da dor [46].

2.2.3 Modelos animais de dor

Modelos animais fornecem sistemas fundamentais para o estudo pré-clínico à medida que permitem investigar fisiopatologia, processos neurobiológicos e tratamento de doenças, sendo, para tanto, necessário elaboração de protocolos de pesquisa e a submissão a comitês de ética institucionais [47]. Os roedores são os mais utilizados pelas suas características: reprodução rápida, pequeno porte, similaridade dos genes com o humano, entre outras.

A utilização de modelos animais de dor é uma ferramenta muito importante para um melhor entendimento dos processos nociceptivos e busca de novas terapêuticas analgésicas e anti-inflamatórias [48]. Estes modelos mimetizam situações de doença ou de lesão, auxiliando na compreensão da etiopatogenia destes fenômenos [20].

Para estudo da dor inflamatória podem ser usadas substâncias algogênicas injetadas em diferentes regiões do corpo do animal (pele, pata, músculos, articulações e vísceras). Exemplo desse tipo é o modelo da formalina, inicialmente descrito por Dubuison e Dennis [49]. Para mimetizar a dor visceral, têm sido utilizados estímulos nocivos como corrente elétrica, trauma mecânico, isquemia e substâncias químicas [48].

Diferentes modelos também foram desenvolvidos para o estudo da dor neuropática, baseados nos multifatores da mesma. Alguns modelos servem para estudar os mecanismos periféricos e outros para os mecanismos centrais, exemplos: axotomias completas ou parciais, excitotóxicos, lesões centrais por contusão, e ligadura de nervos [50]. O modelo animal de dor neuropática utilizado nesta tese foi o da constrição crônica do nervo isquiático por meio de 4 ligaduras frouxas e contínuas ao redor do nervo [51].

2.3 ÁLCOOL

O álcool, como substância psicoativa, tem seu consumo bastante antigo na história da humanidade. No entanto, seu uso crônico leva a dependência física, tornando-se nocivo ao indivíduo, tanto em nível de saúde física e mental quanto em nível social [52]. Os diagnósticos formais relacionados ao uso nocivo do álcool são baseados na última atualizaçã do Manual Diagnóstico e Estatístico de Transtornos Mentais (DSM-V), em Transtornos relacionados a substâncias e Transtornos aditivos, na qual o álcool é uma das 10 classes distintas de drogas [53]. Os diagnósticos formais incluem: Transtorno por uso de álcool, Intoxicação por álcool, Abstinência de álcool, Outros transtornos induzidos por álcool e Transtorno relacionado ao álcool não especificado (ver anexo D).

2.3.1 Epidemiologia e impacto para a saúde do uso nocivo de álcool

O uso nocivo de álcool está associado a uma série de consequências físicas, psiquiátricas e sociais adversas [52]. Globalmente, 3 milhões de mortes por ano resultam do uso nocivo de álcool, representando 5,3% de todas as mortes. Além de ser atribuíveis ao consumo de álcool, a perda de 139 milhões de anos de vida ajustados por incapacidade (*disability-adjusted life years* - DALYs) e 5,1% por doenças e lesões incapacitantes, com a magnitude do dano determinado pelo volume de álcool consumido e o padrão de consumo [54]. Das mortes atribuídas ao álcool mais de 30% são decorrentes de lesões, como as causadas por acidentes de trânsito, autolesão e violência interpessoal; mais de 20% relacionadas a transtornos digestivos; aproximadamente 20% relacionadas a doenças cardiovasculares e o restante a doenças infecciosas, câncer, transtornos mentais e outras condições de saúde [54], [55].

Os efeitos nocivos do uso do álcool dependem de variáveis como, por exemplo: sexo, idade, quantidade consumida, associação com outras drogas, padrão de consumo, entre outras [7], [54]. A exposição pré-natal ao álcool pelo consumo materno pode causar danos ao feto em desenvolvimento, além de ser relacionada a baixo peso ao nascer [56]. É a principal causa evitável de defeitos congênitos e deficiências intelectuais e de desenvolvimento neurológico [57]. A síndrome alcoólica fetal foi descrita pela primeira vez em 1973, e engloba uma série de defeitos inatos à criança, decorrente de consumo materno de álcool durante a gestação. Essa condição pode causar danos cerebrais e problemas de crescimento que não podem ser revertidos [58]. O chamado Transtorno do espectro do álcool fetal (*fetal alcohol spectrum disorders* - FASDs) inclui diagnósticos diversos: síndrome alcoólica fetal (*fetal alcohol syndrome* - FAS), síndrome alcoólica fetal parcial, defeitos congênitos relacionados ao álcool (*alcohol-related birth defects* - ARBD), transtorno do neurodesenvolvimento relacionado ao álcool (*alcohol-related*

neurodevelopmental disorder - ARND), e transtorno neurocomportamental associado à exposição pré-natal ao álcool (*neurobehavioral disorder associated with prenatal alcohol exposure* - ND-PAE) [57]. Além disso, o álcool é um fator de risco para outros resultados adversos de gravidez e parto, como aborto espontâneo e natimorto [59].

O álcool é a droga de escolha para a maioria dos adolescentes e a droga mais frequentemente associada à morte de adolescentes [60], [61]. Os transtornos decorrentes do uso de álcool estão associados a episódios depressivos, ansiedade severa, insônia e suicídio [62]. O comportamento violento sob a influência do álcool é um fenômeno conhecido e causa grande sofrimento às pessoas afetadas, além de enormes custos para a sociedade [63]. Problema no trabalho é outra consequência social e econômica associado ao uso abusivo de álcool [64]. Mudanças fisiológicas relacionadas ao envelhecimento podem alterar a apresentação das complicações médicas relacionadas ao alcoolismo. Por exemplo, em pacientes idosos os efeitos do álcool podem ser aumentados devido às associações farmacológicas próprias da idade [65].

Além de tudo isso, consumidores de altas doses e / ou de uso crônico de álcool também são mais propensos ao consumo de outras drogas [66] e há uma relação causal entre o uso nocivo do álcool e uma série de transtornos mentais e comportamentais [54], [67]. Em uma cidade da Rússia, pesquisadores identificaram que os transtornos mentais e comportamentais causados pelo álcool estão presentes em 6,5% dos pacientes e apresentam longevidade diminuída [68]. Pacientes com problemas de saúde mental e uso de substâncias coexistentes representam um grande problema para os serviços de saúde [69], apesar de muitas vezes o estigma agravar as consequências médicas e sociais do uso nocivo de álcool [70].

2.3.2 Fisiopatologia e a neuroquímica do álcool

Apesar de ser aceito socialmente, o álcool é uma substância psicoativa, seu consumo agudo ou crônico tem efeitos diretos sobre o SNC [71], modulando múltiplos sistemas de neurotransmissores, como GABAérgico, glutamatérgico, dopaminérgico, serotonérgico e opioidérgico [72], [73], e canais iônicos dependentes de voltagem. Cabe ainda salientar que o neuropeptídeo Y (NPY) também parece desempenhar importante papel nas respostas dos mamíferos ao álcool [17], [74], [75].

Altas doses de álcool reduzem agudamente a função do córtex pré-frontal e do lobo temporal, incluindo planejamento, fluência verbal, memória e controle motor complexo, como a função cerebelar [76]. Revisão sobre achados de neuroimagem, neurocognitivos e a toxicidade relacionada ao álcool em adolescentes demostrou que o uso do álcool piora o desempenho neurocognitivo, provocando alterações na estrutura cerebral da substância cinzenta e branca, e padrões de ativação cerebral funcionais [77], [78]. Esse outro estudo em ratos expostos ao álcool durante o início da adolescência (PD 28-42) aumentou a densidade de espinhos dendríticos longos / finos da camada 5 de neurônios piramidais no córtex pré-límbico adulto, induzindo anormalidades celulares e dopaminérgicas, com redução pronunciada na modulação do receptor D1 da dopamina (DA), tanto do disparo intrínseco quanto das correntes NMDA evocadas em células piramidais [79].

A intoxicação aguda por álcool resulta em desinibição comportamental, interrupção do processamento socioemocional e desempenho psicomotor prejudicado [76]. Por outro lado, o uso crônico está associado a várias condições neurológicas graves, uma das quais é a encefalopatia de Wernicke [76]. Este transtorno neurológico agudo caracteriza-se pela tríade clínica: estado mental alterado, disfunções oculomotoras e

cerebelares. Quando não tratado pode progredir para a síndrome de Korsakoff, mais grave e classicamente caracterizada por amnésia anterógrada (e retrógrada), junto a déficits pré-frontais [76].

Estudos neuropatológicos estabeleceram a atrofia cerebelar no uso crônico de álcool [76], [80]. Volume reduzido da amígdala também foi descrito naqueles com consumo elevado de álcool [76], [81]. Até mesmo a abstinência alcoólica tem sido associada à perda de substância branca nos lobos temporais [82]. Para explicar todos esses efeitos deletérios do consumo de álcool, diferentes mecanismos têm sido propostos: o álcool sendo diretamente neurotóxico; a regulação positiva dos receptores NMDA, deixando os neurônios vulneráveis à excitotoxicidade do influxo maciço de cálcio; a morte celular que também pode ser por via neuroinflamatória, indução de mediadores inflamatórios e ativação microglial [76]. O uso crônico de álcool também é responsável por produzir neuropatia periférica dolorosa, que está presente em 25-66% dos pacientes alcoólatras [83]. É potencialmente incapacitante, caracterizada por dor e disestesias, principalmente nas extremidades inferiores [84].

Além de tudo isso, o álcool e seus metabólitos são considerados hepatotóxicos. O fígado, como principal órgão responsável pelo metabolismo, é também o mais afetado pelo uso nocivo do álcool [85]. Existem basicamente três tipos de doenças do fígado determinadas pelo uso de álcool: esteatose (fígado gorduroso), hepatite alcoólica e cirrose alcoólica. Mas o álcool ainda exerce efeito tóxico, direta ou indiretamente: (1) no pâncreas – lesando as células acinares e as células estreladas que acabam induzindo sequências de "necrose-fibrose" e posterior atrofia; e (2) no trato gastrointestinal, provocando dano direto à mucosa do esôfago e do estômago, modificação da pressão esfincterial e comprometimento da motilidade, alteração da produção de ácido gástrico, e

dano direto pelo etanol à mucosa intestinal ou indiretamente, alterando a microflora, levando a prejuízos no sistema imunológico da mucosa [85].

Apesar do esforço de várias pesquisas na busca por delinear uma associação genômica para a condição do uso abusivo do álcool, principalmente no desenvolvimento para a dependência [73], [86], nenhum gene isolado ainda foi conclusivamente associado ao risco de dependência ao álcool [87], [88]. Evidências estabelecem um papel para os microRNAs no desenvolvimento e na patogênese da doença hepática [89]. É provável que vários genes interajam de formas complexas entre si e com experiências únicas de vida dos indivíduos para determinar a suscetibilidade à dependência do álcool [86].

Neste sentido, modelos animais, acessíveis a análises genéticas e farmacológicas, têm sido amplamente utilizados para estudar comportamentos complexos relacionados ao álcool [90]–[92].

2.4 RELAÇÃO ENTRE DOR CRÔNICA E O CONSUMO DE ÁLCOOL

Alterações mal adaptativas nas redes cerebrais e desregulação dos sistemas neuroquímicos são reconhecidas como causas de estados de doença com sintomas persistentes, como a dor crônica e o uso nocivo de álcool [6], [93]. A relação entre essas duas condições muitas vezes se apresenta de forma direta, mas também indireta. E, apesar de muitos estudos na área, ainda não foi atingida uma compreensão clara e completa dos mecanismos envolvidos. Considerando o aspecto multidimensional de ambas, várias são as características comuns entre dor crônica e uso do álcool [94]. E, assim como para desenvolvimento e manutenção da dor crônica existem estágios [94]–[96], o uso nocivo de álcool apresenta-se em ciclos: desejo, abstinência e recaída; com mecanismos específicos fundamentando cada uma das fases [9], [97].

Investigações experimentais e clínicas evidenciam que o uso de álcool e / ou abstinência ao álcool pode levar a hiperalgesia (dor aguda) ou induzir dor crônica por meio de neuropatia alcoólica [11]. Além do efeito do álcool, como recompensa e alívio da dor [98], há os efeitos da abstinência, como hiperexcitabilidade, ansiedade, alterações de sono e disforia, entre outros, que contribuem para o abuso e a recaída [99]. O ciclo é perpetuado pelo aumento constante do consumo do álcool para aliviar os sintomas motivacionais desencadeados pela abstinência [97], [100]. Já os efeitos neuropáticos periféricos do álcool estão associadas à axonopatia e à redução da densidade das fibras nervosas [101]. Devido aos níveis menores de tiamina, induzidos pelo uso do álcool, há interrupção do metabolismo dos carboidratos, aumento do estresse oxidativo ou carga mitocondrial, desencadeando apoptose ou necrose, respectivamente [102]. Assim como, o acúmulo de metabólitos (acetaldeído) pode levar a uma maior produção de citocinas, estresse oxidativo potencializado e / ou aumento da proteína quinase ativada por mitogênio (MAPK) ou sinalização de PKC [11], [83], [103].

Entretanto, a relação é mais intrincada, bidirecional e com disfunções do circuito neuroimunoendócrino comum entre dor crônica e álcool [6], [11]. Os estudos apontam pelo menos três interações que há entre a dor e o álcool: (I) o uso de álcool levando à hiperalgesia, (II) o uso de álcool moderando a dor e a hiperalgesia, e (III) a dor crônica como um fator de risco que predispõe à recaída ao álcool [11]. O desequilíbrio dopaminérgico na via mesocorticolímbica explica parte do efeito [104], [105]. Ainda, como ocorre na dor crônica, há uma reorganização das conexões sinápticas (plasticidade estrutural) em circuitos cerebrais relevantes para a evolução aos transtornos por álcool [11], [106]. Glutamato/GABA, opioides, CRF/UCNs e PKCε são os principais mecanismos moleculares apontados para a comorbidade [6], [11], [107]. Ambas as

doenças envolvem circuitos límbicos ativados [93], [96], sendo o sistema de recompensa atribuído para o estado de cronicidade [108]–[110]. As disfunções emocionais e cognitivas envolvidas na dor crônica como para o uso nocivo do álcool resultam de um processo mal adaptativo na estrutura do encéfalo e alterações de conectividade no córtex pré-frontal, hipocampo, amígdala, hipotálamo e estriado [9], [11]. Estudos clínicos apontam os sintomas de ansiedade como provável fator explicativo subjacente às associações de dor e uso de álcool [111]. A intensidade da dor e a incapacidade relacionada à dor foram associadas à alteração emocional, que por sua vez foi associada à gravidade do uso de álcool [111]. Maior catastrofização da dor foi associada a níveis mais elevados de desejo e sintomas mais elevados de ansiedade [112]. Bebedores moderados a pesados relatam uma maior fissura e intenção de beber quando submetidos a um protocolo experimental de hiperalgesia [113]. Em resumo, a desregulação afetiva e sensorial relacionada a dor crônica e associada às alterações funcionais em resposta ao uso nocivo de álcool, explicam parte da inter-relação da comorbidade e vários são os neurocircuitos que se sobrepõem.

2.5 ESTIMULAÇÃO TRANSCRANIANA POR CORRENTE CONTÍNUA (ETCC)

Estimulação transcraniana por corrente contínua (ETCC) é uma técnica de estimulação não invasiva baseada em uma modulação do potencial de repouso neuronal (sem induzir potenciais de ação) [114], [115], na qual uma corrente elétrica contínua e de baixa intensidade (entre 1 e 2 mA) é aplicada em áreas corticais com o objetivo de facilitar ou inibir a atividade neuronal espontânea [116]. Os efeitos da ETCC em sessões repetidas podem ser do tipo neuromodulatórios e também plásticos [18], [39], [117]–[119] produzindo mudanças em nível central e periférico [120]. Esses efeitos dependem de

variados parâmetros, como: região cerebral da aplicação, distância e orientação dos axônios ou corpos celulares dos neurônios em relação ao campo elétrico [121] e disposição dos eletrodos (anodal, catodal e bimodal) [122]. O ânodo possui uma carga positiva em relação ao cátodo [123]. Estudo propõe que as alterações na polarização da membrana neuronal são o resultado da atividade (atração e repulsão) de íons de sódio e potássio, principalmente no meio extracelular [123]. ETCC altera a atividade em áreas corticais localizadas diretamente abaixo dos eletrodos, e também em áreas remotas do encéfalo, provavelmente devido à comunicações primárias entre a área estimulada e outras estruturas encefálicas [124]. O protocolo de aplicação mais frequentemente usado em humanos prevê aplicação da corrente por períodos de até 30 minutos por dia [125].

Os efeitos da ETCC envolvem uma cascata de eventos em níveis celular e molecular, além de estarem associados à modulação de atividade glutamatérgica, gabaérgica, dopaminérgica, serotonérgica e colinérgica [39], [40], [126]–[128]. Como exemplo, em um trabalho o bloqueio de receptores NMDA, os quais são ativados pelo glutamato, produz inibição dos efeitos da ETCC [129]. Estudos em modelos animais, modelos computacionais e culturas celulares têm sido desenvolvidos com a finalidade de aprofundar o conhecimento acerca dos mecanismos de ação [130], [131]. Como técnica de estimulação cerebral não invasiva, acessível, de fácil aplicação e baixo risco ao paciente, a ETCC é promissora no tratamento de dor crônica e do uso nocivo de álcool, bem como para outras doenças neuropsiquiátricas e neurológicas [132]. Os eventos adversos relatados incluem queimaduras leves na pele devido ao contato ineficiente com o eletrodo, além de cefaleia e fadiga após a estimulação, e sensação de queimação durante o procedimento [116], [133]. Entretanto, ainda se fazem necessários mais estudos que

objetivem ampliar uma melhor compreensão dos mecanismos neurobiológicos envolvidos, bem como, que sustentem sua aplicação em uma configuração clínica.

2.5.1 ETCC no tratamento da dor e do uso nocivo de álcool

Uma revisão epidemiológica de 2019 sobre a prevalência de dor crônica demonstrou que 30% da população mundial é acometida [27], sendo igualmente prevalente os problemas relacionados ao consumo de álcool [7]. Ambas as condições impõem altos custos à economia e saúde pública global [134]. Embora existam alguns tratamentos farmacológicos para a dor crônica [31], [43], [135] e o uso nocivo do álcool [136], grande parte dos pacientes são refratários aos tratamentos [137]. Assim, a busca de novas estratégias para o tratamento da dor crônica e do uso nocivo do álcool se faz necessária, sendo nesse contexto que as terapias adjuvantes, como a estimulação cerebral, desempenham significativo papel [138].

Um exemplo de terapia não farmacológica que vem sendo empregada em várias doenças do SNC é a técnica de estimulação cerebral – ETCC – que modula a atividade cerebral dependente da polaridade do eletrodo aplicado [139], [140]. Estudos do nosso grupo de pesquisa têm demonstrado efeitos imediatos e duradouros sobre a nocicepção após tratamento com a ETCC [39], [119], [127], [141]. Os efeitos analgésicos da ETCC também foram demonstrados em estudos clínicos, reduzindo os escores de dor e a frequência do uso de analgésicos em diferentes estados de dor crônica [142], [143], provavelmente devido à sua capacidade de modular estruturas corticais e subcorticais, direta ou indiretamente envolvidas na inibição descendente do controle da dor [127], [143], [144]. Os efeitos analgésicos da ETCC envolvem mecanismos como a participação do BDNF e de receptores glutamatérgicos do tipo NMDA, relacionados ao processo de plasticidade sináptica [39], [119], [126]. Assim como na dor, a ETCC tem sido

investigada na neuromodulação dos efeitos ao álcool [140], [145]. Um estudo clinico demonstrou que a ETCC anódica aplicada à DLPFC esquerda melhorou as funções cognitivas e reduziu sintomas de depressão e *craving* por álcool [146]. Ainda em 2008, Boggio et al., já demostraram que o ETCC ativo diminui o desejo por álcool em pacientes adictos. ETCC bilateral aplicada no córtex pré-frontal dorsolateral em bebedores de altas doses e / ou de uso crônico alterou o comportamento em relação a recompensa e consumo de álcool [148]. Outro estudo clínico mostrou que 10 sessões de ETCC bilateral sobre o córtex DLPFC (catódico direito e anódico esquerdo) diminuiu o desejo e as recaídas do uso de álcool em pacientes com problemas graves relacionados ao álcool, mas não chegaram a reduzir os sintomas de ansiedade e nem melhoraram o desempenho cognitivo [138]. Estudos pré-clínicos do grupo também demostraram que a ETCC inibe o *craving* alimentar [144], [149].

Ensaio clínico randomizado controlado por placebo avaliou sessões múltiplas de ETCC bilateral sobre o DLPFC no transtorno por uso de álcool, e sugeriu a ETCC como ferramenta adjunta promissora para reduzir fissura e recaída ao uso de álcool, facilitando a cessação do alcoolismo. ETCC é uma técnica bem tolerada, sem eventos adversos significativos [138]. A estimulação neuromodulatória do encefálo demonstra que a alteração da neuroplasticidade no DLPFC em pacientes alcoolistas é promissora como um tratamento para a dependência de álcool, mas os parâmetros neuromodulatórios ideais ainda não foram identificados [150]. Prévios estudos do grupo de pesquisa têm focado na plasticidade mal adaptativa associada à dor crônica e nos efeitos do tratamento com ETCC. Nosso grupo de pesquisa tem demonstrado em ensaios pré-clínicos utilizando ratos, que o tratamento repetitivo com ETCC reverteu a hiperalgesia e alodinia induzidas por estresse crônico [40], por dor crônica inflamatória (Laste et al., 2012) e por dor

neuropática (Cioato et al., 2016). Além disso, demonstramos o efeito profilático do ETCC no estabelecimento de hiperalgesia e alodinia induzida por estresse crônico (Fregni et al, 2018). Estudos clínicos utilizando uma única sessão de ETCC sobre a área motora esquerda mostram que esta reduz significativamente a dor neuropática associada à lesão da medula espinhal [151]. Na busca dos mecanismos neuroquímico envolvidos na ETCC, usando a estimulação invasiva do córtex motor, demonstrou que a liberação de aminoácidos glicina e GABA na área cinzenta periaquedutal pode estar envolvida no efeito analgésico [152]. O tratamento com ETCC modula o sistema dopaminérgico no córtex frontal, promovendo a liberação de dopamina [128], [153].

Atual diretriz baseada em evidências e meta-análise secundária para o uso da ETCC, indica o tratamento com a técnica para dor neuropática e dependência de álcool como sendo provavelmente eficaz (Nível B) [19]. Os mediadores inflamatórios e as neurotrofinas, como veremos no tópico seguinte dessa tese, podem exercer funções diversas, tanto adaptativas, quanto mal adaptativas no processamento e desenvolvimento da dor crônica, assim como do uso nocivo do álcool. Especificamente, a ETCC parece neuromodular os principais circuitos neurológicos, readequando a plasticidade aberrante subjacente às fisiopatologias dessas doenças. Trabalho do nosso grupo demostrou que o efeito antialodínico da ETCC foi associado a diferentes sistemas de neurotransmissores, incluído o opioidérgico, noradrenérgico, gabaérgico e glutamatérgico, entre outros [128].

2.6 BIOMARCADORES

Biomarcadores são utilizados como parâmetros de confirmação de diagnóstico, de relação causa-efeito e de efetividade de tratamento. Vários estudos buscam marcadores que possam estar relacionados com processos de doenças, entre elas a dor crônica e o uso

nocivo do álcool [93], [96], [97], [154], que podem ser avaliados tanto em nível periférico quanto central. Tanto as progressões para dor crônica como as condições relacionadas ao uso nocivo de álcool estão associadas a estados disfuncionais caracterizadas por desregulação em vias neuroinflamatórias e neuroendócrinas [155], [156]. E, estes mecanismos moleculares e celulares parecem ser responsáveis pela natureza progressiva e perpetuadora desses transtornos, especialmente quando são sub-tratados ou em pacientes refratários ao tratamento [9], [155].

Revisões sobre o assunto destacam alguns dos principais mecanismos moleculares implicados tanto na dor crônica quanto no uso nocivo do álcool [6], [11]. O álcool inibe a atividade dos receptores ionotrópicos do glutamato e intensifica a inibição mediada por GABA em todo o SNC. Alterações nestes sistemas também podem ser observadas em condições de dor crônica, levando ao aumento dos níveis de glutamato e diminuição dos níveis de GABA [11]. Agonistas do receptor delta (DOR), um dos três subtipos principais de receptores opioides, são responsáveis pela analgesia em quadros de dor crônica [157] e apresentam também atividade protetora nos comportamentos relacionados ao álcool, por exemplo, na abstinência, diminuindo os comportamentos de ansiedade [158]. Já a via mesocorticolímbica, responsável por aspectos emocionais e cognitivos, tais como o reforço a estímulos prazerosos e a organização de funções executivas como atenção, planejamento e flexibilidade comportamental também é alterada em ambas as doenças [96], [97], [159]. Revisão sobre os efeitos do álcool no habenula lateral (LHb), uma estrutura epitalâmica, sugerem que canais de potássio do tipo M e a transmissão glutamatérgica, são alteradas pela exposição aguda e repetida ao álcool [160]. O uso nocivo do álcool também mostra relação com um desequilíbrio dopaminérgico na via mesocorticolímbica [105]. Esta via é composta por neurônios cujos corpos celulares se

originam na área tegmental ventral que inervam o estriado ventral, núcleo accumbens (NAc) e córtex pré-frontal (CPF). Por outro lado, a exposição ao ETCC está associada a diferentes sistemas de neurotransmissores (como o opioidérgico, gabaérgico e glutamatérgico, etc) e a duração dos efeitos posteriores depende do tempo de exposição ao tratamento [126], [128]. Em um estudo clínico, Taylor et al. (2012) indicaram alterações no sistema opioide endógeno induzidas pela modulação cerebral por estimulação elétrica (Taylor et al., 2012).

Estudo que enfoca os efeitos das mudanças crônicas destaca vários neuropeptídeos pró-estresse (CRF, dinorfina) e anti-estresse (NPY, oxitocina) [156]. O NPY, amplamente distribuído no SNC e periférico dos mamíferos [15] é expresso em neurônios específicos do hipotálamo e sistema límbico, aparecendo alterado tanto na condição da dor neuropática como no uso nocivo do álcool [16], [17]. Há evidencias sobre o NPY em processos neurodegenerativos abordando-o como um alvo terapêutico [161]. O NPY, co-secretado com outros neurotransmissores como GABA e glutamato [15], [74], [162], atua em 4 receptores específicos: Y1, Y2, Y4 e Y5. A estimulação do receptor Y1 parece ter efeito ansiolítico e do receptor Y2, ansiogênico [161]. Como um importante promotor da plasticidade neuronal, o NPY tem impacto na regulação de estados afetivos negativos, comportamento semelhante à ansiedade, nocicepção e recompensa. Diversas pesquisas pré-clínicas sugerem o papel anti-hiperalgésico do NPY em vários modelos de dor inflamatória e neuropática [163]. A infusão de NPY nos ventrículos ou na amígdala produz antinocicepção em modelo de dor neuropática em ratos, e esses efeitos são sinérgicos aos efeitos antinociceptivos da morfina [164], [165]. Um estudo prévio em roedores utilizando ablação seletiva ou inibição do receptor Y1 espinhais produziu diminuição da dor e preveniu a transição para dor crônica [163]. Outro estudo investigou

as cascatas em que NPY atua, evidenciou uma via de sinalização molecular intracelular envolvendo o NMDAR➔ adenilil ciclase tipo 1 (AC1) e induzida pela lesão de nervo [166]. Estudos pré-clínicos demonstram que os receptores Y1 e Y2 apresentam ações diferentes sobre o uso de álcool (uso agudo e crônico e abstinência) com padrões divergentes dependente da região do cérebro [74]. Postula-se que, no NAc, por meio de interações com a DA, o NPY module o comportamento de recompensa associado à resposta ao álcool [167]–[170]. Plescia et al., (2014) mostraram que mudanças plásticas complexas ocorrem no NPY durante a intoxicação (redução na expressão de NPY) por acetaldeído (o primeiro metabólito do álcool) e subsequente retirada (aumento dos níveis de NPY) nas regiões do hipocampo e NAcc de rato [171].

Vários mediadores interagem nos processos de dor crônica e de uso nocivo do álcool, como citocinas e fatores neurotróficos. Estudos anteriores em animais, demonstraram a complexidade da regulação da dor neuropática por fatores imunológicos endógenos [10], [14], [95], e nos quadros de uso nocivo de álcool [172], [173]. Por exemplo, as citocinas pró-inflamatórias liberadas durante o processo inflamatório podem causar hiperexcitabilidade nos neurônios transmissores da dor, levando à hiperalgesia e alodinia. Estudos do nosso grupo evidenciam alterações que acontecem no sistema neuroimunológico (no eixo córtex-tronco e medula espinhal-encéfalo) após modelos de lesão inflamatória e de dor crônica [39], [119], [141] e a ETCC também desempenha um papel neuroimmunomodulador semelhante [18], [118]. Estudo pré-clínico demonstrou que ETCC anodal reverte a hiperalgesia e alodinia induzidas por estresse crônico e diminui os níveis de TNFα em hipocampo [40]. Outro estudo, também em ratos expostos ao modelo de dor neuropática por CCI, investigou os efeitos da ETCC na resposta nociceptiva que mostrou-se eficaz em promover analgesia e reverter o aumento dos níveis

de IL-1β, IL-10 e TNF-α produzido pelo quadro de dor [118]. Portanto, a analgesia induzida por ETCC pode estar relacionada à neuroimunomodulação espinhal em longo prazo [118]. Esse estudo utilizando o mesmo modelo de dor neuropática em ratos demostrou alteração no comportamento do tipo ansioso e dos níveis de BDNF periféricos e centrais (diminuiu os níveis de BDNF em soro e córtex cerebral e aumentou em medula espinhal), na qual a ETCC reverteu as alterações [39]. Outro estudo do grupo utilizando ratas ovariectomizadas e tratadas com ETCC apresentou uma reversão da hiperalgesia e alteração nos de níveis de BDNF séricos e hipotalâmicos [174]. O BDNF é um importante regulador da plasticidade sináptica, formação de memória [175] e seu papel correlaciona à estrutura analisada. NGF por sua vez, outra neurotrofina também crucial, participa da gênese da dor neuropática aumentando a transmissão excitatória, reduzindo a transmissão inibitória e promovendo reforço da facilitação descendente no corno dorsal da medula espinhal [176]. Além disso, o consumo crônico de grandes quantidades de álcool em ratos leva a um aumento transitório dos níveis de NGF em regiões distintas do encéfalo [177]. Outro estudo demostrou que a exposição crônica ao álcool diminuiu os níveis de NGF, mas que esse efeito é dependente do tempo e do local, com efeitos variando dependendo da duração da exposição ao álcool e das estruturas analisadas [178]. No SNC, o NGF é responsável por uma regulação positiva de vários genes, como o BDNF nos neurônios nociceptivos, modulando e sensibilizando uma população representativa de neurônios envolvidos no processo de dor neuropática, mas também do uso nocivo do álcool [176].

Quanto às citocinas, elas podem ser pró-inflamatórias, que incluem IL-6 e IL-1β, ou anti-inflamatórias, como a IL-10 e o antagonista do receptor de IL-1 (IL-1Ra); suas funções são dependentes de estruturas específicas. Estudo visando avaliar a contribuição do sistema purinérgico na modulação dos níveis de interleucinas após CCI, demostrou

que a administração de um antagonista do receptor P2X4 reduziu os níveis de IL-1β, IL-18, IL-6 e regulou positivamente o IL-1Ra [179]. O sistema IL-1 inclui três ligantes solúveis: dois agonistas conhecidos como IL-1α e IL-1β, e um antagonista natural o IL-1Ra [10], [14], [180], [181]. A IL-1β é conhecida por desempenhar um papel importante na nocicepção na medida em que modula a excitabilidade neuronal ao afetar receptores neuronais como TRPV1, canais de sódio e GABA e receptores NMDA [14], [182]. Por outro lado, a IL-1α é um membro incomum da família IL-1 e uma citocina de função dupla, pois desencadeia a inflamação por ácidos nucléicos e padrões moleculares associados a danos (DAMPs), enquanto os demais membros das citocinas da família IL-1 desencadeiam apenas a inflamação inata por meio da família de receptores IL-1 [181]. Ainda, IL-1α nuclear funciona para aumentar a expressão gênica (como fator de transcrição) e pode ser encontrada em indivíduos saudáveis, enquanto IL-1β é induzida apenas nas condições de doença [181], [183]. Trabalho que investigou a influência dos polimorfismos na família de genes de IL-1 (*Il1a, Il1b* e no antagonista do receptor *Il1*) em pacientes caucasianos com câncer, indicou que a variação genética no gene *Il1b* pode influenciar os níveis séricos com consequências proporcionais na resposta relacionada à dor [184]. Há associação entre alterações em sistemas inflamatórios e oxidativos, vários mediadores imunológicos liberados e que interagem com a dor crônica e o uso nocivo do álcool, como fator de necrose tumoral alfa (TNF-α), citocinas, óxido nítrico (NO), bradicinina e NGF que atuam, por exemplo, ligando aos receptores acoplados à proteína G e ativando as proteínas quinase A e C responsáveis pela fosforilação de receptores, ou aumentando a expressão desses [185], [186].

III. MARCO CONCEITUAL

3.1 Da dor

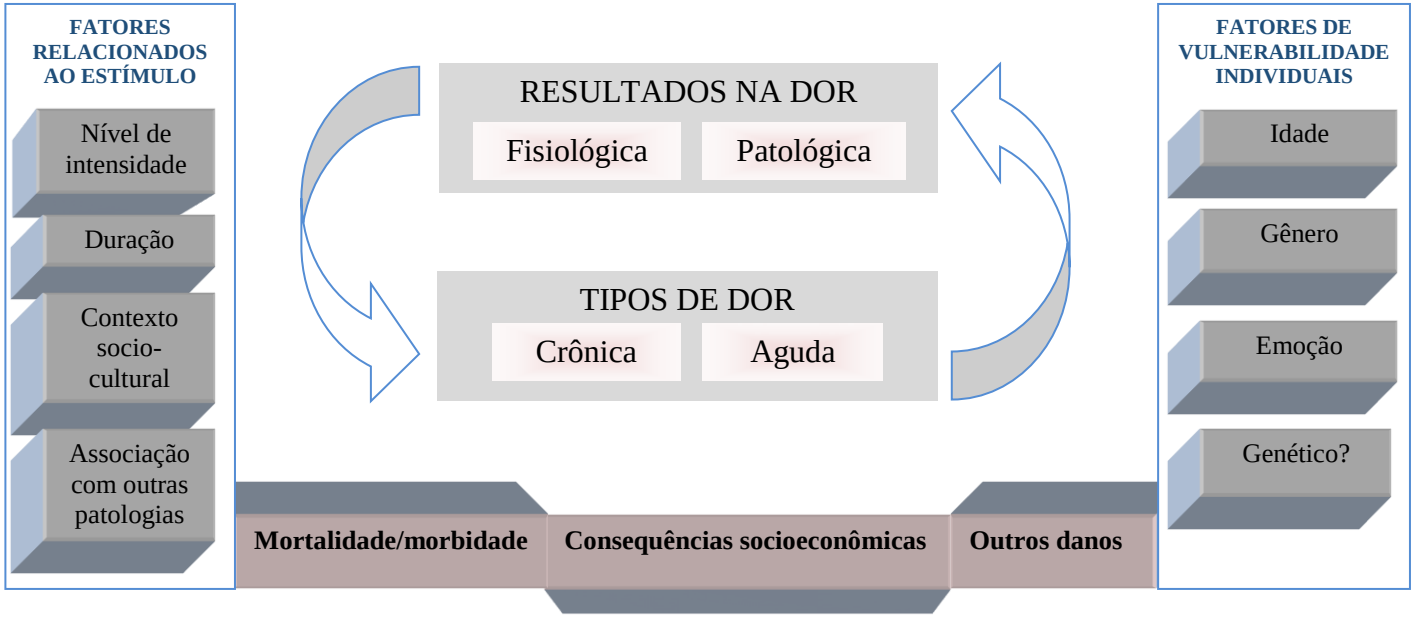

Figura 2. Marco conceitual da dor.

3.2 Do álcool

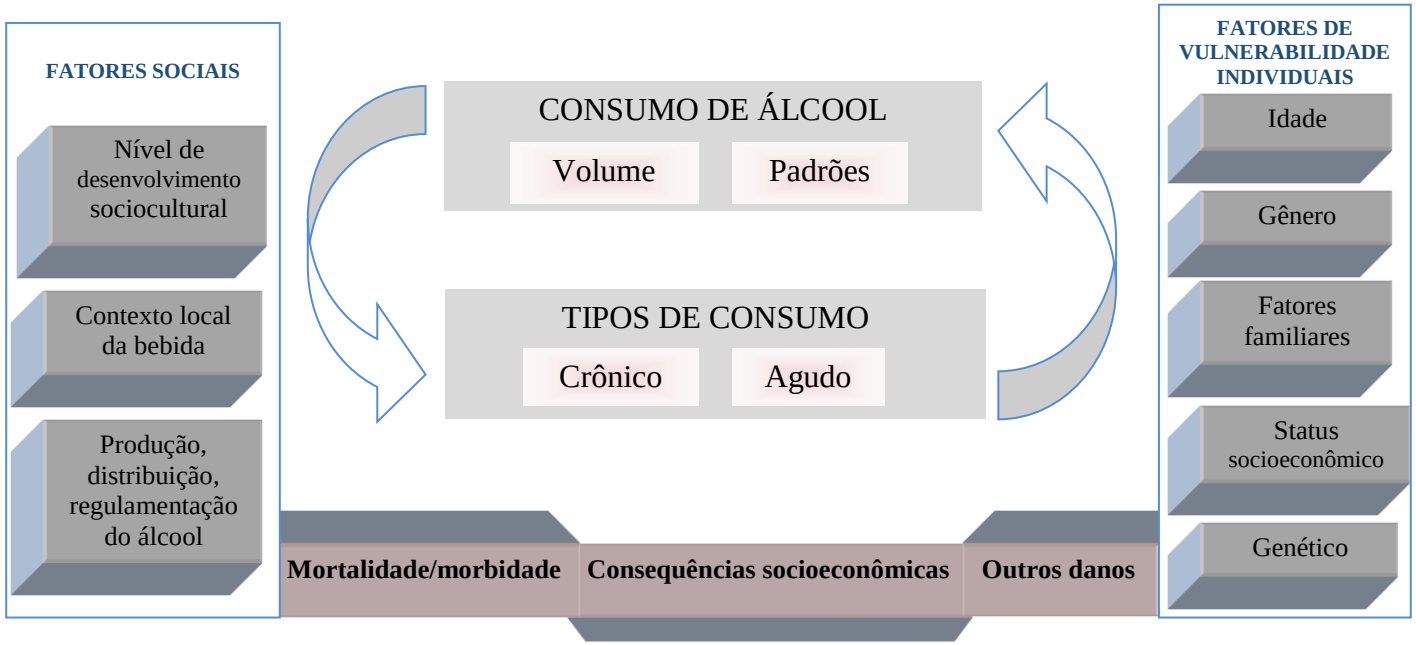

Figura 3. Marco conceitual do álcool (adaptado de Organização Mundial da Saúde (OMS). Relatório Global sobre Álcool e Saúde – 2018. Genebra – Disponível https://www.who.int/news-room/fact-sheets/detail/alcohol).

IV. JUSTIFICATIVA

4 **JUSTIFICATIVA**

Dor crônica é uma doença debilitante que afeta o SNC, com consequências nefastas para a condição física, psicológica e comportamental. Por outro lado, o consumo do álcool também tem efeitos diretos no SNC, alterando parâmetros bioquímicos e comportamentais, com consequências negativas para o indivíduo e para a sociedade. Tratamentos farmacológicos convencionais apresentam um grande número de efeitos colaterais e muitas vezes, são ineficientes, tanto no tratamento da dor crônica quanto no uso nocivo de álcool. Desta forma, a técnica neuromodulatória, ETCC, por ser de aplicação simples e de baixo custo e risco, pode ser uma ferramenta terapêutica não farmacológica eficaz no tratamento destes transtornos. No entanto são necessários estudos para melhor elucidação de seus mecanismos de ação. Considerando que ambas as condições têm altas prevalências e elevados custos sociais e econômicos, o objetivo deste projeto foi investigar o efeito do tratamento repetido com a ETCC sobre parâmetros comportamentais e neurais induzidos por um modelo animal de dor crônica e / ou uso de álcool. Assim como, ampliar o conhecimento dos mecanismos que estão intrinsicamente ligados a essas doenças, dor crônica e uso nocivo de álcool.

V. OBJETIVOS

5.1 Objetivo Geral

O objetivo geral desta Tese foi investigar o efeito do tratamento repetido com a ETCC sobre parâmetros comportamentais e neurais induzidos por um modelo animal de dor crônica e / ou exposição ao álcool.

5.2 Objetivos Específicos

Esta tese objetivou avaliar o efeito do tratamento repetido com a ETCC em ratos machos adultos submetidos ao modelo animal de dor crônica e / ou exposição ao álcool (ativa / passiva). Os resultados foram compilados em três manuscritos científicos. Os objetivos específicos seguem abaixo:

Artigo I

- ✓ Investigar o efeito do tratamento com ETCC como uma ferramenta neuromodulatória sobre a associação da dor neuropática e abstinência de álcool nos limiares nociceptivos;
- ✓ Investigar o efeito do tratamento repetido com a ETCC em ratos machos adultos submetidos ao modelo animal de dor neuropática e / ou exposição ativa ao álcool nos níveis de NGF em córtex e tronco cerebral;
- ✓ Avaliar mudanças nos níveis centrais de IL-1α, IL-1β e IL-10, em córtex e tronco cerebral, a partir do uso do ETCC como uma ferramenta neuromodulatória em ratos machos adultos submetidos ao modelo animal de dor neuropática e / ou exposição ativa ao álcool;

Artigo II

- ✓ Investigar o efeito do tratamento com ETCC como uma ferramenta neuromodulatória sobre o comportamento do tipo ansioso em ratos machos adultos submetidos ao modelo animal de dor neuropática e / ou exposição ativa ao álcool;
- ✓ Avaliar mudanças nos níveis de centrais de IL-1α e IL-1β em estriado, hipocampo, medula espinhal e cerebelo, a partir do uso do ETCC como uma ferramenta neuromodulatória em ratos machos adultos submetidos ao modelo animal de dor neuropática e / ou exposição ativa ao álcool;
- ✓ Investigar a expressão gênica de Il1a e Il1b em medula espinhal, cerebelo e hipocampo em ratos com lesão nervosa e exposição ativa ao álcool e tratados com ETCC;
- ✓ Investigar o efeito do tratamento da ETCC em ratos com lesão nervosa e exposição ativa ao álcool, nos níveis de neuropeptídio Y em hipotálamo.

Artigo III

- ✓ Avaliar o efeito do tratamento repetido com ETCC no consumo de álcool associado ou não a presença de dor crônica;
- ✓ Avaliar o efeito do tratamento repetido com ETCC em ratos machos adultos submetidos ao modelo animal de dor crônica e / ou exposição passiva ao álcool nos níveis de neuropeptídio Y em córtex pré-frontal, amígdala e estriado;

- ✓ Avaliar o efeito do tratamento repetido com a ETCC em ratos machos adultos submetidos ao modelo animal de dor crônica e / ou exposição passiva ao álcool nos níveis de BDNF e NGF em cerebelo e estriado;
- ✓ Avaliar o efeito do tratamento repetido com a ETCC em ratos machos adultos submetidos ao modelo animal de dor crônica e / ou exposição passiva ao álcool nos níveis de IL-10 e IL-6 no cerebelo e estriado.

VI. REFERÊNCIAS DA REVISÃO DA LITERATURA

REFERÊNCIAS DA REVISÃO DA LITERATURA

[1] C. Harstall and M. Ospina, "How Prevalent Is Chronic Pain?," *Pain Clin. Updat.*, 2003, doi: 10.1016/j.jpain.2010.07.002.

[2] A. C. D. C. Williams and K. D. Craig, "Updating the definition of pain," *Pain*. 2016, doi: 10.1097/j.pain.0000000000000613.

[3] J. Dahlhamer *et al.*, "Prevalence of Chronic Pain and High-Impact Chronic Pain Among Adults - United States, 2016.," *MMWR. Morb. Mortal. Wkly. Rep.*, 2018, doi: 10.15585/mmwr.mm6736a2.

[4] M. P. Duckworth, T. Iezzi, and G. Carlson, "Chronic Pain," in *Principle-Based Stepped Care and Brief Psychotherapy for Integrated Care Settings*, 2018, pp. 129–142.

[5] T. W. Vanderah, "Pathophysiology of pain.," *Med. Clin. North Am.*, vol. 91, no. 1, pp. 1–12, Jan. 2007, doi: 10.1016/j.mcna.2006.10.006.

[6] M. Egli, G. F. Koob, and S. Edwards, "Alcohol dependence as a chronic pain disorder," *Neuroscience and Biobehavioral Reviews*. 2012, doi: 10.1016/j.neubiorev.2012.07.010.

[7] M. G. Griswold *et al.*, "Alcohol use and burden for 195 countries and territories, 1990-2016: A systematic analysis for the Global Burden of Disease Study 2016," *Lancet*, 2018, doi: 10.1016/S0140-6736(18)31310-2.

[8] World Health Organisation, *Global Health TB Report*. 2018.

[9] C. Vilpoux, V. Warnault, O. Pierrefiche, M. Daoust, and M. Naassila, "Ethanol-sensitive brain regions in rat and mouse: A cartographic review, using immediate early gene expression," *Alcoholism: Clinical and Experimental Research*, vol. 33, no. 6. 2009, doi: 10.1111/j.1530-0277.2009.00916.x.

[10] M. Zychowska *et al.*, "Participation of pro- and anti-nociceptive interleukins in botulinum toxin A-induced analgesia in a rat model of neuropathic pain," *Eur. J. Pharmacol.*, vol. 791, 2016, doi: 10.1016/j.ejphar.2016.09.019.

[11] M. T. Robins, M. M. Heinricher, and A. E. Ryabinin, "From Pleasure to Pain, and Back Again: The Intricate Relationship between Alcohol and Nociception," *Alcohol and Alcoholism*, vol. 54, no. 6. 2019, doi: 10.1093/alcalc/agz067.

[12] R. R. Patel *et al.*, "IL-1β expression is increased and regulates GABA transmission following chronic ethanol in mouse central amygdala.," *Brain. Behav. Immun.*, vol. 75, pp. 208–219, Jan. 2019, doi: 10.1016/j.bbi.2018.10.009.

[13] B. Pradier, E. Erxlebe, A. Markert, and I. Rácz, "Microglial IL-1β progressively increases with duration of alcohol consumption," *Naunyn. Schmiedebergs. Arch. Pharmacol.*, vol. 391, no. 4, 2018, doi: 10.1007/s00210-018-1475-7.

[14] J. Mika *et al.*, "Interleukin-1alpha has antiallodynic and antihyperalgesic activities in a rat neuropathic pain model," *Pain*, 2008, doi: 10.1016/j.pain.2008.02.015.

[15] N. W. Gilpin, "Neuropeptide Y (NPY) in the extended amygdala is recruited during the transition to alcohol dependence," *Neuropeptides*. 2012, doi:

10.1016/j.npep.2012.08.001.

[16] M. Diaz-delCastillo, D. P. D. Woldbye, and A. M. Heegaard, "Neuropeptide Y and its Involvement in Chronic Pain," *Neuroscience*. 2018, doi: 10.1016/j.neuroscience.2017.08.050.

[17] A. Thorsell and A. A. Mathé, "Neuropeptide Y in alcohol addiction and affective disorders," *Frontiers in Endocrinology*. 2017, doi: 10.3389/fendo.2017.00178.

[18] M. A. Nitsche and W. Paulus, "Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans," *Neurology*, vol. 57, no. 10, 2001, doi: 10.1212/WNL.57.10.1899.

[19] F. Fregni *et al.*, "Evidence-based guidelines and secondary meta-analysis for the use of transcranial direct current stimulation (tDCS) in neurological and psychiatric disorders," *Int. J. Neuropsychopharmacol.*, Jul. 2020, doi: 10.1093/ijnp/pyaa051.

[20] L. M. Ferreira, B. Hochman, and M. V. J. Barbosa, "[Experimental models in research].," *Acta Cir. Bras.*, 2005, doi: /S0102-86502005000800008.

[21] S. Finger and W. T. Clower, "Victor Horsley on 'trephining in pre-historic times'.," *Neurosurgery*, 2001.

[22] S. N. Raja *et al.*, "The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises.," *Pain*, vol. 161, no. 9, pp. 1976–1982, Sep. 2020, doi: 10.1097/j.pain.0000000000001939.

[23] and E. Institute of Medicine (US) Committee on Advancing Pain Research, Care, "Relieving Pain in America: A Blueprint for Transforming Prevention, Care, Education, and Research - PubMed - NCBI," *Natl. Acad. Collect. Reports funded by Natl. Institutes Heal.*, 2011.

[24] T. Neogi, "The epidemiology and impact of pain in osteoarthritis," *Osteoarthr. Cartil.*, 2013, doi: 10.1016/j.joca.2013.03.018.

[25] F. A. E. F. Sousa, "Dor: o quinto sinal vital," *Rev. Lat. Am. Enfermagem*, 2005, doi: 10.1590/s0104-11692002000300020.

[26] A. C. D. C. Williams and K. D. Craig, "Updating the definition of pain," *Pain*. 2016, doi: 10.1097/j.pain.0000000000000613.

[27] S. E. E. Mills, K. P. Nicolson, and B. H. Smith, "Chronic pain: a review of its epidemiology and associated factors in population-based studies," *Br. J. Anaesth.*, 2019, doi: 10.1016/j.bja.2019.03.023.

[28] D. S. Goldberg and S. J. McGee, "Pain as a global public health priority.," *BMC Public Health*, vol. 11, p. 770, Oct. 2011, doi: 10.1186/1471-2458-11-770.

[29] H. Breivik, B. Collett, V. Ventafridda, R. Cohen, and D. Gallacher, "Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment.," *Eur. J. Pain*, 2006, doi: 10.1016/j.ejpain.2005.06.009.

[30] C. B. Johannes, T. K. Le, X. Zhou, J. A. Johnston, and R. H. Dworkin, "The Prevalence of Chronic Pain in United States Adults: Results of an Internet-Based Survey," *J. Pain*, 2010, doi: 10.1016/j.jpain.2010.07.002.

[31] S. P. Cohen and J. Mao, "Neuropathic pain: mechanisms and their clinical implications.," *BMJ*, vol. 348, p. f7656, Feb. 2014, doi: 10.1136/bmj.f7656.

[32] R. Abdullayev, O. Uludag, and B. Celik, "Analgesia Nociception Index: assessment of acute postoperative pain," *Brazilian J. Anesthesiol.*, vol. 69, no. 4, 2019, doi: 10.1016/j.bjan.2019.01.003.

[33] H.-G. Schaible, "[Pathophysiology of pain].," *Orthopade*, vol. 36, no. 1, pp. 8,10-12,14-16, Jan. 2007, doi: 10.1007/s00132-006-1022-y.

[34] X. Zhang and L. Bao, "The development and modulation of nociceptive circuitry.," *Curr. Opin. Neurobiol.*, vol. 16, no. 4, pp. 460–466, Aug. 2006, doi: 10.1016/j.conb.2006.06.002.

[35] P. Dinakar and A. M. Stillman, "Pathogenesis of Pain," *Seminars in Pediatric Neurology*. 2016, doi: 10.1016/j.spen.2016.10.003.

[36] S. M. Sherman and R. W. Guillery, "The role of the thalamus in the flow of information to the cortex," 2002, doi: 10.1098/rstb.2002.1161.

[37] H. Merskey and N. Bogduk (Eds), "Classification of Chronic Pain. Descriptions of Chronic Pain Syndromes and Definitions of Pain Terms," *IASP Press*, 1994, doi: 10.1002/ana.20394.

[38] J.-P. J. Pozek, D. Beausang, J. L. Baratta, and E. R. Viscusi, "The Acute to Chronic Pain Transition: Can Chronic Pain Be Prevented?," *Med. Clin. North Am.*, vol. 100, no. 1, pp. 17–30, Jan. 2016, doi: 10.1016/j.mcna.2015.08.005.

[39] P. R. M. Filho *et al.*, "Transcranial direct current stimulation (tDCS) reverts behavioral alterations and brainstem BDNF level increase induced by neuropathic pain model: Long-lasting effect," *Prog. Neuro-Psychopharmacology Biol. Psychiatry*, 2016, doi: 10.1016/j.pnpbp.2015.06.016.

[40] L. N. Spezia Adachi *et al.*, "Reversal of chronic stress-induced pain by transcranial direct current stimulation (tDCS) in an animal model," *Brain Res.*, 2012, doi: 10.1016/j.brainres.2012.10.009.

[41] L. M. Botelho *et al.*, "A Framework for Understanding the Relationship between Descending Pain Modulation, Motor Corticospinal, and Neuroplasticity Regulation Systems in Chronic Myofascial Pain," *Front. Hum. Neurosci.*, 2016, doi: 10.3389/fnhum.2016.00308.

[42] P. J. Ibor, I. Sanchez-Magro, J. Villoria, A. Leal, and A. Esquivias, "Mixed Pain Can Be Discerned in the Primary Care and Orthopedics Settings in Spain: A Large Cross-Sectional Study.," *Clin. J. Pain*, vol. 33, no. 12, pp. 1100–1108, Dec. 2017, doi: 10.1097/AJP.0000000000000491.

[43] S. S. C. on H. T. A. (SBU); 2006 O. SBU Yellow Report No.177/1+2., *Health Technology Assessment. Methods of Treating Chronic Pain: A Systematic Review*. Stockholm, 2006, p. https://www.ncbi.nlm.nih.gov/books/NBK447986/.

[44] N. B. Finnerup, S. H. Sindrup, and T. S. Jensen, "Recent advances in pharmacological treatment of neuropathic pain," *F1000 Med. Rep.*, vol. 2, no. 1, 2010, doi: 10.3410/M2-52.

[45] L. R. Watkins and S. F. Maier, "Glia: A novel drug discovery target for clinical

pain," *Nature Reviews Drug Discovery*. 2003, doi: 10.1038/nrd1251.

[46] J. O. de Oliveira Júnior, C. S. A. Portella Junior, C. P. Cohen, J. O. de Oliveira Júnior, C. S. A. Portella Junior, and C. P. Cohen, "Inflammatory mediators of neuropathic pain," *Rev. Dor*, 2016, doi: 10.5935/1806-0013.20160045.

[47] L. X. Wang and Z. J. Wang, "Animal and cellular models of chronic pain," *Adv. Drug Deliv. Rev.*, 2003, doi: 10.1016/S0169-409X(03)00098-X.

[48] S. Kaliyaperumal, K. Wilson, F. Aeffner, and C. J. Dean, "Animal Models of Peripheral Pain: Biology Review and Application for Drug Discovery.," *Toxicol. Pathol.*, p. 192623319857051, Jul. 2019, doi: 10.1177/0192623319857051.

[49] D. Dubuisson and S. G. Dennis, "The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats.," *Pain*, vol. 4, no. 2, pp. 161–174, Dec. 1977.

[50] A. M. Sousa, G. V. Lages, C. L. Pereira, and A. Slullitel, "Experimental models for the study of neuropathic pain," *Rev. Dor*, 2016, doi: 10.5935/1806-0013.20160043.

[51] G. J. Bennett and Y. K. Xie, "A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man," *Pain*, 1988, doi: 10.1016/0304-3959(88)90209-6.

[52] L. Degenhardt *et al.*, "The global burden of disease attributable to alcohol and drug use in 195 countries and territories, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016," *The Lancet Psychiatry*, 2018, doi: 10.1016/S2215-0366(18)30337-7.

[53] American Psychiatry Association Apa, *DSM-V-TR - Manual diagnóstico e estatístico de transtornos mentais*. 2013.

[54] World Health Organization, "Global status report on alcohol and health 2018: executive summary," *World Heal. Organ.*, 2018.

[55] C. Holst, J. S. Tolstrup, H. J. Sorensen, and U. Becker, "Alcohol dependence and risk of somatic diseases and mortality: a cohort study in 19 002 men and women attending alcohol treatment.," *Addiction*, vol. 112, no. 8, pp. 1358–1366, Aug. 2017, doi: 10.1111/add.13799.

[56] M. dos A. Mesquita, "The effects of alcohol in newborns," *Einstein (São Paulo)*, vol. 8, no. 3, 2010, doi: 10.1590/s1679-45082010rw1624.

[57] J. F. Williams and V. C. Smith, "Fetal Alcohol Spectrum Disorders.," *Pediatrics*, vol. 136, no. 5, pp. e1395-406, Nov. 2015, doi: 10.1542/peds.2015-3113.

[58] K. L. Jones, D. W. Smith, C. N. Ulleland, and P. Streissguth, "Pattern of malformation in offspring of chronic alcoholic mothers.," *Lancet (London, England)*, vol. 1, no. 7815, pp. 1267–1271, Jun. 1973, doi: 10.1016/s0140-6736(73)91291-9.

[59] B. A. Bailey and R. J. Sokol, "Prenatal alcohol exposure and miscarriage, stillbirth, preterm delivery, and sudden infant death syndrome.," *Alcohol Res. Health*, vol. 34, no. 1, pp. 86–91, 2011.

[60] P. D. Rogers, J. Harris, and J. Jarmuskewicz, "Alcohol and adolescence.," *Pediatr.*

Clin. North Am., vol. 34, no. 2, pp. 289–303, Apr. 1987, doi: 10.1016/s0031-3955(16)36215-0.

[61] I. Licanin, A. Redzic, and E. Ibrahimagic, "Alcoholism during adolescence.," *Med. Arh.*, vol. 60, no. 4, pp. 225–229, 2006.

[62] M. A. Schuckit, "Alcohol-use disorders.," *Lancet (London, England)*, vol. 373, no. 9662, pp. 492–501, Feb. 2009, doi: 10.1016/S0140-6736(09)60009-X.

[63] M. G. Proescholdt, M. Walter, and G. A. Wiesbeck, "[Alcohol and violence: a current review].," *Fortschr. Neurol. Psychiatr.*, vol. 80, no. 8, pp. 441–449, Aug. 2012, doi: 10.1055/s-0031-1282018.

[64] J. A. Vaingankar *et al.*, "Prevalence of Chronic Mental and Physical Disorders, Impact on Work Productivity and Correlates of Alcohol Use Disorders and Nicotine Dependence across Occupations," *Ann. Acad. Med. Singapore*, vol. 44, no. 4, 2015.

[65] S. K. Rigler, "Alcoholism in the elderly.," *Am. Fam. Physician*, vol. 61, no. 6, pp. 1710–6, 1883–4, 1887–8 passim, Mar. 2000.

[66] F. Lewin *et al.*, "Smoking tobacco, oral snuff, and alcohol in the etiology of squamous cell carcinoma of the head and neck: a population-based case-referent study in Sweden.," *Cancer*, vol. 82, no. 7, pp. 1367–1375, Apr. 1998, doi: 10.1002/(sici)1097-0142(19980401)82:7<1367::aid-cncr21>3.0.co;2-3.

[67] G. T. Sutherland, D. Sheedy, and J. J. Kril, "Using Autopsy Brain Tissue to Study Alcohol-Related Brain Damage in the Genomic Age," *Alcoholism: Clinical and Experimental Research*. 2014, doi: 10.1111/acer.12243.

[68] A. G. Soloviev, A. M. Vyazmin, E. A. Mordovsky, and V. V. Nikulichev, "The structure of mortality of patients under dispensary supervision diagnosed with alcohol-related mental and behavioral disorders," *Zhurnal Nevrol. i Psihiatr. Im. S.S. Korsakova*, vol. 2015, no. 11, 2015, doi: 10.17116/jnevro201511511197-102.

[69] N. Latt *et al.*, "Alcohol and substance use by patients with psychosis presenting to an emergency department: Changing patterns," *Australasian Psychiatry*, vol. 19, no. 4. 2011, doi: 10.3109/10398562.2011.579971.

[70] G. Schomerus, M. Lucht, A. Holzinger, H. Matschinger, M. G. Carta, and M. C. Angermeyer, "The stigma of alcohol dependence compared with other mental disorders: A review of population studies," *Alcohol and Alcoholism*, vol. 46, no. 2. 2011, doi: 10.1093/alcalc/agq089.

[71] R. Nardone *et al.*, "Non-invasive brain stimulation in the functional evaluation of alcohol effects and in the treatment of alcohol craving: A review," *Neuroscience Research*. 2012, doi: 10.1016/j.neures.2012.08.003.

[72] J. D. Jentsch and J. R. Taylor, "Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli.," *Psychopharmacology (Berl).*, vol. 146, no. 4, pp. 373–390, Oct. 1999.

[73] M. J. Kreek, D. A. Nielsen, E. R. Butelman, and K. S. LaForge, "Genetic influences on impulsivity, risk taking, stress responsivity and vulnerability to drug abuse and addiction.," *Nat. Neurosci.*, vol. 8, no. 11, pp. 1450–1457, Nov. 2005,

doi: 10.1038/nn1583.

[74] S. L. Robinson and T. E. Thiele, "The Role of Neuropeptide Y (NPY) in Alcohol and Drug Abuse Disorders," in *International Review of Neurobiology*, 2017.

[75] T. E. Thiele, D. J. Marsh, L. Ste. Marie, I. L. Bernstein, and R. D. Palmiter, "Ethanol consumption and resistance are inversely related to neuropeptide Y levels," *Nature*, vol. 396, no. 6709, pp. 366–369, 1998, doi: 10.1038/24614.

[76] A. Topiwala and K. P. Ebmeier, "Effects of drinking on late-life brain and cognition.," *Evid. Based. Ment. Health*, vol. 21, no. 1, pp. 12–15, Feb. 2018, doi: 10.1136/eb-2017-102820.

[77] J. Jacobus and S. F. Tapert, "Neurotoxic effects of alcohol in adolescence," *Annual Review of Clinical Psychology*, vol. 9. 2013, doi: 10.1146/annurev-clinpsy-050212-185610.

[78] L. M. Squeglia *et al.*, "Neural predictors of initiating alcohol use during adolescence," *Am. J. Psychiatry*, vol. 174, no. 2, 2017, doi: 10.1176/appi.ajp.2016.15121587.

[79] H. Trantham-Davidson *et al.*, "Binge-Like Alcohol Exposure during Adolescence Disrupts Dopaminergic Neurotransmission in the Adult Prelimbic Cortex," *Neuropsychopharmacology*, vol. 42, no. 5, 2017, doi: 10.1038/npp.2016.190.

[80] I. Ferrer, I. Fabregues, M. Pineda, I. Gracia, and T. Ribalta, "A Golgi study of cerebellar atrophy in human chronic alcoholism.," *Neuropathol. Appl. Neurobiol.*, vol. 10, no. 4, pp. 245–253, 1984.

[81] I. Alvarez, L. M. Gonzalo, and J. Llor, "Effects of chronic alcoholism on the amygdaloid complex. A study in human and rats.," *Histol. Histopathol.*, vol. 4, no. 2, pp. 183–192, Apr. 1989.

[82] E. V Sullivan, L. Marsh, D. H. Mathalon, K. O. Lim, and A. Pfefferbaum, "Relationship between alcohol withdrawal seizures and temporal lobe white matter volume deficits.," *Alcohol. Clin. Exp. Res.*, vol. 20, no. 2, pp. 348–354, Apr. 1996.

[83] K. Chopra and V. Tiwari, "Alcoholic neuropathy: Possible mechanisms and future treatment possibilities," *Br. J. Clin. Pharmacol.*, vol. 73, no. 3, 2012, doi: 10.1111/j.1365-2125.2011.04111.x.

[84] H. Koike *et al.*, "Alcoholic neuropathy is clinicopathologically distinct from thiamine-deficiency neuropathy," *Ann. Neurol.*, vol. 54, no. 1, 2003, doi: 10.1002/ana.10550.

[85] A. Rocco, D. Compare, D. Angrisani, M. Sanduzzi Zamparelli, and G. Nardone, "Alcoholic disease: Liver and beyond," *World Journal of Gastroenterology*, vol. 20, no. 40. 2014, doi: 10.3748/wjg.v20.i40.14652.

[86] L. H. Tecott and U. Heberlein, "Y Do We Drink?," *Cell*, vol. 95, no. 6, pp. 733–735, Dec. 1998, doi: 10.1016/S0092-8674(00)81695-5.

[87] K. C. Young-Wolff, P. Wang, C. Tuvblad, L. A. Baker, A. Raine, and C. A. Prescott, "Drinking experience uncovers genetic influences on alcohol expectancies across adolescence," *Addiction*, vol. 110, no. 4, 2015, doi: 10.1111/add.12845.

[88] P. A. Vernon, D. Lee, J. A. Harris, and K. L. Jang, "Genetic and environmental contributions to individual differences in alcohol expectancies," *Pers. Individ. Dif.*, vol. 21, no. 2, 1996, doi: 10.1016/0191-8869(96)00068-2.

[89] J. L. Torres *et al.*, "Role of microRNAs in alcohol-induced liver disorders and non-alcoholic fatty liver disease," *World J. Gastroenterol.*, vol. 24, no. 36, 2018, doi: 10.3748/wjg.v24.i36.4104.

[90] J. C. Crabbe, J. K. Belknap, and K. J. Buck, "Genetic animal models of alcohol and drug abuse.," *Science*, vol. 264, no. 5166, pp. 1715–1723, Jun. 1994, doi: 10.1126/science.8209252.

[91] S. Carnicella, D. Ron, and S. Barak, "Intermittent ethanol access schedule in rats as a preclinical model of alcohol abuse.," *Alcohol*, vol. 48, no. 3, pp. 243–52, May 2014, doi: 10.1016/j.alcohol.2014.01.006.

[92] G. A. Deehan *et al.*, "Selective breeding for high alcohol consumption and response to nicotine: locomotor activity, dopaminergic in the mesolimbic system, and innate genetic differences in male and female alcohol-preferring, non-preferring, and replicate lines of high-alcohol drinking and low-alcohol drinking rats," *Psychopharmacology (Berl).*, vol. 235, no. 9, 2018, doi: 10.1007/s00213-018-4970-0.

[93] M. M. Sperry, E. J. Granquist, and B. A. Winkelstein, "Early changes in brain network topology and activation of affective pathways predict persistent pain in the rat," *Pain*, vol. 162, no. 1, pp. 45–55, Jan. 2021, doi: 10.1097/j.pain.0000000000002010.

[94] M. Llorca-Torralba *et al.*, "Chemogenetic Silencing of the Locus Coeruleus–Basolateral Amygdala Pathway Abolishes Pain-Induced Anxiety and Enhanced Aversive Learning in Rats," *Biol. Psychiatry*, vol. 85, no. 12, 2019, doi: 10.1016/j.biopsych.2019.02.018.

[95] D. Pilat *et al.*, "IL-1 receptor antagonist improves morphine and buprenorphine efficacy in a rat neuropathic pain model," *Eur. J. Pharmacol.*, 2015, doi: 10.1016/j.ejphar.2015.05.058.

[96] M. N. Baliki and A. V. Apkarian, "Nociception, Pain, Negative Moods, and Behavior Selection," *Neuron*, vol. 87, no. 3. 2015, doi: 10.1016/j.neuron.2015.06.005.

[97] G. F. Koob and N. D. Volkow, "Neurocircuitry of addiction," *Neuropsychopharmacology*. 2010, doi: 10.1038/npp.2009.110.

[98] C. Horn-Hofmann, P. Buscher, S. Lautenbacher, and J. Wolstein, "The effect of nonrecurring alcohol administration on pain perception in humans: a systematic review.," *J. Pain Res.*, vol. 8, pp. 175–187, 2015, doi: 10.2147/JPR.S79618.

[99] J. L. Riley and C. King, "Self-Report of Alcohol Use for Pain in a Multi-Ethnic Community Sample," *J. Pain*, 2009, doi: 10.1016/j.jpain.2009.03.005.

[100] G. F. Koob, "Alcoholism: allostasis and beyond.," *Alcohol. Clin. Exp. Res.*, vol. 27, no. 2, pp. 232–243, Feb. 2003, doi: 10.1097/01.ALC.0000057122.36127.C2.

[101] H. Koike *et al.*, "Painful alcoholic polyneuropathy with predominant small-fiber loss and normal thiamine status," *Neurology*, vol. 56, no. 12, 2001, doi:

10.1212/WNL.56.12.1727.

[102] C. K. Singleton and P. R. Martin, "Molecular mechanisms of thiamine utilization.," *Curr. Mol. Med.*, vol. 1, no. 2, pp. 197–207, May 2001, doi: 10.2174/1566524013363870.

[103] C. C. Chen, C. H. Yang, C. C. Huang, and K. Sen Hsu, "Acute stress impairs hippocampal mossy fiber-CA3 long-term potentiation by enhancing cAMP-specific phosphodiesterase 4 activity," *Neuropsychopharmacology*, vol. 35, no. 7, 2010, doi: 10.1038/npp.2010.33.

[104] M. A. Nitsche *et al.*, "Consolidation of human motor cortical neuroplasticity by D-cycloserine.," *Neuropsychopharmacology*, vol. 29, no. 8, pp. 1573–8, Aug. 2004, doi: 10.1016/S0193-953X(03)00113-8.

[105] T. E. Wilens, "Attention-deficit/hyperactivity disorder and the substance use disorders: the nature of the relationship, subtypes at risk, and treatment issues.," *Psychiatr. Clin. North Am.*, vol. 27, no. 2, pp. 283–301, Jun. 2004, doi: 10.1016/S0193-953X(03)00113-8.

[106] T. E. Robinson and B. Kolb, "Structural plasticity associated with exposure to drugs of abuse," *Neuropharmacology*, vol. 47, no. SUPPL. 1, 2004, doi: 10.1016/j.neuropharm.2004.06.025.

[107] A. V. Apkarian *et al.*, "Neural mechanisms of pain and alcohol dependence," *Pharmacology Biochemistry and Behavior*, vol. 112. 2013, doi: 10.1016/j.pbb.2013.09.008.

[108] Y. Tu, Y. Bi, L. Zhang, H. Wei, and L. Hu, "Mesocorticolimbic pathways encode cue-based expectancy effects on pain," *J. Neurosci.*, vol. 40, no. 2, 2020, doi: 10.1523/JNEUROSCI.1082-19.2019.

[109] T. M. Le, S. Zhornitsky, S. Zhang, and C. S. R. Li, "Pain and reward circuits antagonistically modulate alcohol expectancy to regulate drinking," *Transl. Psychiatry*, vol. 10, no. 1, 2020, doi: 10.1038/s41398-020-00909-z.

[110] Y. W. Shih *et al.*, "Effects of positive and negative expectations on human pain perception engage separate but interrelated and dependently regulated cerebral mechanisms," *J. Neurosci.*, vol. 39, no. 7, 2019, doi: 10.1523/JNEUROSCI.2154-18.2018.

[111] D. J. Paulus *et al.*, "Emotion dysregulation in the context of pain and alcohol use among latinos in primary care," *J. Stud. Alcohol Drugs*, vol. 78, no. 6, 2017, doi: 10.15288/jsad.2017.78.938.

[112] E. T. Kneeland, M. L. Griffin, N. Taghian, R. D. Weiss, and R. K. McHugh, "Associations between pain catastrophizing and clinical characteristics in adults with substance use disorders and co-occurring chronic pain," *Am. J. Drug Alcohol Abuse*, vol. 45, no. 5, 2019, doi: 10.1080/00952990.2019.1581793.

[113] D. Moskal, S. A. Maisto, M. De Vita, and J. W. Ditre, "Effects of experimental pain induction on alcohol urge, intention to consume alcohol, and alcohol demand," *Exp. Clin. Psychopharmacol.*, vol. 26, no. 1, 2018, doi: 10.1037/pha0000170.

[114] F. Fregni *et al.*, "Transcranial direct current stimulation of the prefrontal cortex

modulates the desire for specific foods," *Appetite*, 2008, doi: 10.1016/j.appet.2007.09.016.

[115] A. C. Rosen, M. Ramkumar, T. Nguyen, and F. Hoeft, "Noninvasive transcranial brain stimulation and pain," *Current Pain and Headache Reports*. 2009, doi: 10.1007/s11916-009-0004-2.

[116] A. Antal *et al.*, "Low intensity transcranial electric stimulation: Safety, ethical, legal regulatory and application guidelines," *Clinical Neurophysiology*, vol. 128, no. 9. Elsevier Ireland Ltd, pp. 1774–1809, Sep. 01, 2017, doi: 10.1016/j.clinph.2017.06.001.

[117] J. G. Rohan, K. A. Carhuatanta, S. M. McInturf, M. K. Miklasevich, and R. Jankord, "Modulating hippocampal plasticity with in Vivo brain stimulation," *J. Neurosci.*, vol. 35, no. 37, 2015, doi: 10.1523/JNEUROSCI.2376-15.2015.

[118] S. G. Cioato *et al.*, "Long-Lasting Effect of Transcranial Direct Current Stimulation in the Reversal of Hyperalgesia and Cytokine Alterations Induced by the Neuropathic Pain Model," *Brain Stimul.*, 2016, doi: 10.1016/j.brs.2015.12.001.

[119] G. Laste *et al.*, "After-effects of consecutive sessions of transcranial direct current stimulation (tDCS) in a rat model of chronic inflammation," *Exp. Brain Res.*, 2012, doi: 10.1007/s00221-012-3149-x.

[120] E. Plow, A. Pascual-Leone, and A. Machado, "Brain Stimulation in the Treatment of Chronic Neuropathic and Non-Cancerous Pain," *J Pain*, 2012, doi: 10.1016/j.jpain.2012.02.001.

[121] A. J. Woods, V. Bryant, D. Sacchetti, F. Gervits, and R. Hamilton, "Effects of electrode drift in transcranial direct current stimulation," *Brain Stimul.*, vol. 8, no. 3, 2015, doi: 10.1016/j.brs.2014.12.007.

[122] S. Raghavan, S. Eldabe, and R. Strachan, "Novel trends in pain management - Neuromodulation," *Curr. Anaesth. Crit. Care*, 2008, doi: 10.1016/j.cacc.2008.07.005.

[123] M. A. Nitsche *et al.*, "Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans," *J. Physiol.*, vol. 553, no. 1, 2003, doi: 10.1113/jphysiol.2003.049916.

[124] N. Lang *et al.*, "How does transcranial DC stimulation of the primary motor cortex alter regional neuronal activity in the human brain?," *Eur. J. Neurosci.*, 2005, doi: 10.1111/j.1460-9568.2005.04233.x.

[125] A. R. Brunoni *et al.*, "Clinical research with transcranial direct current stimulation (tDCS): challenges and future directions.," *Brain Stimul.*, vol. 5, no. 3, pp. 175–195, Jul. 2012, doi: 10.1016/j.brs.2011.03.002.

[126] L. F. Medeiros *et al.*, "Neurobiological effects of transcranial direct current stimulation: A review," *Frontiers in Psychiatry*. 2012, doi: 10.3389/fpsyt.2012.00110.

[127] S. G. Cioato *et al.*, "Long-Lasting Effect of Transcranial Direct Current Stimulation in the Reversal of Hyperalgesia and Cytokine Alterations Induced by the Neuropathic Pain Model," *Brain Stimul.*, 2016, doi: 10.1016/j.brs.2015.12.001.

[128] A. Souza *et al.*, "Neurobiological mechanisms of antiallodynic effect of transcranial direct current stimulation (tDCS) in a mice model of neuropathic pain," *Brain Res.*, 2018, doi: 10.1016/j.brainres.2017.12.005.

[129] D. Liebetanz, M. A. Nitsche, F. Tergau, and W. Paulus, "Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability," *Brain*, vol. 125, no. 10, 2002, doi: 10.1093/brain/awf238.

[130] A. Fertonani and C. Miniussi, "Transcranial electrical stimulation: What we know and do not know about mechanisms," *Neuroscientist*, vol. 23, no. 2, 2017, doi: 10.1177/1073858416631966.

[131] M. P. Jackson *et al.*, "Animal models of transcranial direct current stimulation: Methods and mechanisms," *Clinical Neurophysiology*, vol. 127, no. 11. 2016, doi: 10.1016/j.clinph.2016.08.016.

[132] R. Dengler, "Guidelines for low intensity transcranial electrical stimulation – An overdue step in a fairly uncontrolled field," *Clinical Neurophysiology*, vol. 128, no. 9. Elsevier Ireland Ltd, pp. 1770–1771, Sep. 01, 2017, doi: 10.1016/j.clinph.2017.06.242.

[133] J. P. Lefaucheur *et al.*, "Evidence-based guidelines on the therapeutic use of transcranial direct current stimulation (tDCS)," *Clinical Neurophysiology*, vol. 128, no. 1. pp. 56–92, 2017, doi: 10.1016/j.clinph.2016.10.087.

[134] M. H. Ossipov, K. Morimura, and F. Porreca, "Descending pain modulation and chronification of pain," *Current Opinion in Supportive and Palliative Care*, vol. 8, no. 2. 2014, doi: 10.1097/spc.0000000000000055.

[135] L. A. Roeckel, G. M. Le Coz, C. Gavériaux-Ruff, and F. Simonin, "Opioid-induced hyperalgesia: Cellular and molecular mechanisms," *Neuroscience*, vol. 338. 2016, doi: 10.1016/j.neuroscience.2016.06.029.

[136] J. C. Ipser, D. Wilson, T. O. Akindipe, C. Sager, and D. J. Stein, "Pharmacotherapy for anxiety and comorbid alcohol use disorders," *Cochrane Database of Systematic Reviews*, vol. 2017, no. 6. 2015, doi: 10.1002/14651858.CD007505.pub2.

[137] A. J. Shackman and T. D. Wager, "The emotional brain: Fundamental questions and strategies for future research," *Neuroscience Letters*, vol. 693. 2019, doi: 10.1016/j.neulet.2018.10.012.

[138] J. Klauss, Q. S. Anders, L. V. Felippe, M. A. Nitsche, and E. M. Nakamura-Palacios, "Multiple sessions of transcranial direct current stimulation (tDCS) reduced craving and relapses for alcohol use: A randomized placebo-controlled trial in alcohol use disorder," *Front. Pharmacol.*, 2018, doi: 10.3389/fphar.2018.00716.

[139] M. A. Nitsche *et al.*, "Transcranial direct current stimulation: State of the art 2008," *Brain Stimulation*. 2008, doi: 10.1016/j.brs.2008.06.004.

[140] T. E. Den Uyl, T. E. Gladwin, and R. W. Wiers, "Transcranial direct current stimulation, implicit alcohol associations and craving," *Biol. Psychol.*, vol. 105, 2015, doi: 10.1016/j.biopsycho.2014.12.004.

[141] D. S. Santos *et al.*, "Transcranial Direct Current Stimulation (tDCS) Induces

Analgesia in Rats with Neuropathic Pain and Alcohol Abstinence," *Neurochem. Res.*, vol. 45, no. 11, 2020, doi: 10.1007/s11064-020-03116-w.

[142] M. da Graca-Tarragó *et al.*, "Intramuscular electrical stimulus potentiates motor cortex modulation effects on pain and descending inhibitory systems in knee osteoarthritis: A randomized, factorial, sham-controlled study," *J. Pain Res.*, 2019, doi: 10.2147/JPR.S181019.

[143] A. P. Brietzke *et al.*, "Large treatment effect with extended home-based transcranial direct current stimulation over dorsolateral prefrontal cortex in fibromyalgia: A Proof of Concept Sham-Randomized Clinical Study," *J. Pain*, 2019, doi: 10.1016/j.jpain.2019.06.013.

[144] C. de Oliveira *et al.*, "Transcranial direct current stimulation (tDCS) modulates biometric and inflammatory parameters and anxiety-like behavior in obese rats," *Neuropeptides*, 2019, doi: 10.1016/j.npep.2018.09.006.

[145] E. M. Nakamura-Palacios *et al.*, "Ventral medial prefrontal cortex (vmPFC) as a target of the dorsolateral prefrontal modulation by transcranial direct current stimulation (tDCS) in drug addiction," *J. Neural Transm.*, vol. 123, no. 10, pp. 1179–1194, 2016, doi: 10.1007/s00702-016-1559-9.

[146] M. C. da Silva *et al.*, "Behavioral effects of transcranial Direct Current Stimulation (tDCS) induced dorsolateral prefrontal cortex plasticity in alcohol dependence," *J. Physiol. Paris*, 2013, doi: 10.1016/j.jphysparis.2013.07.003.

[147] P. S. Boggio *et al.*, "Prefrontal cortex modulation using transcranial DC stimulation reduces alcohol craving: A double-blind, sham-controlled study," *Drug Alcohol Depend.*, 2008, doi: 10.1016/j.drugalcdep.2007.06.011.

[148] M. A. Vanderhasselt, J. Allaert, R. De Raedt, C. Baeken, R. M. Krebs, and S. Herremans, "Bifrontal tDCS applied to the dorsolateral prefrontal cortex in heavy drinkers: Influence on reward-triggered approach bias and alcohol consumption," *Brain Cogn.*, vol. 138, 2020, doi: 10.1016/j.bandc.2019.105512.

[149] I. C. Macedo *et al.*, "Repeated transcranial direct current stimulation reduces food craving in Wistar rats," *Appetite*, 2016, doi: 10.1016/j.appet.2016.03.014.

[150] G. Loheswaran, M. S. Barr, T. K. Rajji, R. Zomorrodi, B. Le Foll, and Z. J. Daskalakis, "Brain Stimulation in Alcohol Use Disorders: Investigational and Therapeutic Tools," *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*, vol. 1, no. 1. 2016, doi: 10.1016/j.bpsc.2015.09.005.

[151] N. Ngernyam *et al.*, "The effects of transcranial direct current stimulation in patients with neuropathic pain from spinal cord injury," *Clin. Neurophysiol.*, vol. 126, no. 2, 2015, doi: 10.1016/j.clinph.2014.05.034.

[152] E. M. De Andrade *et al.*, "Neurochemical effects of motor cortex stimulation in the periaqueductal gray during neuropathic pain," *J. Neurosurg.*, vol. 132, no. 1, 2020, doi: 10.3171/2018.7.JNS173239.

[153] T. Tanaka *et al.*, "Transcranial direct-current stimulation increases extracellular dopamine levels in the rat striatum," *Front. Syst. Neurosci.*, no. MARCH 2013, 2013, doi: 10.3389/fnsys.2013.00006.

[154] R. Z. Goldstein and N. D. Volkow, "Drug addiction and its underlying

neurobiological basis: Neuroimaging evidence for the involvement of the frontal cortex," *American Journal of Psychiatry*, vol. 159, no. 10. 2002, doi: 10.1176/appi.ajp.159.10.1642.

[155] V. Maletic and C. L. Raison, "Neurobiology of depression, fibromyalgia and neuropathic pain," *Front. Biosci.*, vol. 14, no. 14, 2009, doi: 10.2741/3598.

[156] H. C. Becker, "Influence of stress associated with chronic alcohol exposure on drinking," *Neuropharmacology*, vol. 122. 2017, doi: 10.1016/j.neuropharm.2017.04.028.

[157] A. Vicente-Sanchez, L. Segura, and A. A. Pradhan, "The delta opioid receptor tool box," *Neuroscience*, vol. 338. 2016, doi: 10.1016/j.neuroscience.2016.06.028.

[158] R. M. Van Rijn, D. I. Brissett, and J. L. Whistler, "Emergence of functional spinal delta opioid receptors after chronic ethanol exposure," *Biol. Psychiatry*, vol. 71, no. 3, 2012, doi: 10.1016/j.biopsych.2011.07.015.

[159] P. W. Kalivas and N. D. Volkow, "The neural basis of addiction: A pathology of motivation and choice," *American Journal of Psychiatry*. 2005, doi: 10.1176/appi.ajp.162.8.1403.

[160] A. Shah *et al.*, "The lateral habenula and alcohol: Role of glutamate and M-type potassium channels," *Pharmacology Biochemistry and Behavior*, vol. 162. 2017, doi: 10.1016/j.pbb.2017.06.005.

[161] F. Reichmann and P. Holzer, "Neuropeptide Y: A stressful review," *Neuropeptides*, vol. 55. 2016, doi: 10.1016/j.npep.2015.09.008.

[162] N. W. Gilpin, "Corticotropin-releasing factor (CRF) and neuropeptide Y (NPY): effects on inhibitory transmission in central amygdala, and anxiety- & alcohol-related behaviors.," *Alcohol*, vol. 46, no. 4, pp. 329–337, Jun. 2012, doi: 10.1016/j.alcohol.2011.11.009.

[163] T. S. Nelson and B. K. Taylor, "Targeting spinal neuropeptide Y1 receptor-expressing interneurons to alleviate chronic pain and itch," *Progress in Neurobiology*, vol. 196. 2021, doi: 10.1016/j.pneurobio.2020.101894.

[164] M. A. Upadhya, M. P. Dandekar, D. M. Kokare, P. S. Singru, and N. K. Subhedar, "Involvement of neuropeptide Y in the acute, chronic and withdrawal responses of morphine in nociception in neuropathic rats: Behavioral and neuroanatomical correlates," *Neuropeptides*, 2009, doi: 10.1016/j.npep.2009.05.003.

[165] J.-Z. Wang, "Microinjection of neuropeptide Y into periaqueductal grey produces anti-nociception in rats with mononeuropathy.," *Sheng Li Xue Bao*, 2004.

[166] W. Fu, C. R. Wessel, and B. K. Taylor, "Neuropeptide Y tonically inhibits an NMDAR➔AC1➔TRPA1/TRPV1 mechanism of the affective dimension of chronic neuropathic pain," *Neuropeptides*, vol. 80, 2020, doi: 10.1016/j.npep.2020.102024.

[167] A. G. Davies, J. C. Bettinger, T. R. Thiele, M. E. Judy, and S. L. McIntire, "Natural variation in the npr-1 gene modifies ethanol responses of wild strains of C. elegans," *Neuron*, 2004, doi: 10.1016/j.neuron.2004.05.004.

[168] J. Chen, Y. Zhang, and P. Shen, "A protein kinase C activity localized to

neuropeptide Y-like neurons mediates ethanol intoxication in Drosophila melanogaster," *Neuroscience*, 2008, doi: 10.1016/j.neuroscience.2008.07.008.

[169] G. Shohat-Ophir, K. R. Kaun, R. Azanchi, and U. Heberlein, "Sexual deprivation increases ethanol intake in Drosophila," *Science (80-.).*, 2012, doi: 10.1126/science.1215932.

[170] C. M. Brown, D. V. Coscina, and P. J. Fletcher, "The rewarding properties of neuropeptide Y in perifornical hypothalamus vs. nucleus accumbens," *Peptides*, 2000, doi: 10.1016/S0196-9781(00)00270-9.

[171] F. Plescia, A. Brancato, R. A. M. Marino, C. Vita, M. Navarra, and C. Cannizzaro, "Effect of acetaldehyde intoxication and withdrawal on NPY expression: Focus on endocannabinoidergic system involvement," *Front. Psychiatry*, vol. 5, no. OCT, 2014, doi: 10.3389/fpsyt.2014.00138.

[172] G. Chastain, "Alcohol, neurotransmitter systems, and behavior," *J. Gen. Psychol.*, 2006, doi: 10.3200/GENP.133.4.329-335.

[173] R. V. A. Schunck *et al.*, "Protracted alcohol abstinence induces analgesia in rats: Possible relationships with BDNF and interleukin-10," *Pharmacol. Biochem. Behav.*, 2015, doi: 10.1016/j.pbb.2015.05.011.

[174] S. F. Da Silva Moreira *et al.*, "Transcranial direct current stimulation (tDCS) neuromodulatory effects on mechanical hyperalgesia and cortical BDNF levels in ovariectomized rats," *Life Sci.*, 2016, doi: 10.1016/j.lfs.2015.10.011.

[175] P. Bekinschtein, M. Cammarota, and J. H. Medina, "BDNF and memory processing," *Neuropharmacology*. 2014, doi: 10.1016/j.neuropharm.2013.04.024.

[176] J. M. Conner *et al.*, "NGF is essential for hippocampal plasticity and learning," *J. Neurosci.*, 2009, doi: 10.1523/JNEUROSCI.2594-09.2009.

[177] C. A. Gericke, O. Schulte-Herbrüggen, T. Arendt, and R. Hellweg, "Chronic alcohol intoxication in rats leads to a strong but transient increase in NGF levels in distinct brain regions," *J. Neural Transm.*, 2006, doi: 10.1007/s00702-005-0361-x.

[178] M. W. Miller, "Repeated episodic exposure to ethanol affects neurotrophin content in the forebrain of the mature rat," *Exp. Neurol.*, 2004, doi: 10.1016/j.expneurol.2004.05.026.

[179] A. M. Jurga, A. Piotrowska, W. Makuch, B. Przewlocka, and J. Mika, "Blockade of P2X4 receptors inhibits neuropathic pain-related behavior by preventing MMP-9 activation and, consequently, pronociceptive interleukin release in a rat model," *Front. Pharmacol.*, vol. 8, no. FEB, 2017, doi: 10.3389/fphar.2017.00048.

[180] J. F. Hojen *et al.*, "IL-1R3 blockade broadly attenuates the functions of six members of the IL-1 family, revealing their contribution to models of disease.," *Nat. Immunol.*, vol. 20, no. 9, pp. 1138–1149, Sep. 2019, doi: 10.1038/s41590-019-0467-1.

[181] C. A. Dinarello, "Overview of the IL-1 family in innate inflammation and acquired immunity," *Immunological Reviews*, vol. 281, no. 1. 2018, doi: 10.1111/imr.12621.

[182] M. Schäfers and L. Sorkin, "Effect of cytokines on neuronal excitability," *Neurosci. Lett.*, vol. 437, no. 3, 2008, doi: 10.1016/j.neulet.2008.03.052.

[183] C. A. Dinarello, "Overview of the interleukin-1 family of ligands and receptors," *Seminars in Immunology*, vol. 25, no. 6. 2013, doi: 10.1016/j.smim.2013.10.001.

[184] A. Oliveira *et al.*, "Interleukin-1β genotype and circulating levels in cancer patients: Metastatic status and pain perception," *Clin. Biochem.*, vol. 47, no. 13–14, 2014, doi: 10.1016/j.clinbiochem.2014.04.009.

[185] M. Więdłocha *et al.*, "Effect of antidepressant treatment on peripheral inflammation markers – A meta-analysis," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 80. 2018, doi: 10.1016/j.pnpbp.2017.04.026.

[186] R. P. Yezierski and P. Hansson, "Inflammatory and Neuropathic Pain From Bench to Bedside: What Went Wrong?," *Journal of Pain*, vol. 19, no. 6. 2018, doi: 10.1016/j.jpain.2017.12.261.

VII. ARTIGOS CIENTÍFICOS

7.1 Artigo 1

Transcranial Direct Current Stimulation (tDCS) Induces Analgesia in Rats with Neuropathic Pain and Alcohol Abstinence.

Periódico: Neurochemical Research

Status: Published online: 25 August 2020

https://doi.org/10.1007/s11064-020-03116-w

Transcranial Direct Current Stimulation (tDCS) Induces Analgesia in Rats with Neuropathic Pain and Alcohol Abstinence

Daniela Silva Santos[a,b,g], Bettega Costa Lopes[a,c,g], Liciane Fernandes Medeiros[a,d,f], José Antônio Fagundes Assumpção[a,b,g], Andressa de Souza[a,f,] Artur Alban Salvi[a,g], Lisiane Santos da Silva[a,b,g], Felipe Fregni[e], Wolnei Caumo[b], Iraci L. S. Torres[*a,b,c,d,g]

[a]Laboratório de Farmacologia da Dor e Neuromodulação: Investigações Pré-Clínicas, Centro de Pesquisa Experimental, Hospital de Clínicas de Porto Alegre, Porto Alegre/RS, Brazil.

[b]Programa de Pós-Graduação em Medicina: Ciências Médicas, Faculdade de Medicina, Universidade Federal Rio Grande do Sul, Porto Alegre/RS, Brazil.

[c]Programa de Pós-Graduação em Ciências Biológicas: Fisiologia, Instituto de Ciências Básicas da Saúde (ICBS), Universidade Federal do Rio Grande do Sul, Porto Alegre/RS, Brazil.

[d]Programa de Pós-Graduação em Ciências Biológicas: Farmacologia e Terapêutica, Instituto de Ciências Básicas da Saúde (ICBS), Universidade Federal Rio Grande do Sul, Porto Alegre/RS, Brazil.

[e]Laboratory of Neuromodulation, Department of Physical Medicine & Rehabilitation, Spaulding Rehabilitation Hospital and Massachusetts General Hospital, Harvard University, Boston/MA, United States of America

[f]Programa de Pós-Graduação em Saúde e Desenvolvimento Humano, Universidade La Salle, Canoas/RS, Brazil.

[g]Unidade de Experimentação Animal, Grupo de Pesquisa e Pós-Graduação, Hospital de Clínicas de Porto Alegre, Porto Alegre/RS, Brazil.

CORRESPONDING AUTHOR

Iraci L. S. Torres (ORCID: 0000-0002-3081-115X)

Laboratory of Pain Pharmacology and Neuromodulation: Pre-clinical Research, Center of Experimental Research, Hospital de Clínicas de Porto Alegre - Porto Alegre/RS, 90035-007, Brazil.

Email: iltorres@hcpa.edu.br, Phone: 0055 51 3359-8937.

ACKNOWLEDGEMENTS

The authors thank the Biomedical Engineering group from the Hospital de Clínicas de Porto Alegre (HCPA) for the development of the tDCS equipment used in this study.

DECLARATIONS

FUNDING

This work was supported by the following Brazilian funding agencies: Brazilian Federal Agency for Support and Evaluation of Graduate Education—CAPES/MD-PhD (D.S. Santos, B.C. Lopes, L.S. Santos); National Council for Scientific and Technological

Development — CNPq (Dr. I.L.S. Torres, Dr. W. Caumo); Graduate Research Group of the Hospital de Clínicas de Porto Alegre – GPPG (I.L.S. Torres, Grant No.: 15-0501) MCT/FINEP – COENG/2013; and the Support Program for Emerging Nuclei PRONEM (Paulo Roberto Sanches, Grant No. 16/2551-0000249-5).

CONFLICT OF INTEREST

No conflict declared.

ETHICS APPROVAL

All experiments and procedures were approved by the Institutional Animal Care and Use Committee of the Hospital de Clínicas de Porto Alegre/HCPA (GPPG-HCPA protocol no. 15.0501). The experimental protocol complied with the ethical and methodological standards of the ARRIVE guidelines (Kilkenny *et al*., 2013).

ABSTRACT

Background: Neuromodulatory techniques have been studied to treat drug addiction or compulsive eating as well as different chronic pain conditions, such as neuropathic and inflammatory pain in the clinical and preclinical settings. In this study, we aimed to investigate the effect of transcranial direct current stimulation (tDCS) on the association of alcohol withdrawal with neuropathic pain based on nociceptive and neurochemical parameters in rats. **Methods**: Thirty-six adult male Wistar rats were randomized into five groups: control, neuropathic pain, neuropathic pain+tDCS, neuropathic pain+alcohol, and neuropathic pain+alcohol+tDCS. The neuropathic pain model was induced by chronic constriction injury (CCI) to the sciatic nerve. Rats were then exposed to alcohol (20%) by oral gavage administration for 15 days (beginning 24 h after CCI). tDCS was started on the 17th day after surgery and lasted for 8 consecutive days. The nociceptive test (hot plate) was performed at baseline, 16 days after CCI, and immediately and 24 h after the last session of tDCS. Rats were killed by decapitation, and structures were removed and frozen for biochemical analysis (nerve growth factor and interleukin (IL-1α, IL-1β, and IL-10 measurements). **Results**: Neuropathy-induced thermal hyperalgesia was reversed by tDCS, an effect that was delayed by alcohol abstinence. In addition, tDCS treatment induced modulation of central levels of IL-1α, IL-1ß, and IL-10 and neurotrophic growth factor. **Conclusion**: We cannot rule out that the antinociceptive effect of tDCS could be related to increased central levels of IL-1α and IL-10. Therefore, tDCS may be a promising non-pharmacological therapeutic approach for chronic pain treatment.

Keywords*:* tDCS, alcohol withdrawal, neuropathic pain, analgesia, rats

INTRODUCTION

Neuropathic pain (NP) is a relevant clinical problem since it is severely debilitating and largely resistant to treatment because its mechanisms are poorly understood. Similarly, alcohol abuse is an extremely serious public health problem because alcohol is the most widely used addictive substance worldwide [2]. Alcohol consumption is also a risk factor for many chronic diseases, with its effects dependent on volume, alcohol content, and frequency of use [2, 3]. Taken together, these conditions change neurotransmitter levels, leading to changes in widespread modulation of neuronal activity [4].

Chronic alcohol exposure or withdrawal may cause detrimental impairment of the nociceptive system. A systematic review and meta-analysis [5] reported an association between chronic pain and alcohol use, which can be related to dysfunction in the neuro-immuno-endocrine circuitry, leading to greater susceptibility to substance abuse, including alcohol abuse. It is related to dopaminergic imbalance in the mesocorticolimbic pathway [6] and changes in the reward system through dopaminergic signaling pathways between the ventral tegmental area, nucleus accumbens, and medial prefrontal cortex as observed in conditioned place preferences for pain in rats [7]. This modulation is similar to those reported in brain areas activated by alcohol-induced analgesic effects [8, 9]. This hypothesis is supported by reports showing that 25% of individuals who use alcohol aim to relieve some type of pain [10, 11, 12, 13]. . The use of alcohol induces analgesia in humans and animals due to changes in the central and peripheral nervous systems. However, these effects can lead to positive feedback loops and contribute to alcohol abuse

[14]. It is important to note that the previous preclinical study of the research group demonstrated that protracted alcohol withdrawal produced an analgesic effect indexed via an increased nociceptive threshold, which can be related to the increased central levels of BDNF and IL-10 [15]. On the other hand, some studies showed that alcohol withdrawal can trigger hyperalgesia as a component of withdrawal syndrome [16, 17]. The side effects of alcohol abstinence (hyperexcitability, anxiety, sleep disorders, and dysphoria, among others) also contribute to alcohol abuse as well as relapse [12, 18, 19]. In such instances, alcohol is consumed in increasing quantities to alleviate the motivational symptoms triggered by withdrawal [20, 21]. Paradoxically, hyperalgesic alcoholics respond better to the analgesic effects of alcohol than non-users, and this can be attributed to the belief that alcohol normalizes perceptions of pain and discomfort [22]. Additionally, a greater tendency toward familial alcoholism in the presence of chronic pain has been suggested [23].

It should also be stressed that both chronic pain and alcohol exposure/withdrawal lead to modifications in both central and peripheral neuroinflammatory patterns [2, 15, 24]. For example, previous studies have reported altered patterns of cytokines and neurotrophic factors following inflammatory and chronic pain injury models throughout the cortex-brainstem-spinal cord axis [25, 26]. Additionally, binge drinking and binge-like alcohol exposure induced the production of several cytokines, such as interleukins IL-10, IL-1α, and IL-1β, showing that both interventions can cause broad neuroimmune signaling throughout the peripheral and central nervous systems [2, 15, 27, 28]. Thus, it should be emphasized that an intermingled relationship between neuroimmunomodulatory and behavioral changes is involved in neuropathic pain and

symptoms of alcohol withdrawal, with new therapeutic approaches required to better understand and treat these conditions.

Non-invasive brain stimulation techniques (NIBS) have been used to treat different conditions such as inflammatory and neuropathic pain in clinical [29] and preclinical settings [26, 30]. These neuromodulatory techniques can reduce cravings in individuals with drug addiction [31] or compulsive eating [32, 33]. Transcranial direct current stimulation (tDCS) is notable in that it is considered a safe, low-cost, and an easily applied technique for modulating the neuronal membranes' resting potential using a weak electrical current applied through the scalp [34, 35 The analgesic effects of tDCS have also been demonstrated in clinical studies by reducing pain scores and the frequency of analgesic use in different chronic pain states [29, 36] probably due to its ability to modulate cortical and subcortical structures directly or indirectly involved in inhibitory descending pain control [29, 32, 37]. The neuromodulatory features of tDCS corroborate its use as a non-pharmacological approach modulating behavior and neuroimmune alterations induced by chronic pain and alcohol abstinence.

Thus, we aimed to investigate the effect of tDCS treatment on the association between neuropathic pain and alcohol withdrawal based on the nociceptive and neurochemical parameters of rats. Furthermore, we hypothesized that the use of tDCS as a neuromodulatory tool would lead to modification of pain thresholds accompanied by changes in central biomarker levels.

METHODS

Animals

Thirty-six male Wistar rats (weight 200–250 g) were randomized by weight and kept in groups of three or four animals per home cage (49 × 34 × 16 cm). Rats were maintained in a room under controlled temperature (22±2°C), on a standard 12 h light/dark cycle (lights on at 7 a.m.), with access to water and chow ad libitum during the whole experiment. All experiments and procedures were approved by the Institutional Animal Care and Use Committee (GPPG-HCPA protocol no. 20150501). The experimental protocol complied with the ethical and methodological standards of the ARRIVE guidelines [38].

Experimental Design

The rats were assigned into five groups: control (CT), neuropathic pain (NP), neuropathic pain plus transcranial direct current stimulation (NPtDCS), neuropathic pain plus alcohol (NPAL), and neuropathic pain plus alcohol plus tDCS (NPAL tDCS). During the establishment of NP (from 1 to 15th after the surgery procedure), the rats were given oral alcohol gavage. After that, the rats were subjected to a daily tDCS session for eight consecutive days. The nociceptive test (hot plate) was performed at baseline, 16 days after the CCI surgical procedure, immediately after the last session of tDCS, and 24 hours after the last session of tDCS. The rats were killed by decapitation 48 h post-treatment (Figure 1). For all procedures (nociceptive and biochemical assays), the experimenter was blinded to the group of rats being tested.

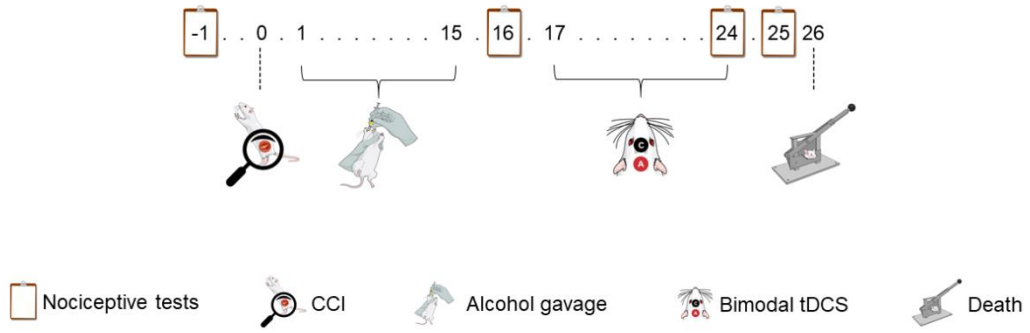

Fig 1 Experimental Design. CCI: Chronic Constriction Injury; tDCS: Transcranial Direct Current Stimulation.

Transcranial direct current stimulation

The rats were subjected to bimodal tDCS (0.5 mA) for 20 min per day for 8 days under immobilization from the 17th- to 24th-day post-CCI surgery [37, 39]. The cathode was positioned at the midpoint between the lateral angles of both eyes (supraorbital area) and the anode was placed on the head using landmarks of the neck and shoulder lines as a guide (the anterior and posterior regions in the midline between the two hemispheres of the parietal cortex, as described by Takano et al [40]. Adapted electroencephalogram electrodes (1.5 cm^2) with a conductive hydrogel were fixed to their heads with an adhesive tape to prevent removal and connected to a battery-driven stimulator to deliver a constant electrical current. The rats had their heads shaved for better adherence. To deliver the current, animals had to be immobilized using a soft cloth during stimulation. The stimulation was performed at the same time of day (11 a.m.) by the same researcher. This technique has been applied by our research group and has been found to show long-lasting effects and is able to mirror human tDCS protocols used in pain treatment [35, 41].

Neuropathic pain model: Chronic constriction injury (CCI) of the sciatic nerve

Chronic constriction injury (CCI) was induced as described by Bennett [42]. Briefly, each rat was anesthetized by isoflurane inhalation (5% for induction and 2.5% for maintenance). The common sciatic nerve was then exposed and freed from the adherent tissue at the mid-thigh by blunt dissection of the biceps femoris muscle. Three loose ligatures were placed 1 mm apart using a chromic gut suture vicryl 4.0. After the procedure, the wound was closed with non-absorbable mono nylon yarn 4.0. Rats undergoing surgery received intraperitoneal tramadol (5mg/kg) for pain relief immediately after surgery and once every 12 hours for 2 days after CCI induction [43].

Model of Exposure to Alcohol

For alcohol administration, the ethanol was diluted daily with distilled water to prepare a 20% v/v solution. It was then delivered by oral gavage in a volume of 4 g/kg body weight according to previous studies [15, 44]. Administrations were performed from the 1st until the 15th day after the surgical procedure, between 8 a.m. and 10 a.m. The rats were weighed every three days to allow for adjustment of the volume/weight administered.

Behavioral Tests

The behavior test (hot plate) was performed at baseline, 16 days post-surgery, as well as immediately and 24 hours after the last tDCS session.

Thermal Hyperalgesia (Hot Plate Test)

This test was carried out to determine changes in latency such as licking or jumping responses, which resulted from supraspinal sensory integration and indicate

modifications in the supraspinal process [45, 46, 47]. The rats were acclimated 24 h prior to the test for a period of 5 min inside the switched apparatus. During the test, the plate temperature was maintained at 55°C ± 0.1. The rats were placed in a transparent polypropylene funnel on the heated surface. The time between placing the animals and the beginning of paw withdrawal or "tapping" was recorded as response latency in seconds, with each being a single measurement in each evaluation period [48, 49].

Sample Collection

The rats were killed by decapitation 48 h after the last treatment session with tDCS, and the central nervous system structures (cerebral cortex and brainstem) were removed and frozen at −80°C for further analysis.

Biochemical Assays

Nerve growth factor (NGF), IL-1α, IL-1β, and IL-10 levels were determined by sandwich ELISA using monoclonal antibodies specific for each measurement (R&D Systems, Minneapolis, United States). Total protein was measured using the Bradford's method using bovine serum albumin as a standard [50] Results were expressed as pg/mg of protein.

Statistical Analysis

The behavioral tests were analyzed using generalized estimated equations (GEE) by Bonferroni. The biomarkers data were analyzed through a one-way ANOVA followed by a Student-Newman-Keuls test. P-values < 0.05 were considered statistically significant. SPSS 19.0 for Windows was used for statistical analyses.

RESULTS

Thermal Hyperalgesia

There was no difference between the groups at baseline (GEE: Wald $\chi 2 = 79.99$, $P = 0.60$). There was an interaction between group and time (GEE/Bonferroni; Wald $\chi 2 = 79.99$, $P = 0.001$). The neuropathic pain groups displayed thermal hyperalgesia 16 days after CCI surgery as indexed by a decrease in the nociceptive threshold (Figure 2), thus confirming the effectiveness of neuropathic pain induction. This effect was immediately reversed 24 h after the end of tDCS treatment. However, this analgesic tDCS effect was only observed 24 h post tDCS treatment in the neuropathic pain + alcohol + tDCS group (Figure 2).

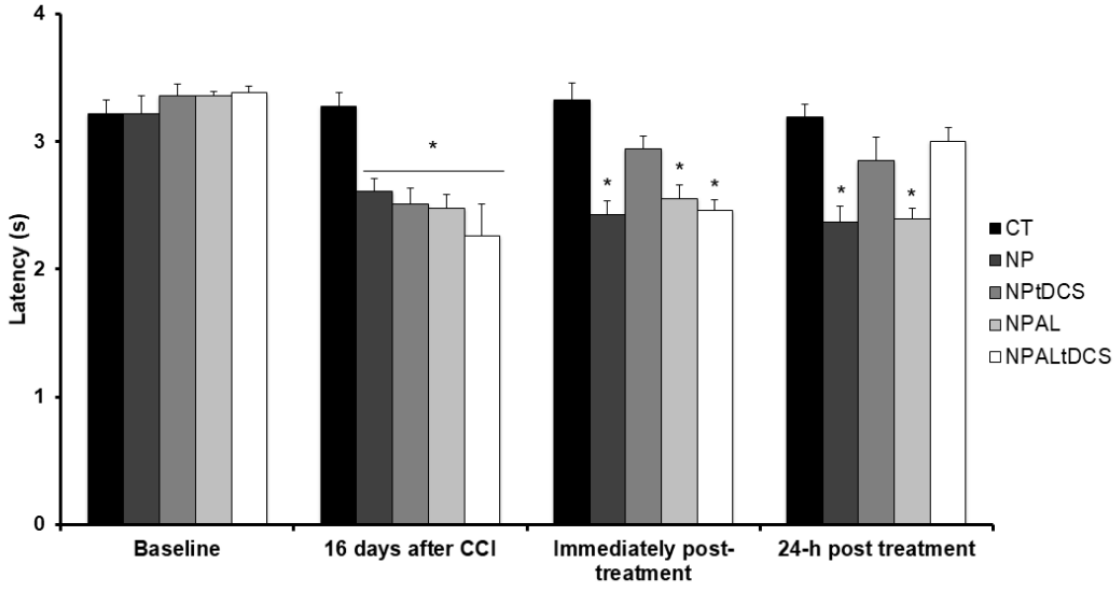

Fig 2 Thermal hyperalgesia assessed by hot plate test at baseline, 16 days after the CCI model, immediately, and 24 hours after bicephalic tDCS treatment (n=36). Data are presented as mean ±standard error of the mean (SEM) of paw withdrawal latency (s). Control-Group (CT), Neuropathic pain (NP), Neuropathic pain+tDCS (NPtDCS), Neuropathic pain+Alcohol (NPAL) and Neuropathic pain+Alcohol+tDCS (NPALtDCS).

*There was an interaction between group and time (GEE/Bonferroni, Wald $\chi 2 = 79.99$, P = 0.001).

Central NGF Levels

There was an increase in cerebral cortex NGF levels in the neuropathic pain + alcohol group, when compared to the control group. In addition, neuropathic pain + tDCS and neuropathic pain + alcohol + tDCS groups displayed increased NGF levels compared to the control and neuropathic pain groups (one-way ANOVA/SNK, $F_{(4,27)} = 7.876$, P = 0.001; Figure 3, Panel A). There were no differences among the groups in terms of NGF levels in the brainstem (one-way ANOVA, $F_{(4,27)} = 2.371$; P = 0.07, Figure 3, Panel B).

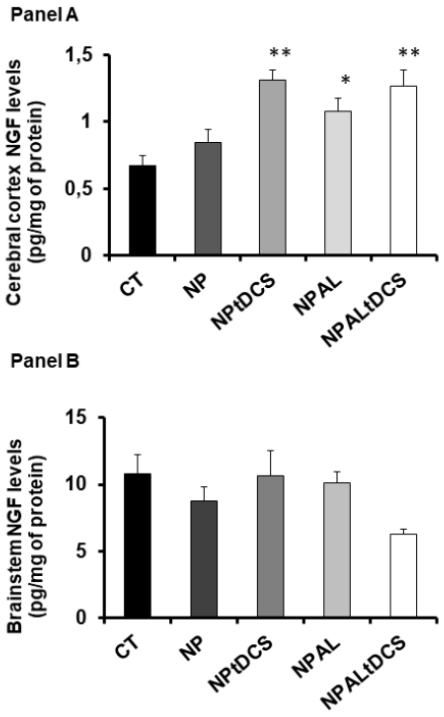

Fig 3 NGF levels in the cerebral cortex (Panel A) and brainstem (Panel B) of rats subjected to CCI and alcohol abstinence as well as tDCS treatment. Data are presented as mean ± standard error of the mean (SEM) of pg/mg of protein. Control-Group (CT),

Neuropathic pain (NP), Neuropathic pain+tDCS (NPtDCS), Neuropathic pain+Alcohol (NPAL) and Neuropathic pain+Alcohol+tDCS (NPALtDCS).

Panel A: There were significant differences among groups in terms of cerebral cortex NGF levels (one-way ANOVA/SNK, P = 0.001).* - significant difference from CT group, and ** - significant difference from CT and NP group Panel B: There were no differences among the groups in terms of brainstem NGF levels (one-way ANOVA, $F_{(4,27)}$ = 2.371; P = 0.07).

Central IL-1α Levels

There was an increase in IL-1α levels in the cerebral cortex in the neuropathic pain + tDCS and neuropathic pain + alcohol + tDCS groups compared to the other groups (one-way ANOVA/SNK, $F_{(4,27)}$ = 5.364, P = 0.003; Figure 4, Panel A). There were no differences among groups in terms of IL-1α levels in the brainstem (one-way ANOVA, $F_{(4,27)}$ = 1.035, P = 0.40; Figure 4, Panel B).

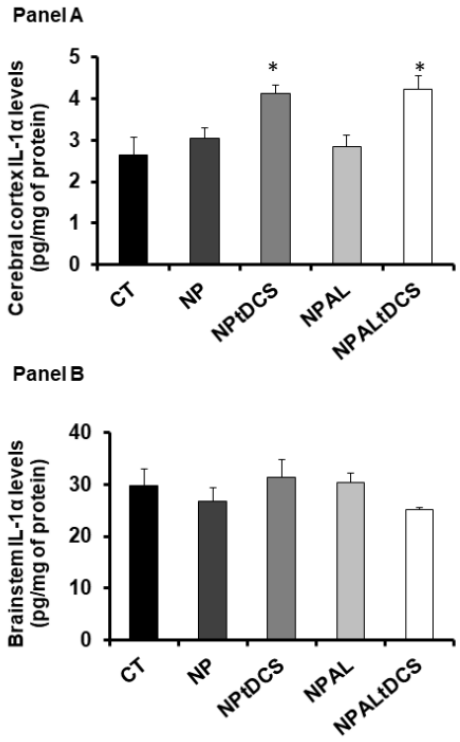

Fig 4 IL-1α levels in the cerebral cortex (Panel A) and brainstem (Panel B) of rats subjected to CCI and alcohol abstinence as well as tDCS treatment. Data are presented as mean ± standard error of the mean (SEM) of pg/mg of protein. Control-Group (CT), Neuropathic pain (NP), Neuropathic pain+tDCS (NPtDCS), Neuropathic pain+Alcohol (NPAL) and Neuropathic pain+Alcohol+tDCS (NPALtDCS).

Panel A: There was a significant difference in IL-1α levels in the cerebral cortex between groups (one-way ANOVA/SNK, $F_{(4,27)} = 5.364$, $P = 0.003$)

* Significant difference from CT, NP, and NPAL groups

Panel B: There was no difference among the groups in terms of IL-1α levels in the brainstem (one-way ANOVA, $F_{(4,27)} = 1.035$, $P = 0.40$).

Central IL-1β Levels

There was increase in IL-1β levels in the cerebral cortex in the neuropathic pain + tDCS and neuropathic pain + alcohol + tDCS groups compared to the control group

(one-way ANOVA/SNK, $F_{(4,27)} = 3.391$, P = 0.02; Figure 5, Panel A). There were no differences among groups in terms of IL-1β levels in the brainstem (one-way ANOVA, $F_{(4,27)} = 1.776$, P = 0.16; Panel B).

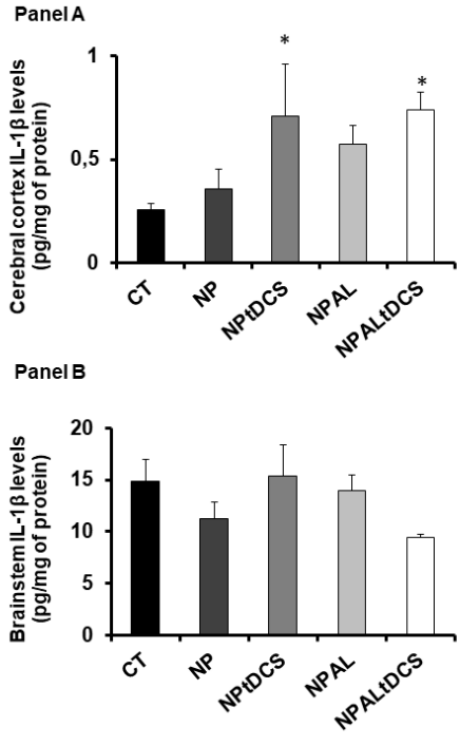

Fig 5 IL-1β levels in the cerebral cortex (Panel A) and brainstem (Panel B) of rats subjected to CCI and alcohol abstinence as well as tDCS treatment. Data are presented as mean ± standard error of the mean (SEM) of pg/mg of protein. Control-Group (CT), Neuropathic pain (NP), Neuropathic pain+tDCS (NPtDCS), Neuropathic pain+Alcohol (NPAL) and Neuropathic pain+Alcohol+tDCS (NPALtDCS).

Panel A: There were differences among the groups in terms of IL-1β levels in the cerebral cortex (one-way ANOVA/SNK, P = 0.02).

* Statistically significant difference from the CT group.

Panel B: There were no differences among the groups in terms of IL-1β levels in the brainstem (one-way ANOVA, $F_{(4,27)} = 1.776$, P = 0.16).

Central IL-10 Levels

There were no differences among the groups in terms of IL-10 levels in the cerebral cortex (one-way ANOVA, $F_{(4,27)} = 2.335$, P = 0.08; Figure 6, Panel A). In the brainstem, there was an increase in IL-10 levels in the neuropathic pain + tDCS group compared to other groups (one-way ANOVA/SNK, $F_{(4,27)} = 5.686$, P = 0.002; Figure 6, Panel B).

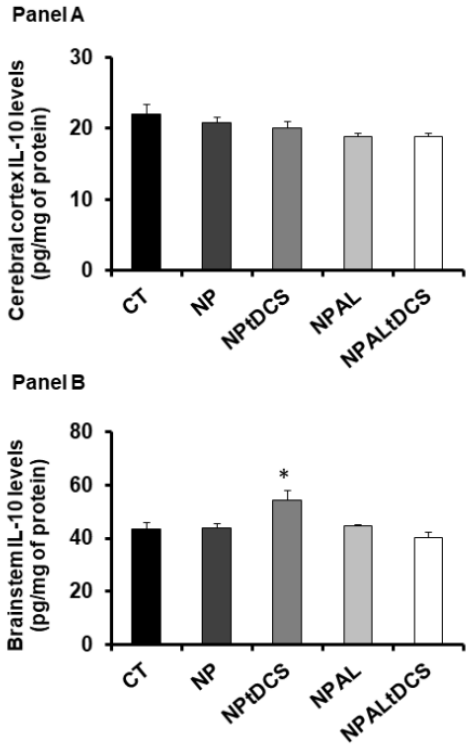

Fig 6 IL-10 levels in the cerebral cortex (Panel A) and brainstem (Panel B) of rats subjected to CCI and alcohol abstinence as well as tDCS treatment. Data are presented as mean ± standard error of the mean (SEM) of pg/mg of protein. Control-Group (CT), Neuropathic pain (NP), Neuropathic pain+tDCS (NPtDCS), Neuropathic pain+Alcohol (NPAL) and Neuropathic pain+Alcohol+tDCS (NPALtDCS).

Panel A: There were no differences among the groups in terms of IL-10 levels in the cerebral cortex (one-way ANOVA, $F_{(4,27)} = 2.335$, $P = 0.08$).

Panel B: There was a significant difference in IL-10 levels in the neuropathic pain + tDCS group compared to other groups (one-way ANOVA/SNK, $F_{(4,27)} = 5.686$, $P = 0.002$).

* Significant difference from CT, NP, NPAL, and NPALtDCS groups

DISCUSSION

The present study showed that bimodal tDCS induced short- and long-term antinociceptive effects in rats with neuropathic pain. However, when alcohol abstinence was associated with CCI, only long-term effects were observed. These results corroborate our previous research which showed that tDCS may have analgesic effects in different chronic pain models [26, 37, 39]. Besides, tDCS treatment induced modulation of central levels of interleukins (IL-1α, IL-1ß, and IL-10). Cerebral cortex NGF levels were increased by tDCS treatment and alcohol withdrawal, with the effect of the latter intensified when simultaneously administered with the former.

This study interestingly found that tDCS-induced analgesia was delayed by alcohol withdrawal. It is well known that alcohol consumption induces depressant effects in the central nervous system, modulates pain thresholds, and displays analgesic and anti-inflammatory effects [51, 52]. As such, its withdrawal associated with chronic pain drives an imbalance between excitatory/inhibitory neurotransmission in different cortical and subcortical brain regions, such as the medial prefrontal cortex, nucleus accumbens, and amygdala, which are connected to important brain regions related to nociception/analgesia, including the periaqueductal gray and rostral ventromedial medulla. The delayed tDCS-induced analgesia may be due to its neuromodulatory role, after-effects, as well as its non-specific modes of action [53]. Alcohol is a psychoactive substance that acts on multiple important neurotransmitter systems, including the GABAergic, glutamatergic, serotonergic, and opioidergic systems [54-58]. Indeed, $GABA_A$ and NMDA receptors participate in alcohol-induced analgesia and alcohol withdrawal-induced hyperalgesia/hyperexcitability [59-64]. In this way, we can suggest

that the imbalance between these neurotransmitter systems could be involved in the delayed response observed in tDCS-induced analgesia.

Despite a previous study showing an increase in the thermal nociceptive threshold induced by alcohol withdrawal in rats not experiencing pain as evaluated by the tail-flick latency test [15], effects of alcohol withdrawal effect upon thermal hyperalgesia were not observed in the current study. This difference can be interpreted in two ways: a) different status of the animals, with a group experiencing pain and the other not experiencing pain; and b) differences in evaluated behavior, nociceptive thresholds, and the degree of hyperalgesia. While the nociceptive threshold is the latency of response to the nociception stimulus, hyperalgesia is an abnormal increase in the sensitivity to nociceptive stimuli, including different activation of fibers and supraspinal responses triggered by each test [65]. In addition, our data are in agreement with those of previous studies, which suggested that alcohol withdrawal exacerbates the symptoms of mechanical hyperalgesia without affecting the thermal response [66]. In contrast, previous studies have shown mechanical and thermal hypersensitivity in Sprague-Dawley rats subjected to alcohol withdrawal [67, 68]. On the other hand, studies have suggested pain relief induced by alcohol, but the mechanisms of action as well as the variables involved are still unclear [69-72]. It is important to note that the inconsistencies found in the literature may be related to the protocol for alcohol use, withdrawal times, as well as the strains and baseline status of the animals. Currently, it is believed that neurotransmitter and inflammatory systems are common pathways involved in the pathologies of both chronic pain [24, 37] and alcohol withdrawal [15, 73]. In this study, we showed that tDCS improved thermal hyperalgesia and modulated central biomarker levels, corroborating previous studies from our research group [24, 30, 32, 36, 37]. tDCS treatment increased IL-1α in the cerebral

cortex in rats with chronic pain and alcohol abstinence. Previous studies have shown that IL-1α has antiallodynic and antihyperalgesic effects in a rat neuropathic pain model [74]. Thus, it is likely that an increase in central IL-1α levels might be the mechanism underlying the pain relief induced by tDCS treatment.

It is important to note that the increased brainstem IL-10 levels found in the current study in the neuropathic pain group may have also contributed to the tDCS-induced antinociceptive effect once it takes effect on key centers for pain modulation [25, 37, 75]. In contrast, alcohol abstinence attenuated the effects of tDCS on IL-10 levels without leading to changes in long-lasting tDCS antinociceptive effects. On the other hand, a previous study using a different alcohol protocol showed an increase of IL-10 levels in the hippocampus, prefrontal cortex, and brainstem in rats after alcohol abstinence [15]. Altogether, these findings highlight an important interaction between the immune system and alcohol exposure/withdrawal.

It is interesting to note that IL-1α and IL-1ß act on the same receptor to differentially influence nociceptive transmission and neuropathic pain responses [74, 76, 77]. As such, neurochemical measures were performed 48 h after the end of tDCS treatment or 26 days after CCI Model induction in the current study. This corroborates our previous findings that the levels of substances associated with neuropathic pain did not change when measured at the same time point. However, 29 days after CCI, neuropathic pain rats showed an increase in IL-1ß levels in the cerebral cortex. [37]. On the other hand, the tDCS group showed an increased level of IL-1 ß in rats with neuropathic pain independent of alcohol abstinence. Previous studies have shown that intrathecal IL-1ß administration in normal and inflamed rats led to different effects [78] without changing the latencies of paw withdrawal in normal rats while producing

significant antinociception when administered intrathecally in rats with peripheral inflammation (carrageenan model). Considering the dual effect of IL-1ß, we cannot disregard the involvement of this interleukin in the observed antinociceptive effect in the current study. Analysis of the results of interleukin modulation should thus be related to the neuroimmunomodulatory effects of tCDS.

NGF mediates neuronal activity as well as the synaptic plasticity of neurons [79]. In the current study, tDCS was able to cause analgesia in rats with neuropathic pain, with this effect linked to elevated levels of NGF in the cerebral cortex. In addition, we found that alcohol withdrawal also increased NGF levels in the cerebral cortex of rats with neuropathic pain, with the alcohol withdrawal effect intensified when associated with tDCS. A previous study showed that chronic exposure to ethanol decreased NGF levels but that this effect was time- and site-dependent, with effects varying depending on the length of alcohol exposure and structures analyzed [80]. Besides, chronic consumption of high amounts of alcohol in rats leads to a transient increase in NGF levels in distinct brain regions [81]. We also highlight that the changes in NGF levels observed in the study may have been influenced by the length of alcohol exposure or withdrawal. tDCS also triggers a central neuromodulatory effect once it modulates NGF levels independent of the alcohol withdrawal effect.

CONCLUSION

The rationale of the current study was that pain from chronic conditions can be relieved by alcohol consumption. However, this substance is highly addictive for humans and animals. In this context, it is important to understand the central effects induced by alcohol exposure or withdrawal. In the same line, tDCS as a central neuromodulatory

technique may benefit patients suffering both from alcohol abuse and chronic neuropathic pain. Besides, alcohol abuse and neuropathic pain treatments are oftentimes refractive to pharmacological treatment. Thus, tDCS may be a promising non-pharmacological therapeutic approach for both chronic conditions. This study showed that bimodal tDCS was able to effectively induce analgesia in rats with neuropathic pain, which was delayed by alcohol abstinence. We suppose that the analgesic effect of tDCS might be related to increased central levels of IL-1α, IL-10, and NGF, since its antinociceptive role has been well described in key pain pathways likely due to its capacity to neuromodulate immune signaling. Concerning alcohol exposure/withdrawal, the increase in central NGF levels suggests that alcohol-induced neuroplasticity might contribute to this dependence taking into consideration its broad interference upon biological processes. Overall, further research is needed to improve and broaden existing knowledge regarding tDCS and the effects of alcohol on pain.

REFERENCES

1) Cohen, S. P., Mao, J., 2014. Neuropathic pain: mechanisms and their clinical implications. *BMJ*. https://doi.org/10.1136/bmj.f7656

2) Chang, S. L., Huang, W., Han, H., Sariyer, I. K., 2019. Binge-like exposure to ethanol enhances morphine's anti-nociception in B6 mice. *Frontiers in Psychiatry*. https://doi.org/10.3389/fpsyt.2018.00756

3) Shield, K. D., Parry, C., Rehm, J., 2013. Chronic diseases and conditions related to alcohol use. *Alcohol Research : Current Reviews*.

4) Yamada, A., Koga, K., Kume, K., Ohsawa, M., Furue, H., 2018. Ethanol-induced enhancement of inhibitory synaptic transmission in the rat spinal substantia gelatinosa. *Molecular Pain*. https://doi.org/10.1177/1744806918817969

5) Thompson, T., Oram C., Correll, C.U., Tsermentseli S., Stubbs, B., 2017. Analgesic Effects of Alcohol: A Systematic Review and Meta-Analysis of Controlled Experimental Studies in Healthy Participants. *The Journal of Pain*. https://doi.org/10.1016/j.jpain.2016.11.009

6) Wilens, T. E., 2004. Attention-deficit/hyperactivity disorder and the substance use disorders: the nature of the relationship, subtypes at risk, and treatment issues. *The Psychiatric Clinics of North America*. https://doi.org/10.1016/S0193-953X(03)00113-8

7) Natraviola, E., Xie, J. Y., Okun, A., Qu, C., Eyde, N., Ci, S., Ossipov, M. H., King, T., Fields, H. L., Porreca, F., 2012. Pain relief produces negative reinforcement through activation of mesolimbic reward–valuation circuitry. *Proceedings of the National Academy of Sciences*. https://doi.org/10.1073/pnas.1214605109

8) Yu, W., Hwa, L. S., Makhijani, V. H., Besheer, J., Kash, T. L., 2019. Chronic inflammatory pain drives alcohol drinking in a sex-dependent manner for C57BL/6J mice. *Alcohol*. https://doi.org/10.1016/j.alcohol.2018.10.002

9) Kerns R. T., Ravindranathan A., Hassan S., Cage M. P., York T., Sikela J. M., Williams R. W., Miles M. F., 2005. Ethanol-responsive brain region expression networks: implications for behavioral responses to acute ethanol in DBA/2J versus C57BL/6J mice. *The Journal of Neuroscience*. https://doi.org/10.1523/JNEUROSCI.4372-04.2005

10) Gatch, M. B., 2009. Ethanol withdrawal and hyperalgesia. *Current Drug Abuse Reviews*. https://doi.org/10.2174/1874473710902010041

11) Jochum, T., Boettger, M. K., Burkhardt, C., Juckel, G., Bär, K., 2012. Increased pain sensitivity in alcohol withdrawal syndrome. *European Journal of Pain*. https://doi.org/10.1016/j.ejpain.2009.11.008

12) Riley, J. L., King, C., 2009. Self-report of alcohol use for pain in a multi-ethnic community sample. *The Journal of Pain*. https://doi.org/10.1016/j.jpain.2009.03.005

13) Brennan, P. L., Schutte, K. K., Moos, R. H., 2005. Pain and use of alcohol to manage pain: prevalence and 3-year outcomes among older problem and non-problem drinkers. *Addiction*. https://doi.org/10.1111/j.1360-0443.2005.01074.x

14) Mellion, M., Gilchrist, J. M., De La Monte, S., 2011. Alcohol-related peripheral neuropathy: Nutritional, toxic, or both? *Muscle & Nerve*. https://doi.org/10.1002/mus.21946

15) Schunck, R. V. A., Torres, I. L. S., Laste, G., De Souza, A., Macedo, I. C., Valle, M. T. C., Leal, M. B., 2015. Protracted alcohol abstinence induces analgesia in rats: Possible relationships with BDNF and interleukin-10. *Pharmacology Biochemistry and Behavior*. https://doi.org/10.1016/j.pbb.2015.05.011

16) Egli, M., Koob, G. F., Edwards, S., 2012. Alcohol dependence as a chronic pain disorder. *Neuroscience and Biobehavioral Reviews*. https://doi.org/10.1016/j.neubiorev.2012.07.010

17) Li, J., Fu, C., Liu, H., Fu, R., Zuo, W., Kang, S., Chen, P., Gregor, D., Paulose, R., Bekker, A., Ye, J., 2017. Electroacupuncture Attenuates Hyperalgesia in Rats Withdrawn from Chronic Alcohol Drinking via Habenular Mu Opioid Receptors. *Alcoholism: Clinical and Experimental Research*. https://doi.org/10.1111/acer.13332

18) Castillo, R. C., MacKenzie, E. J., Wegener, S.T., Bosse, M.J., Group, L. S., 2006. Prevalence of chronic pain seven years following limb threatening lower extremity trauma. *Pain*. https://doi.org/10.1016/j.pain.2006.04.020

19) Holmes, A., Williamson, O., Hogg, M., Arnold, C., Prosser, A., Clements, J., O'Donnell, M., 2010. Predictors of pain severity 3 months after serious injury. *Pain Medicine*. https://doi.org/10.1111/j.1526-4637.2010.00890.x

20) Gilpin, N. W., Koob, G. F., 2008. Neurobiology of alcohol dependence: focus on motivational mechanisms. Alcohol Research: Current Reviews. *Alcohol Research & Health : The Journal of the National Institute on Alcohol Abuse and Alcoholism*. PMID: PMC2770186

21) Koob, G. F., 2003. Alcoholism: allostasis and beyond. *Alcoholism, Clinical and Experimental Research*. https://doi.org/10.1097/01.alc.0000057122.36127.c2

22) Stewart, S., Finn, P. R., Pihl, R. O., 1995. A dose–response study of the effects of alcohol on the perception of pain and discomfort due to electric shock in men at high familial-genetic risk for alcoholism. *Psychopharmacology*. https://doi.org/10.1007/bf02246289

23) Katon, W., Egan, K., Miler, D., 1985. Chronic pain: lifetime psychiatric diagnoses and family history. *The American Journal of Psychiatry*. https://doi.org/10.1176/ajp.142.10.1156

24) Filho, P. R. M., Vercelino, R., Cioato, S. G., Medeiros, L. F., de Oliveira, C., Scarabelot, V. L., Torres, I. L. S., 2016. Transcranial direct current stimulation (tDCS) reverts behavioral alterations and brainstem BDNF level increase induced by neuropathic pain model: Long-lasting effect. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. https://doi.org/10.1016/j.pnpbp.2015.06.016

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

26) Laste, G., Caumo, W., Adachi, L. N. S., Rozisky, J. R., De Macedo, I. C., Filho, P. R. M., Torres, I. L. S., 2012. After-effects of consecutive sessions of transcranial direct current stimulation (tDCS) in a rat model of chronic inflammation. *Experimental Brain Research*. https://doi.org/10.1007/s00221-012-3149-x

27) Lehnert, M., Kovacs, E. J., Molina, P. E., Relja, B., 2014. Modulation of inflammation by alcohol exposure. *Mediators of Inflammation*. https://doi.org/10.1155/2014/283756

28) Molina, P. E., Happel, K. I., Zhang, P., Kolls, J. K., Nelson, S., 2010. Focus on: Alcohol and the immune system. *Alcohol Research & Health : The Journal of the National Institute on Alcohol Abuse and Alcoholism*. PMID: 23579940

29) Brietzke, A. P., Zortea, M., Carvalho, F., Sanches, P. R. S., Silva, J. D. P., Torres, I. L. da S., Caumo, W, 2019. Large treatment effect with extended home-based transcranial

direct current stimulation over dorsolateral prefrontal cortex in fibromyalgia: A Proof of Concept Sham-Randomized Clinical Study. *The Journal of Pain*. https://doi.org/10.1016/j.jpain.2019.06.013

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

31) Nakamura-Palacios, E. M., Lopes, I. B. C., Souza, R. A., Klauss, J., Batista, E. K., Conti, C. L., de Souza, R. S. M.. 2016. Ventral medial prefrontal cortex (vmPFC) as a target of the dorsolateral prefrontal modulation by transcranial direct current stimulation (tDCS) in drug addiction. *Journal of Neural Transmission*. https://doi.org/10.1007/s00702-016-1559-9

32) de Oliveira, C., de Freitas, J. S., Macedo, I. C., Scarabelot, V. L., Ströher, R., Santos, D. S., Torres, I. L. S., 2019. Transcranial direct current stimulation (tDCS) modulates biometric and inflammatory parameters and anxiety-like behavior in obese rats. *Neuropeptides*. https://doi.org/10.1016/j.npep.2018.09.006

33) Song, S., Zilverstand, A., Gui, W., Li, H. Jie, Zhou, X., 2019. Effects of single-session versus multi-session non-invasive brain stimulation on craving and consumption in individuals with drug addiction, eating disorders or obesity: A meta-analysis. *Brain Stimulation*. https://doi.org/10.1016/j.brs.2018.12.975

34) Lefaucheur, J. P., Antal, A., Ayache, S. S., Benninger, D. H., Brunelin, J., Cogiamanian, F., Paulus, W., 2017. Evidence-based guidelines on the therapeutic use of transcranial direct current stimulation (tDCS). *Clinical Neurophysiology*, Vol. 128, pp. 56–92. https://doi.org/10.1016/j.clinph.2016.10.087

35) Nitsche, M. A., Cohen, L. G., Wassermann, E. M., Priori, A., Lang, N., Antal, A., Pascual-Leone, A., 2008a. Transcranial direct current stimulation: State of the art 2008. *Brain Stimulation*. https://doi.org/10.1016/j.brs.2008.06.004

36) da Graca-Tarragó, M., Lech, M., Angoleri, L. D. M., Santos, D. S., Deitos, A., Brietzke, A. P., Caumo, W., 2019. Intramuscular electrical stimulus potentiates motor cortex modulation effects on pain and descending inhibitory systems in knee osteoarthritis: A randomized, factorial, sham-controlled study. *Journal of Pain Research*. https://doi.org/10.2147/JPR.S181019

37) Cioato, S. G., Medeiros, L. F., Filho, P. R. M., Vercelino, R., De Souza, A., Scarabelot, V. L., Torres, I. L. S., 2016. Long-Lasting Effect of Transcranial Direct Current Stimulation in the Reversal of Hyperalgesia and Cytokine Alterations Induced by the Neuropathic Pain Model. *Brain Stimulation*. https://doi.org/10.1016/j.brs.2015.12.001

38) Kilkenny, C., Browne, W. J., Cuthill, I. C., Emerson, M., Altman, D. G., 2013. Improving bioscience research reporting: The arrive guidelines for reporting animal research. *Animals*. https://doi.org/10.3390/ani4010035

39) Spezia Adachi, L. N., Caumo, W., Laste, G., Fernandes Medeiros, L., Ripoll Rozisky, J., De Souza, A., Torres, I. L. S., 2012. Reversal of chronic stress-induced pain by transcranial direct current stimulation (tDCS) in an animal model. *Brain Research*. https://doi.org/10.1016/j.brainres.2012.10.009

40) Takano, Y., Yokawa, T., Masuda, A., Niimi, J., Tanaka, S., Hironaka, N., 2011. A rat model for measuring the effectiveness of transcranial direct current stimulation using fMRI. *Neuroscience Letters*. https://doi.org/10.1016/j.neulet.2011.01.004

41) Boggio, P. S., Sultani, N., Fecteau, S., Merabet, L., Mecca, T., Pascual-Leone, A., Fregni, F., 2008. Prefrontal cortex modulation using transcranial DC stimulation reduces alcohol craving: A double-blind, sham-controlled study. *Drug and Alcohol Dependence*. https://doi.org/10.1016/j.drugalcdep.2007.06.011

42) Bennett, G. J., Xie, Y. K., 1988. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain*. https://doi.org/10.1016/0304-3959(88)90209-6

43) Guzman-silva, M. A., Pollastri, C. E., Augusto, J., Pantaleão, S., Carolina, A., Carvalho, B. De, Boaventura, G. T., 2008. Tramadol minimizes potential pain during post-oophorectomy in Wistar rats. *Aatex*, (14), 91–92.

44) Overstreet, D. H., Knapp, D. J., Breese, G. R., 2002. Accentuated decrease in social interaction in rats subjected to repeated ethanol withdrawals. *Alcoholism, Clinical and Experimental Research*. https://doi.org/10.1097/01.ALC.0000023983.10615.D7

45) Caggiula, A. R., Perkins, K. A., Saylor, S., Epstein, L. H., 1995. Different methods of assessing nicotine-induced antinociception may engage different neural mechanisms. *Psychopharmacology*. https://doi.org/10.1007/BF02246552

46) Ossipov, M. H., Kovelowski, C. J., Nichols, M. L., Hruby, V. J., Porreca, F., 1995. Characterization of supraspinal antinociceptive actions of opiod delta agonists in the rat. *Pain*. https://doi.org/10.1016/0304-3959(94)00231-3

47) Rubinstein, M., Mogil, J. S., Japon, M., Chan, E. C., Allen, R. G., Low, M. J., 2002. Absence of opioid stress-induced analgesia in mice lacking beta-endorphin by site-directed mutagenesis. *Proceedings of the National Academy of Sciences*. https://doi.org/10.1073/pnas.93.9.3995

48) Spindola, H. M., Servat, L., Denny, C., Rodrigues, R. A. F., Eberlin, M. N., Cabral, E., Foglio, M. A., 2010. Antinociceptive effect of geranylgeraniol and 6alpha,7beta-dihydroxyvouacapan-17beta-oate methyl ester isolated from Pterodon pubescens Benth. *BMC Pharmacology*. https://doi.org/10.1186/1471-2210-10-1

49) Woolfe, G., Macdonald, A., 1944. The Evaluation of the Analgesic Action of Pethidine Hydrochloride (Demerol*). Journal of Pharmacology and Experimental Therapeutics*.

50) Bradford, M. M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein dye binding. *Analytical Biochemistry*. https://doi.org/10.1016/0003-2697(76)90527-3

51) Emamuzo, E. D., Miniakiri, S. I., Tedwin, E. J. O., Ufouma, O., Lucky, M., 2010. Analgesic and anti—inflammatory activities of the ethanol extract of the leaves of Helianthus Annus in Wistar rats. *Asian Pacific Journal of Tropical Medicine*. https://doi.org/10.1016/S1995-7645(10)60083-1

52) Gatch, M. B., Lal, H., 1999a. Effects of ethanol and ethanol withdrawal on nociception in rats. *Alcoholism: Clinical and Experimental Research*. https://doi.org/10.1111/j.1530-0277.1999.tb04118.x

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

54) Davies, M., 2003. The role of GABAA receptors in mediating the effects of alcohol in the central nervous system. *Journal of Psychiatry & Neuroscience*. PMID: 12921221

55) Rao, P. S. S., Bell, R. L., Engleman, E. A., Sari, Y., 2015. Targeting glutamate uptake to treat alcohol use disorders. *Frontiers in Neuroscience*. doi: 10.3389/fnins.2015.00144

56) LeMarquand, D., Pihl, R. O., Benkelfat, C., 1994. Serotonin and alcohol intake, abuse, and dependence: Clinical evidence. *Biological Psychiatry*. https://doi.org/10.1016/0006-3223(94)90630-0

57) McBride, W. J., Murphy, J. M., Yoshimoto, K., Lumeng, L., Li, T., 1993. Serotonin Mechanisms in Alcohol Drinking Behavior. *Drug Development Research*. https://doi.org/10.1002/ddr.430300309

58) Font, L., Luján, M. Á., Pastor, R., 2013. Involvement of the endogenous opioid system in the psychopharmacological actions of ethanol: the role of acetaldehyde. *Frontiers in Behavioral Neuroscience*. https://doi.org/10.3389/fnbeh.2013.00093

59) Gatch, M. B., 1999b. Effects of benzodiazepines on acute and chronic ethanol-induced nociception in rats. *Alcoholism: Clinical and Experimental Research*. https://doi.org/10.1111/j.1530-0277.1999.tb04068.x

60) Mogil, J. S., Marek, P., Yirmiya, R., Balian, H., Sadowski, B., Taylor, A. N., Liebeskind, J. C., 1993. Antagonism of the non-opioid component of ethanol-induced analgesia by the NMDA receptor antagonist MK-801. *Brain Research*. https://doi.org/10.1016/0006-8993(93)90251-h

61) Khanna, J. M., Mayer, J. M., Lê, D. A., Kalant, H., 1984. Differential response to ethanol, pentobarbital and morphine in mice selectively bred for ethanol sensitivity. *Alcohol*. https://doi.org/10.1016/0741-8329(84)90029-6

62) Newman, L. M., Curran, M. A., Becker, G. L., 1986. Effects of chronic alcohol intake on anesthetic responses to diazepam and thiopental in rats. *Anesthesiology*. https://doi.org/10.1097/00000542-198608000-00012

63) Lovinger, D. M., White, G., Weight, F. F., 1989. Ethanol inhibits NMDA-activated ion current in hippocampal neurons. *Science*. https://doi.org/10.1126/science.2467382

64) Hoffman, P. L., Rabe, C. S., Moses, F., Tabakoff, B., 1989. N-methyl-D-aspartate receptors and ethanol: inhibition of calcium flux and cyclic GMP production. *Journal of Neurochemistry*. https://doi.org/10.1111/j.1471-4159.1989.tb07280.x

65) Le Bars, D., Gozariu, M., Cadden, W., 2001. Animal Models of Nociception. *Pharmacological Reviews*. PMID: 11734620

66) Shumilla, J. A., Liron, T., Mochly-Rosen, D., Kendig, J. J., Sweitzer, S. M., 2005. Ethanol withdrawal-associated allodynia and hyperalgesia: age-dependent regulation by protein kinase C epsilon and gamma isoenzymes. *The Journal of Pain*. https://doi.org/10.1016/j.jpain.2005.03.005

67) Fu, R., Gregor, D., Peng, Z., Li, J., Bekker, A., Ye, J. H., 2015. Chronic intermittent voluntary alcohol drinking induces hyperalgesia in Sprague-Dawley rats. *International Journal of Physiology, Pathophysiology and Pharmacology*. PMID: 26823962

68) Li, J., Chen, P., Han, X., Zuo, W., Mei, Q., Bian, E. Y., Umeugo, J., Ye, J., 2019. Differences between male and female rats in alcohol drinking, negative affects and neuronal activity after acute and prolonged abstinence. *International Journal of Physiology, Pathophysiology and Pharmacology*. PMID: 31523363

69) Duarte, R., McNeill, A., Drummond, G., Tiplady, B., 2008. Comparison of the sedative, cognitive, and analgesic effects of nitrous oxide, sevoflurane, and ethanol. *British Journal of Anaesthesia*. https://doi.org/10.1093/bja/aem369

70) Perrino Jr, A. C., Ralevski, E., Acampora, G., Edgecombe, J., Limoncelli, D., Petrakis, I. L., 2008. Ethanol and pain sensitivity: effects in healthy subjects using an

acute pain paradigm. *Alcoholism, Clinical and Experimental Research*. https://doi.org/10.1111/j.1530-0277.2008.00653.x

71) Saddler, J. M., James, M. F., Harington, A. P., 1985. Naloxone does not reverse ethanol analgesia in man. *Clinical and Experimental Pharmacology & Physiology*. https://doi.org/10.1111/j.1440-1681.1985.tb00883.x

72) Horn-Hofmann, C., Buscher, P., Lautenbacher, S., Wolstein, J., 2015. The effect of nonrecurring alcohol administration on pain perception in humans: a systematic review. *Journal of Pain Research*, 8, 175–187. https://doi.org/10.2147/JPR.S79618

73) Chastain, G., 2006. Alcohol, neurotransmitter systems, and behavior. *Journal of General Psychology*. https://doi.org/10.3200/GENP.133.4.329-335

74) Mika, J., Korostynski, M., Kaminska, D., Wawrzczak-Bargiela, A., Osikowicz, M., Makuch, W., Przewlocka, B., 2008. Interleukin-1alpha has antiallodynic and antihyperalgesic activities in a rat neuropathic pain model. *Pain*. https://doi.org/10.1016/j.pain.2008.02.015

75) Dai, W., Sun, J., Li, C., Mao, W., Huang, Y., Zhao, Z., Zhang, Y., Lü, N., 2019. Involvement of Interleukin-10 in Analgesia of Electroacupuncture on Incision Pain. *Evidence-Based Complementary and Alternative Medicine*. https://doi.org/10.1155/2019/8413576

76) Stemkowski, P. L., Garcia-Caballero, A., Gadotti, V. M., M'Dahoma, S., Chen, L., Souza, I. A., Zamponi, G. W., 2017. Identification of interleukin-1 beta as a key mediator in the upregulation of Cav3.2–USP5 interactions in the pain pathway. *Molecular Pain*. https://dx.doi.org/10.1177%2F1744806917724698

77) Ren, K., Torres, R., 2009. Role of interleukin-1β during pain and inflammation. *Brain Research Reviews*. https://dx.doi.org/10.1016%2Fj.brainresrev.2008.12.020

78) Souter, A. J., Garry, M. G., 2000. Spinal interleukin-1β reduces inflammatory pain. Pain. https://doi.org/10.1016/S0304-3959(99)00315-2

79) Conner, J. M., Franks, K. M., Titterness, A. K., Russell, K., Merrill, D. A., Christie, B. R., Tuszynski, M. H., 2009. NGF is essential for hippocampal plasticity and learning. *Journal of Neuroscience*. https://doi.org/10.1523/jneurosci.2594-09.2009

80) Miller, M. W., Mooney, S. M., 2004. Chronic exposure to ethanol alters neurotrophin content in the basal forebrain-cortex system in the mature rat: Effects on autocrine-paracrine mechanisms. *Journal of Neurobiology*. https://doi.org/10.1002/neu.20059

81) Gericke, C. A., Schulte-Herbrüggen, O., Arendt, T., Hellweg, R., 2006. Chronic alcohol intoxication in rats leads to a strong but transient increase in NGF levels in distinct brain regions. *Journal of Neural Transmission*. https://doi.org/10.1007/s00702-005-0361-x

7.2 Artigo 2

Transcranial Direct Current Stimulation alters anxious-like behavior and neural parameters in rats with chronic pain exposed to alcohol

Periódico: Psychiatric research

Status: Under Review

Transcranial Direct Current Stimulation alters anxious-like behavior and neural parameters in rats with chronic pain exposed to alcohol

Daniela Silva Santos[1,2], Helouise Richardt Medeiros[1,2], Fernanda dos Santos Pereira[1], Dirson João Stein[1,2], Isabel Cristina de Macedo[1,4], Felipe Fregni[3], Wolnei Caumo[2], Iraci L. S. Torres[1,2]

[1]Laboratory of Pain Pharmacology and Neuromodulation: Preclinical Investigations, Center of Experimental Research, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS 90035-007, Brazil.
[2]Postgraduate Program in Medical Sciences, School of Medicine, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.
[3]Laboratory of Neuromodulation, Department of Physical Medicine & Rehabilitation, Spaulding Rehabilitation Hospital and Massachusetts General Hospital, Harvard University, Boston, MA, USA.
[4]Universidade Federal do Pampa, Uruguaiana, RS, Brazil.

*Corresponding author:
Iraci L.S. Torres, PhD
Hospital de Clínicas de Porto Alegre.
Rua Ramiro Barcelos, 2350, Bairro Santa Cecília
90035-903 - Porto Alegre, RS, Brazil.
e-mail: iltorres@hcpa.edu.br

ABSTRACT

The aim of this study was to evaluate the effects of transcranial direct current stimulation (tDCS) on anxiety-like behavior and neural parameters of rats with chronic pain and exposed to alcohol. Thirty-six adult male Wistar rats were randomized by weight and assigned to one of five groups: control (CT), neuropathic pain (NP), NPtDCS, NP plus alcohol (NPAL), and NPALtDCS. The rats were exposed to chronic constriction injury (CCI) of the sciatic nerve and alcohol (20% v/v solution, 4 g/kg) or vehicle (distilled water) by gavage for 15 days. Afterward, rats were treated using bimodal tDCS (0.5mA / 20 min / 8 days) and tested twice in the open field (OF). Rats were killed 24h after the last behavioral assessment, and brain tissue samples were collected and processed for NPY immunohistochemistry, expression of *IL1a* and *IL1b* in the spinal cord, cerebellum, and hippocampus, and levels of IL-1α and IL-1β in the same brain structures and the striatum. Both, CCI and alcohol exposure, induced anxiety-like behavior, indexed by the lower number of central crossings and rearings in the OF. These behaviors were reversed by tDCS, which also modulated the hypothalamic NPY-immunoreactivity. NP decreased the levels of IL-1α in the striatum. The association of NP, AL, and tDCS increased the levels of IL-1α in the hippocampus. The alcohol-induced an increase in the expression of *IL1a* in the spinal cord, which is reverted by tDCS. The alcohol and tDCS increase the expression of *IL1b* in the spinal cord. The alcohol increased the expression of *IL1b* in the cerebellum. Thus, tDCS may be considered as a treatment option for anxiety symptoms induced by NP and alcohol consumption. Thus, the current study showed that tDCS changes anxiety-like behavior induced by chronic neuropathic pain, which could be related to its modulatory effect in the immunoreactivity of NPY and spinal cord expression of *IL1a* and *IL1b*.

KEYWORDS: Anxiety; tDCS; NPY; IL-1α; IL-1β.

1. INTRODUCTION

Neural diseases may occur due to the alterations and adaptations of brain networks, promoting persistent symptoms, such as chronic pain and alcohol dependence (Sperry et al., 2021). Chronic pain triggers functional changes that are responsible for affective and cognitive dysfunctions (anxiety, emotional decision-making, and memory) (Llorca-Torralba et al., 2019), just as activation of specific brain regions can occur due to ethanol dependence at particular stages of development (Vilpoux et al., 2009).

The relationship between harmful alcohol use and pain is known to be bidirectional: on one hand, alcohol intake modulates pain and, on the other hand, acute and chronic pain influence alcohol-related behaviors (Robins et al., 2019). Both conditions seem to involve activated limbic circuits (Sperry et al., 2021), while brain regions such as the amygdala, the hippocampus, and the hypothalamus are activated by alcohol relapse-inducing stressors (Vilpoux et al., 2009).

Pain intensity and pain-related disability have been associated with emotional dysregulation, which in turn is associated with the severity of alcohol use (Paulus et al., 2017). It is important to highlight that alcohol dependence, which is a chronic condition with repeated cycles of withdrawal, craving, and relapse, is associated with a progressive (allostatic) dysfunctional state characterized by changes in neuroendocrine and brain stress pathways that underlie the expression of withdrawal symptoms reflecting a negative affective state (dysphoria, anxiety) (Becker, 2017).

Moreover, chronic pain affects sensory and emotional aversive responses causing anxiety-related illnesses (Llorca-Torralba et al., 2019). Additionally, it is known that the inflammatory pathways dysregulation alters brain circuits that modulate mood, pain, and

the response to stress (Maletic & Raison, 2009). Neuroinflammation is hypothesized to be influenced by alcohol consumption and also by chronic pain. In particular, the interleukin-1 (IL-1) system has been recognized as a key regulator of neuroimmune responses in several conditions, including alcohol intake and tissue injury (Mika et al., 2008; Patel et al., 2019). Considering that neuroinflammation plays an important role in pain and alcohol conditions, many efforts have been combined to unravel its mechanisms and to develop new therapeutic strategies with anti-inflammatory properties.

Besides, among the various neurotransmitter and neuropeptides involved in neuropathic pain and alcohol consumption, the neuropeptide Y (NPY) has implications in neurodegenerative diseases with stress-relieve, anxiolytic and neuroprotective properties (Reichmann & Holzer, 2016). NPY is expressed in specific neurons in the limbic system, brain stem, and hypothalamus, which may explain why NPY impacts stress-related changes in affective-emotional behavior (Reichmann & Holzer, 2016). A better understanding of the neural circuits affected by chronic pain and alcohol exposure and their adaptations and their relationship with the NPY system will provide new opportunities for the development of appropriate therapies.

In this context and considering that individuals with neuropathic pain and alcohol-related problems are oftentimes refractive to pharmacological treatment, transcranial direct current stimulation (tDCS) emerges as a non-invasive neuromodulatory technique that may benefit these patients. tDCS is a safe and low-cost tool that can be easily applied (Iannone et al., 2016; Schulze-Bonhage, 2017), and it has been used to treat different conditions. In our previous study, we showed that treatment with bimodal tDCS induced analgesia in rats with neuropathic pain, which was delayed by alcohol abstinence, and was associated with an increase in the central levels of IL-1α, IL-10, and NGF (Santos et

al., 2020). In other studies conducted in our group using different animal models we showed short- and long-term effects of tDCS treatment on anxiety-like behavior (de Oliveira et al., 2019; Filho et al., 2016), nociceptive parameters, and cytokines levels (Cioato et al., 2016; Laste et al., 2012; Lopes et al., 2020; Santos et al., 2020; Spezia Adachi et al., 2012, 2015).

Thus, we aimed in the present study to investigate the effects of repeated tDCS over the anxious-like behavior and neurochemical parameters in nerve-injured rats exposed or not to alcohol consumption.

2. MATERIALS AND METHODS

2.1 Animals

Thirty-six adult male Wistar rats (200–250 g / 45 to 60 days old) were randomized by weight and assigned to one of the five following groups: control (CT), NP, NP plus tDCS (NPtDCS), NP plus alcohol (NPAL), and NP plus AL plus tDCS (NPALtDCS). Rats were maintained in a room under controlled temperature (22 ± 2 °C), on a standard 12 h light/dark cycle, with *ad libitum* access to water and rodent chow. All experiments and procedures were approved by the Institutional Animal Care and Use Committee (GPPG-HCPA protocol #150501) and complied with the ethical and methodological standards of the ARRIVE guidelines (Kilkenny et al., 2013).

2.2 Experimental Design

On day zero the rats were submitted to the chronic constriction injury (CCI) surgery, and subsequently started on alcohol passive drinking for the next 15 days (Figure 1). For alcohol administration, the ethanol was diluted daily with distilled water to prepare a 20 %

v/v solution and delivered in a volume of 4 g/kg body weight according to previous studies (Santos et al., 2020; Schunck et al., 2015). One day after the end of alcohol administration, rats were tested in the open field (OF). Subsequently, the rats received eight consecutive days of treatment with tDCS and were tested in the OF for a second time twenty-four hours after the end of treatment. A day after the second OF test, the rats were killed by decapitation and brain tissue samples were collected and stored at −80 °C for further analysis. For all procedures, the experimenter was unaware of the experimental group each rat belonged to.

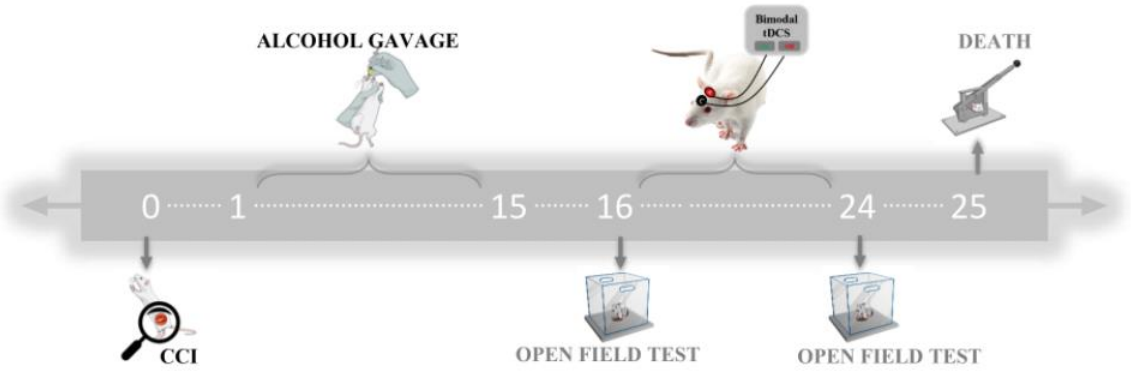

Figure 1 - Experimental timeline. CCI = chronic constriction injury surgery. tDCS = transcranial direct current stimulation.

2.3 Open field test (OF)

The behavioral assessment was performed in a 60 × 40 × 50 cm varnished wooden cage with glass-lined inside walls. The linoleum floor was divided by dark lines into twelve 13 × 13 cm squares. The OF test is often used to assess anxiety, exploration, and locomotion (Prut & Belzung, 2003). Each trial started immediately after the animals were placed in the back left corner and allowed to explore the surroundings for 5 min (Medeiros et al., 2012). The box was cleaned between trials to remove odor from previously tested subjects. Three parameters were evaluated during the test: (1) number of total crossings, (2) number of internal square crossings; and (3) number of rearings (i.e., vertical activity).

The number of crossings of internal squares (all paws crossing the boundary into an adjacent marked-out area) assessed anxiety-like behaviors (Vuralli et al., 2019). Anxious rodents are afraid to explore and prefer to stay in a safer place, which is the outer perimeter of the OF. The number of rearing behaviors (standing upright on its hind legs) was used to assess exploratory activity and as an additional measure of anxiety in rodents (Sturman et al., 2018).

2.4 Chronic constriction injury (CCI) of the sciatic nerve

CCI was induced according to the method of Bennett and Xie (Bennett & Xie, 1988). Rats were anesthetized by isoflurane inhalation at doses of 5 % for induction and 2.5 % for maintenance. Four loose ligatures were made in the common sciatic nerve at the mid-thigh, 1 mm apart using vicryl 4.0 suture. After the procedure, the incision site was sutured with non-absorbable mononylon yarn 4.0. Rats undergoing surgery received intraperitoneal tramadol (5 mg/kg) for pain relief immediately after surgery, and once every 12 hours for 2 additional days (Guzman-silva et al., 2008).

2.5 Transcranial Direct Current Stimulation (tDCS)

Our group has expertise using the tDCS technique in experimental models (de Oliveira et al., 2019; Filho et al., 2016; Laste et al., 2012; Santos et al., 2020). This technique mirrors the tDCS protocols for humans (da Graca-Tarragó et al., 2019; Fregni et al., 2006). The tDCS treatment started 16 days after the CCI model induction and confirmation of nociception by the hot plate test. It consisted of a bimodal stimulation by low-intensity constant current (0.5 mA) applied for 20 min during 8 days under immobilization using a soft cloth. The cathode was positioned at a point between the lateral angles of the eyes (supraorbital region) and the anode over the parietal cortex (Takano et al., 2011). All rats had their heads shaved for better adherence to the adapted electrocardiogram electrodes

(1.5 cm^2) with a conductive hydrogel. Electrodes were fixed to the head with an adhesive tape (Micropore™) and connected to a battery-driven stimulator. The stimulation was performed at the same time of the day (11 AM), and by the same blind researcher in all eight sessions.

2.6 Determination of IL-1α and IL-1β central levels

The rats were killed by decapitation 24 hours after the last treatment session, and central nervous system structures (Spinal cord + Hippocampus + Cerebellum + Striatum) were removed and frozen at −80 °C. The levels of IL-1 alpha and beta were determined by sandwich ELISA using monoclonal antibodies specific for each measurement (R&D Systems, Minneapolis, United States). Total protein was determined by Bradford's method using bovine serum albumin as the standard (Bradford, 1976).

2.7 Immunohistochemistry NPY

The hypothalamus was fixed in 10 % buffered formaldehyde, processed, and embedded in paraffin. Afterward, brains were sliced (4 μm) using a microtome (Microm/HM360) and mounted on previously silanized glass slides. To perform immunohistochemistry, the slides were heated in an oven at 80 °C for 30 minutes, deparaffinized in xylol, and rehydrated in ethyl alcohol followed by distilled water. Antigen retrieval was performed in a citrate buffer (pH 6.0) in a 95 °C water bath for 20 minutes. The specimens were then cooled for 10 minutes in the buffer itself. Endogenous peroxidase activity was blocked using 5 % hydrogen peroxide solution in methanol for 20 minutes. Protein blocking was performed for 20 minutes using powdered skim milk diluted to 5 % in PBS. The sections were then incubated overnight at 2-8 °C with an anti-NPY primary antibody (Sigma, N9528) at 1:8000 dilution. Next, a rabbit IgG secondary antibody (Millipore, AP132P) was applied at 1:200 dilution for 90 min at room temperature. The reaction was then

visualized using Liquid Dab (Dako, K3468), as recommended by the manufacturer. The slides were counterstained in Harris hematoxylin for 10 seconds and differentiated in 2 % ammoniacal water for 30 seconds. The sections were dehydrated in absolute alcohol and placed in xylol to allow assembly of resin-type Entellan slides. For the number of cells, the total area, and the average size, the counts were stereologically made in 6 fields/slides for 2 rats/group. The results were calculated by the percentage of control and expressed as delta variation of control (value of group - 100). The numbers of cells indicate an increase or decrease of staining in comparison to the control group (% of control of immunoreactive cells/field).

2.8 Analysis of gene expression by qRT-PCR

After decapitation, the central nervous system structures (Spinal cord + Hippocampus + Cerebellum) of each animal were collected and individually stored in tubes at -80 °C. For RNA extraction, 100 mg of marrow and cerebellum and 50 mg of hippocampus were used. For the striatum, extraction was not possible due to the small size of the structure. RNA was extracted using TRIzol Reagent (Ambion, Inc - cat. number 15596026), according to manufacturers' instructions. RNA integrity for each sample was measured using the Nanodrop 1000 spectrophotometer (Thermo Fisher, Waltham, Massachusetts, USA) and stored in a -20 °C freezer. 100 nanograms of RNA was reverse transcribed into complementary DNA (cDNA) using the M-MLV Reverse Transcriptase (Invitrogen, Carlsbad, California, USA - cat. number 28025-013) enzyme, according to manufacturers' instructions. Reverse transcription was performed in the Veriti PCR System Thermal Cycling Block (Applied Biosystems, Foster City, California, USA) for 5 minutes at 65 °C, 2 minutes at 37 °C, 50 minutes at 37 °C, and 15 minutes at 70 °C. The gene expression reaction was performed in 10 µL reactions containing 5 µL of

TaqMan Universal Master Mix II with UNG, 1 µL cDNA product, 0.5 µL gene expression assay (20X) of interest, 0.5 µL of gene expression assay (20X) housekeeping gene, and 3 µL nuclease-free water. The following thermal cycling specifications were performed on the StepOne Real-Time PCR System Thermal Cycling Block (Applied Biosystems, Foster City, California, USA): 2 minutes at 50 °C, 10 minutes at 95 °C (polymerase activation), and 40 cycles each for 15 seconds at 95 °C (denature) and 1 minute at 60 °C (anneal/extend). qPCR gene expression was performed using a customized TaqMan probe. The fluorescent dye 6-carboxy-fluorescein (FAM) was used for detecting the genes of interest, and the fluorescent dye victoria (VIC) was used for detecting the reference gene GAPDH. All reactions were performed in triplicate in 48-well plates sealed with adhesive film. Relative fold change was computed using the $2^{-\Delta\Delta Ct}$ method, in which each sample's ΔΔCt is used to calculate a fold change relative to controls. ΔCt values were first calculated by subtracting the Ct value of GAPDH normalizer gene from Ct of interest gene from each sample (ΔCt = gene interest Ct – GAPDH Ct), and ΔΔCt values were calculated by subtracting the average of the control ΔCt values from each ΔCt sample (ΔΔCt = ΔCt sample – ΔCt control average). The value obtained from this calculation is applied to the formula $2^{-\Delta\Delta CT}$ and, thus, we obtained the relative expression of the gene of interest normalized to a reference gene (normalizer gene).

2.9 Data analysis

Data were expressed as the mean ± standard error of the mean (S.E.M.). The behavioral analysis in the OF was performed using Generalized Estimated Equations (GEEs) followed by Bonferroni's correction. The data of immunohistochemical detection of NPY were calculated as the percentage of control of the number of immunoreactive cells per field and expressed as delta variation of control. Expressive changes were conventionally

considered when there was an increase or decrease in NPY staining over 40 % of the control group. The levels and expression of ILs were analyzed using one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls (SNK) method. P–values ≤ 0.05 were reported as statistically significant. SPSS 26.0 was used for statistical analysis.

3. RESULTS

3.1 Open field test

The OF is a largely used test for detecting anxiety-like behavior in rodents. In this experiment, behaviors similar to anxiety were evaluated at two time points, before and after treatment with tDCS (Figure 1). Regarding the number of crossings of internal squares and the number of rearings, there was interaction between group and time (GEE: Wald $\chi2 = 17.510$, $P < 0.001$), and significant effects of time (GEE: Wald $\chi2 = 9.690$, $P < 0.05$) and group (GEE: Wald $\chi2 = 23.021$, $P < 0.001$). The Bonferroni post hoc analysis revealed that animals submitted to the CCI / alcohol presented a lower number of central crossings (Figure 2 a) and a lower number of rearings (Figure 2 b) in the pre-treatment test in the OF, compared to controls. On the other hand, tDCS treatment reversed the decrease of the number of central crossings and rearings as observed in the post-treatment test in the OF (Figure 2 a-b), suggesting an anxiolytic effect of tDCS. The number of total crossings was similar for all groups (GEE, $P > 0.05$), suggesting that there was no effect on the locomotor activity.

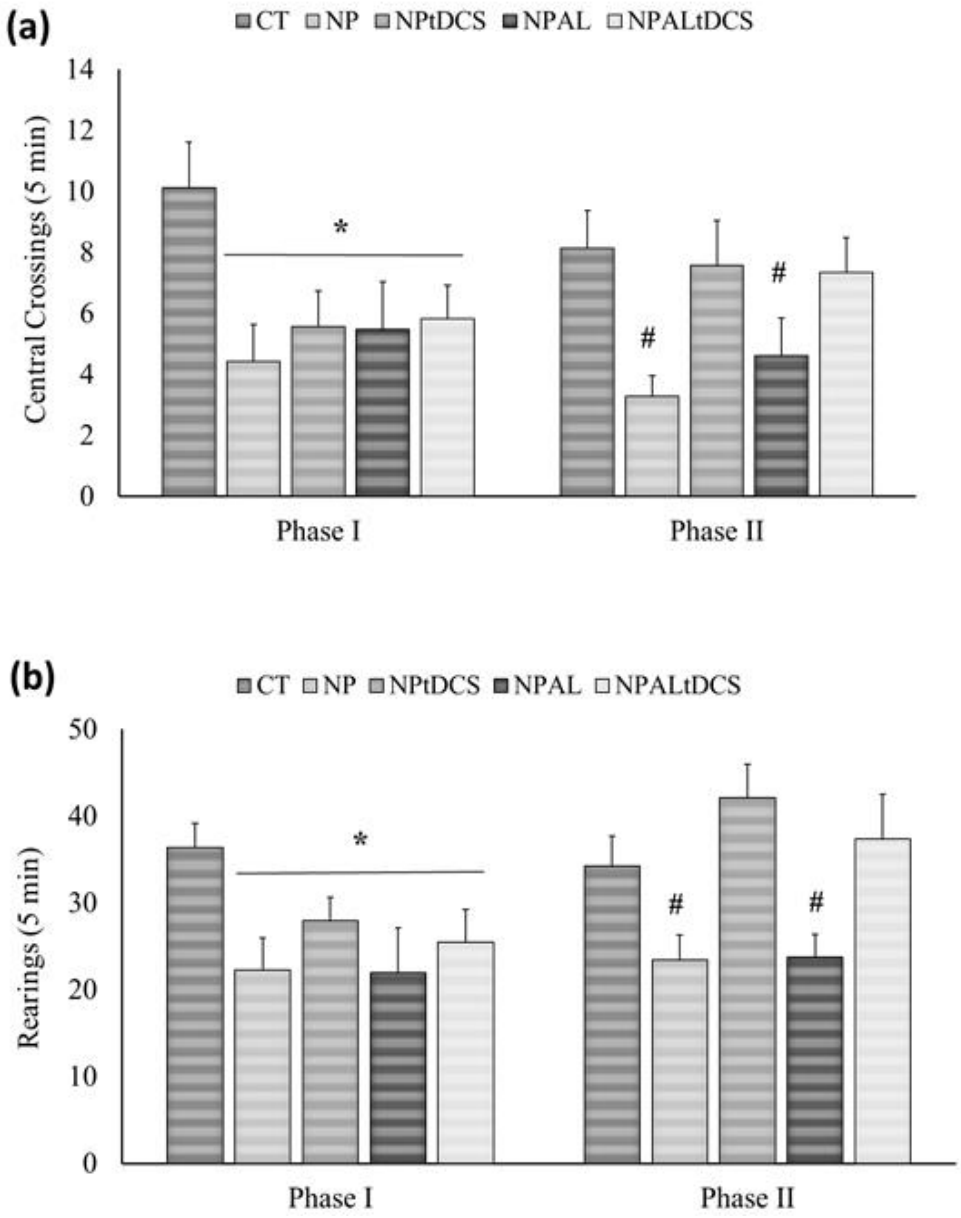

Figure 2 - Anxiety-like behavior parameters in the open field (OF) test (5 min sessions). (a) number of central crossings; (b) number of rearings. Data are expressed as mean ± SEM (GEE / Bonferroni, $P<0.05$). Phase I = test before treatment; Phase II = test after treatment. (*) Significant difference from CT; (#) Significant difference from CT, NPtDCS and NPALtDCS. n = 6-8/group.

3.2 Central levels of IL-1α and IL-1β in striatum, hippocampus, spinal cord, and cerebellum

In the striatum, it is possible to observe a pain effect in the levels of IL-1α. All groups subjected to CCI presented lower IL-1α levels in comparison with the control group (one-

way ANOVA, $F_{(4,24)} = 7.491$, $P < 0.001$; Table 1); there are no significant differences between groups in the striatal IL-1β levels (one-way ANOVA, $F_{(4,27)} = 01.933$, $P > 0.05$; Table 1).

Regarding the hippocampus, the NPALtDCS group presented higher IL-1α levels in comparison with control group (one-way ANOVA, $F(4,27) = 2.656$, $P \leq 0.05$; Table 1); without significant differences between groups regarding hippocampal IL-1β levels (one-way ANOVA, $F(4,27) = 0.806$, $P > 0.05$; Table 1).

In the spinal cord and cerebellum, no significant differences were observed between groups regarding IL-1α and IL-1β levels (one-way ANOVA, $F_{(4,27)} = 2.122$ and $F_{(4,27)} = 1.984$, respectively for spinal cord; $F_{(4,27)} = 2.187$ and $F_{(4,27)} = 0.456$, respectively for cerebellum, $P > 0.05$ for all; Table 1).

Table 1 – IL-1α and IL-1β levels in central structures.

Experimental Groups	Brain Structure *Variable*							
	Striatum		Hippocampus		Spinal Cord		Cerebellum	
	IL-1α	*IL-1β*	*IL-1α*	*IL-1β*	*IL-1α*	*IL-1β*	*IL-1α*	*IL-1β*
Control (CT)	391.07 ± 24.06	156.12 ± 12.17	61.57 ± 8.18	91.17 ± 23.56	258.67 ± 51.91	75.59 ± 11.33	93.06 ± 2.87	43.78 ± 5.96
Neuropathic Pain (NP)	**259.50 ± 31.15***	99.42 ± 7.18	103.52 ± 7.62	95.71 ± 24.38	245.61 ± 64.36	122.95 ± 19.61	110.87 ± 2.53	50.09 ± 7.08
Neuropathic Pain + tDCS (NPtDCS)	**258.84 ± 44.90***	143.20 ± 46.76	89.26 ± 19.20	50.24 ± 9.76	243.15 ± 28.31	145.53 ± 29.32	109.57 ± 6.12	47.41 ± 0.65
Neuopathic Pain + Alcohol (NPAL)	**221.68 ± 10.46***	96.59 ± 15.17	100.04 ± 17.04	103.90 ± 20.47	124.77 ± 28.51	150.79 ± 20.23	99.83 ±5.01	49.74 ± 7.18
Neuropathic Pain + Alcohol + tDCS (NPALtDCS)	**190.38 ± 12,36***	110.30 ± 13.28	**128.67 ± 21.92***	97.21 ± 21.85	128.42 ± 36.12	144.55 ± 33.15	105.54 ± 8.25	57.12 ± 10.23

Data are expressed as mean ± SEM of pg/mg of protein. (*) Indicate a significant difference from CT (one-way ANOVA/SNK. $P \leq 0.05$). tDCS = Transcranial Direct Current Stimulation. n = 6-8/group.

3.3 Expression of *IL1a* and *IL1b* in spinal cord, cerebellum, and hippocampus

In the spinal cord, we observed an increase in the *IL1a* mRNA induced by alcohol; the NPAL group was different from the NP and NPtDCS groups (one-way ANOVA, $F_{(4,25)}$ = 4.992, $P < 0.05$; Figure 3 a). In addition, there was an increase in the *IL1b* mRNA induced by alcohol and/or tDCS; the NPAL, NPtDCS, NPALtDCS groups were different from the CT and NP groups (one-way ANOVA, $F_{(4,25)} = 4.106$, $P < 0.05$; Figure 3 b).

Regarding the cerebellum, there was no significant difference observed regarding cerebellar *IL1a* mRNA between groups (one-way ANOVA, $F_{(4,27)} = 1.514$, $P > 0.05$; Figure 3 c). On the other hand, there was an increase in the *IL1b* mRNA induced by alcohol observed in the NPAL and NPALtDCS groups (one-way ANOVA, $F_{(4,25)} = 6.004$, $P < 0.05$; Figure 3 d).

In the hippocampus, there was no difference between groups in the *IL1a* and *Il1b* mRNA (one-way ANOVA, $F_{(4,25)} = 0.556$, $F_{(4,26)} = 2.042$, respectively, $P > 0.05$; Figure 3 e and f).

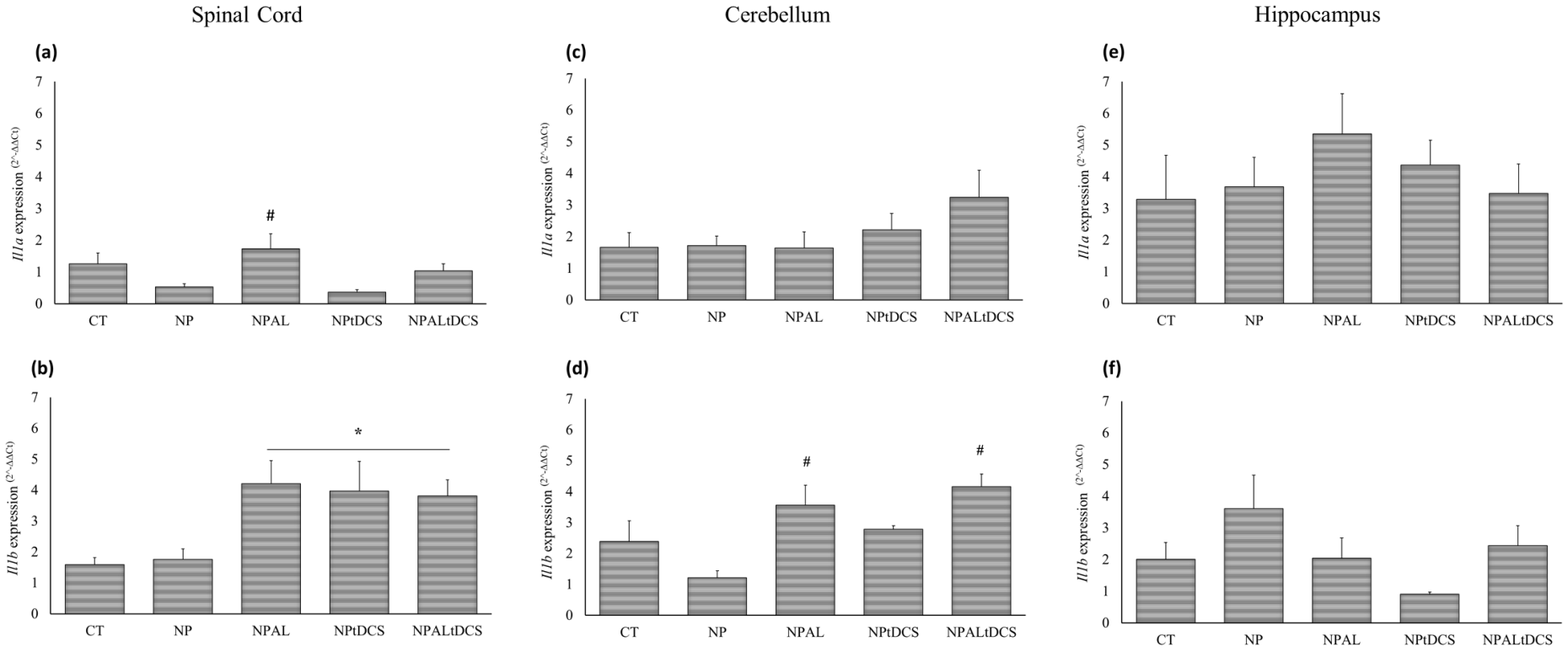

Figure 3 - Gene expression of *Il1a* and *Il1b*, respectively, in the Spinal Cord (a, b), Cerebellum (c, d), and Hippocampus (e, f). Data are expressed as mean ± SEM, (one-way ANOVA/SNK, P<0.05). (*) Significant difference from CT and NP; (#) Significant difference from NP. n = 6/group.

3.4 Neuropeptide Y detection

The analysis of hypothalamic immunohistochemical detection of NPY antibody (Figure 4) showed a tDCS treatment modulatory effect (Table 2). The NPtDCS group showed an increase in the immunoreactivity for NPY in the number of cells (123.19%) and total area marked (117.02%). Also, the NPALtDCS group showed an increase in the immunoreactivity for NPY in total area (66.31%) and average cell size (42.34%). However, NPY-immunoreactivity in untreated rats was similar to controls.

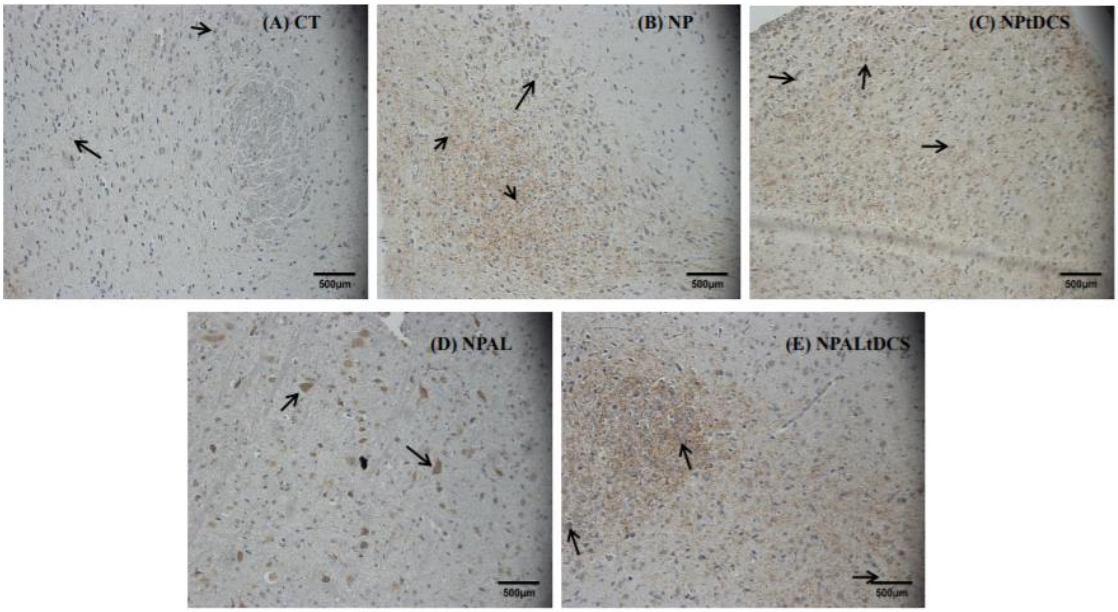

Figure 4 - Illustrative images from NPY immunodetection in the hypothalamus of rats (Image-J; Magnification: 40X; Scale bars: 500 µm). (A) control, CT; (B) neuropathic pain, NP; (C) neuropathic pain plus transcranial direct current stimulation, NPtDCS; (D) neuropathic pain plus alcohol, NPAL; (E) neuropathic pain plus alcohol plus tDCS, NPALtDCS. Arrows point to NPY immunodetection in cell bodies and neurites.

Table 2 – NPY immunodetection in the hypothalamus.

Experimental Groups	**Hypothalamus** *Variable*		
	Number of Cells	*Total Area*	*Average Size*
Neuropathic Pain (NP)	- 14.34	- 13.19	39.00
Neuropathic Pain + tDCS (NPtDCS)	**123.19***	**117.02***	- 37.39
Neuopathic Pain + Alcohol (NPAL)	- 35.83	- 24.52	2.07
Neuropathic Pain + Alcohol + tDCS (NPALtDCS)	37.54	**66.31***	**42.34***

Data were calculated as % of control (CT) of immunoreactive cells/field and expressed as delta variation of control (value of control - 100). NPY = Neuropeptide Y; tDCS = Transcranial Direct Current Stimulation. (*) Expressive changes were considered: a decrease or an increase of over 40% of control (bold). n = 2/group.

DISCUSSION

The current study shows that rats exposed to a model of neuropathic pain, associated or not with alcohol consumption, present anxiety-like behaviors. These behaviors, indexed by a decrease in the spontaneous rearing and in the number of central crossings in the open field test (Sturman et al., 2018; Vuralli et al., 2019), were reverted by tDCS treatment over the course of eight days. Besides that, we showed that these behavioral alterations were associated with changes in the IL-1α and IL-1β levels, in the *IL1a and IL1b* expression in the spinal cord, hippocampus, striatum, and cerebellum, and in the hypothalamic NPY immunoreactivity.

Empirical evidence supports the hypothesis that alcohol dependence is among the pathologies arising from aberrant neurobiological substrates of pain (Egli et al., 2012). In a previous study using the same protocols, we showed the effectiveness of the CCI pain model in inducing hypernociception (Santos et al., 2020). The interrelation between chronic pain and alcohol consumption is of increasing interest since both conditions have a high impact on public health (Zale et al., 2015). However, despite evidence that alcohol has effects on pain processes and symptoms (Thompson et al., 2017), and that pain contributes to alcohol consumption (Egli et al., 2012), not enough attention has been devoted to the mechanistic understanding of this relationship, paramount for preventing and treating both problems. Among other emotional problems, symptoms of anxiety are highly prevalent in NP and alcohol consumption conditions, as has been reported in both preclinical and clinical studies (Lee et al., 2015; Radat et al., 2013; Sieberg et al., 2018; Smith & Randall, 2012). We have previously demonstrated that rats submitted to the CCI pain model exhibit anxiety-like symptoms for at least 21 days after surgery (Filho et al., 2016; Lopes et al., 2021).

In the current study, we demonstrate that CCI rats develop an anxious-like state that was reversed by repeated tDCS treatment. Some previous studies have already shown that tDCS has anxiolytic effects in rodents under several conditions (Filho et al., 2016; Lopes et al., 2021;

Macedo et al., 2016). Furthermore, the literature on the use of tDCS to treat anxiety in humans has been recently reviewed by us (Stein et al., 2020), and shows that, despite the small number of clinical studies to date, tDCS is a well-tolerated method of neuromodulation that holds promise for the treatment of this condition.

Chronic pain and alcohol are known to change the levels of cytokines in different tissues (Crews et al., 2006). Among several cytokines, IL-1 is considered to have diverse physiological functions and pathological significance in health and disease (Kaneko et al., 2019). Furthermore, IL-1α and β play a fundamental role in the development and progression of anxiety-like states (Murray et al., 2013; Rossi et al., 2012). Here, we mainly show that rats under CCI, regardless of whether they were exposed to alcohol or treated with tDCS, have reduced IL-1α - but not IL-1β - levels in the striatum. In contrast, IL-1α levels were significantly increased in the hippocampus of rats with CCI exposed to alcohol and treated with tDCS. No changes were observed in IL-1α levels in the spinal cord and cerebellum, nor in the IL-1β levels in any of the analyzed brain structures. Our previous studies demonstrate the complexity of regulating neuropathic pain by endogenous immunological factors (Callai et al., 2019; Cioato et al., 2016; Lopes et al., 2020; Santos et al., 2020). Also, exposure to alcohol produces changes in the immune function, as shown in a study involving prenatal alcohol exposure in combination with neuropathic pain, resulting in increased protein expression of IL-1β, TNF-α, and IL-6 (Noor et al., 2017). It is important to note that ILs have important homeostatic functions in normal organisms; however, overproduction of pro-inflammatory cytokines is implicated in the pathophysiological changes that occur during different disease states (Furman et al., 2019), including neuropathic pain (Gonçalves dos Santos et al., 2020) and alcohol exposure (Neupane, 2016). However, in the current study, we did find a significant reduction of IL-1α level in the striatum induced by CCI, without the effect of alcohol or tDCS. Previous studies have shown that IL-1α has antiallodynic and antihyperalgesic effects in a rat neuropathic pain model (Mika

et al., 2008), Thus, we can suggest that a decrease in central IL-1α levels might be the mechanism underlying the neuropathic pain.

Moreover, the interaction between nociception and anxiety-like states in rodents may also be regulated by endogenous anxiolytic neuropeptides and neurotransmitters (Zhang et al., 2014). An important neuropeptide is NPY, which has appeared as a relevant component in nociceptive signaling modulation through its action on several receptors in the central nervous system (Gibbs et al., 2004; Gupta et al., 2019). In addition, NPY (co-secreted with other neurotransmitters, GABA and glutamate) and its receptors are related to the etiology and pathophysiology of mood and anxiety disorders, and the central regulation of drug/alcohol use, nociception, reward, and energy homeostasis (Baliki & Apkarian, 2015; Pilat et al., 2015; Reichmann & Holzer, 2016; Robinson & Thiele, 2017; Vilpoux et al., 2009). NPY is a highly conserved endogenous peptide in the central and peripheral nervous systems of all mammals, which has been implicated in both pro- and antinociceptive effects (Diaz-delCastillo et al., 2018). The analgesic effects of NPY was previously demonstrated by Yalamury and colleagues (2012); using pharmacological modulation of NPY Y1 and Y2 receptors in a postoperative model of pain, these authors show that a single dose of intrathecally administered NPY was sufficient to attenuate postoperative pain behaviors for 48 h and that the administration of a Y2 receptor antagonist has anti-hyperalgesic effects (Yalamuri et al., 2013). Accordingly, selective ablation or inhibition of spinal Y1 interneurons produced changes in the endogenous NPY-Y1 signaling cascade, generating pain reduction and preventing the transition to chronic pain (Nelson & Taylor, 2021). Furthermore, the anxiolytic activity of NPY is primarily mediated by Y1 receptors. For instance, the administration of a selective Y1 receptor antagonist before or together with NPY abolishes its anxiolytic effect (Lach & de Lima, 2013). Unpublished data from our lab using CCI and alcohol consumption in rats suggest a modulatory role of tDCS in the immunoreactivity of NPY in central structures (PFC, amygdala, and striatum) (Santos et al,

personal communication). In the present study, we show that the CCI or alcohol consumption did not change the expression of NPY in the hypothalamus; however, its expression was increased by tDCS treatment. Therefore, we can suggest that this effect could be related to the tDCS anxiolytic effects, which could be a result of the neuromodulation-induced neuroplasticity (Monai et al., 2016; Nitsche et al., 2012). In addition, the peptide's regulation of disease states suggest that modulation of the activity of the NPY system via receptor agonists/antagonists may be a putative treatment mechanism in affective disorders as well as alcohol use disorders (Thorsell & Mathé, 2017).

We recognize that the present study has some limitations. First, the causal relationship between alcohol consumption and comorbidity with neuropathic pain has not been defined, as it was not our primary objective at this point (we focused specifically on the effects of the association of both conditions). Future studies still need to clarify this causal relationship that involves complex brain circuits. Second, the sample size was limited, especially for the assessment of central NPY immunoreactivity, and it should be further amplified. Third, other brain regions, such as the amygdala, crucial in both NP and alcohol consumption processes, need to be further investigated to unravel their role in both conditions. Beyond that, future studies should investigate the effects of brain modulatory interventions in chronic pain and alcohol consumption conditions through the dopamine and the endogenous opioid system.

Despite the limitations, the results of the current study offer insights into how limbic structures may be involved in the establishment and the modulation of both diseases. The current findings also support the understanding of the modulatory characteristics of transcranial direct current stimulation. Since chronic pain and harmful use of alcohol reduce the quality of life and they are important clinical and economic problems, promising modulatory therapies, including tDCS, must be encouraged.

CONCLUSION

Our results collaborate with the better molecular understanding involved in the paradigm of the association of chronic pain and alcohol consumption. An important result was the robust decrease in the IL-1α levels observed in the striatum induced by CCI, suggesting a role of the IL-1α in the neurobiology of NP. Thus, the current study showed that tDCS changes anxiety-like behavior induced by chronic neuropathic pain, which could be related to its modulatory effect in the immunoreactivity of NPY and spinal cord expression of *IL1a* and *IL1b*.

ACKNOWLEDGEMENTS

This work was supported by the following Brazilian funding agencies: Brazilian Federal Agency for Support and Evaluation of Graduate Education—CAPES/MD-PhD (Santos, DS); National Council for Scientific and Technological Development—CNPq (Dra. ILS Torres, Dr. W. Caumo). We also want to thank the Engineering Division from the HCPA for having developed the tDCS stimulator; the Graduate Research Group of the Hospital de Clínicas de Porto Alegre – GPPG (I.L.S. Torres, grant number 15-0501).

CONFLICT OF INTEREST: There was no financial interest between any of the authors or any commercial interest in the outcome of this study.

REFERENCES

Baliki, M. N., & Apkarian, A. V. (2015). Nociception, Pain, Negative Moods, and Behavior Selection. In *Neuron* (Vol. 87, Issue 3). https://doi.org/10.1016/j.neuron.2015.06.005

Becker, H. C. (2017). Influence of stress associated with chronic alcohol exposure on drinking. In *Neuropharmacology* (Vol. 122). https://doi.org/10.1016/j.neuropharm.2017.04.028

Bennett, G. J., & Xie, Y. K. (1988). A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain*. https://doi.org/10.1016/0304-3959(88)90209-6

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*. https://doi.org/10.1016/0003-2697(76)90527-3

Callai, E. M. M., Scarabelot, V. L., Fernandes Medeiros, L., de Oliveira, C., de Souza, A., Macedo, I. C., Cioato, S. G., Finamor, F., Caumo, W., Quevedo, A. da S., & Torres, I. L. S. (2019). Transcranial direct current stimulation (tDCS) and trigeminal pain: A preclinical study. *Oral Diseases*, *25*(3), 888–897. https://doi.org/10.1111/odi.13038

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

Crews, F. T., Bechara, R., Brown, L. A., Guidot, D. M., Mandrekar, P., Oak, S., Qin, L., Szabo, G., Wheeler, M., & Zou, J. (2006). Cytokines and alcohol. *Alcoholism: Clinical and Experimental Research*. https://doi.org/10.1111/j.1530-0277.2006.00084.x

da Graca-Tarragó, M., Lech, M., Angoleri, L. D. M., Santos, D. S., Deitos, A., Brietzke, A. P., Torres, I. L. S., Fregni, F., & Caumo, W. (2019). Intramuscular electrical stimulus potentiates motor cortex modulation effects on pain and descending inhibitory systems in knee osteoarthritis: A randomized, factorial, sham-controlled study. *Journal of Pain Research*. https://doi.org/10.2147/JPR.S181019

de Oliveira, C., de Freitas, J. S., Macedo, I. C., Scarabelot, V. L., Ströher, R., Santos, D. S., Souza, A., Fregni, F., Caumo, W., & Torres, I. L. S. (2019). Transcranial direct current stimulation (tDCS) modulates biometric and inflammatory parameters and anxiety-like behavior in obese rats. *Neuropeptides*. https://doi.org/10.1016/j.npep.2018.09.006

Diaz-delCastillo, M., Woldbye, D. P. D., & Heegaard, A. M. (2018). Neuropeptide Y and its Involvement in Chronic Pain. In *Neuroscience*. https://doi.org/10.1016/j.neuroscience.2017.08.050

Egli, M., Koob, G. F., & Edwards, S. (2012). Alcohol dependence as a chronic pain disorder. In *Neuroscience and Biobehavioral Reviews*. https://doi.org/10.1016/j.neubiorev.2012.07.010

Filho, P. R. M., Vercelino, R., Cioato, S. G., Medeiros, L. F., de Oliveira, C., Scarabelot, V. L., Souza, A., Rozisky, J. R., Quevedo, A. da S., Adachi, L. N. S., Sanches, P. R. S., Fregni, F., Caumo, W., & Torres, I. L. S. (2016). Transcranial direct current stimulation (tDCS) reverts behavioral alterations and brainstem BDNF level increase induced by neuropathic

pain model: Long-lasting effect. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. https://doi.org/10.1016/j.pnpbp.2015.06.016

Fregni, F., Boggio, P. S., Nitsche, M. A., Rigonatti, S. P., & Pascual-Leone, A. (2006). Cognitive effects of repeated sessions of transcranial direct current stimulation in patients with depression. *Depression and Anxiety*. https://doi.org/10.1002/da.20201

Furman, D., Campisi, J., Verdin, E., Carrera-Bastos, P., Targ, S., Franceschi, C., Ferrucci, L., Gilroy, D. W., Fasano, A., Miller, G. W., Miller, A. H., Mantovani, A., Weyand, C. M., Barzilai, N., Goronzy, J. J., Rando, T. A., Effros, R. B., Lucia, A., Kleinstreuer, N., & Slavich, G. M. (2019). Chronic inflammation in the etiology of disease across the life span. *Nature Medicine*, *25*(12), 1822–1832. https://doi.org/10.1038/s41591-019-0675-0

Gibbs, J., Flores, C. M., & Hargreaves, K. M. (2004). Neuropeptide Y inhibits capsaicin-sensitive nociceptors via a Y1-receptor-mediated mechanism. *Neuroscience*, *125*(3). https://doi.org/10.1016/j.neuroscience.2004.01.044

Gonçalves dos Santos, G., Delay, L., Yaksh, T. L., & Corr, M. (2020). Neuraxial Cytokines in Pain States . In *Frontiers in Immunology* (Vol. 10, p. 3061). https://www.frontiersin.org/article/10.3389/fimmu.2019.03061

Gupta, S., Gautam, M., Prasoon, P., Kumar, R., Ray, S. B., & Kaler Jhajhria, S. (2019). Involvement of Neuropeptide y in Post-Incisional Nociception in Rats. *Annals of Neurosciences*, *25*(4). https://doi.org/10.1159/000495130

Guzman-silva, M. A., Pollastri, C. E., Augusto, J., Pantaleão, S., Carolina, A., Carvalho, B. De, Henriques, H. N., Camara, N. R., Pacheco, J. T., & Boaventura, G. T. (2008). Tramadol minimizes potential pain during post-oophorectomy in Wistar rats. *Aatex*, *14*, 91–92.

Iannone, A., Cruz, A. P. de M., Brasil-Neto, J. P., & Boechat-Barros, R. (2016). Transcranial magnetic stimulation and transcranial direct current stimulation appear to be safe neuromodulatory techniques useful in the treatment of anxiety disorders and other neuropsychiatric disorders. *Arquivos de Neuro-Psiquiatria*. https://doi.org/10.1590/0004-282X20160115

Kaneko, N., Kurata, M., Yamamoto, T., Morikawa, S., & Masumoto, J. (2019). The role of interleukin-1 in general pathology. In *Inflammation and Regeneration* (Vol. 39, Issue 1). https://doi.org/10.1186/s41232-019-0101-5

Kilkenny, C., Browne, W. J., Cuthill, I. C., Emerson, M., & Altman, D. G. (2013). Improving bioscience research reporting: The arrive guidelines for reporting animal research. *Animals*. https://doi.org/10.3390/ani4010035

Lach, G., & de Lima, T. C. M. (2013). Role of NPY Y1 receptor on acquisition, consolidation and extinction on contextual fear conditioning: Dissociation between anxiety, locomotion and non-emotional memory behavior. *Neurobiology of Learning and Memory*, *103*. https://doi.org/10.1016/j.nlm.2013.04.005

Laste, G., Caumo, W., Adachi, L. N. S., Rozisky, J. R., De MacEdo, I. C., Filho, P. R. M., Partata, W. A., Fregni, F., & Torres, I. L. S. (2012). After-effects of consecutive sessions of transcranial direct current stimulation (tDCS) in a rat model of chronic inflammation. *Experimental Brain Research*. https://doi.org/10.1007/s00221-012-3149-x

Lee, K. M., Coehlo, M., McGregor, H. A., Waltermire, R. S., & Szumlinski, K. K. (2015). Binge alcohol drinking elicits persistent negative affect in mice. *Behavioural Brain Research*, *291*. https://doi.org/10.1016/j.bbr.2015.05.055

Llorca-Torralba, M., Suárez-Pereira, I., Bravo, L., Camarena-Delgado, C., Garcia-Partida, J. A., Mico, J. A., & Berrocoso, E. (2019). Chemogenetic Silencing of the Locus Coeruleus–Basolateral Amygdala Pathway Abolishes Pain-Induced Anxiety and Enhanced Aversive Learning in Rats. *Biological Psychiatry*, *85*(12). https://doi.org/10.1016/j.biopsych.2019.02.018

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

Lopes, B. C., Medeiros, L. F., Stein, D. J., Cioato, S. G., de Souza, V. S., Medeiros, H. R., Sanches, P. R. S., Fregni, F., Caumo, W., & Torres, I. L. S. (2021). tDCS and exercise improve anxiety-like behavior and locomotion in chronic pain rats via modulation of neurotrophins and inflammatory mediators. *Behavioural Brain Research*, *404*, 113173. https://doi.org/10.1016/j.bbr.2021.113173

Macedo, I. C., de Oliveira, C., Vercelino, R., Souza, A., Laste, G., Medeiros, L. F., Scarabelot, V. L., Nunes, E. A., Kuo, J., Fregni, F., Caumo, W., & Torres, I. L. S. (2016). Repeated transcranial direct current stimulation reduces food craving in Wistar rats. *Appetite*. https://doi.org/10.1016/j.appet.2016.03.014

Maletic, V., & Raison, C. L. (2009). Neurobiology of depression, fibromyalgia and neuropathic pain. *Frontiers in Bioscience*, *14*(14). https://doi.org/10.2741/3598

Medeiros, L. F., Souza, A. C. de, Souza, A. de, Cioato, S. G., Scarabelot, V. L., Caumo, W., Fernandes, L. C., & Torres, I. L. S. (2012). Fentanyl administration in infant rats produces long-term behavioral responses. *International Journal of Developmental Neuroscience*. https://doi.org/10.1016/j.ijdevneu.2011.10.004

Mika, J., Korostynski, M., Kaminska, D., Wawrzczak-Bargiela, A., Osikowicz, M., Makuch, W., Przewlocki, R., & Przewlocka, B. (2008). Interleukin-1alpha has antiallodynic and antihyperalgesic activities in a rat neuropathic pain model. *Pain*. https://doi.org/10.1016/j.pain.2008.02.015

Monai, H., Ohkura, M., Tanaka, M., Oe, Y., Konno, A., Hirai, H., Mikoshiba, K., Itohara, S., Nakai, J., Iwai, Y., & Hirase, H. (2016). Calcium imaging reveals glial involvement in transcranial direct current stimulation-induced plasticity in mouse brain. *Nature Communications*, *7*. https://doi.org/10.1038/ncomms11100

Murray, C. L., Obiang, P., Bannerman, D., & Cunningham, C. (2013). Endogenous IL-1 in cognitive function and anxiety: A study in IL-1RI-/-mice. *PLoS ONE*, *8*(10). https://doi.org/10.1371/journal.pone.0078385

Nelson, T. S., & Taylor, B. K. (2021). Targeting spinal neuropeptide Y1 receptor-expressing interneurons to alleviate chronic pain and itch. In *Progress in Neurobiology* (Vol. 196). https://doi.org/10.1016/j.pneurobio.2020.101894

Neupane, S. P. (2016). Neuroimmune interface in the comorbidity between alcohol use disorder and major depression. In *Frontiers in Immunology* (Vol. 7, Issue DEC). https://doi.org/10.3389/fimmu.2016.00655

Nitsche, M. A., Müller-Dahlhaus, F., Paulus, W., & Ziemann, U. (2012). The pharmacology of neuroplasticity induced by non-invasive brain stimulation: Building models for the clinical

use of CNS active drugs. In *Journal of Physiology* (Vol. 590, Issue 19). https://doi.org/10.1113/jphysiol.2012.232975

Noor, S., Sanchez, J. J., Vanderwall, A. G., Sun, M. S., Maxwell, J. R., Davies, S., Jantzie, L. L., Petersen, T. R., Savage, D. D., & Milligan, E. D. (2017). Prenatal alcohol exposure potentiates chronic neuropathic pain, spinal glial and immune cell activation and alters sciatic nerve and DRG cytokine levels. *Brain, Behavior, and Immunity*, *61*. https://doi.org/10.1016/j.bbi.2016.12.016

Patel, R. R., Khom, S., Steinman, M. Q., Varodayan, F. P., Kiosses, W. B., Hedges, D. M., Vlkolinsky, R., Nadav, T., Polis, I., Bajo, M., Roberts, A. J., & Roberto, M. (2019). IL-1β expression is increased and regulates GABA transmission following chronic ethanol in mouse central amygdala. *Brain, Behavior, and Immunity*, *75*, 208–219. https://doi.org/10.1016/j.bbi.2018.10.009

Paulus, D. J., Bakhshaie, J., Ditre, J. W., Viana, A. G., Garza, M., Valdivieso, J., Ochoa-Perez, M., Lemaire, C., & Zvolensky, M. J. (2017). Emotion dysregulation in the context of pain and alcohol use among latinos in primary care. *Journal of Studies on Alcohol and Drugs*, *78*(6). https://doi.org/10.15288/jsad.2017.78.938

Pilat, D., Rojewska, E., Jurga, A. M., Piotrowska, A., Makuch, W., Przewlocka, B., & Mika, J. (2015). IL-1 receptor antagonist improves morphine and buprenorphine efficacy in a rat neuropathic pain model. *European Journal of Pharmacology*. https://doi.org/10.1016/j.ejphar.2015.05.058

Prut, L., & Belzung, C. (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: A review. In *European Journal of Pharmacology* (Vol. 463, Issues 1–3). https://doi.org/10.1016/S0014-2999(03)01272-X

Radat, F., Margot-Duclot, A., & Attal, N. (2013). Psychiatric co-morbidities in patients with chronic peripheral neuropathic pain: A multicentre cohort study. *European Journal of Pain (United Kingdom)*, *17*(10). https://doi.org/10.1002/j.1532-2149.2013.00334.x

Reichmann, F., & Holzer, P. (2016). Neuropeptide Y: A stressful review. In *Neuropeptides* (Vol. 55). https://doi.org/10.1016/j.npep.2015.09.008

Robins, M. T., Heinricher, M. M., & Ryabinin, A. E. (2019). From Pleasure to Pain, and Back Again: The Intricate Relationship between Alcohol and Nociception. In *Alcohol and Alcoholism* (Vol. 54, Issue 6). https://doi.org/10.1093/alcalc/agz067

Robinson, S. L., & Thiele, T. E. (2017). The Role of Neuropeptide Y (NPY) in Alcohol and Drug Abuse Disorders. In *International Review of Neurobiology*. https://doi.org/10.1016/bs.irn.2017.06.005

Rossi, S., Sacchetti, L., Napolitano, F., De Chiara, V., Motta, C., Studer, V., Musella, A., Barbieri, F., Bari, M., Bernardi, G., Maccarrone, M., Usiello, A., & Centonze, D. (2012). Interleukin-1β causes anxiety by interacting with the endocannabinoid system. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *32*(40), 13896–13905. https://doi.org/10.1523/JNEUROSCI.1515-12.2012

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

Schulze-Bonhage, A. (2017). Brain stimulation as a neuromodulatory epilepsy therapy. In *Seizure*. https://doi.org/10.1016/j.seizure.2016.10.026

Schunck, R. V. A., Torres, I. L. S., Laste, G., De Souza, A., Macedo, I. C., Valle, M. T. C., Salomón, J. L. O., Moreira, S., Kuo, J., Arbo, M. D., Dallegrave, E., & Leal, M. B. (2015). Protracted alcohol abstinence induces analgesia in rats: Possible relationships with BDNF and interleukin-10. *Pharmacology Biochemistry and Behavior*. https://doi.org/10.1016/j.pbb.2015.05.011

Sieberg, C. B., Taras, C., Gomaa, A., Nickerson, C., Wong, C., Ward, C., Baskozos, G., Bennett, D. L. H., Ramirez, J. D., Themistocleous, A. C., Rice, A. S. C., Shillo, P. R., Tesfaye, S., Edwards, R. R., Andrews, N. A., Berde, C., & Costigan, M. (2018). Neuropathic pain drives anxiety behavior in mice, results consistent with anxiety levels in diabetic neuropathy patients. *Pain Reports*, *3*(3). https://doi.org/10.1097/PR9.0000000000000651

Smith, J. P., & Randall, C. L. (2012). Anxiety and alcohol use disorders: Comorbidity and treatment considerations. *Alcohol Research: Current Reviews*, *34*(4).

Sperry, M. M., Granquist, E. J., & Winkelstein, B. A. (2021). Early changes in brain network topology and activation of affective pathways predict persistent pain in the rat. *Pain*, *162*(1), 45–55. https://doi.org/10.1097/j.pain.0000000000002010

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

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

Stein, D. J., Medeiros, L. F., Caumo, W., & Torres, I. L. S. (2020). Transcranial direct current stimulation in patients with anxiety: Current perspectives. In *Neuropsychiatric Disease and Treatment* (Vol. 16). https://doi.org/10.2147/NDT.S195840

Sturman, O., Germain, P. L., & Bohacek, J. (2018). Exploratory rearing: a context- and stress-sensitive behavior recorded in the open-field test. *Stress*, *21*(5). https://doi.org/10.1080/10253890.2018.1438405

Takano, Y., Yokawa, T., Masuda, A., Niimi, J., Tanaka, S., & Hironaka, N. (2011). A rat model for measuring the effectiveness of transcranial direct current stimulation using fMRI. *Neurosci Lett*. https://doi.org/10.1016/j.neulet.2011.01.004

Thompson, T., Oram, C., Correll, C. U., Tsermentseli, S., & Stubbs, B. (2017). Analgesic Effects of Alcohol: A Systematic Review and Meta-Analysis of Controlled Experimental Studies in Healthy Participants. In *Journal of Pain* (Vol. 18, Issue 5). https://doi.org/10.1016/j.jpain.2016.11.009

Thorsell, A., & Mathé, A. A. (2017). Neuropeptide Y in alcohol addiction and affective disorders. In *Frontiers in Endocrinology*. https://doi.org/10.3389/fendo.2017.00178

Vilpoux, C., Warnault, V., Pierrefiche, O., Daoust, M., & Naassila, M. (2009). Ethanol-sensitive brain regions in rat and mouse: A cartographic review, using immediate early

gene expression. In *Alcoholism: Clinical and Experimental Research* (Vol. 33, Issue 6). https://doi.org/10.1111/j.1530-0277.2009.00916.x

Vuralli, D., Wattiez, A. S., Russo, A. F., & Bolay, H. (2019). Behavioral and cognitive animal models in headache research Cenk Ayata. In *Journal of Headache and Pain* (Vol. 20, Issue 1). https://doi.org/10.1186/s10194-019-0963-6

Yalamuri, S. M., Brennan, T. J., & Spofford, C. M. (2013). Neuropeptide y is analgesic in rats after plantar incision. *European Journal of Pharmacology*, *698*(1–3). https://doi.org/10.1016/j.ejphar.2012.10.036

Zale, E. L., Maisto, S. A., & Ditre, J. W. (2015). Interrelations between pain and alcohol: An integrative review. In *Clinical Psychology Review*. https://doi.org/10.1016/j.cpr.2015.02.005

Zhang, S., Jin, X., You, Z., Wang, S., Lim, G., Yang, J., McCabe, M., Li, N., Marota, J., Chen, L., & Mao, J. (2014). Persistent nociception induces anxiety-like behavior in rodents: Role of endogenous neuropeptide S. *Pain*, *155*(8). https://doi.org/10.1016/j.pain.2014.04.026

7.3 Artigo 3

Bimodal transcranial direct current stimulation reduces alcohol consumption and induces long-term neurochemical changes in rats with neuropathic pain.

Periódico: Neuroscience Letters

Status: Published online: 07 june 2021

https://doi.org/10.1016/j.neulet.2021.136014

BIMODAL TRANSCRANIAL DIRECT CURRENT STIMULATION REDUCES ALCOHOL CONSUMPTION AND INDUCES LONG-TERM NEUROCHEMICAL CHANGES IN RATS WITH NEUROPATHIC PAIN

DANIELA SILVA SANTOS[1,2,5], LICIANE FERNANDES MEDEIROS[1,2,6], DIRSON JOÃO STEIN[1,2,5], ISABEL CRISTINA DE MACEDO[1,5], DIEGO EVANDRO DA SILVA RIOS[1,5], CARLA DE OLIVEIRA[1,5], ROBERTA STRÖHER TOLEDO[1,3,5], FELIPE FREGNI[4], WOLNEI CAUMO[2], IRACI L. S. TORRES[1,2,3,5*]

[1] Laboratory of Pain Pharmacology and Neuromodulation: Preclinical Investigations - Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil
[2] Postgraduate Program in Medical Sciences, School of Medicine, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil
[3] Postgraduate Program in Biological Sciences: Pharmacology and Therapeutics, Institute of Basic Health Sciences, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil
[4] Laboratory of Neuromodulation, Department of Physical Medicine & Rehabilitation, Spaulding Rehabilitation Hospital and Massachusetts General Hospital, Harvard University, Boston, MA, USA
[5] Animal Experimentation Unit and Graduate Research Group, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil
[6] Postgraduate Program in Health and Human Development, Universidade La Salle, Canoas, RS, Brazil

*Corresponding Author: iltorres@hcpa.edu.br
**Send all co-authors email ID for communications: [Note- all authors agree for publication of their names and author list, order is correct. All authors agree that no modification to the author list can be made without the formal approval letter from all authors]
* Article is original work and not having self-plagiarism
All authors have participated in the research of this article, and have read, reviewed, agree and approved with the content of the article
*Author taken: Laboratory / Institute Approval: Approval letter / NOC email / email / confirmation from all Co-Authors / Principle Investigator/ Major Advisor/ Research Guide / Chairperson of research / Faculty Participant
**The research protocol / methodology was approved by the institutional review committee / Principle Investigator/ Major Advisor/ Research Guide / Chairperson of research / Faculty Participant
If need: Laboratory / Institute Approval: Approval letter / NOC email / email from all Co-Authors / Principle Investigator / Research Guide / Chairperson of research

Abstract- This study aimed to evaluate the effects of repeated bimodal transcranial direct current stimulation (tDCS) on alcohol consumption and immunohistological and neurochemical parameters in nerve-injured rats. Forty-eight adult male Wistar rats were distributed into six groups: control, neuropathic pain (NP)+sham-tDCS, NP+alcohol+sham-tDCS, alcohol+sham-tDCS, alcohol+tDCS, and NP+alcohol+tDCS. NP is induced by chronic sciatic nerve constriction (CCI). The rats were exposed to a 10% alcohol solution by voluntary consumption for 14 days. From the 16th day after surgery, bimodal tDCS was applied for 20 minutes/day for 8 days. Brain structures were collected to evaluate the number of neuropeptide Y (NPY)-positive neurons, neurites, and argyrophilic grains by immunohistochemistry, and brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), interleukin (IL)-6, and IL-10 by ELISA. Nerve-injured rats showed a progressive increase in alcohol consumption compared to the non-injured rats. In addition, there was a reduction in voluntary alcohol consumption over time induced by tDCS. Alcohol exposure, chronic pain, and tDCS treatment modulated the central NPY immunoreactivity. tDCS increased the cerebellar levels of IL-6 and IL-10, and CCI and/or tDCS reduced striatal BDNF levels. The current data suggest that tDCS could be a promising non-pharmacological adjuvant to treat patients with chronic pain who use alcohol to relieve their symptoms.

Keywords- Chronic pain, Alcohol, tDCS, Neuropeptide Y, Cytokines, Rats

Introduction

Alcohol provides an accessible means for chronic pain patients to relieve their symptoms, despite its potential consequences for long-term health, such as alcohol dependence and alcohol withdrawal syndrome. Approximately 25% of patients with chronic pain consume alcohol to relieve their symptoms [1]. However, chronic alcohol intake is known to induce selective neuronal damage [2] and exacerbates pain, with marked increases in sensitivity occurring after

a period of abstinence [3,4]. Both pain and chronic alcohol consumption result in the interaction of a broad range of physiological mechanisms in the nervous system [5,6]. Several biochemical markers seem to be involved in both chronic alcohol consumption and the development of chronic pain.

The development of alcohol dependency induces an adaptive process in the amygdala-NPY system, as well as in the levels of brain-derived neurotrophic factor (BDNF) in areas such as the hippocampus, cerebral cortex, and striatum [7]. In rodents, NPY regulates negative affective states, anxiety-like behavior, nociception, and reward [7]. Furthermore, through its action on the dopaminergic system, NPY may modulate reward circuitry in animals subjected to alcohol consumption [7]. Preclinical studies have shown significant and temporally dynamic changes in the central and peripheral cytokines under pain conditions [8,9] and alcohol exposure [10].

Considering the complexity of chronic pain and alcohol dependence, it is important to search for new therapeutic options. Neuromodulatory techniques have been used to relieve chronic pain and drug cravings, including alcohol dependence [11]. Particularly, transcranial direct current stimulation (tDCS) is a safe and low-cost tool that can be easily applied to such therapeutic options [12]. Our previous preclinical studies have shown short- and long-term effects on nociceptive parameters after tDCS treatment in different chronic pain models, altering central cytokine (IL-1β, IL-10, TNF-α), and neurotrophin (BDNF) levels [8,13,14,15,16,17]. However, there have been no studies on the effects of tDCS on chronic pain associated with alcohol exposure. In this way, it has been suggested that noninvasive brain stimulation may represent a promising alternative treatment when these conditions are associated.

Thus, in the current study, considering that chronic pain can trigger alcohol consumption in individuals to relieve their symptoms and induce alcohol dependence, we aimed to evaluate the

effects of repeated bimodal transcranial direct current stimulation (tDCS) on alcohol consumption and immunohistological and neurochemical parameters in nerve-injured rats.

Materials and Methods

An expanded version of all methods is available in the Supplementary Methods section.

Animals

Forty-eight adult male Wistar rats (200–250 g) from the Center for Reproduction and Experimentation of Laboratory Animals at Universidade Federal do Rio Grande do Sul/Brazil were used in this study. Initially, animals were randomized by weight and maintained in groups of four per cage (49 × 34 × 16 cm). Rats were housed in a vivarium under standard environmental conditions under a 12-hour light/dark cycle (lights on at 7 a.m.). The animals had ad libitum access to water and rodent chow. All experimental procedures were approved by the Institutional Animal Care and Use Committee (GPPG-HCPA#15.0501). The experimental protocol complied with the ethical and methodological standards of the ARRIVE guidelines [18].

Experimental design

The rats were acclimated to the vivarium for 15 d. The rats were randomly assigned by weight to six groups (n = 8/group): control (CT) - no manipulation; neuropathic pain (NP) - chronic sciatic nerve constriction injury (CCI) plus sham tDCS; NP+alcohol (NPAL) - CCI and alcohol administration plus sham-tDCS; alcohol (AL) - alcohol administration plus sham-tDCS; AL+tDCS (ALtDCS) - alcohol administration and tDCS treatment; and NP+AL+tDCS (NPALtDCS) - CCI, alcohol administration, and tDCS treatment. The sequence of steps and experimental group sizes are elaborated in the experimental design (Figure 1). All investigators were blinded to the treatment so that the bias between the groups receiving active or sham tDCS treatment could be diminished. Three researchers analyzed the results; importantly, these

evaluators were unaware of the experimental protocol. Rats were killed by decapitation 7 days after the end of the tDCS treatment and alcohol withdrawal.

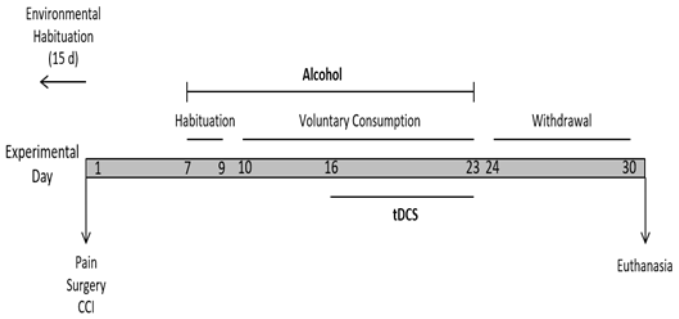

Figure 1. Experimental timeline. CCI: chronic constriction injury; tDCS: transcranial direct current stimulation.

Neuropathic pain model

NP was induced by CCI of the sciatic nerve according to the method described by Bennett and Xie [19].

Model of alcohol exposure

The protocol for voluntary ethanol consumption in the two-bottle choice model was adapted from Carnicella et al. [20].

Transcranial direct current stimulation (tDCS)

Rats were subjected to bimodal tDCS (0.5 mA) under immobilization for 20 min per day for 8 consecutive days, starting on the 16th day after CCI surgery [14,8,17].

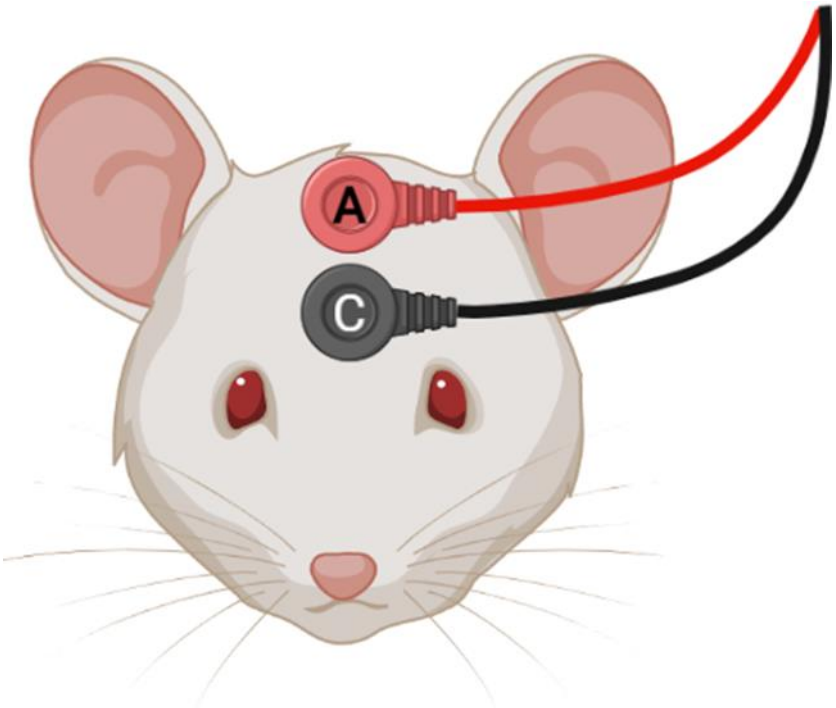

Figure 2. Electrode placement on the rat scalp during tDCS sessions. A = Anode; C = Cathode. Created with BioRender.com

Immunohistochemistry

Two animals were used for evaluating the brain structures (PFC, amygdala, and striatum) that had been fixed in 10% buffered formaldehyde, processed, and embedded in paraffin. An average of 16 fields were used to obtain the immunoreactivity of NPY in neurons, neurites, and argyrophilic grains per field per rat, and the average was obtained between two rats per group. Numbers indicate an increase or decrease of staining in comparison to the control group (% of control of immunoreactive cells/field) (Table 1). Expressive changes were conventionally considered when there was an increase or decrease in NPY staining in over 40% of the control group. The results were calculated as the percentage of control and expressed as delta variation of control (value of group -100).

Determination of biomarker central levels

The levels of BDNF, NGF, IL-6, and IL-10 were determined using a sandwich enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, USA), which uses specific monoclonal antibodies.

Statistical analysis

Data are expressed as the mean ± standard error of the mean (SEM). NP establishment was detected by the von Frey test at baseline and 14 days after surgery, and alcohol consumption analysis was carried out using generalized estimated equations (GEEs) followed by Bonferroni's correction. The levels of biomarkers were analyzed using one-way ANOVA followed by the Student–Newman–Keuls (SNK) method. The immunohistochemical detection of NPY was calculated as the percentage of control of the number of immunoreactive cells per field and expressed as delta variation of the control. Statistical significance was set at $P < 0.05$. Statistical analysis was performed using SPSS version 26.0.

Results

Data from the nociceptive tests were reported in the Supplementary Results section (Supplementary Figure 1).

Alcohol consumption

There was an interaction between group and time (GEE: Wald $\chi 2 = 9929.081$, $P < 0.001$). On day 11, nerve-injured rats consumed less alcohol than non-injured rats. On days 13 and 15, alcohol consumption decreased in all the groups. On day 17, a peak of alcohol consumption was observed in all groups, except in the non-injured groups subjected to tDCS. On day 19, there was a decrease in alcohol consumption in all groups; however, the consumption of injured rats increased from this point on. On day 23, the nerve-injured rats exposed to alcohol (NPAL) increased their consumption, while both groups subjected to tDCS (ALtDCS and NPALtDCS) showed less alcohol consumption, and the AL group presented stabilized levels of alcohol

consumption. It is interesting to note that rats in pain increased alcohol consumption over time, while pain-free rats showed decreased alcohol consumption (Figure 3; Supplementary Figure 2; Supplementary Table 1).

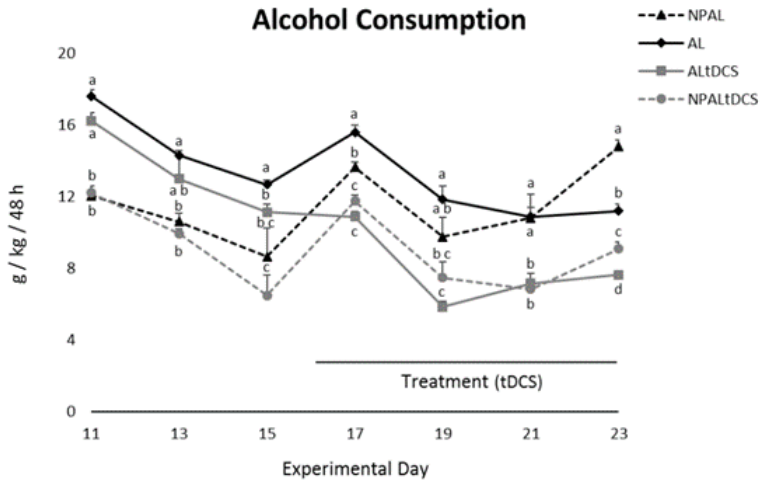

Figure 3. Measurement of alcohol consumption. Data were expressed as mean ± S.E.M. Different letters show significant differences between groups. There was a significant effect of time (GEE, P < 0.05) and group (GEE, P < 0.001). There was an interaction between group and time (GEE, P < 0.001). AL: alcohol group; NPAL: neuropathic pain plus alcohol group; ALtDCS: alcohol plus tDCS group; NPALtDCS: neuropathic pain plus alcohol plus tDCS group. n = 8 / group.

Neuropeptide Y

Alcohol exposure, nerve injury, and tDCS treatment modulated central NPY immunoreactivity (Table 1). Analysis of the PFC showed that the CCI induced an increase in the immunoreactivity for NPY in the argyrophilic grains (169.38%); the NPAL group showed an increase in neurites (91.14%) and argyrophilic grains (205.26%); and the AL group showed increased immunoreactivity in neurites (100.24%) and argyrophilic grains (255.98%). The rats subjected to alcohol consumption plus tDCS treatment (ALtDCS group) showed an increase in NPY immunoreactivity in argyrophilic grains (184.78%); the NPALtDCS group showed increased

NPY in neurites (54.73%) and argyrophilic grains (96.17%) (Figure 4; Table 1).

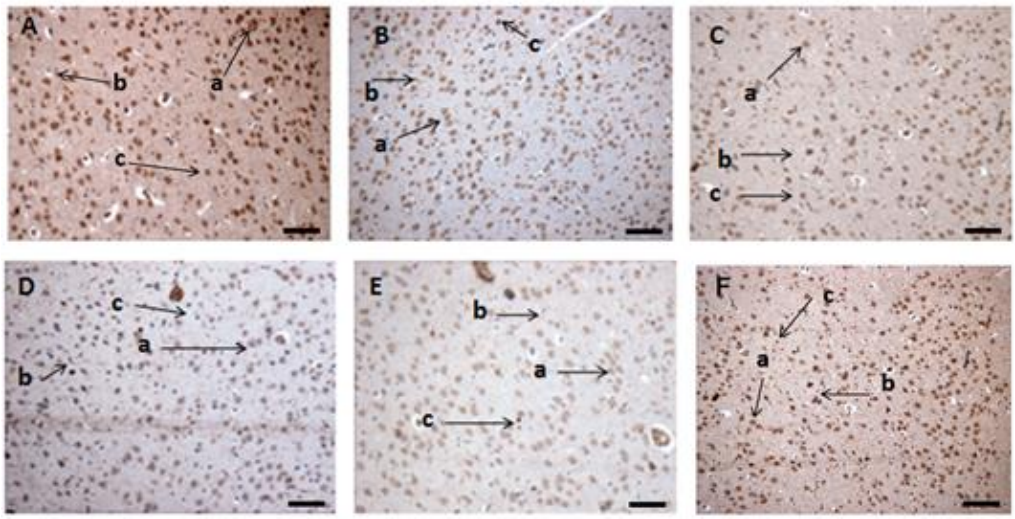

Figure 4. Representative images of NPY immunodetection in neurons (a), neurites (b), and argyrophilic grains (c) in the Prefrontal Cortex (Image-Pro Plus 7.0, Media Cybernetics, Rockville, MD, USA). (A) CT: control group; (B) NP: neuropathic pain group; (C) NPAL: neuropathic pain plus alcohol group; (D) AL: alcohol group; (E) ALtDCS: alcohol plus tDCS group; (F) NPALtDCS: neuropathic pain plus alcohol plus tDCS group. Magnification: 40X; Scale bars: 500μm.

The amygdala analysis showed that the NP group presented an increase in the number of NPY-positive neurons (117.29%), neurites (115.66%), and argyrophilic grains (66.97%). The NPAL group showed an increase in NPY immunoreactivity in neurites (129.38%); however, the increase in neurons induced by CCI was reversed when the rats were exposed to alcohol (a decrease of 12.91%), while the argyrophilic grains (an increase of 12%) were similar to control levels. The AL group showed an increase in immunoreactivity in neurites (89.40%), while the NPALtDCS group showed a decrease in neurons (46.19%), reverting the pain effect, and an increase in the immunoreactivity for NPY in neurites (136.70%) and argyrophilic grains

(42.71%) (Figure 5; Table 1).

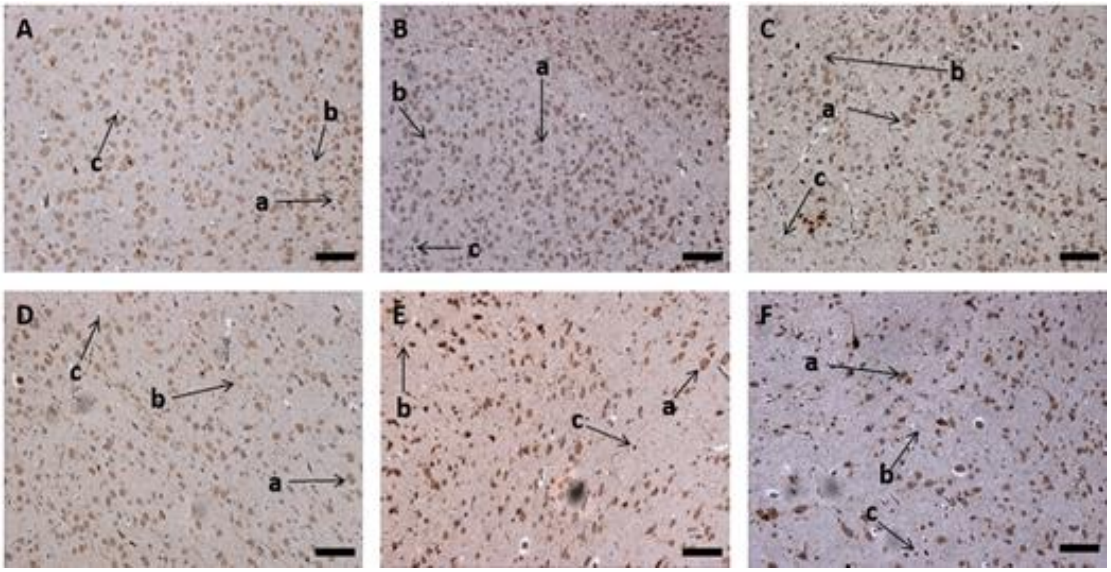

Figure 5. Representative images of NPY immunodetection in neurons (a), neurites (b), and argyrophilic grains (c) in the amygdala (Image-Pro Plus 7.0, Media Cybernetics, Rockville, MD, USA). (A) CT: control group; (B) NP: neuropathic pain group; (C) NPAL: neuropathic pain plus alcohol group; (D) AL: alcohol group; (E) ALtDCS: alcohol plus tDCS group; (F) NPALtDCS: neuropathic pain plus alcohol plus tDCS group. Magnification: 40X; Scale bars: 500µm.

The striatum analysis showed that NPY immunoreactivity decreased in the NP group (56.46% in neurons; 61.19% in neurites), AL group (57.21% in neurons), and ALtDCS group (60.36% in neurons; 56.72% in neurites). The animals exposed to CCI, alcohol, and tDCS presented a

reversal of these decreases in immunoreactivity (Figure 6; Table 1).

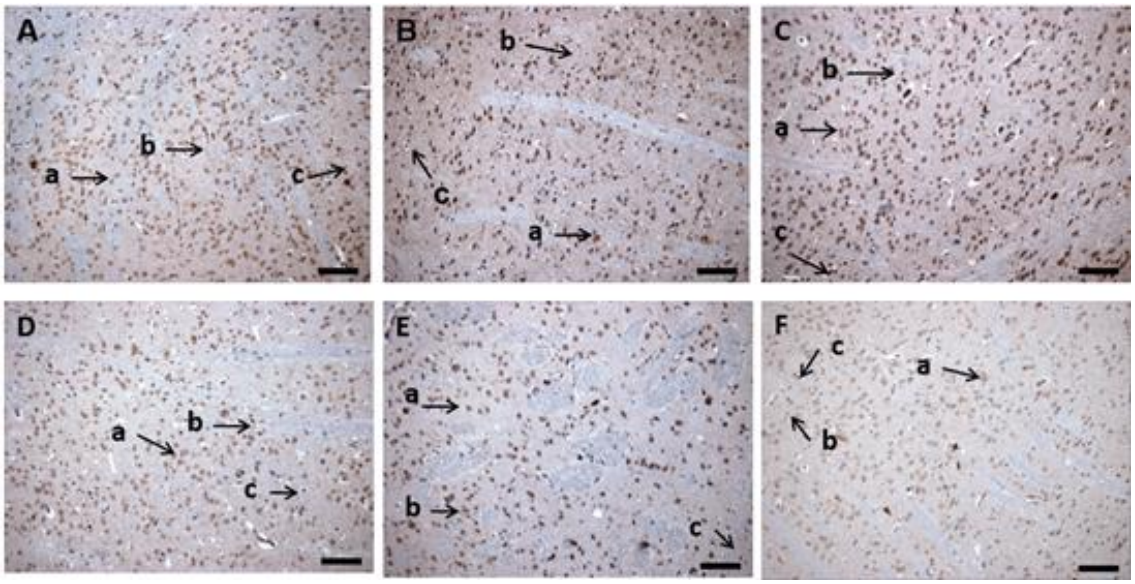

Figure 6. Representative images of NPY immunodetection in neurons (a), neurites (b), and argyrophilic grains (c) in the striatum (Image-Pro Plus 7.0, Media Cybernetics, Rockville, MD, USA). (A) CT: control group; (B) NP: neuropathic pain group; (C) NPAL: neuropathic pain plus alcohol group; (D) AL: alcohol group; (E) ALtDCS: alcohol plus tDCS group; (F) NPALtDCS: neuropathic pain plus alcohol plus tDCS group. Magnification: 40X; Scale bars: 500μm.

BDNF, NGF, IL-6, and IL-10 levels in the cerebellum and striatum

In the cerebellum, the ALtDCS group presented an increase in IL-6 levels compared to the NPAL group (one-way ANOVA/SNK, $F(5,42) = 2.61$, $P < 0.05$), and IL-10 levels compared to the other groups (one-way ANOVA/SNK: $F(5,42) = 2.92$, $P < 0.05$). There were no differences in cerebellar BDNF and NGF levels between groups (one-way ANOVA: $P > 0.05$, for both) (Table 2).

In the striatum, the NPAL, ALtDCS, and NPALtDCS groups showed lower BDNF levels than the CT group (one-way ANOVA/SNK, $F(5,42) = 5.24$, $P < 0.05$). In addition, the NP group showed a significant decrease in the CT and AL groups (one-way ANOVA/SNK: $F(5,42) =$

5.24, $P < 0.001$). The striatal NGF, IL-10, and IL-6 levels were not different between groups (one-way ANOVA, $P > 0.05$, for all) (Table 2).

Discussion

In the current study, we showed that tDCS decreases alcohol consumption, an effect that is more pronounced in non-injured rats. The alcohol consumption level is dependent on the state of the animal; while the non-injured animals consumed more alcohol initially, during the treatment, it was the injured animals that consumed more alcohol, as observed on day 23 (NPAL group). The peak of alcohol consumption was observed on day 17, except for the non-injured rats subjected to tDCS treatment. Alcohol exposure, CCI, and tDCS treatment modulated NPY immunoreactivity in the PFC, amygdala, and striatum. Moreover, only the association between alcohol withdrawal and tDCS treatment increased the cerebellar levels of IL-6 and IL-10. The striatal BDNF levels were decreased by CCI and tDCS treatment, demonstrating the long-term effect of tDCS (7 days after the end of treatment).

The increased alcohol consumption over time induced by CCI may be related to its analgesic effect, as suggested previously [21], and corroborated by a systematic review suggesting alcohol-inducing pain relief [22]. A study showed an inverse relationship between drinking levels and hyperalgesia over four weeks in rats with inflammatory pain, without an increase in the absolute value of alcohol consumption over time, corroborating the analgesic effect [23]. However, the mechanisms of action remain unclear [24]. Currently, it is believed that the neurotransmitter systems and inflammation are common pathways involved in both pathologies: chronic pain [8] and alcohol withdrawal [25]. In addition, previous studies have shown that the affective and sensory dimensions of pain may be important factors in alcohol abstinence syndrome, and the relief of negative emotional states can trigger alcohol consumption [26,27]. Our results corroborate clinical studies, where repeated tDCS applied bilaterally over the PFC is a promising adjunctive tool to reduce alcohol craving and relapse,

facilitating alcoholism cessation [28,29]. We have also shown that tDCS inhibits food cravings in rats [30]. Both drugs and food are known to develop addiction by activation of brain circuitries involved in reward, motivation, and decision-making processes [31].

The current study showed that CCI promoted a more pronounced decrease in striatal BDNF levels than other conditions, and alcohol consumption and tDCS increased the cerebellar levels of IL-6 and IL-10. Like cytokines, BDNF is an important regulator of synaptic plasticity and memory formation [32]. Our previous study showed an increase in PFC BDNF levels and IL-10 levels in the hippocampus, PFC, and brainstem in rats even after 11 days of alcohol withdrawal [25]. Proinflammatory cytokines, such as IL-6, play a major role in initiating and sustaining inflammatory events, and their roles are tissue-specific and can be altered by alcohol [33], and by tDCS [8,9,13,14]. The striatum and cerebellum participate in the accessory motor system, acting in fine-tuning motor movements, which can be related to the tDCS effects and alcohol abstinence observed in the current study. A previous study showed that patients abstaining from alcohol had reduced inhibitory control and higher trait impulsivity, which characterized a dysfunction in the neural inhibitory ability during movement preparation [34]. Thus, we suggest that tDCS over the cerebral cortex of rats can modify inhibitory processes, decrease alcohol consumption, and alter biomarkers.

To the best of our knowledge, this is the first study to demonstrate the effects of tDCS treatment on NPY-immunoreactivity in alcohol consumption/withdrawal. NPY has been shown to modulate both alcohol consumption and aversion-resistant intake, which may be a secondary effect of prolonged alcohol consumption [35]. PFC, amygdala, and striatum were chosen by their involvement in the rewarding system [36], and drug addiction [37]. NPY is associated with both alcohol consumption/withdrawal and chronic pain [38,39]. We observed that CCI modulated the NPY-immunoreactivity in the PFC and in the amygdala, increasing its levels, and decreasing it in the striatum; however, the association with alcohol reversed this effect in

the amygdala. In addition, tDCS reversed the increased NPY-immunoreactivity by alcohol consumption/withdrawal in the PFC, demonstrating the modulatory effect of tDCS. Moreover, the association between alcohol and tDCS induced a decrease in NPY immunoreactivity in the amygdala. These results suggest a modulatory effect of tDCS and alcohol consumption in a maladaptive state induced by CCI.

In the PFC, alcohol, CCI, and tDCS treatment altered NPY immunoreactivity, characterizing a greater sensitivity of argyrophilic grains to these interventions, and cells' specific NPY response. The PFC participates in decision-making, executive function, and reward circuitry [40,41,42]. In addition, individuals with chronic pain have been hypothesized to present deficits in PFC functioning and may be more susceptible to alcohol misuse and poor pain management [26]. Considering that argyrophilic grains are granular or punctuate deposits related to neurodegenerative disorders [43], the increase in NPY immunoreactivity in the grains from the PFC may be involved in these harmful effects.

Finally, some limitations of our study should be pointed out: (1) we did not evaluate the cause-effect relationship between the central NPY immunoreactivity and alcohol exposure or tDCS; (2) the groups of non-injured rats did not undergo surgery, anesthesia, or analgesic procedures. Thus, the possible effect on alcohol consumption cannot be discarded; (3) there is no sham-tDCS group; however, to replace this, we opted to immobilize rats from all groups except for the control.

Conclusion

Our results show that the level of alcohol consumption is dependent on the state of the animal, and bimodal tDCS treatment decreases alcohol consumption independent of the presence of CCI in rats. Considering our results, and given that the changes in the NPY and BDNF levels were modulated in the striatum, we believe that an investigation of the relationship between NPY and BDNF may clarify aspects of pain and tDCS effects. Thus, these results suggest that

tDCS can be a non-pharmacological adjuvant for treating alcohol consumption or withdrawal symptoms in patients with chronic pain who use alcohol to relieve their symptoms.

References

[1] Riley, J.L., King, C., 2009. Self-Report of Alcohol Use for Pain in a Multi-Ethnic Community Sample. J. Pain. https://doi.org/10.1016/j.jpain.2009.03.005

[2] Jung, M.E., Jacobs, S., Rewal, M., Wilson, A., Simpkins, J.W., 2005. Estradiol protects against alteration of protein kinase C (epsilon) in a binge model of ethanol dependence and withdrawal. Eur. J. Pharmacol. 16, 62–72. https://doi.org/10.1016/j.ejphar.2005.03.038

[3] Boissoneault, J., Lewis, B., Nixon, S.J., 2019. Characterizing chronic pain and alcohol use trajectory among treatment-seeking alcoholics. Alcohol. https://doi.org/10.1016/j.alcohol.2018.05.009

[4] Edwards, S., Vendruscolo, L.F., Schlosburg, J.E., Misra, K.K., Wee, S., Park, P.E., Schulteis, G., Koob, G.F., 2012. Development of mechanical hypersensitivity in rats during heroin and ethanol dependence: Alleviation by CRF1 receptor antagonism. Neuropharmacology. https://doi.org/10.1016/j.neuropharm.2011.11.006

[5] Diatchenko, L., Fillingim, R.B., Smith, S.B., Maixner, W., 2013. The phenotypic and genetic signatures of common musculoskeletal pain conditions. Nat. Rev. Rheumatol. https://doi.org/10.1038/nrrheum.2013.43

[6] Nardone, R., Bergmann, J., Christova, M., Lochner, P., Tezzon, F., Golaszewski, S., Trinka, E., Brigo, F., 2012. Non-invasive brain stimulation in the functional evaluation of alcohol effects and in the treatment of alcohol craving: A review. Neurosci. Res. https://doi.org/10.1016/j.neures.2012.08.003

[7] Gilpin, N.W., 2012. Neuropeptide Y (NPY) in the extended amygdala is recruited during the transition to alcohol dependence. Neuropeptides. https://doi.org/10.1016/j.npep.2012.08.001

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

[9] Filho, P.R.M., Vercelino, R., Cioato, S.G., Medeiros, L.F., de Oliveira, C., Scarabelot, V.L., Souza, A., Rozisky, J.R., Quevedo, A. da S., Adachi, L.N.S., Sanches, P.R.S., Fregni, F., Caumo, W., Torres, I.L.S., 2016. Transcranial direct current stimulation (tDCS) reverts behavioral alterations and brainstem BDNF level increase induced by neuropathic pain model: Long-lasting effect. Prog. Neuro-Psychopharmacology Biol. Psychiatry. https://doi.org/10.1016/j.pnpbp.2015.06.016

[10] Doremus-Fitzwater, T.L., Buck, H.M., Bordner, K., Richey, L., Jones, M.E., Deak, T., 2014. Intoxication- and Withdrawal-Dependent Expression of Central and Peripheral Cytokines Following Initial Ethanol Exposure. Alcohol. Clin. Exp. Res. https://doi.org/10.1111/acer.12481

[11] Lefaucheur, J.P., Antal, A., Ayache, S.S., Benninger, D.H., Brunelin, J., Cogiamanian, F., Cotelli, M., De Ridder, D., Ferrucci, R., Langguth, B., Marangolo, P., Mylius, V., Nitsche, M.A., Padberg, F., Palm, U., Poulet, E., Priori, A., Rossi, S., Schecklmann, M., Vanneste, S., Ziemann, U., Garcia-Larrea, L., Paulus, W., 2017. Evidence-based guidelines on the therapeutic use of transcranial direct current stimulation (tDCS). Clin. Neurophysiol. https://doi.org/10.1016/j.clinph.2016.10.087

[12] Schulze-Bonhage, A., 2017. Brain stimulation as a neuromodulatory epilepsy therapy. Seizure. https://doi.org/10.1016/j.seizure.2016.10.026

[13] Santos, D.S., Lopes, B.C., Medeiros, L.F. et al., 2020. Transcranial Direct Current Stimulation (tDCS) Induces Analgesia in Rats with Neuropathic Pain and Alcohol Abstinence. Neurochem. Res. 45, 2653–2663. https://doi.org/10.1007/s11064-020-03116-w

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

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

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

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

[18] Kilkenny, C., Browne, W.J., Cuthill, I.C., Emerson, M., Altman, D.G., 2013. Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. Animals. https://doi.org/10.3390/ani4010035

[19] Bennett, G.J., Xie, Y.K., 1988. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain. https://doi.org/10.1016/0304-3959(88)90209-6

[20] Carnicella, S., Ron, D., Barak, S., 2014. Intermittent ethanol access schedule in rats as a preclinical model of alcohol abuse. Alcohol 48, 243–52. https://doi.org/10.1016/j.alcohol.2014.01.006

[21] Scott, J.R., Hassett, A.L., Schrepf, A.D., Brummett, C.M., Harris, R.E., Clauw, D.J., Harte, S.E., 2018. Moderate Alcohol Consumption Is Associated with Reduced Pain and Fibromyalgia Symptoms in Chronic Pain Patients. Pain Med. https://doi.org/10.1093/pm/pny032

[22] Thompson, T., Oram, C., Correll, C.U., Tsermentseli, S., Stubbs, B., 2017. Analgesic Effects of Alcohol: A Systematic Review and Meta-Analysis of Controlled Experimental Studies in Healthy Participants. J Pain. https://doi.org/10.1016/j.jpain.2016.11.009

[23] McGinn, A.M., Edwards, K.N., Edwards, S., 2020. Chronic inflammatory pain alters alcohol-regulated frontocortical signaling and associations between alcohol drinking and thermal sensitivity. Neurobiol. Pain. https://doi.org/10.1016/j.ynpai.2020.100052

[24] Horn-Hofmann, C., Büscher, P., Lautenbacher, S., Wolstein, J., 2015. The effect of nonrecurring alcohol administration on pain perception in humans: a systematic review. J. Pain Res. https://doi.org/10.2147/JPR.S79618

[25] Schunck, R.V.A., Torres, I.L.S., Laste, G., De Souza, A., Macedo, I.C., Valle, M.T.C., Salomón, J.L.O., Moreira, S., Kuo, J., Arbo, M.D., Dallegrave, E., Leal, M.B., 2015. Protracted alcohol abstinence induces analgesia in rats: Possible relationships with BDNF and interleukin-10. Pharmacol. Biochem. Behav. https://doi.org/10.1016/j.pbb.2015.05.011

[26] Egli, M., Koob, G.F., Edwards, S., 2012. Alcohol dependence as a chronic pain disorder. Neurosci. Biobehav. Rev. https://doi.org/10.1016/j.neubiorev.2012.07.010

[27] Heilig, M., Egli, M., Crabbe, J.C., Becker, H.C., 2010. Acute withdrawal, protracted abstinence, and negative affect in alcoholism: Are they linked? Addict. Biol. https://doi.org/10.1111/j.1369-1600.2009.00194.x

[28] Klauss, J., Anders, Q.S., Felippe, L. V., Nitsche, M.A., Nakamura-Palacios, E.M., 2018. Multiple sessions of transcranial direct current stimulation (tDCS) reduced craving and relapses for alcohol use: A randomized placebo-controlled trial in alcohol use disorder. Front. Pharmacol. https://doi.org/10.3389/fphar.2018.00716

[29] Boggio, P.S., Sultani, N., Fecteau, S., Merabet, L., Mecca, T., Pascual-Leone, A., Basaglia, A., Fregni, F., 2008. Prefrontal cortex modulation using transcranial DC stimulation reduces alcohol craving: A double-blind, sham-controlled study. Drug Alcohol Depend. https://doi.org/10.1016/j.drugalcdep.2007.06.011

[30] Macedo, I.C., de Oliveira, C., Vercelino, R., Souza, A., Laste, G., Medeiros, L.F., Scarabelot, V.L., Nunes, E.A., Kuo, J., Fregni, F., Caumo, W., Torres, I.L.S., 2016. Repeated transcranial direct current stimulation reduces food craving in Wistar rats. Appetite. https://doi.org/10.1016/j.appet.2016.03.014

[31] Murdaugh, D.L., Cox, J.E., Cook, E.W., Weller, R.E., 2012. FMRI reactivity to high-calorie food pictures predicts short- and long-term outcome in a weight-loss program. Neuroimage. https://doi.org/10.1016/j.neuroimage.2011.10.071

[32] Bekinschtein, P., Cammarota, M., Medina, J.H., 2014. BDNF and memory processing. Neuropharmacology. https://doi.org/10.1016/j.neuropharm.2013.04.024

[33] Crews, F.T., Bechara, R., Brown, L.A., Guidot, D.M., Mandrekar, P., Oak, S., Qin, L., Szabo, G., Wheeler, M., Zou, J., 2006. Cytokines and alcohol, in: Alcoholism: Clinical and Experimental Research. https://doi.org/10.1111/j.1530-0277.2006.00084.x

[34] Zhou, L., Zhan, B., He, W., Luo, W., 2019. Commentary: Deficient Inhibition in Alcohol-Dependence: Let's Consider the Role of the Motor System! Front. Neurosc. https://doi.org/10.3389/fnins.2019.00876

[35] Lesscher, H.M., Houthuijzen, J.M., Groot Koerkamp, M.J., Holstege, F.C., Vanderschuren, L.J., 2012. Amygdala 14-3-3ζ as a novel modulator of escalating alcohol intake in mice. PloS One. https://doi.org/10.1371/journal.pone.0037999

[36] Volkow, N.D., Wang, G.J., Fowler, J.S., Tomasi, D., Telang, F., 2011. Addiction: Beyond dopamine reward circuitry. Proc. Natl. Acad. Sci. U. S. A. https://doi.org/10.1073/pnas.1010654108

[37] Koob, G.F., Volkow, N.D., 2010. Neurocircuitry of addiction. Neuropsychopharmacology. https://doi.org/10.1038/npp.2009.110

[38] Diaz-delCastillo, M., Woldbye, D.P.D., Heegaard, A.M., 2018. Neuropeptide Y and its Involvement in Chronic Pain. Neuroscience. https://doi.org/10.1016/j.neuroscience.2017.08.050

[39] Thorsell, A., Mathé, A.A., 2017. Neuropeptide Y in alcohol addiction and affective disorders. Front. Endocrinol. (Lausanne). https://doi.org/10.3389/fendo.2017.00178

[40] D'Esposito, M., Detre, J.A., Alsop, D.C., Shin, R.K., Atlas, S., Grossman, M., 1995. The neural basis of the central executive system of working memory. Nature. https://doi.org/10.1038/378279a0

[41] Fuster, J.M., 2000. Executive frontal functions. Exp. Brain Res. https://doi.org/10.1007/s002210000401

[42] Hyman, S.E., Malenka, R.C., Nestler, E.J., 2006. Neural Mechanisms of Addiction: The Role of Reward-Related Learning and Memory. Annu. Rev. Neurosci. https://doi.org/10.1146/annurev.neuro.29.051605.113009

[43] Braak, H., & Braak, E., 1998. Argyrophilic grain disease: frequency of occurrence in different age categories and neuropathological diagnostic criteria. Journal of Neural Transmission 105, 801–819. https://doi.org/10.1007/s007020050096

VIII. CONSIDERAÇÕES FINAIS

CONSIDERAÇÕES FINAIS

A ineficácia das terapias atuais para as condições de dor crônica e uso nocivo do álcool, pelo menos em parte, resulta da pouca compreensão dos vários mecanismos envolvidos em cada uma dessas condições, assim como na associação delas. Considerando que são transtornos prevalentes, com piora na qualidade de vida, os tratamentos atuais insuficientes e os altos gastos com a saúde e para a economia, há uma busca contínua por novas terapêuticas. Nesta tese buscamos avaliar os efeitos da ETCC como estratégia terapêutica promissora para tratar ambas as doenças, uma vez que é de baixo custo e com poucos efeitos adversos. O uso de modelos animais adequados é essencial para compreensão dos mecanismos e translacionalidade da técnica.

Nesta tese demonstramos que um protocolo de estimulação com ETCC por 8 dias é capaz de, 1. reverter a hiperalgesia térmica induzida pelos modelos de CCI e / ou exposição ao álcool; 2. reverter o comportamento do tipo ansioso induzido pela dor neuropática crônica e dor neuropática associada ao álcool, 3. reduzir o consumo voluntário de álcool independente da presença de CCI. Além disso, vimos que CCI aumentou o consumo de álcool.

Levando em consideração a ampla interferência sobre os processos biológicos das doenças e do tratamento, sugerimos: que a modulação do NPY avaliada por meio da imunoreatividade no hipotálamo e outras estruturas do sistema límbico, a expressão gênica e os níveis das proteínas IL-1α e β em áreas distintas do SNC podem ser mecanismos de ação envoltos na estimulação com a ETCC; que o aumento nos níveis centrais de NGF pode estar relacionado com a neuroplasticidade induzida pelo álcool contribuindo assim para sua cronicidade.

Em resumo, nossos resultados aumentam a compreensão molecular envolta no paradigma da associação da dor neuropática crônica e uso nocivo de álcool e que a ETCC se

caracteriza como uma alternativa terapêutica promissora para estas condições. Apesar dos resultados interessantes obtidos nesta tese, entendemos que ainda há campo a ser melhor explorado tanto na interface da comorbidade dor crônica / uso de álcool quanto nos mecanismos da técnica de ETCC.

IX. PERSPECTIVAS

9. PERSPECTIVAS

Acreditamos que nossos resultados expandem o conhecimento presente no campo de pesquisa sobre a dor crônica e uso nocivo de álcool. Como perspectivas futuras podemos caracterizar o perfil oxidativo em diferentes estruturas cerebrais envoltos para essas doenças. Incluir a investigação mais pormenorizada dos mecanismos de ação envoltos nos receptores – opioidérgico, glutamatérgico e dopaminérgico – através de estimulação ou antagonismo farmacológico dos mesmos. Quanto ao tratamento com a ETCC, a ínsula pode ser alvo para a estimulação cerebral, visto ter muitas funções críticas relacionadas ao vício/sistema de recompensa, mas para isso seria necessário estudar um meio que permita o direcionamento da estimulação à esta estrutura.

X. ANEXOS

*A) **APROVAÇÃO DO COMITÊ DE ÉTICA***

HCPA - HOSPITAL DE CLÍNICAS DE PORTO ALEGRE
GRUPO DE PESQUISA E PÓS-GRADUAÇÃO

COMISSÃO DE ÉTICA NO USO DE ANIMAIS

A Comissão de Ética no Uso de Animais (CEUA/HCPA) analisou o projeto:

Projeto: 150501
Data da Versão do Projeto: 04/11/2015

Pesquisadores:
IRACI LUCENA DA SILVA TORRES
DANIELA SILVA SANTOS

Título: Impacto da Estimulação Transcraniana por Corrente Contínua (ETCC) em ratos Wistar machos submetidos a um modelo de dor crônica e/ou à exposição ao álcool.

Este projeto foi APROVADO em seus aspectos éticos e metodológicos de acordo com as Diretrizes e Normas Nacionais e Internacionais, especialmente a Lei 11.794 de 08/10/2008, que estabelece procedimentos para o uso científico de animais.

- Os membros da CEUA/HCPA não participaram do processo de avaliação de projetos 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.

Porto Alegre, 24 de novembro de 2015.

Biol. Michael Everton Andrades
Coordenador CEUA/HCPA

B) CARTAS RECOMENDAÇÕES

HOSPITAL DE CLÍNICAS
PORTO ALEGRE RS

UFRGS
UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

Porto Alegre, 16 de maio de 2016,

Assunto: Carta de recomendação para aluna Daniela Silva Santos

Recomendo a aluna **Daniela Silva Santos** para o Programa de Bolsa Especial para Doutorado em Pesquisa Médica, disponibilizado pela Capes.

Conheço a candidata desde 2014 quando fui seu professor de farmacologia clínica no 5º semestre da graduação em medicina da Universidade Federal do Rio Grande do Sul. Naquela época, já entusiasmada pela neurociência, a aluna me procurou interessada em participar das reuniões do meu grupo de pesquisa, às quais ela tem comparecido com frequência. Considero a candidata uma ótima aluna e preparada para ingressar no programa.

A Daniela trabalha sob a orientação da professora Dra. Iraci Lucena da Silva Torres, há três anos, em diferentes projetos na área da neuromodulação, usando modelo animal. Através de parcerias, também trabalha em pesquisas clínicas. A aluna demostra destreza na execução de todas as fases de um projeto cientifico, deste a revisão da literatura, a realização de testes comportamentais e ensaios bioquímicos, até a análise estatística e boa interpretação dos resultados. A candidata propôs ainda estudo original sobre a associação dor crônica e o consumo de álcool, bem como a utilização da Estimulação Transcraniana por Corrente Contínua (ETCC), como tratamento não-farmacológico para ambas condições, projeto com o qual pretende receber a bolsa de doutorado em pesquisa médica. Saliento, entretanto, que a candidata já possui resultados importantes nessa área que estão sendo registrado em forma de artigo científico, demonstrando a viabilidade do referido projeto.

Além de dedicada e com aptidão para pesquisa, ela é uma jovem com qualidades humanas e de relacionamento interpessoal. A Daniela apresenta ótimo desempenho acadêmico e atua desde cedo em diferentes aspectos da vida acadêmica. Assim, posso recomendar seu ingresso no programa.

Atenciosamente,

Prof. Dr. Rafael Roesler

Departamento de Farmacologia

Instituto de Ciências Básicas da Saúde

Porto Alegre, 11 de maio de 2015,

De: Prof. Lavínia Schuler Faccini

Assunto: Carta de recomendação da aluna Daniela Silva Santos

Recomendo a aluna **Daniela Silva Santos** para o Programa de Bolsa Especial para Doutorado em Pesquisa Médica, disponibilizado pela Capes.

A candidata foi minha aluna da graduação na Faculdade de Medicina e também bolsista de Extensão no Projeto SIAT - Gravidez Segura da Universidade Federal do Rio Grande do Sul (UFRGS) desde 2013 quando estava em seu terceiro trimestre da faculdade. Além do período de bolsa, desenvolveu também atividades voluntárias ao integrar o grupo de pesquisa de neuromodulação com a professora Iraci Torres.

Durante seu período no SIAT – Sistema de Informação sobre Teratógenos, sob minha coordenação, Daniela teve envolvimento tanto com pacientes como com pesquisa. Publicou diversos resumos em congresso dos trabalhos que desenvolveu conosco enfocando aspectos teratogênicos de anestésicos, retinóides, cosméticos, além de medicamentos na amamentação e a descrição geral do SIAT. Um destes trabalhos está como manuscrito em preparo para ser submetido em breve.

Daniela sempre mostrou interesse na questão dos efeitos do álcool durante a gravidez e está participando de um projeto multidisciplinar sobre a síndrome fetal do álcool em escolares da cidade de Porto Alegre.

Além de sua capacidade de trabalho científica, mostra ainda empenho, dedicação e excelente disposição para trabalhar em grupo. Tenho a satisfação de recomendá-la fortemente para esta modalidade de pós-graduação.

Estou disponível para maiores informações

Prof. Dra. Lavínia Schuler Faccini
Departamento de Genética / Programa de Pós-graduação em Genética e Biologia Molecular
Universidade Federal do Rio Grande do Sul
Serviço de Genética Medica – Hospital de Clinicas de Porto Alegre
lavinia.faccini@ufrgs.br
55 51 9975-6770

C) OUTRAS PUBLICAÇÔES

Capítulos de livros

1. Costa, A.L.C.; Borba, M.A.M.; **Santos, D.S.**; Farias, T.P. Tracheostomy and Obesity. In: Terence Pires de Farias. (Org.). Tracheostomy. 1ed.: Springer International Publishing, 2018, v. 1, p. 161-

2. Borba, M.A.M.; Costa,A.L.C.; **Santos, D.S.**; Farias, T.P.. Bronchoscopy Before and After Tracheostomy. In: Terence Pires de Farias. (Org.). Tracheostomy A Surgical Guide. 1ed.: Springer International Publishing, 2018, v. 1, p. 363-376.

3. **Santos, D.S.**; Freitas, N.M.S.; Morais, S.G. Novas tecnologias e possíveis influências na fundamentação da sentença. In: Denise Pires Fincato - Leiliane Piovesani Vidaletti (Orgs.). (Org.). NOVAS TECNOLOGIAS, PROCESSO E RELAÇÕES DE TRABALHO II. 1ed.: EDITORA CRV, 2017, v., p. 111-

Artigos Publicados

1. Silva, A.A.; Leopoldino, M.; Rocha, A.; Goldani, B.F.; Gomes, D.; **Santos, D.S.**; Vianna, F.S.L.; Matuella, G.; Metzdorf, L.; Bellaver, P.; Abeche, A.M.; Sanseverino, M.T.; Schuler-Faccini, L. Warfarin: A retrospective analysis of consultations to a Brazilian Teratology Information Service. REPRODUCTIVE TOXICOLOGY, v. 57, p. 224, 2015. DOI: https://doi.org/10.1016/j.reprotox.2015.06.035.

2. de Oliveira, C.; de Freitas, J.S.; Macedo, I.C.; Scarabelot, V.L.; Ströher, R.; **Santos, D.S**.; Souza, A.; Fregni, F.; Caumo, W.; Torres, I.L.S. Transcranial direct current stimulation (tDCS) modulates biometric and inflammatory parameters and anxiety-like behavior in obese rats. 2018. doi: 10.1016/j.npep.2018.09.006

3. da Graca-Tarragó, M.; Lech, M.; Dal Moro Angoleri, L.; **Santos, D.S.**; Deitos, A.; Brietzke, A.P.; Torres, I.L.S.; Fregni, F.; Caumo, W. Intramuscular electrical stimulus

potentiates the motor cortex modulation effects on pain and descending inhibitory systems in knee osteoarthritis: a randomized, factorial, sham-controlled study. 2019. doi: https://doi.org/10.2147/JPR.S181019

4. Schüler-Faccini, L.; Sanseverino, M.T.V.; Abeche, A.M.; Vianna, F.S.L; Fraga, L.R.; Rocha, A.G.; da Silva, A.A.; de Souza, P.R.A.; Hilgert, A.H.; Barbosa, C.P.; Kauppinem, C.G.; Martins, D.F.; **Santos, D.S**.; Colpes, G.H.; Ecco, G.;da Silva, M.F.S.H.; Penteado, L.P.; dos Santos, T. From abortion-inducing medications to Zika Virus Syndrome: 27 years experience of the First Teratogen Information Service in Latin America. 2019. DOI: http://dx.doi.org/10.1590/1678-4685-GMB-2018-0111.

Artigo submetido

1. de Oliveira, C.; Scarabelot, V.L; Vercelino, R.; Lopes, B.C.; de Souza, A.; de Freitas, J.S.; Macedo, I.C.; Moreira, S.F.S.; **Santos, D.S**.; Visioli, F.; Caumo, W.; Torres, I.L.S. Effects of Transcranial Direct Current Stimulation on the Inflammatory Responses of Ovariectomized Rats. Submetido.

Resumos expandidos publicados em anais de congressos

1. **Santos, D.S.**; TORRE, I.L.S. Estimulação Elétrica Transcraniana por Corrente Contínua (ETCC) e dor crônica. In: Latin-American Workshop on Computational Neuroscience, 2017, PORTO ALEGRE. Anais LAWCN 2017. PORTO ALEGRE, 2017.

2. Mariluce Anderle; Cristianne Maria Famer Rocha; **Daniela Silva Santos**; et.al. PET observatório de saúde da gerência glória/cruzeiro/cristal: uma construção coletiva e integrada entre ensino e serviço do município de Porto Alegre, RS. 11º Congresso Internacional da Rede Unida. Revista Interface - Comunicação, Saúde, Educação ISSN 1807-5762. 2014.

3. Silva, AA; Leopoldino, MAA; Rocha, AG; Goldani, BF; Gomes, DRC; **Santos, DS**; Vianna, FSL; Matuella, GF; Metzdorf, L; Bellaver, P; Abeche, AM; Sanseverino, MTV; Faccini, LS. Warfarin: a retrospective analysis of consultations to a Brazilian Teratology Information Service. 43th Annual Conference of the European Teratology Society. 2015.

Outras participações em eventos, congressos e outros relacionados

1. II SEMANA NACIONAL DO CÉREBRO. 2013. (congresso)

2. II jornada da Liga de Anestesiologia da PUCRS - Anestesia e Especialidades. 2013. (evento)

3. "**A aplicação da genética forense na elucidação criminal**". VII Semana Acadêmica da Biomedicina UFRGS. 2013. (congresso)

4. Liga de Anestesiologia da PUCRS. 2013-2015

5. **Efeito da estimulação transcraniana por corrente contínua (ETCC) sobre os níveis centrais e periféricos de BDNF de ratos submetidos a um modelo de dor crônica orofacial.** Daniela Silva Santos, Vanessa Leal Scarabelot, Liciane Fernandes Medeiros, Carla de Oliveira, Lauren Naomi Spezia Adachi, Stefania Giotti Cioato, Andressa de Souza, Wolnei Caumo, Iraci Lucena da Silva Torres. 35ª Semana Científica do HCPA. 2015.

6. Prestação de Serviços: ação social e comunitária - Sistema de informação sobre teratógenos (SIAT) – HCPA: Departamento de Genética. Coordenadora: Lavinia Schuler Faccini. 2015 - 2017.

7. **Exposições durante a lactação: uma experiência do Sistema de Informações sobre Agentes Teratogênicos do Hospital de Clínicas de Porto Alegre (SIAT-HCPA).** Katherine Krieser, Artur Hartmann Hilgert, Camila Pocharski Barbosa, Daniela Silva Santos, Luiza Metzdorf, Priscila Bellaver, Victória Campos Dornelles, Equipe SIAT, Maria Teresa Sanseverino, Fernanda Sales Luiz Vianna. 35ª Semana Científica do HCPA. 2015.

8. **Psicofármacos: uma análise retrospectiva de consultas ao Sistema de Informações sobre Agentes Teratogênicos (SIAT).** Victória d'Azevedo Silveira, Georgea Malfatti, Priscila Bellaver, Luísa Grave Gross, Eduardo de Araújo Silva, Daniela Silva Santos, Equipe SIAT, André Anjos da Silva, Alberto Mantovani Abeche. 35ª Semana Científica do HCPA. 2015.

9. Prestação de Serviços: PET Gestão e Educação na Rede de Urgência/Emergência. 260 horas. 2015.

10. **Validação e tradução das escalas de avaliação da incapacidade funcional e de catastrofismo em crianças com dor crônica para o português do brasil.** Luciana Paula Cadore Stefani; Wolnei Caumo; Daniela Silva Santos; Cibelle de Abreu Evaldt; Larissa Schneider; Leticia Kraemer. 63º Congresso Brasileiro de Anestesiologia. 2016. (Estudo Observacional - Poster)

11. **Uso de anestésicos durante a gestação e riscos para o bebê**. Daniela Silva Santos, Marcela Metzdorf, Luiza Metzdorf, Fernanda Salles Luiz Vianna, André Anjos da Silva. Congresso paulista de Anestesiologia – COPA. 2016.

12. **Isoflurano potencializa o efeito analgésico da acupuntura e da eletroacupuntura em ratos.** Daniela Silva Santos, Lauren Naomi Spezia Adachi, Rafael Vercelino, Carla de Oliveira, Iraci Lucena da Silva Torres. Congresso paulista de Anestesiologia – COPA. 2016.

13. **Síndrome do álcool fetal: uma análise clínica e características associadas.** Rocha, AG; Hilgert, AH; Santos, DS; Gross, LG; Souza, PRA; Vianna, FSL; Sanseverino, MTV; Abeche, AM; Silva, AA; Faccini, LS. XIX Congresso gaúcho de ginecologia e obstetrícia. 2016. (Pôster)

14. **Ensaio clínico randomizado com tDCS e Estimulação Intramuscular Elétrica ou grupo Sham na osteartrite de joelho.** Mateus Correa Lech; Daniela Silva Santos; Maria da Graça Lopes Tarragó; Wolnei Caumo. 36ª Semana Científica do HCPA. 2016.

15. **Adaptação e validação para o português do Brasil das escalas de avalição da incapacidade funcional e de catastrofismo em crianças com dor crônica.** Larissa Schneider;

Cibele de Abreu Evaldt; Daniela Silva Santos; Tahiris Martinez Castro; Wolnei Caumo; Luciana Cadore Stefani. 36ª Semana Científica do HCPA. 2016.

16. **Estratégias de otimização do ambulatório de avaliação pré-anestésica ambulatorial (APA) do hospital de clínicas de Porto Alegre: resultados de 1 ano após implementação.** Cibelle de Abreu Evaldt; Daniela Silva Santos; Carolina Alboim; Ronaldo David Costa; Gustavo Somm; Roberta Machado Vidal; Gilmara Souza; Vanda Regina Machado; Luciana Cadore Stefani. 36ª Semana Científica do HCPA. 2016.

17. **Estimulação transcraniana por corrente contínua reverte hiperalgia desencadeada por dor crônica e/ou exposição ao álcool em ratos wistar machos.** Daniela Silva Santos; Isabel Cristina de Macedo; Carla de Oliveira; Lauren Naomi Adachi; Rafael Vercelino; Lisiane Santos da Silva; Natália de Paula Silveira; Diego Evandro da Silva Rios; Camila Silva Muneretto; Iraci Lucena da Silva Torres. 36ª Semana Científica do HCPA. 2016.

18. **Ovariectomia exacerba respostas inflamatórias e sofre modulação pela Estimulação Transcraniana por Corrente Contínua (ETCC) em nível central.** Guilherme Campos Ferreira; Carla de Oliveira; Rafael Vercelino; Vanessa Leal Scarabelot; Lauren Naomi Spezia Adachi; Andressa de Souza; Daniela Silva Santos; Natalia de Paula Silveira; Camila Silva Muneretto; Iraci Lucena da Silva Torres. 36ª Semana Científica do HCPA. 2016.

19. **Análise do espectro clínico da Síndrome do Álcool Fetal e distúrbios associados na população brasileira.** Anastácia Guimarães Rocha; Artur Hrtmann Hilgert; Daniela Silva Santos; Luisa Grave Gross; Paulo Ricardo Assis de Souza; Maria Tereza Vieira Sanseverino; André Anjos da Silva; Lavínia Schuler Faccini. 36ª Semana Científica do HCPA. 2016.

20. **Avaliação da relação entre uso de álcool e malformações congênitas no Hospital de clínicas de Porto Alegre.** Júlio César Loguercio Leite; Bábara Zanetti Patrício de Macedo; Gabriela Peitot Rezende; Daniela Silva Santos; Eduarda Chiesa Ghisleni; Gabriela Raimann;

Janine Alessi; Laura Vedana; Thais Soares Ferreira; Juliano Fockink Guimalhães. 36ª Semana Científica do HCPA. 2016.

21. Liga de anestegiologia HCPA-UFRGS. 2016-2018.

22. Monitora em epidemiologia - 20h semanais. Conceito A. 2016-2017. Orientador: Ricardo de Souza Kuchenbecker.

23. Estagiária no Hospital Nossa Senhora da Conceição no serviço de Anestesiologia em Porto Alegre - RS. 2016: setembro - 40 h/semanais.

24. **Comportamentos catastróficos estão associados ao fator neurotrófico derivado do cérebro (BDNF) em diferentes amostras de pacientes com dor crônica**. Daniela Silva Santos; Luciana da Conceição Antunes; Joice Dickel Segabinazi; Maria da Graça Lopes Tarragó; Andressa Souza; Hugo Ribeiro; Ana Claudia de Souza; Iraci Lucena da Silva Torres; Wolnei Caumo. 37ª Semana Científica do HCPA. 2017. (E-POSTER)

25. Integrante da Comissão científica. 38ª Semana Científica do HCPA. 2018.

26. Estagiária no Centro Hospitalar Universitário Cova da Beira – Covilhã e no Hospital de São Teotónio - Viseu nos serviços de Anestesiologia em Portugal. 2018: março/abril - 40 h/semanais.

27. **Efeito da ETCC na dor neuropática e na exposição/ abstinência alcoólica em ratos wistar sobre o consumo e parâmetros bioquímicos.** Jeovana Ceresa, Daniela Silva Santos, Bettega Costa Lopes, Roberta Ströher, Iraci LS Torres. II SIMPOSIO GAÚCHO DE FARMACOLOGIA. 2018.

28. **Investigação imuno-histológica do NPY envolvido na dor neuropática e na exposição/ abstinência alcoólica em ratos Wistar tratados com ETCC.** Luana X. Marques, Daniela Silva Santos, Isabel Cristina de Macedo, Liciane Fernandes Medeiros, Carla de Oliveira, Iraci LS Torres. II SIMPOSIO GAÚCHO DE FARMACOLOGIA. 2018.

29. Estagiária no Hospital Santa Casa de Misericórdia - UFCSPA no serviço de Anestesiologia em Porto Alegre - RS. 2018: maio - 40 h/semanais.

30. Avaliadora de trabalhos. 40ª Semana Científica do HCPA. 2020.

D) DIAGNOSTICOS DE USO NOCIVO DO ÁLCOOL CONFORME DSM-5

a. TRANSTORNO POR USO DE ÁLCOOL

Transtorno por Uso de Álcool

Critérios Diagnósticos

A. Um padrão problemático de uso de álcool, levando a comprometimento ou sofrimento clinicamente significativos, manifestado por pelo menos dois dos seguintes critérios, ocorrendo durante um período de 12 meses:
   1. Álcool é frequentemente consumido em maiores quantidades ou por um período mais longo do que o pretendido.
   2. Existe um desejo persistente ou esforços malsucedidos no sentido de reduzir ou controlar o uso de álcool.
   3. Muito tempo é gasto em atividades necessárias para a obtenção de álcool, na utilização de álcool ou na recuperação de seus efeitos.
   4. Fissura ou um forte desejo ou necessidade de usar álcool.
   5. Uso recorrente de álcool, resultando no fracasso em desempenhar papéis importantes no trabalho, na escola ou em casa.
   6. Uso continuado de álcool, apesar de problemas sociais ou interpessoais persistentes ou recorrentes causados ou exacerbados por seus efeitos.
   7. Importantes atividades sociais, profissionais ou recreacionais são abandonadas ou reduzidas em virtude do uso de álcool.
   8. Uso recorrente de álcool em situações nas quais isso representa perigo para a integridade física.
   9. O uso de álcool é mantido apesar da consciência de ter um problema físico ou psicológico persistente ou recorrente que tende a ser causado ou exacerbado pelo álcool.
   10. Tolerância, definida por qualquer um dos seguintes aspectos:
      a. Necessidade de quantidades progressivamente maiores de álcool para alcançar a intoxicação ou o efeito desejado.
      b. Efeito acentuadamente menor com o uso continuado da mesma quantidade de álcool.
   11. Abstinência, manifestada por qualquer um dos seguintes aspectos:
      a. Síndrome de abstinência característica de álcool (consultar os Critérios A e B do conjunto de critérios para abstinência de álcool, p. 499-500).
      b. Álcool (ou uma substância estreitamente relacionada, como benzodiazepínicos) é consumido para aliviar ou evitar os sintomas de abstinência.

b. INTOXICAÇÃO POR ÁLCOOL

Intoxicação por Álcool

Critérios Diagnósticos

A. Ingestão recente de álcool.
B. Alterações comportamentais ou psicológicas clinicamente significativas e problemáticas (p. ex., comportamento sexual ou agressivo inadequado, humor instável, julgamento prejudicado) desenvolvidas durante ou logo após a ingestão de álcool.
C. Um (ou mais) dos seguintes sinais ou sintomas, desenvolvidos durante ou logo após o uso de álcool:
   1. Fala arrastada.
   2. Incoordenação.
   3. Instabilidade na marcha.
   4. Nistagmo.
   5. Comprometimento da atenção ou da memória.
   6. Estupor ou coma.
D. Os sinais ou sintomas não são atribuíveis a outra condição médica nem são mais bem explicados por outro transtorno mental, incluindo intoxicação por outra substância.

c. ABSTINÊNCIA DE ÁLCOOL

Abstinência de Álcool

Critérios Diagnósticos

A. Cessação (ou redução) do uso pesado e prolongado de álcool.
B. Dois (ou mais) dos seguintes sintomas, desenvolvidos no período de algumas horas a alguns dias após a cessação (ou redução) do uso de álcool descrita no Critério A:
1. Hiperatividade autonômica (p. ex., sudorese ou frequência cardíaca maior que 100 bpm).
2. Tremor aumentado nas mãos.
3. Insônia.
4. Náusea ou vômitos.
5. Alucinações ou ilusões visuais, táteis ou auditivas transitórias.
6. Agitação psicomotora.
7. Ansiedade.
8. Convulsões tônico-clônicas generalizadas.

C. Os sinais ou sintomas do Critério B causam sofrimento clinicamente significativo ou prejuízo no funcionamento social, profissional ou em outras áreas importantes da vida do indivíduo.
D. Os sinais ou sintomas não são atribuíveis a outra condição médica nem são mais bem explicados por outro transtorno mental, incluindo intoxicação por ou abstinência de outra substância.

d. OUTROS TRANSTORNOS INDUZIDOS POR ÁLCOOL

Outros Transtornos Induzidos por Álcool

Os seguintes transtornos induzidos por álcool são descritos em outros capítulos do Manual, juntamente aos transtornos com os quais compartilham fenomenologia (ver transtornos mentais induzidos por substância/medicamento nestes capítulos): transtorno psicótico induzido por álcool ("Espectro da Esquizofrenia e Outros Transtornos Psicóticos"); transtorno bipolar induzido por álcool ("Transtorno Bipolar e Transtornos Relacionados"); transtorno depressivo induzido por álcool ("Transtornos Depressivos"); transtorno de ansiedade induzido por álcool ("Transtornos de Ansiedade"); transtorno do sono induzido por álcool ("Transtornos do Sono-Vigília"); disfunção sexual induzida por álcool ("Disfunções Sexuais"); e transtorno neurocognitivo maior ou leve induzido por álcool ("Transtornos Neurocognitivos"). Para *delirium* por intoxicação por álcool e *delirium* por abstinência de álcool, ver os critérios e a abordagem de *delirium* no capítulo "Transtornos Neurocognitivos". Esses transtornos induzidos por álcool são diagnosticados em lugar de intoxicação por álcool ou abstinência de álcool apenas quando os sintomas são suficientemente graves para justificar atenção clínica independente.

e. TRANSTORNO RELACIONADO AO ÁLCOOL NÃO ESPECIFICADO

Esta categoria aplica-se a apresentações em que sintomas característicos de um transtorno relacionado ao álcool que causam sofrimento clinicamente significativo ou prejuízo no funcionamento social, profissional ou em outras áreas importantes da vida do indivíduo predominam, mas não satisfazem todos os critérios para qualquer transtorno relacionado ao álcool específico nem para outro transtorno na classe diagnóstica de transtornos relacionados a substâncias e transtornos aditivos.