Faculdade de Medicina

Programa de Pós-Graduação em Medicina: Ciências Médicas

IMPACTO DA ESTIMULAÇÃO TRANSCRANIANA POR CORRENTE CONTÍNUA (ETCC) NA RESPOSTA COMPORTAMENTAL E NEUROQUÍMICA DE RATOS SUBMETIDOS A UM MODELO DE DOR NEUROPÁTICA

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Orientadora: Prof. Dra. Iraci Lucena da Silva Torres

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"A força não provém da capacidade física. Provém de uma vontade indomável."

Mahatma Gandhi

A minha Avó

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RESUMO

A Associação Internacional para Estudos da Dor (IASP) define a dor neuropática como a dor que surge diretamente de uma lesão ou doença que afeta o sistema somatossensorial. Os sintomas mais característicos são a hiperalgesia e a alodinia. Além disso, alterações comportamentais como transtornos de ansiedade são comorbidades comuns associadas à dor crônica com características neuropáticas. Os métodos de neuromodulação transcraniana como a ETCC tem se mostrado promissores no tratamento da dor e de alguns transtornos neuropsiquiátricos, uma vez que parecem promover alterações neuroplásticas em nível central. Sendo assim, neste estudo avaliamos o efeito da ETCC na atividade locomotora e exploratória, no comportamento do tipo ansioso e na plasticidade medular e cortical em ratos submetidos a um modelo de dor neuropática. Foram utilizados 144 ratos machos Wistar com 55-65 dias de idade, divididos em 6 grupos: Sham Cirurgia (Sc), Sham Cirurgia+Sham ETCC (SsSE), Sham Cirurgia+ETCC (ScE), Dor Neuropática (Dn), Dor Neuropática+Sham ETCC (DnSE) e Dor Neuropática+ETCC (DnE). O modelo de dor neuropática foi realizado a partir da compressão parcial do nervo isquiático e no 14º dia após a cirurgia iniciou-se o tratamento. A ETCC foi aplicada durante 8 dias com sessões de 20 minutos e foi utilizada uma corrente de 0,5 mA de intensidade. O aparato de Campo Aberto e o Labirinto de Cruz Elevado foram avaliados em dois momentos 24h (Fase I) e sete dias (Fase II) após o tratamento. Os níveis de BDNF foram quantificados em dois momentos 48h (Fase I) e sete dias (Fase II) após a última sessão de tratamento. Nossos resultados demonstram que a dor neuropática induz a uma menor atividade locomotora e exploratória associado a um aumento do comportamento do tipo ansioso em ratos. Por outro lado, o tratamento com ETCC provoca aumento na locomoção e na atividade exploratoria associados à diminuição do comportamento do tipo ansioso. A ETCC mostrou ser capaz de induzir mudanças neuroplásticas alterando níveis de BDNF periférico e central. Concluindo, a ETCC foi capaz de alterar parâmetros comportamentais e neuroplásticos. Podendo ser uma técnica promissora para o tratamento de comorbidades associadas à dor neuropática.

***Palavras-chave**:* Dor neuropática; Estimulação Transcraniana por Corrente Contínua (ETCC); Atividades Locomotora e Exploratória; Ansiedade; BDNF, ratos.

ABSTRACT

The IASP defines neuropathic pain as pain that arises directly from an injury or disease affecting the somatosensory system. The most characteristic symptoms are hyperalgesia and allodynia. Furthermore, behavior changes such as anxiety disorders are common comorbidities associated with chronic pain with characteristics neuropathic. Methods for Neuromodulation transcranial as tDCS are promising in the treatment of pain and some neuropsychiatric disorders, since they seem to further neuroplastic changes in the central level. In this study we evaluate the effect of tDCS on locomotor and exploratory activities, anxiety-like behavior and medullary and cortical plasticity in rats submitted to a neuropathic pain model. A total of 144 male Wistar rats (55-65 days-old; weighing 200–250 g) were divided into 6 groups: Sham Surgery (Ss), Sham Surgery+Sham tDCS (SsS), Sham Surgery+tDCS (SsT), Neuropathic Pain (Np), Neuropathic Pain+Sham tDCS (NpS) and Neuropathic Pain+tDCS (NpT). The model of neuropathic pain was performed by partial sciatic nerve compression and on the 14^{th} day after surgery began tDCS treatment. The tDCS was applied for 8 days with 20-minute sessions and a current intensity of 0.5 mA was used. Open Field and the Plus Maze tests were evaluated at two times 24 (Phase I) and seven days (Phase II) after end of treatment. BDNF levels were quantified in two at 48h (Phase I) and seven days (Phase II) after the last treatment session. Our results demonstrate that neuropathic pain induced a decreased in the locomotor activity and exploratory activity associated with an increase in anxiety-like behavior in rats. On the other hand, treatment with tDCS causes an increase in locomotion and exploratory activity associated with a reduction in anxiety-like behavior. The tDCS proved able to induce neuroplastic changes in BDNF levels by altering the peripheral and central. In conclusion, tDCS changes behavior and neuroplastic parameters; thus it can be a promising technique for the treatment of comorbid conditions associated with neuropathic pain.

***Keywords**:* Neuropathic Pain; Transcranial Direct Current Stimulation (tDCS); Locomotor and Exploratory Activities; Anxiety; BDNF, rats

LISTA DE FIGURAS

Revisão da literatura:

Manuscrito:

LISTA DE SIGLAS E ABREVIATURAS

AMPA = Ácido α-amino-3-hidróxi-5-metil-4-isoxazolepropiônico

AMPC = Monofosfato de Adenosina

ASIC 3 = Canal iônico ácido sensível

ATP = Adenosina 5'-trifosfato

BDNF = Fator Neurotrófico Derivado do Cérebro

Ca^{++} = Íon Cálcio

CB1 = Receptor canabinoide tipo 1

CCI = Constrição Crônica do nervo Isquiático (*Chronic Constriction Sciatic)*

PRGC = Peptídeo Relacionado ao Gene da Calcitonina (*Calcitonin Gene-Related Peptide)*

DBS = Estimulação cerebral profunda (*Deep brain stimulation)*

EAV = Escala analógica visual

ECG = Eletrocardiograma

EMT = Estimulação Magnética Transcraniana

EPM = Labirinto de Cruz Elevado *(Elevated Pluz Maze)*

ETCC = Estimulação Transcraniana por Corrente Contínua

H^+ = Íon Hidrogênio

HIV = Vírus da imunodeficiência humana (*Human Immunodeficiency Virus*)

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

LDH = Lactato Desidrogenase

MAPK = Proteína Quinase Ativada por Mitógeno

NGF = Fator de Crescimento Neuronal (*Neural Growth Fator)*

NK 1 = Receptor de Neurocinina 1

NMDA = N-metil-D-aspartato

NSE = Enolase Neurônio Especifíca

NT-3 = Neurotrofina 3

NT-4/5 = Neurotrofina 4/5

PGE = Prostaglandina endoperóxido

SCS = Estimulação medular epidural (*Spinal cord stimulation)*

SNC = Sistema Nervoso Central

SNP = Sistema Nervoso Periférico

SP = Substância P

TNF-α = Fator de Necrose Tumoral Alfa-α (*Tumoral Necrosis Factor α*)

TrkA = Receptor Quinase Relacionado à Tropomiosina A

TrkB = Receptor Quinase Relacionado à Tropomiosina B

TrkC = Receptor Quinase Relacionado à Tropomiosina C

TRPV = Receptor vaniloide de potencial transitório (*Transient Receptor Potential Vaniloid)*

WDR= Neurônios de Amplo Espectro (*Wide-Dinamic Range)*

SUMÁRIO

I. INTRODUÇÃO

1 INTRODUÇÃO

Sintoma comum de várias condições clínicas, a dor é ainda é considerada uma das grandes preocupações da humanidade e mesmo com toda a revolução tecnológica e farmacêutica, ainda continua sendo uma das principais razões de procura de auxílio médico e de demais profissionais da saúde. A dor é uma experiência subjetiva e individual que envolve diversos fatores, incluindo aspectos culturais e psíquicos, podendo ser alterada por elementos internos e externos ao indivíduo (1). O controle da dor é uma importante prioridade terapêutica, mesmo assim, limitações ainda estão relacionadas ao seu alívio, e incluem: subestimação da dor relatada pelo paciente, equívocos na prescrição farmacológica, ocorrência de efeitos adversos e influência de transtornos afetivos (2,3). Esse controle é ainda mais complexo quando há presença de dores relacionadas a dano ou alteração do sistema nervoso central ou periférico. As neuropatias periféricas, como as ocasionadas por diabetes mellitus ou Herpes Zoster, podem ser acompanhadas por sintomas de dor, e neste caso são conceituadas como dor de origem neuropática. Sendo assim, a dor neuropática pode surgir de lesão ou doença do sistema nervoso periférico ou central, induzindo a alterações no sistema somatossensorial (4), sendo uma importante causa de morbidade e incapacidade em longo prazo (5,6).

A compressão de um nervo como, por exemplo, o nervo isquiático, pode afetar não apenas os nervos periféricos, mas também diversos níveis do sistema nervoso central (SNC), caracterizando a dor neuropática pelo desenvolvimento de hipersensibilidade a estímulos tanto mecânicos como térmicos (7). A principal sintomatologia clínica das dores neuropáticas inclui dor espontânea ou hipersensibilidade da área afetada, levando à hiperalgesia ou à alodinia, que ocorrem principalmente devido às alterações ocorridas na medula espinhal (8,9). Além disso, na dor neuropática, é frequente a presença de alterações comportamentais como sintomas de ansiedade e de depressão (10,11). Experimentalmente, tem sido demonstrado que esta condição está envolvida em mudanças neuroplásticas (12), que podem contribuir para o desenvolvimento de sintomas ansiosos e depressivos, geralmente presentes em síndromes dolorosas crônicas em humanos (13,14).

É importante ressaltar que a dor neuropática pode ser classificada com base na localização da lesão, podendo ser de origem central ou periférica. As lesões periféricas englobam mudanças na excitabilidade do nervo periférico e do gânglio da raiz dorsal, no entanto, as lesões centrais estão relacionadas a alterações neuroplasticas em medula espinhal e

em córtex cerebral, e alterações em sistemas descendentes inibitórios (15). Mais especificamente, os processos periféricos estão envolvidos com atividade ectópica do nervo lesionado, expressão anormal dos canais de cálcio, supersensibilidade noradrenérgica, mudanças fenotípicas em neurônios do gânglio da raiz dorsal, onde se destacam alterações na expressão de neurotransmissores, neuromoduladores, receptores e canais iônicos (16). Adicionalmente, essas lesões também induzem a mudanças na expressão de neurotrofinas e de seus receptores, assim como em todos os componentes neurais (17).

Entre as neurotrofinas, o fator neurotrófico derivado do cérebro (BDNF) desempenha um papel fundamental como mediador/modulador da transmissão nociceptiva (18,19) e também na sensibilização central (20). O BDNF atua facilitando a transmissão sináptica glutamatérgica, sendo este um importante mecanismo para a indução da neuroplasticidade induzida pela dor (21). O processo de neuroplasticidade envolve mudanças em áreas corticais, principalmente em córtex somatossensorial, bem como em áreas que envolvem a dimensão afetiva da dor (22).

A dor neuropática é comumente crônica e incapacitante, e em boa parte resistente aos tratamentos farmacológicos, estando entre as condições dolorosas mais difíceis de tratar (8,23). Os tratamentos farmacológicos incluem bloqueadores dos canais de cálcio e de sódio, antidepressivos, anticonvulsivantes e opióides (24), porém ainda são insatisfatórios. Estes fármacos oferecem benefícios terapêuticos limitados e são relacionados à tolerância e/ou efeitos adversos (25,26). Sendo assim, há necessidade de novas terapias não farmacológicas que complementem as terapias farmacológicas (27). Considerando as possibilidades não farmacológicas não-invasivas, a estimulação transcraniana por corrente contínua (ETCC) tem demonstrado efeitos significativos na modulação de diversos quadros álgicos (28,29), assim como em distúrbios psiquiátricos e neurológicos (30). O princípio da ETCC baseia-se na utilização de uma corrente elétrica fraca aplicada no couro cabeludo (31). A estimulação ânodal tipicamente despolariza (aumenta excitação) e estimulação catodal hiperpolariza (diminui excitação) neurônios do cortéx (32,33). Esta técnica induz mudanças na excitabilidade cortical em áreas especificas do cérebro, de modo reversivo e além de apresentar baixo risco e pouco desconforto pode produzir um efeito duradouro (28).

A intricada rede de conexões neurais envolvidas na dor comporta tanto o aspecto somato-sensorial, como o afetivo-motivacional (34), o que justifica a inversão do quadro ansioso dos pacientes portadores de dor neuropática (13) quando ocorre um controle adequado da dor (35). Neste contexto, na busca de uma melhor compreensão dos efeitos da ETCC em quadros de dor de origem neuropática, este trabalho objetivou avaliar o efeito desta

terapia no comportamento do tipo ansioso e nas atividades exploratória e de locomoção em modelo animal de dor neuropática. Adicionalmente avaliaremos marcadores de neuroplasticidade buscando obter um melhor entendimento do mecanismo da ETCC.

II. REVISÃO DA LITERATURA

2 REVISÃO DA LITERATURA

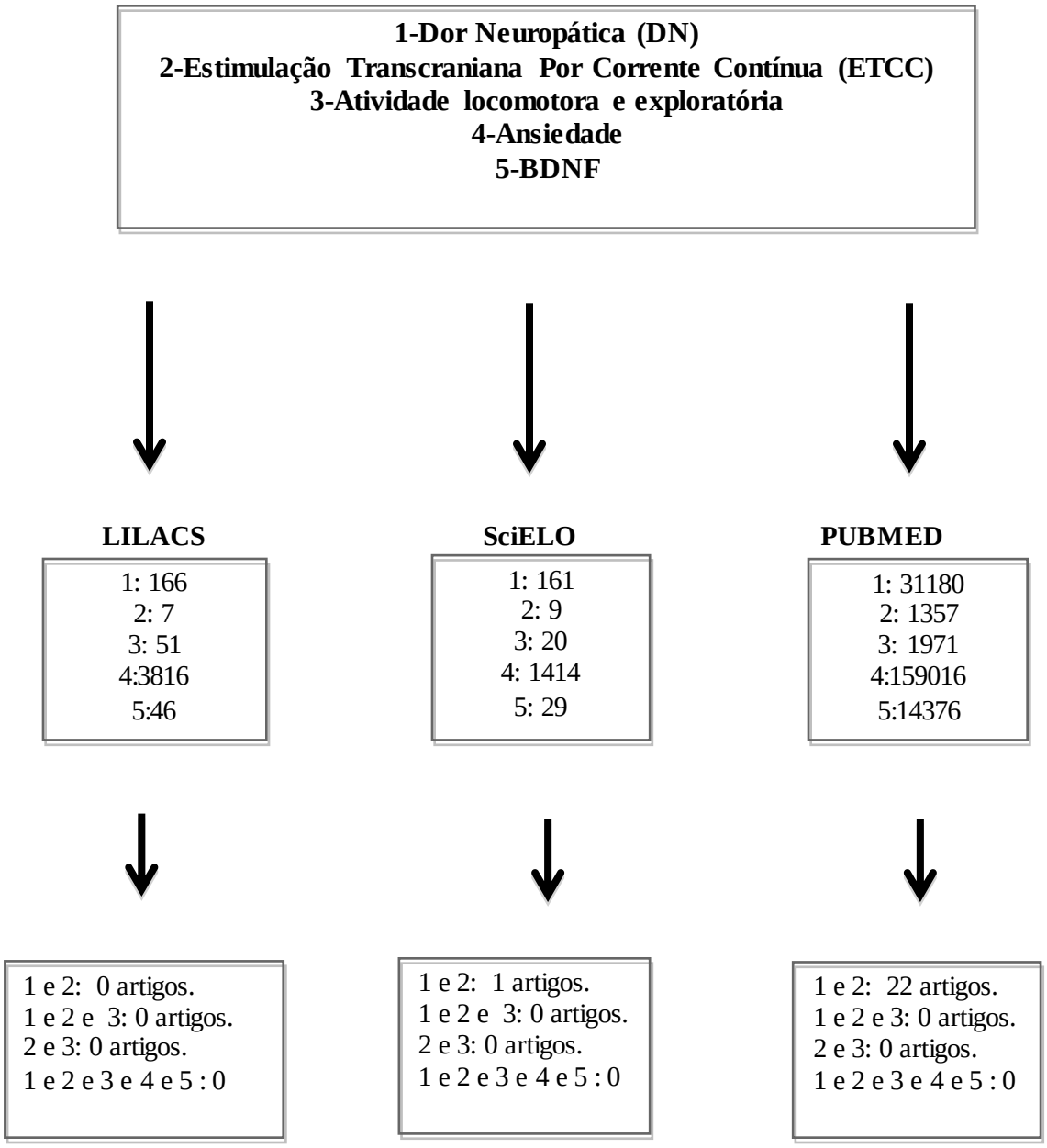
2.1 Estratégias para localização de informações

Na revisão literária, buscamos ressaltar os principais aspectos relacionados com a dor neuropática, estimulação transcraniana por corrente contínua, alterações comportamentais e marcadores de neuroplasticidade. A estratégia de busca envolveu as seguintes bases de dados: LILACS, SciElo e PubMed. Utilizaram-se artigos com datas de publicação entre 1990 e 09\2014, essas referências foram revisadas para localizar outros estudos não contemplados nesta busca.

Nos sites LILACS, SciElo e PubMed foram realizadas buscas por meio dos seguintes termos: dor neuropática, estimulação transcraniana por corrente contínua, ansiedade, atividade locomotora e exploratória e BDNF,. Em relação ao termo "dor neuropática" foram encontrados 166 artigos no site LILACS, 161 artigos no SciElo e 31180 artigos no PubMed. Quando utilizado o termo "estimulação transcraniana por corrente contínua" foram encontrados 7 artigos no LILACS, 9 artigos no site SciElo e no site PubMed 1357 artigos. Em relação ao termo "atividade locomotora e exploratória" foram encontrados 51 artigos no site LILACS, 20 no site SciElo e 1971 artigos no portal PubMed. No termo "ansiedade" 3816 artigos no site LILACS, 1414 no site SciElo e 159016 no portal PubMed.. Realizou-se a busca utilizando o termo "BDNF" foram encontrados 46 artigos no site LILACS, 29 no site SciElo e 14736 artigos no portal PubMed. Cruzando-se a busca, com cruzamentos entre as palavras-chave deste projeto de pesquisa foram encontrados no portal LILACS 0 artigos, no site SciElo foram encontrados 0 artigos e no portal PubMed foram encontrados 8 artigos.

PALAVRAS-CHAVE

Figura 1: Fluxograma da pesquisa realizada sobre o tema nas principais bases de dados.



2.2. Dor

A Associação Internacional para o Estudo da Dor (*International Association for Study of Pain* – IASP) conceitua a dor como "uma experiência sensorial e emocional desagradável, relacionada com lesão tecidual real ou potencial, ou descrita em termos deste tipo de dano" (36,37). Segundo o critério de classificação temporal, a dor pode ser aguda ou crônica. A dor aguda exerce uma função de proteção, associada a estímulos nocivos potencialmente prejudiciais, com uma sensação desagradável (9). É causada por traumas, doenças subjacentes ou alterações funcionais musculares ou viscerais e na maioria dos casos, cessa em alguns dias ou semanas, sendo responsiva a analgésicos clássicos (38).

Para dor crônica, um dos critérios diagnósticos preconizado pela IASP é a duração por pelo menos três meses, no entanto alguns autores sugerem que a dor crônica pode variar de um a seis meses (39,40). Quando a dor é persistente, ela perde seu caráter protetor, onde neste caso o organismo não é mais capaz de controlar a quadro álgico, ou são estabelecidos mecanismos adaptativos inadequados que acarretam prejuízos ao organismo (41). Os quadros de dor persistente podem ser em decorrência de dano periférico tecidual e/ou inflamação, crescimento tumoral, alterações na nocicepção periférica, disfunções do sistema nervoso periférico (SNP) ou sistema nervoso central (SNC) (42–44).

A dor crônica é de difícil tratamento e está associada a um substancial sofrimento psicológico, prejuízo funcional e diminuição da capacidade física (45,46). É relatado que 10% da população mundial (~60 milhões de pessoas) sofre dessa condição, contudo, a prevalência encontrada nos Estados Unidos e Europa é de 12 a 25% e 20%, respectivamente (47).

De modo geral, a dor, seja aguda ou crônica, leva o indivíduo a manifestar alterações de comportamento, como redução do sono e de apetite, manifestações de irritabilidade, diminuição da capacidade de concentração, restrições na execução de atividades profissionais e sociais. A dor, além de induzir a anormalidades físicas, também altera o equilíbrio psicológico do indivíduo (48). As disfunções psicológicas e sociais podem ser causadas por déficits de neurotransmissores, alterações em seus receptores, transtornos em ritmos biológicos, anormalidades neuroendócrinas ou fatores genéticos (49–51). Indivíduos com dor crônica apresentam uma maior prevalência de quadros depressivos associados, diretamente à ansiedade (52,53). Por conseguinte, a presença desses fatores interfere na ativação de sistemas opioidérgicos, noradrenérgicos e serotonérgicos (54).

2.3 Dor neuropática

A IASP define a dor neuropática como a dor que surge diretamente de uma lesão ou doença que afeta o sistema somatossensorial (55). Pode ser classificada em central e periférica, sendo a dor central provenientes de lesões ou doenças que acometem o sistema nervoso central, que incluem a esclerose múltipla, lesão na medula espinhal, acidente vascular encefálico, entre outras (23). Apesar disso, a etiologia mais comum da dor neuropática está associada às lesões periféricas provocadas por traumas de diversas causas, como lacerações por materiais cortantes, arma de fogo ou procedimentos cirúrgicos (56,57), podendo ser desencadeadas também por outras patologias, como infecções virulentas (Herpes Zoster e HIV), terapias anti-retroviral, tumores, quimioterápicos, procedimentos cirúrgicos (amputações de regiões corporais) e por doenças sistêmicas (44,58–64).

A prevalência da dor neuropática ainda é contraditória, pois há falta de instrumentos validados, que possam identificar as características da dor neuropática (65). A dor crônica com características neuropáticas em grandes comunidades indica uma prevalência de 7-8% (23,56).

O sintoma mais característico da dor neuropática é alodinia (respostas dolorosas aos estímulos táteis normalmente inócuos), porém muitas vezes pode estar presente a hiperalgesia (aumento da resposta a estímulos nocivos) para estímulos tanto mecânicos como térmicos, assim como a hipoestesia (perda ou diminuição de sensibilidade), e a parestesia (sensações cutâneas subjetivas espontâneas) (66,67).

A dor neuropática ocasionada por lesão de nervo periférico tem seu início a partir de um insulto traumático ou patológico do nervo, ocorrendo uma sucessão de eventos como resultado do processo reparador, causando modificações estruturais e funcionais que vão levar à alterações da condução nervosa, ocasionando sensibilização periférica (43,68,69), que incluem descargas ectópicas e espontâneas, alterações nos canais de íons, surgimento colateral de neurônios aferentes primários, surgimento de neurônios simpáticos no gânglio da raiz dorsal e sensibilização nociceptiva (70).

Após a lesão do nervo, muitos axônios e corpos celulares associados ao gânglio da raiz dorsal sofrem um aumento da excitabilidade elétrica intrínseca, resultando na geração de descargas espontâneas a estímulos ligados ao sítio da lesão (71). Este fenômeno é denominado como descargas ectópicas, onde são originadas a partir do sistema nervoso

periférico, que gera impulsos elétricos evocados para o sistema nervoso central (72). As atividades ectópicas também podem desencadear e manter a sensibilização central, além disso, geram oscilações no potencial de membrana em neurônios sensoriais primários, podendo levar a alterações na função dos canais de sódio no gânglio da raiz dorsal (73). Descargas anormais podem surgir no local da lesão do nervo, em outros pontos ao longo dos nervos ou no corpo celular, levando a um aumento da atividade ectópica desses neurônios (74).

Os pacientes apresentam uma clínica bastante variada, havendo modificação em diferentes indivíduos e no mesmo paciente, que depende de fatores ambientais (75), a intensidade dos sintomas se difere de maneira espontânea e de acordo com a influência de fatores externos (alterações de temperatura, pressão atmosférica, umidade do ar, alterações psicológicas, estímulos musculoesqueléticos, entre outras) (16). A dor pode se apresentar de forma constante ou intermitente com breves períodos de melhora espontânea ou evocada, paroxística, superficial ou profunda (76). Muitas vezes a neuropatia pode apresentar diversos tipos de dor, sendo utilizados vários termos para caracterizar o sintoma: choque, queimação, dolorido, compressão, agulhada, podendo ser prolongada ou permanente, pode haver remissão espontânea ou com o tratamento, além disso, podemos observar alteração sensorial para estímulos como frio e calor (27). O tratamento das síndromes dolorosas neuropáticas é de grande complexibilidade, necessitando da combinação de diversas modalidades terapêuticas com diferentes mecanismos de ações (6).

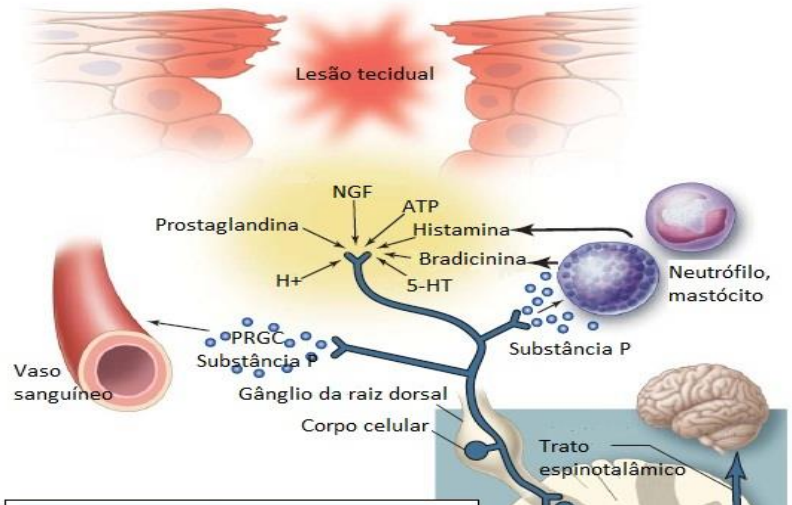
2.4 Transmissão da dor

A nocicepção é uma via neural que consiste dos processos de transdução, transmissão e modulação do estímulo nociceptivo e interpretados no córtex somatossensorial como dor (77). Inicialmente este sinal é transmitido pelo neurônio de primeira ordem que se origina na periferia e projeta-se até o corno dorsal da medula espinhal. Este neurônio faz sinapse com o neurônio de segunda ordem, que ascende pela medula espinhal até o tálamo, onde ocorre a sinapse com o neurônio de terceira ordem, que se projeta para o córtex cerebral (78). O processo inical da dor envolve um sistema que codifica e transmite o sinal ao longo da via ascendente a centros superiores do SNC, a partir do ponto da periferia onde ocorre o estímulo

nocivo (77). Os nociceptores são encontrados na maioria dos órgãos e tecidos, podendo ser ativados por qualquer estímulo nocivo mecânico, térmico ou químico (77,79,80). Por outro lado, a hipóxia ou a lesão tecidual, seguida de inflamação, promove liberação local de inúmeros mediadores (81,82), entre eles, bradicinina, prótons, histamina, serotonina, metabólicos do ácido araquidônico, adenosina trifosfato (ATP), citocinas, aminoácidos excitatórios, óxido nítrico, substância P, neurotrofinas, bombesina, opióides, somastotatina e acetilcolina (81,83). Estes mediadores químicos interagem com canais de íons na membrana plasmática do nociceptor conduzindo a propagação do estímulo nociceptivo por meio de alterações na permeabilidade da membrana da fibra nervosa, gerando potenciais de ação (81,83).

As fibras aferentes levam a informação nociceptiva para medula espinhal, podendo ser de três tipos: Aβ, que são fibras mielinizadas com velocidade de condução 30-100 m/s que respondem a estímulos táteis, fibras Aδ que também são mielinizadas com velocidade de condução de 12-30 m/s, responsáveis pela propagação do estímulo doloroso, e as fibras C, que são amielínicas, com velocidade de condução 0,5-2 m/s responsáveis pela condução dolorosa lenta, onde as fibras C correspondem à maioria das fibras sensoriais (figura 3) (84–86).

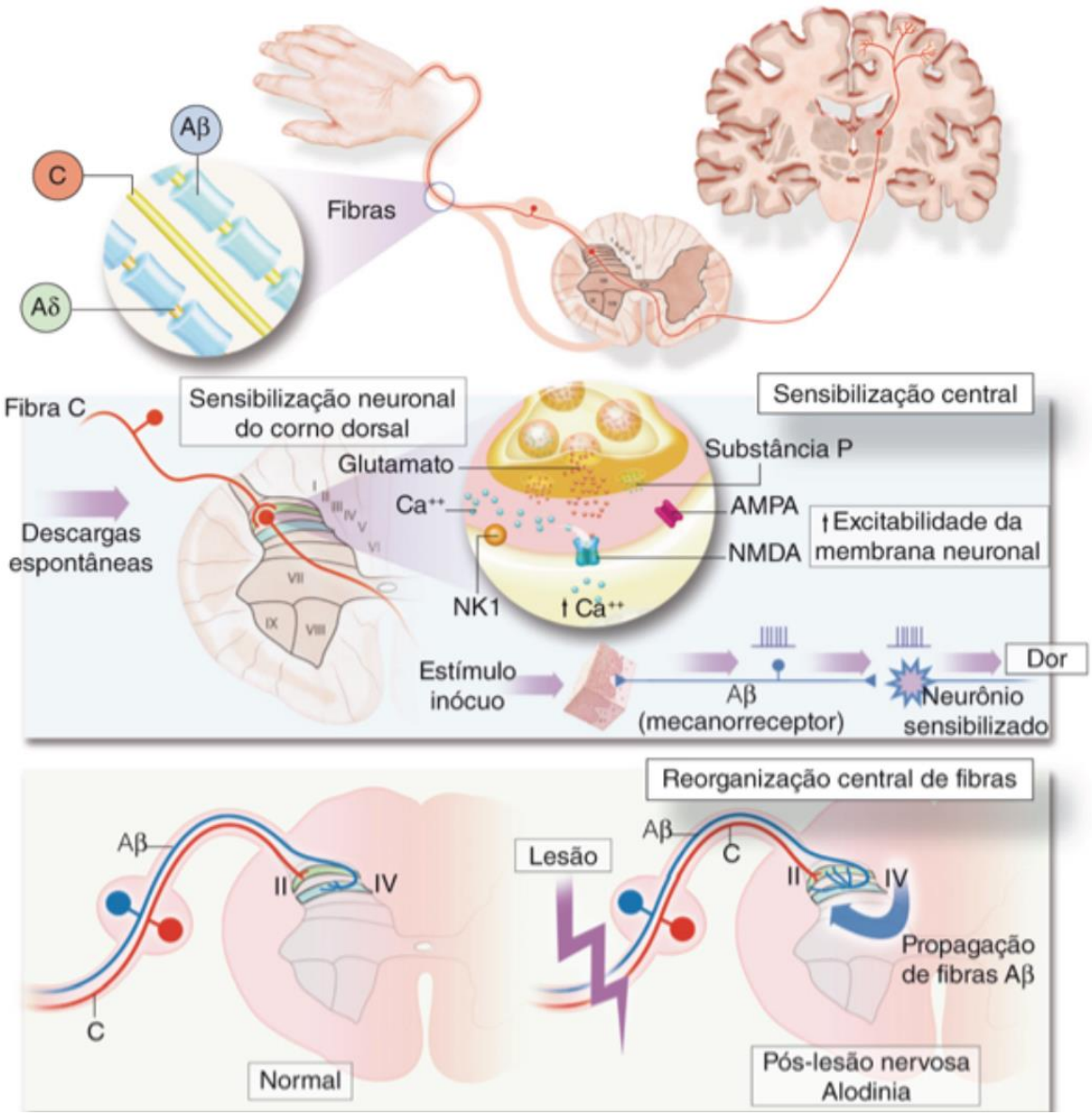
Figura 2: Nociceptores e influência das condições teciduais.



Estímulo	Receptor representativo
NO	TRPV1, TRPV2
NGF	Receptor de tirosina quinase (TrkA), p75
Bradicinina	Receptor de bradicinina B2
Serotonina	Receptor de serotonina (5-HT3)
ATP	Purinorreceptores P2X, família P2Y
Próton (H+)	Canal iônico ácido-sensível (ASIC3) Receptor vaniloide TPPV1
Lipídios	Prostaglandina endoperóxido 2 (PGE2) Receptor de canabinoide de tipo 1 (CB1) Receptor vaniloide TPPV1
Calor	TRPV1-4
Refrigeração	TRPM8
Frio	TRPA1

Fonte: www.medicinanet.com.br/

Figura 3: Transmissão do estímulo nociceptivo pela via ascendente da dor.



Fonte: www.rgrpublicacoes.com.br/

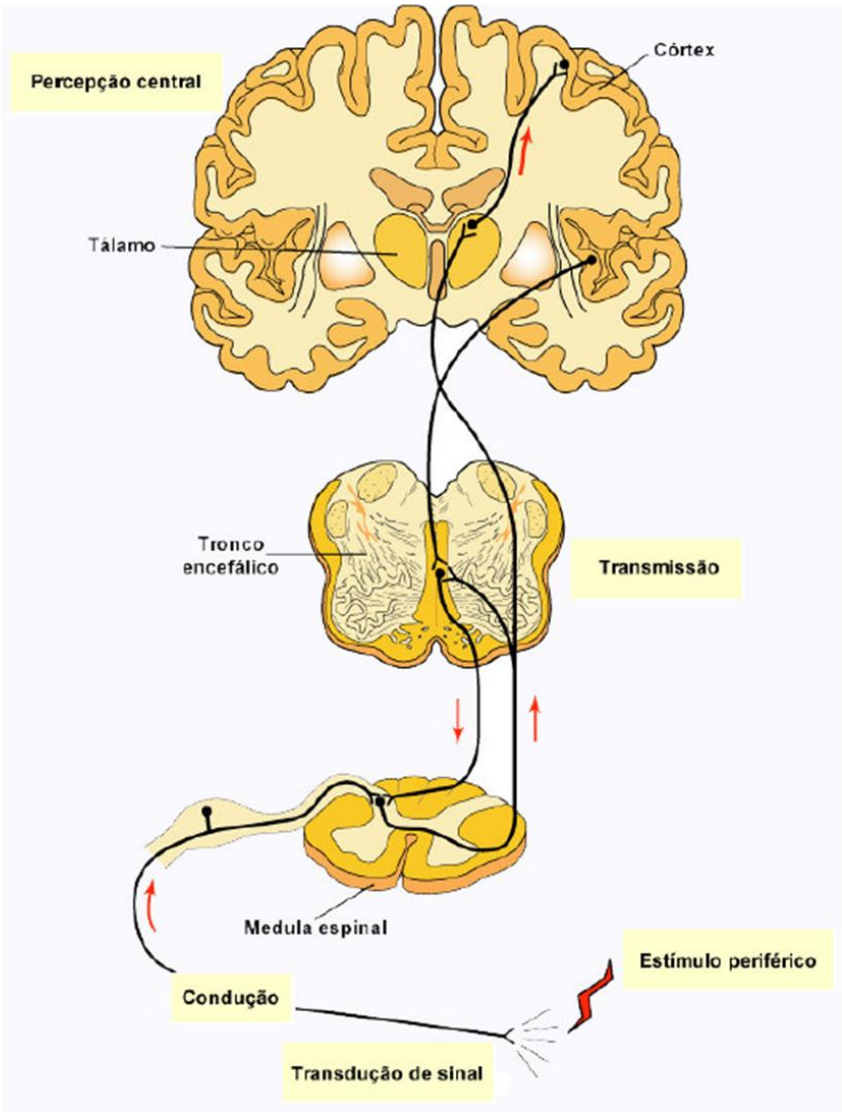
Podemos classificar a sensação de dor em duas fases, uma dor primária rápida e, forte e aguda e após uma dor secundária, lenta e contínua, esse padrão de dor se dá pela diferença de propagação do impulso nervoso de acordo com os tipos de fibras descritas anteriormente (87).

Para ocasionar a ativação dos nociceptores, o estímulo nociceptivo precisa atingir o limiar de despolarização das terminações nervosas, abrindo os canais de sódio voltagem

dependente para deflagrar o impulso nervoso (78). O estímulo nociceptivo, alcança a medula espinhal por meio dos terminais axonais dos nociceptores que fazem sinapse no corno dorsal da medula. A região do corno dorsal da medula espinhal é dividida em camadas (lâminas) chamada de Lâminas de Rexed. A lâmina I é correspondente a estímulos nocivos, a lâmina II é formada por interneurônios excitatórios e inibitórios que respondem somente a aferentes nociceptivos e a lâmina V recebe estímulos das fibras Aβ, Aδ e fibras C (88). O glutamato é o principal mediador da transmissão sináptica entre as fibras aferentes primárias e neurônios do corno dorsal, onde ativa receptores como alfa-amino-3-hidroxi-metil-5-4-isoxazolpropiónico (AMPA) e N-metil-D-aspartato (NMDA), além de neurotransmissores diversas outras substâncias podem interferir e modular a transmissão sináptica na medula como o BDNF, prostaglandinas, ATP e a substância P (89). Informações nociceptivas da periferia seguem pelos neurônios do trato ascendente, que ativam o sistema tálamo cortical que traduzem a sensação de dor (85).

As vias ascedentes envolvidas na transmissão nociceptiva até o córtex cerebral é denominada do trato espinotalâmico (figura 4), que é composto por neurônios nociceptivos específicos e axônios de neurônios de ampla faixa dinâmica (*wide-dinamic range* [WDR]) da lâmina I, V e VII da medula espinhal, além dos tratos espinorreticular, espinomesencefálico e o trato espionomesencefálico (89,90). A região talâmica é a responsável pela recepção, integração e passagem do potencial nociceptivo para o córtex, onde acontece o processamento da dor (88). O sinal nociceptivo ao atingir o córtex cerebral, onde ocorre a integração, interpretação cognitiva e emocional do estímulo nocivo (79,81). Dentro das regiões corticais há uma complexa rede de interconexões que incluem tálamo e estruturas límbicas (91). Estas estruturas encefálicas são responsáveis pelo sensório discriminativo (percepção, intensidade, localização, duração, padrão temporal) e parte afetiva (relação entre dor e humor, atenção, tolerância e racionalização) e os componentes de experiência da dor (92–94).

Figura 4: Representação da via espinotalâmica, a principal via ascendente que conduz a informação nociceptiva.



Fonte: (Adaptado pelo autor a partir de Costigan et al, 2006)

2.5 Sensibilização periférica e central

Após a sequência de eventos que originam o fenômeno sensitivo-doloroso, ocorre a transformação dos estímulos nocivos em potenciais de ação, que são transferidos via fibras nervosas periféricas para o SNC (95). Os receptores específicos para a dor estão localizados nas terminações de fibras nervosas Aδ e C (96) e quando ativados, causam alterações na membrana, permitindo a deflagração de potenciais de ação (97).

Os nociceptores são sensibilizados pela ação de inúmeras substâncias químicas, mediadores inflamatórios, denominados algogênicas, como, acetilcolina, bradicinina, histamina, serotonina, leucotrieno, substância P (SP), fator de ativação plaquetária, prostaglandinas, interleucinas, fator de necrose tumoral (TNFα), fator de crescimento nervoso (NGF) e trifosfato de adenosina (ATP), esse fenômeno é conhecido como hiperalgesia primária (98,99). A persistência do estimulo nocivo causa modificações no sistema nervoso periférico sensibilizando fibras nervosas, como consequência desencadeia a hiperalgesia e um aumento nos níveis de AMPc e cálcio nos nociceptores, este fenômeno ocorre por ação de mediadores inflamatórios e consequente atividade espontânea de neurônios, aumento da resposta a estímulos supraliminares e diminuição do limiar de ativação dos nociceptores (Aida et al., 1999), desencadeando o processo de sensibilização periférica amplificando a resposta ao estímulo doloroso (101). Hiperalgesia secundária é caracterizada pela sensibilização de áreas adjacentes a lesão em decorrência da maior capacidade de resposta dos neurônios do corno dorsal que inervam a fonte primária da lesão (102).

A sensibilização central consiste em alterações dos impulsos periféricos, aumento da excitabilidade de neurônios nociceptivos, descargas persistentes após estímulos repetidos e ampliação dos campos receptivos de neurônios do corno dorsal, sendo definida como o aumento da capacidade de resposta dos neurônios centrais à sinalização de nociceptores periféricos, levando a alteração no processamento sensorial cerebral, mal funcionamento do mecanismo antinociceptivo descendente, aumento da atividade da rota facilitatória da dor e aumento do período da segunda dor (somação temporal) (103).

A sensibilização central é considerada o principal mecanismo fisiopatológico em condições de dor neuropática (104). Primeiramente ocorre a sensibilização sináptica causada por uma sequência de estímulos periféricos nociceptivos repetidos aumentando a resposta de fibras Aδ e C e também das fibras Aβ. Este fenômeno ocorre como consequência da liberação de aminoácidos excitatórios, como o glutamato e aspartato, de peptídeos como SP (substancia P) e PRGC (peptideo relacionado ao gene da calcitonina), e de neurotrofinas como NGF (do ingles *Nerve growth factor* - fator de crescimento neural) e BDNF (do ingles *Brain-derived neurotrophic factor* - fator neurotrofico derivado do cerebro) no corno dorsal da medula espinhal (105). Após a liberação destas substâncias e sua interação com receptores específicos, tais como NMDA (N-metil D-Aspartato), receptor de neurocinina 1 (NK1) e receptor quinase relacionado à tropomiosina B (trkB), há a ativação da cascata de segundos mensageiros, promovendo a abertura de canais de cálcio e, consequentemente, produção de prostaglandinas e óxido nítrico, os quais induzem a liberação de glutamato, aspartato, SP e

PRGC, contribuindo para a ampliação do processo álgico (106). O fenômeno de "wind up" é o resultado da somação de potenciais sinápticos lentos após estimulação aferente repetida por tempo prolongado.

As fibras C enviam os impulsos nociceptivos para neurônios de ampla faixa dinâmica -WDR (do inglês *Wide dynamic range*) no corno dorsal da medula, os quais incluem tanto neurônios nociceptivos como não nociceptivos. Com impulsos intensos, ambos os neurônios são ativados de modo que até estímulos não dolorosos, como o toque, sejam percebidos como dolorosos (107). Isto também estimula a liberação dos neurotransmissores excitatórios glutamato e aspartato no corno dorsal da medula espinhal induzindo à despolarização neuronal via receptores NMDA. Ocorre, então, aumento da condutividade ao cálcio e da resposta à dor, a cada estímulo repetido e de mesma intensidade (108).

A potencialização de longo termo (LTP – do ingles *long-term potentiation*) é decorrente de sequência de estímulos nociceptivos breves e de alta frequência, provocando a ativação de receptores AMPA (acido α-amino-3-hidróxi-5-metil-4-isoxazol propiônico) e NK1 e de canais de cálcio, ocorrendo resposta pós-sináptica prolongada e excitatória (109). A LTP envolve a ativação de fatores de transcrição, tais como a expressão de genes de formação imediata, como *c-fos* e *c-jun*, e de genes de resposta lenta que codificam a prodinorfina, o receptor NK1 e trkB no corno dorsal da medula espinhal. Ocorrendo regulação das vias ascendentes para síntese de citocinas, quimiocinas e moléculas de adesão, e uma mudança fenotípica no gânglio da raiz dorsal (110). Por fim, a sensibilização central pode ser clinicamente caracterizada pela hiperalgesia, alodinia, dor irradiada ou dor persistente descrita como desagradável, latejante, em queimação ou dormência (111) .

2.6 Aspectos comportamentais da Dor

De acordo com Melzack & Casey, (1968), "o estímulo nocivo leva a uma experiência reconhecida como dor, envolvendo uma dimensão sensorial, permitindo a localização e discriminação; uma dimensão afetiva, refletindo a relevância emocional e motivacional dos estímulos; e uma dimensão cognitiva relacionada à forma como aspectos da cognição podem esculpir sua própria experiência." Desta forma, a dor envolve a percepção, por meio de

circuitos centrais e periféricos, que são modulados por influências do sistema límbico e áreas corticais sobre o afeto e o comportamento (113).

A complexibilidade da dor e sua multidimensionalidade são determinadas por diferentes fatores, e podem ser de natureza fisiológica, sensorial, afetiva, cognitiva, comportamental e sociocultural (114–116). Essas carcterísticas podem ter um papel determinante no início, na gravidade, na exarcebação e na manutenção da dor (117).

A percepção da dor é mediada por processos neuronais que a amplificam ou inibem, o que contribue para a subjetividade da percepção dolorosa (118). Já os processos inerentes à percepção individual da experiência dolorosa são determinados por acontecimentos da vida (traumas), personalidade e experiências anteriores de dor. Entao, fatores perceptivos, cognitivos e emocional podem contribuir para o aumento da percepção dolorosa e atribuir um significado a dor (119).

Na presença de um estímulo nocivo, é iniciada uma avaliação cognitiva que atribui um significado, que compara com sintomas conhecidos (116). Quem identifica o estímulo como nocivo como tal, é o cérebro (120), por isso, a relação estabelecida com a dor depende do significado que é atribuído no momento da experiência dolorosa, da forma como é sentida e avaliada (121). Essa avaliação é dependente dos seguintes fatores: intra-pessoais (processos psicodinâmicos dos primeiros anos de vida interferem na qualidade/gravidade da dor, assim como promovem a manifestação tardia de uma doença ou algum tipo de sofrimento); inter-pessoais (os comportamentos podem ser reforçados ou inibidos pelo meio social); biológicos (aspectos do foro biológico que estão envolvidos na sensação de dor) (113,122). A experiência dolorosa apresenta uma percepção individual e manifesta determinadas respostas comportamentais, mas quando a dor progride para um estado crônico as suas características se tornam mais acentuadas, porque as diferentes dimensões associadas à resposta dolorosa se tornam mais complexas e dinâmicas (123).

O estímulo doloroso determina o comportamento perante o fator agressor (dor), que envolve alterações orgânicas e respostas emocionais (negação, ansiedade, depressão, impotência, dependência, necessidade de proteção, entre outras) (113). Dentre os aspectos emocionais, a ansiedade é um fator determinante na manutenção da dor crônica (124). Indivíduos com dor crônica facilmente desencadeiam comorbidades relacionada à fatores psicológicos, entre elas, a ansiedade e depressão, que são magnificadas pela intensidade da dor diária (34,125).

A ansiedade é denominada como uma manifestação emocional ou afetiva do individuo, onde predominam sentimentos desagradáveis como: apreensão, preocupação

excessiva e intranquilidade (126,127). A ansiedade surge como expressão patológica representando um sinal de alarme ou defesa, que inclui sintomas emocionais, cognitivos, comportamentais, e também apresenta características físicas que são mediadas pela ativação do sistema simpático (113). Quando provocada pela dor crônica, a ansiedade faz com que o indivíduo diminua sua capacidade de resistência ao sofrimento, tornando insuficiente a resposta de alívio à dor, interferindo na qualidade de vida (128), ao contrário a ansiedade antecipatória, pode contribuir para o desenvolvimento da dor crônica, onde ela representa uma resposta emocional negativa, que aumenta a intensidade e a percepção da dor, das queixas relacionadas e da própria experiência dolorosa (129,130).

Estudos de prevalência relatam que 50% dos pacientes com dores crônicas apresentam perturbações ansiosas e/ou depressivas (12,131), e quando os que apresentam características neuropáticas, exibem 30% dessas comorbidades associadas (132).

Estudos experimentais demonstram que animais com dor persistente, desenvolvem o comportamento do tipo ansioso (7,133,134), e na dor neuropática, os achados evidenciaram uma diminuição do comportamento exploratório, sugerindo que o modelo imita eficazmente muitos aspectos da dor em humanos (135).

2.7. Marcadores neuroquímicos

As Neurotrofinas são representadas por uma família de proteínas composta pelo NGF, BDNF, Neurotrofina-3 (NT-3) e Neurotrofina-4/5 (NT-4/5) que podem se ligar a dois receptores estruturalmente independentes: receptor de neurotrofina p75NTR e o receptor tropomiosina quinase (TrkA, TrkB e TrkC) (136). Todas as NTs se ligam com baixa afinidade em receptores transmembrana p75 e cada uma se liga com alta afinidade em um dos subtipos trk, NGF em trkA, BDNF e NT4/5 em trkB, e NT3 em trkC. A ativação dos receptores trk por seus ligantes leva à fosforilação dos seus diferentes resíduos e a ativação de diferentes vias de sinalização como, por exemplo, as vias da Proteína Quinase Ativada por Mitógeno (MAPK) (137). O BDNF é uma neurotrofina de 12.4 kDa (138), atualmente conhecida como neurotransmissor e neuromodulador (139,140) com importante participação no processo nociceptivo (18,19).

Além de apresentarem importante papel no desenvolvimento e regeneração do sistema nervoso, as NTs também desempenham funções cruciais em situações patológicas, incluindo

condições de dor crônica. Entre elas, o BDNF é a NT que tem sido mais amplamente estudada como moduladora da transmissão nociceptiva em níveis espinhal e supraespinhal (18,140,141), e também foi demonstrado ser fundamental no processo de sensibilização central (20,142). As lesões nervosas periféricas induzem a mudanças nos níveis de expressão das neurotrofinas e em seus receptores (143). Em particular, a sinalização neurotrófica alterada é essencial para inúmeros processos complexos subjacentes a lesão nervosa, bem como o desenvolvimento da dor neuropática (17). Em estudos com modelo experimental de dor neuropática por ligação de nervo periférico, há um aumento dos níveis de BDNF tanto no corno dorsal como no gânglio da raiz dorsal (GRD) da medula espinhal (144,145).

A sinalização mediada pelo BDNF ocorre pela ativação dos receptores TrkB e está envolvida nos mecanismos sinápticos subjacentes de memória e de dor (141). Além disto, existem outras evidências que justificam o envolvimento desta neurotrofina em condições de dor crônica (79,140). O modelo de dor neuropática ativa a rota de BDNF-TrkB, onde é observado um aumento no gradiente iônico que converte correntes sinápticas inibitórias em excitatórias, promovendo o aumento da excitabilidade de neurônios do corno dorsal (146–148).

A facilitação do BDNF sobre a transmissão sináptica glutamatérgica constitui um importante mecanismo para a indução de neuroplasticidade central provocados pela dor crônica (141). O BDNF-TrkB promove o aumento da fosforilação do receptor NMDA no corno dorsal da medula espinhal (149,150). Outros estudos indicam que a potenciação da transmissão excitatória mediada pelo BDNF no corno ventral da medula espinhal está relacionada com a ativação dos receptores NMDA expressos nos motoneurônios (151–153). Dessa forma, sugere-se que o BDNF induz a facilitação do potencial excitatório pós-sináptico (PEPS) evocado pela estimulação da raiz dorsal, uma vez que estes efeitos foram bloqueados com MK-801 (antagonista de receptor NMDA) (154).

Os níveis circulantes de BDNF representam aproximadamente 75% de seus níveis centrais (155). Demonstrou-se uma correlação entre os níveis séricos e centrais de BDNF em ratos (156), bem como os níveis séricos e liquóricos em humanos (157). Tem sido amplamente demonstrada à relação entre níveis de BDNF e diversas patologias. Um estudo experimental de inflamação crônica, realizado em nosso grupo, demonstrou aumento dos níveis de BDNF após o tratamento com dexametasona (158) Evidências clínicas, mostraram o aumento dos níveis de BDNF em pacientes com dores crônicas (159–161) e diminuição em pacientes com depressão maior e epilepsia (162).

O processo de dor neuropática modula sistemas endógenos que são associados a marcadores neuroquímicos, sendo assim o entendimento e a forma como se comportam estes marcadores, podem contribuir para um melhor entendimento dos mecanismos da dor neuropática.

2.8. Terapêutica da dor neuropática

2.8.1 Tratamentos farmacológicos clássicos

O manejo clínico para dor neuropática consiste no tratamento com terapias farmacológicas (59). Numerosas pesquisas clínicas confirmam a eficácia de agentes antiepiléticos, antidepressivos, analgésicos opióides e lidocaína tópica (163). Além disso, diversos outros novos medicamentos como a toxina botulínica, capsaicina e a lacosamida têm sido utilizado para o tratamento da dor neuropática (164).

A gabapentina e a pregabalina são antiepiléticos que são recomendados como tratamento de primeira linha da dor neuropática (165). Sua ação consiste em inibir a liberação de glutamato, noradrenalina e substância P, por meio da ligação seletiva da subunidade $\alpha 2\delta 1$ dos canais de cálcio (166). Os antidepressivos tricíclicos e inibidores seletivos da receptação de serotonina e noradrenalina são os principais antidepressivos utilizados em quadros de dor neuropática (167). Os efeitos analgésicos são atribuídos à inibição da recaptação da serotonina e da noradrenalina a partir dos terminais pré-sinápticos e o alívio da dor é independente das suas propriedades de elevação do humor (168). Portanto, a utilização de antidepressivos pode ser uma boa escolha para os pacientes de dor neuropática com depressão coexistente (167). Lidocaína tópica tem demonstrado um efeito analgésico eficaz em pacientes com dor neuropática e alodinia (5). Apesar dos seus mecanismos de analgesia ainda ser desconhecido, presume-se um bloqueio nos canais de sódio, leva à uma redução da transmissão do sinal nociceptivo (169). Sem uma absorção sistêmica relevante, a aplicação tópica oferece uma boa vantagem para relação de risco com reações locais leves (por exemplo., eritema ou erupção cutânea) (170). Portanto, lidocaína tópica é particularmente adequada para pacientes com dor neuropática periférica localizada (163). Os analgésicos opióides apresentam boa eficácia no

alívio da dor, seus efeitos analgésicos ocorrem devido a inibição da transmissão nociva pelos receptores μ, κ, δ, que são distribuídos pelo sistema nervoso central (171). No entanto, os opióides não são recomendados como tratamento de primeira linha para pacientes com dor neuropática, porque os efeitos colaterais (mau uso, constipação, sonolência e dependência de drogas e desvio) são difíceis de serem evitados (172).

No entanto, apenas 40-60% dos pacientes alcançam o alívio da dor clinicamente significativo com o uso da farmacoterapia, uma vez que alguns tipos de dor neuropática podem não ser responsivos a analgésicos comuns (164).

2.8.2 Neuromodulação

Neuromodulação é a alteração da atividade nervosa por meio de uma aplicação de agentes magnéticos ou elétricos em uma área do cérebro ou periferia (173). Os tratamentos neuromodulatórios centrais são divididos em invasivos e não invasivos (174). Dentre as técnicas invasivas temos a estimulação cerebral profunda (DBS- Deep Brain Stimulation) que consiste no implante de eletrodos em regiões cerebrais (175), estimulação medular epidural (*SCS - Spinal Cord Stimulation*) onde é implantado um eletrodo no espaço epidural da médula acoplado a um gerador de pulsos (176) e a estimulação dos nervos periféricos invasivos que é um tipo de neuromodulação onde são implantados eletrodos subcutâneos que geram correntes fracas em nervos periféricos específicos (177). Em relação aos tratamentos não-invasivos destaca-se a Estimulação Transcraniana por Corrente Contínua (ETCC). Outra técnica não invasiva e neuromodulatória é a estimulação magnética transcraniana (EMT), que consiste na indução eletromagnética gerada pela passagem de uma corrente elétrica por meio de uma bobina posicionada sobre o crânio, e um campo magnético é gerado sobre o córtex cerebral (178).

2.8.3 Estimulação Elétrica por Corrente Contínua (ETCC)

A ETCC é uma técnica de neuromodulação que envolve a alteração da ação central, periférica e autonômica do sistema nervoso, usando estimulação elétrica (179). Além do seu uso na melhora da qualidade de vida dos pacientes que sofrem de dor crônica, esta técnica também é aplicada no tratamento da espasticidade, distúrbios do movimento, epilepsia, doença vascular periférica e de transtornos psiquiátricos (180). A ETCC, por sua vez, envolve a aplicação de corrente direta constante de baixa intensidade (1 – 2 mA) no escalpo por meio de eletrodos (20-35 cm^2) para modular a excitabilidade de áreas corticais. No tratamento da dor crônica, este metódo tem mostrado resultados positivos (181). Knotkova & Cruciani (2010) realizou a aplicação da ETCC em pacientes com dor neuropática por lesão medular e, após o tratamento, estes pacientes apresentaram redução na escala análogo visual (EAV). Em 63% dos pacientes, esse benefício perdurou por cerca de duas semanas. Outro estudo feito pelo mesmo grupo apresentou resultados semelhantes em pacientes com diagnóstico de fibromialgia (28).

A estimulação anódica provoca a despolarização (aumenta a excitação) dos neurônios corticais enquanto aestimulação catódica hiperpolariza (diminui a excitação) (32,183). Além disso, esta técnica apresenta baixo risco e pouco desconforto, e com a utilização em sessões repetidas o efeito pode ser duradouro (33). O efeito da ETCC a curto prazo (efeito imediato) é, respectivamente, devido a uma diminuição (anódica) ou aumento (catódica) do limiar de repouso neuronal (184). No entanto, os efeitos em longo prazo envolvem a participação do BDNF (185), que é considerado um marcador de plasticidade neuronal sendo associado ao processamento da dor (186) e a diversos sistemas de neurotransmissores, como o sistema dopaminérgico, serotonérgico, acetilcolinérgico e gabaérgico (187–189).

Os efeitos da ETCC dependem da área do cérebro que é estimulada (190), além dos efeitos locais também é observado alterações em áreas corticais e subcorticais interconectadas (191).

O tratamento com ETCC para dor crônica tem como foco o córtex motor, região que representa área adjacente à área da dor (28). Embora a aplicação de ETCC seja de corrente fraca, provoca uma alteração na excitabilidade neuronal espontânea em regiões corticais (Plow et al., 2012). Evidências sugerem que o alívio da dor pela ETCC depende da projeção das fibras do córtex motor para outras estruturas envolvidas no processamento da dor, como o tálamo e núcleos do tronco cerebral (192,193).

A ETCC anódica aplicada em córtex aumenta o acoplamento funcional entre a região M1 ipsilateral e tálamo, onde o provável efeito analgésico seja em decorrência da modulação da atividade talâmica (191), mudanças no fluxo sanguíneo cerebral indicam que a estimulação do córtex motor desencadeia uma ativação rápida e fásica do tálamo, giro cingulado, insula e tronco cerebral (194).

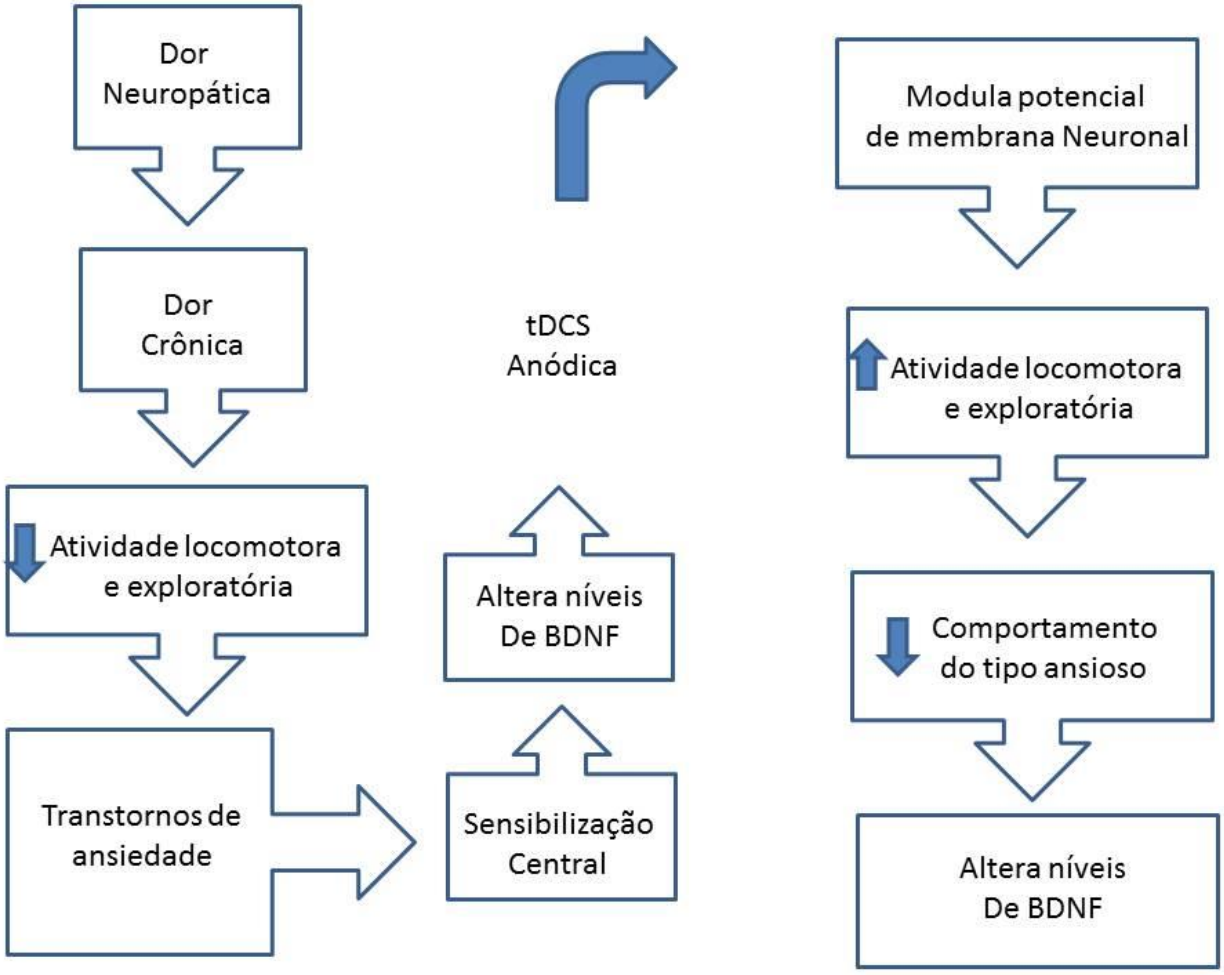
Estudos prévios demonstraram o envolvimento da ETCC anódica com o sistema opioide; foi observado que a estimulação sobre o córtex motor resulta em um aumento da liberação de opioides endógenos, sugerindo um efeito analgésico através desse mecanismo (195).

A segurança dessa técnica também tem sido constantemente observada. A possibilidade de causar lesão cerebral pela formação de produtos tóxicos não existe, pois não há interação dos eletrodos com o córtex cerebral (196). Estudo com ressonância magnética antes e após 30 e 60 minutos da estimulação cerebral, aplicada em córtex motor ou pré-frontal, não indicou alteração patológica, concluindo que a ETCC não induz edema cerebral, alterações da barreira hematoencefálica ou do tecido cerebral (197). Por fim, estudo de Accornero et al. (2007) mostrou que durante e após 20 minutos do término da estimulação não são observadas variações em batimento cardíaco, pressão arterial ou temperatura.

Considerando os mecanismos envolvidos na dor neuropática e suas comorbidades associadas e na busca de um melhor entendimento sobre os mecanismos dessa terapia, esta dissertação visa avaliar o efeito do tratamento repetido com ETCC nos comportamentos do tipo ansioso, locomoção, atividade exploratória e em marcadores neuroquímicos relacionados a esta patologia.

III. MARCO TEÓRICO

3 MARCO TEÓRICO



IV. JUSTIFICATIVA

4 JUSTIFICATIVA

Estudos preclinicos com a utilização da ETCC em quadros de dor crônica (198,199), e nos transtornos neuropsiquiátricos (200–202), vem crescendo de forma exponencial, entretanto há escassez na literatura de estudos que avaliem o efeito da ETCC em parâmetros comportamentais do tipo ansioso e atividades exploratória e de locomoção na dor neuropática. O uso de modelos animais nos permite investigar os efeitos da ETCC no comportamento e nos níveis de mediadores neuroquímicos. Este estudo buscou avaliar além de paramentros comportamentais, um importante marcador de neuroplasticidade como o BDNF. Hipotetiza-se inicialmente que o efeito terapêutico da ETCC reverta as alterações comportamentais e neuroquímicas em ratos Wistar induzidas pelo modelo de dor neuropática. A compreensão do efeito desta terapêutica não-farmacológica na dor neuropática nos permite aprimorar sua aplicalidade no que tange a questão translacional, além de agregar conhecimentos de mecanismos neuroquímicos desta patologia. Desta forma, esta dissertação contribui com ampliação do conhecimento sobre efeitos terapêuticos da ETCC em alterações neuroquímicas e comportamentais induzidos por quadro de dor crônica com característica neuropática.

V. OBJETIVOS

5 OBJETIVOS

5.1 Objetivo Geral

Esta dissertação teve como objetivo avaliar o efeito da ETCC nas atividades locomotora e exploratória, no comportamento do tipo ansioso e nos níveis medular e cortical de BDNF em ratos submetidos a um modelo de dor neuropática. Todos os parâmetros foram avaliados 48 horas e 7 dias após o último dia de tratamento com ETCC.

5.2 Objetivos Específicos

5.2.1 Avaliar as atividades exploratória e de locomoção de ratos submetidos a um modelo de dor neuropática e tratados com ETCC repetida

5.2.2 Avaliar o comportamento do tipo ansioso de ratos submetidos a um modelo de dor neuropática e tratados com ETCC repetida

5.2.3 Quantificar os níveis de BDNF em soro, medula espinhal, cortex cerebral e tronco cerebral de ratos submetidos a um modelo de dor neuropática e tratados com ETCC repetida.

VI. REFERÊNCIAS DA REVISÃO DE LITERATURA

REFERÊNCIAS DA REVISÃO DA LITERATURA

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VII. ARTIGO CIENTÍFICO

LONG-LASTING EFFECT OF TRANSCRANIAL DIRECT CURRENT STIMULATION (tDCS) IN THE REVERSAL OF BEHAVIORAL ALTERATIONS AND BRAINSTEIM BDNF LEVELS INCREASE INDUCED BY NEUROPATHIC PAIN MODEL

Periódico: Brain Stimulation

Status: A ser submetido

LONG-LASTING EFFECT OF TRANSCRANIAL DIRECT CURRENT STIMULATION (tDCS) IN THE REVERSAL OF BEHAVIORAL ALTERATIONS AND BRAINSTEIM BDNF LEVELS INCREASE INDUCED BY NEUROPATHIC PAIN MODEL

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ABSTRACT

Introduction: Neuropathic pain (NP) is a chronic pain modality that usually results of damage in the somatosensory system. NP often shows insufficient response to classic analgesics and remains a challenge to medical treatment. The Transcranial Direct Current Stimulation (tDCS) is a non-invasive technique, which induces neuroplastic changes in central nervous system (CNS) of animals and humans. The brain derived neurotrophic factor (BDNF) plays an important role in synaptic plasticity process. Behavior changes such as decreased locomotor and exploratory activities and anxiety disorders are common comorbidities associated with NP. **Objective:** The aim of this study was evaluate the effect of tDCS treatment on locomotor and exploratory activities, and anxiety-like behavior, and peripheral and central BDNF levels in rats submitted to neuropathic pain model. **Methods:** Rats were randomized divided: Sham Surgery (Ss), Sham Surgery + Sham tDCS (SsS) Sham surgery + tDCS (SsT), Neuropathic Pain (NP), Neuropathic Pain + Sham tDCS (NpS), Neuropathic Pain + tDCS (NpT). The neuropathic pain model was induced by partial sciatic nerve compression at 14 days after surgery; the hot-plate (HP) test was performed for evaluated the nociceptive response (data not show), and after tDCS treatment was initiated. After the establishment of NP (increase the nociceptive response), the animals of treated groups were subjected to a 20 minutes session of anodal tDCS (0.5 mA), every afternoon for eight days. The Open Field (OF) and Elevated Pluz Maze (EPM) tests were applied after 24 hours (phase I) and 7 days (phase II) the end of tDCS treatment for evaluated locomotor and exploratory activities, and anxiety-like behavior, respectively. The serum, spinal cord, brainstem and cerebral cortex BDNF levels were determined 48 hours (phase I) and 8 days (phase II) after tDCS treatment by enzyme-linked immunosorbent assays (ELISA). **Results:** The CCI induced decrease in locomotor and exploratory activities, increase in the behavior-like anxiety, and increased in the brainstem BDNF levels, the last, in the phase II (one-way ANOVA/SNK, $P<0.05$ for all). On the other hand, the tDCS treatment reverted all these effects induced by CCI (one-way ANOVA/SNK, $P<0.05$ for all). In addition, the tDCS treatment decreased serum and cerebral cortex BDNF levels and it increased these levels in the spinal cord in phase II (one-way ANOVA/SNK, $P<0.05$). **Conclusion:** tDCS reverted behavioral alterations associated to neuropathic pain, indicating analgesic and anxiolytic tDCS effects. Furthermore, tDCS treatment induces changes in the BDNF levels in different regions of the CNS, and this effect can be attributed to different cellular signaling activations.

Keywords: Neuropathic pain, Transcranial Direct Current Stimulation (tDCS), Anxiety-like behavior, Locomotor and Exploratory activities, BDNF, rats

1 INTRODUCTION

Neuropathic pain, a state complex chronic pain, is usually resulted of damage in the somatosensory system, which in some cases can paradoxically lead to abnormally increased nerve activity (1), characterized by hyperalgesia, allodynia and spontaneous pain (2).

Studies showed that a large number of patients with chronic pain suffer from depression or anxiety disorders resulting from severe pain (3,4). In addition, anxiety symptoms in patients with neuropathic pain are frequently reported (5). Neuropathic pain animal models have showed that the level of mechanical sensitive is positively correlated with anxiety behavior (6), indexed by increased thigmotaxis in the open field arena (7). A previous study using neuropathic pain models showed that rats exhibited mechanical hypersensitivity and increase in anxiety-like behavior, which was reduced by treatment with analgesic drugs (Roeska, et al. 2009).

Recently it was reported that the activity-dependent synaptic plasticity in the spinal cord dorsal horn might be a mechanism that contributes to the development of chronic pain produced by sciatic nerve ligation in rats (9). It has been proposed that brain derived neurotrophic factor (BDNF) has an important role in the synaptic plasticity process and spinal dorsal horn nociceptive information signaling (Miletic, et al, 2002). Likewise, BDNF acts as a modulator in the nociceptive response following spinal cord lesion assuming an important role in the development of neuropathic pain after peripheral nerve injury (11). Synapses within each relay are under precise regulation in order to provide appropriate behavioral response (12). For example, functional and morphological changes in neuronal circuits during transcranial direct current stimulation (tDCS) seem to require the regulation of this neurotrophin expression (Ganguly, et al, 2013), and for this reason, BDNF has been used as a biomarker for cortical excitability effects on neuronal activity (5).

In addition, the tDCS is a non-invasive technique that modulates cortical excitability in human motor (14) and visual cortex (15). Furthermore, the tDCS not only changes activity of cortical areas located directly under the electrodes, but also from distant areas probably due the primary interconnections (16). The current model of tDCS effects is based on cortico-cortical interactions, with some subcortical components (e.g., anterior cingulate cortex and thalamic nuclei) in these circuits (17). Clinical studies show that tDCS can improve cognition performance (Nitsche et al. 2003; Fregni, et al. 2006), such as in the stroke patients (20) and chronic pain syndromes (21). Some studies using rats presented the tDCS effects on the memory (22), Parkinson's disease (23), and focal epilepsy models (24). Additionally, data

from our research group revealed immediate and long-lasting effects of repeat sessions of anodal tDCS treatment in the chronic inflammation (25) and hyperalgesia induced by chronic restraint stress models (26). Considering the effects of tDCS on pain and psychiatry disorders, the aim of this study was evaluate the effect of tDCS treatment in the locomotor and exploratory activities, and anxiety-like behavior, and peripheral and central BDNF levels in rats submitted to neuropathic pain model.

2 MATERIALS AND METHODS

2.1 Animals

Male Wistar rats (≥ 250g) between 55 and 65 days old (at the beginning of the experiment) were used. Animals (n=144) were randomized by weight and housed in three animals per home cages made of polypropylene material (49 x 34 x 16 cm) with the floor covered with sawdust. All animals were maintained in a controlled environment (22±2°C), under a standard light-dark cycle (lights-on at 07:00 h and lights-off at 19:00 h), with water and chow (Nuvital, Porto Alegre/ Brazil) *ad libitum*. All experiments and procedures were approved by the Institutional Committee for Animal Care and Use (GPPG-HCPA protocol No. 12-0514) and conformed to the Guide for the Care and Use of Laboratory Animals 8th ed. 2011. The maintenance of the animals followed the law 11.794 (Brazil), which establishes procedures for the scientific use of animals. The experimental protocol complied with the ethical and methodological standards of the ARRIVE guidelines (27). The experiment used the number of animals necessary to produce reliable scientific data. To control the possible effect of outliers, animals that did not present behavioral responses were excluded from the analysis.

2.2 Neuropathic pain model: chronic constriction injury of sciatic nerve (CCI)

The chronic constriction injury (CCI) of sciatic nerve described by Bennett and Xie (1988) was used as a model for the induction of neuropathic pain. Briefly, animals were anesthetized using isoflurane (5% for induction, 2.5% maintenance), and placed in dorsal position for the realization of the left thigh trichotomy and skin antisepsis with alcoholic iodine 2%. Using aseptic techniques, the left common sciatic nerve was exposed at the middle third of the thigh by removing part of the biceps femoris muscle. Close to the sciatic

trifurcation, about 7 mm of the first third of the nerve was free of adhering tissue and three ligatures (4/0 Vycril) and separated by an interval of 1 mm. Therefore, the total length of nerve affected was approximately 5mm. Loose ligation was performed in order to minimize nerve constriction and allow epineural blood circulation. To ensure equal level of constriction, the same investigator performed the ligatures in all rats. After the procedure, the surgical incision was closed using 4.0-mononylon threads. To the sham surgery, the sciatic nerve was exposed similarly to CCI model, but not ligated. After surgery and anesthetic recovery, the animals were allowed in their home cages where they will remain until the day of death. The control group did not undergo surgical procedure.

2.3 Transcranial Direct Current Stimulation (tDCS)

Fourteen days after the surgery (CCI or Sham), animals received anodal tDCS therapy, which consists in a constant low intensity current (0.5 mA) applied 20 minutes every afternoon during 8 days as described by Spezia Adachi et al. (2012). Notably, this model of application required neither anesthesia, unlike models used in previous tDCS studies in rats (30). In fact, this lack of anesthesia strengthens the study because volatile anesthesia (such as isoflurane) has been shown to decrease excitatory transmission and to increase inhibitory transmission (31), altering BDNF levels and, therefore, neuroplasticity (32). Thus, we removed this confounding factor by adapting the human model using ECG electrodes (33) in rats.

The direct current was delivered from a battery-driven, constant current stimulator using ECG electrodes with conductive adhesive hydrogel. Rats heads were shaved for better adherence, and the electrodes were trimmed to 1.5 cm^2 for a better fit. Electrodes had a conductive adhesive hydrogel and were fixed onto the head using adhesive tape (Micropore™) and covered with a protective mesh to prevent removal (Fig.1). The cathodal electrode was positioned at the midpoint between the lateral angles of both eyes (supraorbital area) and the anodal electrode was placed between the ears, on the neck of the rat (parietal cortex). This technique mimics tDCS protocols used in humans (34) and has been applied by our research group showing antinociceptive effects (25,26).

For sham condition, the electrodes were placed in the same positions as the real stimulation; however, the stimulator was turned off during the entire time. Therefore, animals could maintain continuity of the physical sensation of real tDCS conditions.

2.4 Experimental design

Animals were acclimated to the study environment for 1 week before the beginning of the experiment. Afterwards, animals were randomly allocated into 6 groups: Sham Surgery (Ss), Sham Surgery + Sham tDCS (SsS), Sham surgery + tDCS (SsT), Neuropathic Pain (Np), Neuropathic Pain + Sham tDCS (NpS), Neuropathic Pain + tDCS (NpT). At this point, surgical groups received the appropriate intervention (CCI or sham surgery). Fourteen days later, the hot plate test was conducted to evaluate thermal pain sensitivity (hyperalgesia) as efficacy control of pain model, confirming the presence of neuropathic pain in the groups submitted to CCI. Subsequently, animals were treated (tDCS, Sham-tDCS, or no treatment) for 8 days according to the group they belonged to.

To test the effects of tDCS on animal behavior, Open Field (OF) and Pluz Maze (EPM) tests were applied at the time of the tDCS after the seventh session (Behavior test I) and one week after the last treatment day (Behavior test II). To perform biochemical analysis, each group of animals was divided into 2 subsets and killed at different time-points after the last tDCS-treatment (2 and 7 days). As the naïve animals obtained the results similar to sham surgery, no significant difference, we chose to use the sham surgery as control of other groups.

2.5 Open field Test (OF)

The behavioral assessment was performed in a varnished wood cage, measuring 60cm x 40cm x 50cm, with the inside lined with glass. The linoleum floor was divided by dark lines into twelve 13x 13-cm squares. Each trial started immediately after the animal was placed in the back left corner and allowed to explore the surroundings for 5 min (35,36). The box was cleaned between each trial.

Three measures were evaluated during the test: (1) latency to leave the first quadrant (seconds); (2) number total crossings; and (3) number of rearing behaviors (i.e. vertical activity). The number of line crossings (all paws crossed the boundary into a different marked-out area) was taken as a measure of locomotor activity (37). The latency to leave the first quadrant assessed anxiety-like behaviors (38). The amount of time that the animal spent rearing (standing upright on its hind legs) (39) was used to evaluate exploratory activity (36). Grooming was defined as the licking/washing of the head and/or body and indicated biological functions of caring for the body surface (Silveira and Portella 2005).

2.6 Elevated Plus-Maze Test (EPM)

The elevated plus-maze test was used mainly to assess the anxiety-like behavior. The maze was constructed from black PVC synthetic material and elevated to a height of 50 cm above floor level. The apparatus comprised two open arms and two closed arms (50cm x 40cm x 10cm), which extended from a common central platform (10x10cm). The animal was placed in the central area of the EPM facing one of the open arms. During a 5-minute session, the following behavioral measures were recorded: (1) number of non-protected head-dipping movements (NPHD); (2) total time spent on the open arms (TOA); (3) total time spent on the closed arms (TCA); NPHD were considered to occur when the animals dipped its head over the sides of the maze while on an open arm. In the EPM, entering a new area was recorded when all four paws crossed into the new arm or onto the central area (41,42). After each test, the apparatus was cleaned to remove any pet scent.

2.7 Tissue Collection

The animals were killed by decapitation forty-eight hours (Fase I) and seven days after the end of tDCS treatment (Fase II). Central nervous system structures (prefrontal cortex, brainstem and spinal cord) were removed and frozen at -80°C for subsequent analysis.

2.8 Analysis of BDNF

The blood samples were collected and centrifuged in plastic tubes for 10 min at 4500 rpm at 4°C, and the serum and plasma were stored at -80°C. The BDNF levels were determined by sandwich-ELISA using monoclonal antibodies specific for BDNF (R&D Systems, Minneapolis, United States). Total protein was measured by Bradford's method using bovine serum albumin as standard. The serum, spinal cord, cortex and brainstem was collected and frozen at −80°C until the time of testing.

2.9 Statistical Analysis

Data were expressed as the mean ± standard error of the mean (S.E.M). To evaluate behavior parameters (EPM and OF) across groups, one-way ANOVA followed by SNK was performed at different time points (24hs and 7 days) after the last tDCS session. One-way analysis of variance (ANOVA) followed by Student-Newman-Keuls (SNK) was performed to compare all groups in BDNF levels. P values less than 0.05 was reported as statistically significant.

3. RESULTS

Data previous from our group shows that tDCS is able to revert the mechanical and thermal hyperalgesia in neuropathic pain induced by CCI model (Cioato et al. 2014, personal communication).

3.1 Open Field Test

The ANOVA showed that in OF test, in phase I, there was a between-group effect on line crossing number ($F_{(6,62)}$=3.76, P<0.05). And, there was an increase this parameter in the NpT group when compared to Np group ($F_{(6,62)}$=3.76, P<0.05). There was no effect on the other parameters (Fs< 1.60, P>0.05 for all) (Figure 1).

In OF test in phase II, the ANOVA showed differences between groups in the total number of crossing ($F_{(5,64)}$= 16.06, P<0.05), the latency to leave the first quadrant ($F_{(5,64)}$= 26.06, P<0.05) and the rearing number ($F_{(5,64)}$= 24.05, P<0.05). NpT group showed a higher number of line crossing than Np group (($F_{(5,64)}$= 16.06, P<0.05) and NpS ($F_{(5,64)}$= 16.06, P<0.05). The NpT group showed a decrease latency to leave the first quadrant compared to Np ($F_{(5,64)}$= 26.06, P<0.05) and NpS groups ($F_{(5,64)}$= 26.06, P<0.05). Similarly, rearing activity in the NpT group was greater than Np groups ($F_{(5,64)}$= 24.05, P<0.05) and NpS ($F_{(5,64)}$= 24.05, P<0.05). There was no effect on the other parameters (Fs< 2.96. P>0.05 for all) (Figure 1).

INSERT FIGURE 1

3.2 Elevate Pluz Maze test

In PM test in phase I, one-way ANOVA showed that NpT and NpS groups presented decreased time closed arms ($F_{(5,53)}$=5.82, P<0.05) compared to all Np groups. Additionally, the NpT and NpS groups showed increased time in the open arms compared to Np group ($F_{(5,53)}$=6.43, P<0.05) (Figure 2). When observed the activity in NDPH the NpT and NpS group showed increase this variable compared to Np group ($F_{(5,53)}$=5.32, P<0.05). There was no effect on the other parameters (Fs< 4.86. P>0.05 for all) (Figure 2).

During the PM test II the NpT group presented increase of time open arms compared to Np ($F_{(5,59)}$=5.29, P<0.05) and NpS groups ($F_{(5,59)}$=5.29, P<0.05). This same effect was observed for other groups compared to Np and NpS groups (all Fs < 4.69. P<0.05). In addition, the NpT group showed decreased time closed arms compared to Np and NpS group ($F_{(5,59)}$=5.25, P<0.05). No differences were observed between NpS and Np ($F_{(5,59)}$=5.29, P>0.05). The NpT group showed frequency increase in the NDPH in relation to Np and NpS groups ($F_{(5,59)}$=3.88, P<0.05). Interestingly, there was no significant difference between the NP and NPS groups compared to all sham groups (Ss, SsS and SsT) ($F_{(5,59)}$=3.88, P>0.05). There was no significant difference on the other parameters (all Fs< 4.78. P>0.05) (Figure 2).

INSERT FIGURE 2

3.3 Peripheral and central BDNF levels

In the phase I, the serum BDNF levels presented no significant difference between groups ($F_{(5,34)}$= 0.88, P>0.05). On the other hand, in the phase II, it showed a decrease in NpT group compared to other groups ($F_{(5,33)}$= 2.89, P<0.05). In spinal cord BDNF levels showed no significant difference between groups ($F_{(5,27)}$= 0.85, P>0.05) in phase I. But unlike, in phase II NpT group presented an increase compared to other groups ($F_{(5,27)}$=5.82, P<0.05).

Additionally, in phase I, cerebral cortex BDNF levels showed significant decrease in the NpT group compared to other groups ($F_{(5,30)}$= 6.12, P<0.05). However, in phase II there was no significant difference between groups ($F_{(5,30)}$= 2.50, P>0.05). In phase I, the brainstem BDNF levels showed a decrease in the NpT group compared to other groups ($F_{(5,30)}$= 9.06, P<0.05). Furthermore, was observed increase in the NpS group compared Np groups ($F_{(5,30)}$= 9.06, P<0.05). In phase II it showed decrease in the NpT group compared to other groups ($F_{(5,30)}$= 19.14, P<0.05) and Np group demonstrated increase compared to other groups

($F_{(5,30)}$= 19.14, P<0.05). Moreover the NpS group observed decrease compared to Np group ($F_{(5,30)}$= 19.14, P<0.05).

INSERT FIGURE 3

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4. DISCUSSION

This study shows for the first time the tDCS therapeutic effects on locomotor and exploratory activities, and anxiety-like behavior in the neuropathic pain model. After 14 days of surgery, CCI rats, in addition the hyperalgesia (Cioato et al. 2014, personal communication) also showed and anxiety-like behavior, even as decreased of locomotor and exploratory activities. This particular kind of behavior is characteristic of persistent neuropathic pain model (43). Overall the results assessing nociceptive behavior show that CCI decreases thermal nociceptive threshold as determined by hot plate test in the previous study (44).

The CCI animals showed a decrease in the locomotor and exploratory activities in phase II corroborating another study that showed the same results after 3 weeks of the neuropathic pain surgical procedure in rats (45,46). In addition, these exhibited a longer latency to leave of the first quadrant. Walsh and Cummins (1976) suggest that the output latency to leave of the first quadrant is a behavior associated to emotional factors, which may indicate an increase in anxiety-like behavior (48). Most importantly, tDCS treatment reverted these effects induced by CCI, thus we can suggest that this effect could be resulted of tDCS action on cortical areas involved in the pain matrix as thalamus, anterior cingulate cortex (ACC), cortex insular, frontal, premotor and primary sensory and motor cortices (49–52). The theory of pain matrix could explain changes behavior in chronic pain syndromes, such as, in the locomotor and exploratory activities observed in this study. The stimulation of cortical areas possibly inhibited active areas involved in the pain process, leading to reversal of the behaviors induced by CCI group after tDCS treatment. Additionally, previous studies in our group using chronic inflammation (25), and hyperalgesia induced by chronic restraint stress models (26) showed antinociceptive effect of tDCS by cortical stimulation. Beyond analgesic effect, human studies with tDCS shows effects on motor control, stress and depression, that could justify our results (53–55). The adequate locomotor activity results from the interaction

of several neurotransmitters (56), as the opioid system, that is an important modulator of the descending pathway of pain; and it could be involved in this behavior, beyond pain mechanism supression (57). This involvement of opioid system is demonstrated by the opioids receptors antagonist administration (naloxone) that causes a decrease in locomotor and exploratory activities in naive rats (58). Corroborating these data, μ-opioid receptor knockout mice show less exploratory activity (59). According Taylor et al. (2012), brain modulation by eletrical stimulation can induce changes endogenous opioid system in humans. Similarly, motor cortex stimulation (MCS) induced an increase of endogenous opioid activity in patients with chronic pain (61) and anodal tDCS induced increased endogenous opioid release in humans healthy (Dos Santos et al, 2012). We suggest that tDCS could modulate the opioid system, and improve the locomotor and exploratory activities in neurophatic pain rats, further reduced pain. The tDCS application in human studies shows that the stimulation can be made on motor or prefrontal cortex to obtain different effects (63). However, in animal studies the tDCS application is not as precise, because possibly several areas are stimulates, including prefrontal cortex. Another hypothesis suggests that the descending projections of the prefrontal cortex exerts an excitatory control in midbrain dopamine neurons, and on ventral striatum and nucleus accumbens (NAc) induces dopamine release (64). The evidence for this interaction arises from neurochemical and electrophysiological studies demonstrating that the stimulation of the prefrontal cortex increases dopamine release in the NAc and increases burst firing of midbrain dopamine neurons (65–69). The involvement of glutamate in the modulation of dopamine levels in nerve terminals has been demonstrated (70). The stimulation of dopaminergic neurons causes increased locomotion (71), demonstrating its importance in the control of locomotor activity. Therefore, another hypothesis to explain the locomotor and exploratory activities improvement in CCI rats observed in this study is that tDCS treatment modulates the dopaminergic system by frontal cortex excitability promoting dopamine release (72,73). The tDCS applied in humans increases the levels of extracellular dopamine (74). In addition, it has demonstrated that the long-term effect of cortical excitability and neuroplasticity in the motor cortex can be induced by D2 receptor inhibition (75).

Neuropathic pain, as a chronic stressor, induces both physiological and psychological changes, which may lead multiple neuropsychiatric disorders (76). In animal studies, the EPM is a validate device to evaluate the anxiety-like behavior (77). The latency to leave of the first quadrant evaluation in OF test is not a specific variable to assess anxiety, but may suggest a change in this parameter. The Np group showed an increase in latency to leave the first

quadrant (OF) corroborating the anxiety-like behavior assessed by EPM. The tDCS reverted the increase in this parameter induced by CCI model. Np group in phase I and phase II showed an increase over time in closed arms, associated with a decrease in time open arms and in frequency NDPH, demonstrating that neuropathic pain triggered an increase in the anxiety-like behavior. Animal studies that evaluated the effect of neuropathic pain in anxiety-like behavior are inconsistent. There is anxiety-like behavior in the EPM for rats until 2 weeks after receiving the spinal nerve ligation (78), and after spared nerve injury, this effect is maintained for several week (45). Wallace et al., (2007) used the model of HIV-induced peripheral neuropathy showed an anxiety-like behavior until 2 weeks after induction. CCI rats showed anxiety-like behavior in the EPM until 3 weeks after the injury, but did not find these effects using the model of partial sciatic nerve ligation (PSNL) (46). In our study, the CCI rats developed anxiety-like behavior at least until 21 days after surgery. After tDCS treatment the animals showed a reduction in this behavior, demonstrating an anxiolytic tDCS effect. In the EPM, test phase I, the NpT showed a decrease in anxiety-like behavior, however, NpS group demonstrated the same effect compared to Np group. These findings suggest that this sham treatment effect was induced by subacute stress caused by movement restraint. Stress situation can activate endogenous opioid system and to promote behavioral changes, such as decreased anxiety levels, a protective mechanism body's resistance to the stress (80,81). This occurs by blockage or down-regulation of the opioid system by second messengers associated to episode of anxiety and agitation (82). Additionally, in the phase II an anxiolytic tDCS effect was observed too, the animals that received tDCS treatment showed a decrease in time closed arms associated to an increase in open arms. NDPH number was observed increase in animals tDCS treatment. We can suggested that these effects can be associated to the mechanisms of tDCS modulate glutamatergic (Radman et al, 2009), GABAergic (84), opiods (62), cholinergic (85,86), serotonergic (86), catecholamines (87) systems. Two structures with high densities of opioid receptors has been studied extensively in relation to anxiolytic states, the periaqueductal gray (PAG) and the hippocampus (88). Studies have shown morphine anti-stress effect when administered directly to the PAG; and this effect was antagonized by naltrexone (an antagonist more selective of μ receptor) and mimicked by DAMGO, an agonist of the μ-selective receptor (89,90). The acute administration of fentanyl in adult rats increased cortical excitability in hippocampal through GABAergic inhibition ((91). In addition, the μ-opioid receptors in the dorsal hippocampus is involved in anxiolytic behavior; and opiate deficient mice are more anxious than naïve animals (92,93). So that, anxiety disorders may be related, at least in part, to a deficiency in the endogenous opioid system (94). Thus, we also

can suggest that the reduction of anxiety-like behavior could be related to possible effect of tDCS in the opioid system.

In addition, the present study demonstrated that CCI increased BDNF levels in brainstem phase II and that treatment with tDCS decreases serum BDNF levels and increased in spinal cord in phase II experiment, however in cerebral cortex, in phase I, there was decreases of its levels. Additionally, the brainstem BDNF levels were decreased in both phases (I and II). BDNF has been implicated in the control of nociceptive neurotransmission, behavioral pharmacological and physiological process (95). Its role in pain is still unclear, studies show that an attenuation of pain behavior has been associated with decreased BDNF levels (96,97); however, there is evidence that BDNF may play a role antinociceptive in different contexts (98). BDNF is a neurotrophin that is involved in the synaptic plasticity, survival of neurons, and central sensitization contributing to the development and maintenance of neuropathic pain by activation of the dorsal horn NMDA receptors (99). Nevertheless, the underlying effect of tDCS on the BDNF levels mechanisms has not been fully elucidated.

Furthermore, BDNF is believed to be involved in the pathogenesis of several neuropsychiatric disorders. And it is used in some studies as a biological indicator for clinical conditions, such as anxiety, depression, fibromyalgia, and schizophrenia (100–102). On the other hand, the findings of BDNF levels in anxiety disorders are inconsistent. BDNF levels appear to be reduced in individuals with an anxiety disorder, this is not consistent across the various anxiety disorders (103). In humans, the relationship between BDNF levels with anxiety disorders is controversial. Previous studies show decrease of serum levels in patients with anxiety disorder (104–108). Another study shows higher BDNF levels in patients compared to controls (109). And in the other two studies, patients with anxiety disorders showed no significant difference in BDNF levels (110,111). In animal studies, Govindarajan et al. (2006) using genetically engineered mice, showed that BDNF overexpression can leads to leads to elevated anxiety. BDNF knockout mice presented a significant decrease in exploratory behavior, associated increase anxiety-like behaviour (113). The increased levels of BDNF in the spinal cord observed in the present study can be related to the spinal synaptic plasticity, since it is considered a marker of neuroplasticity (114). However, BDNF is not defined exclusively as promoter of excitation and inhibition, also it may play a regulatory role that is dependent normalizing activity of neurotransmission (115). It has been shown its great importance in synaptic scale that is essential for the stability of excitatory synapses dependent neural activity (116). Several signaling pathways can be activated for BDNF binding to its

receptor TrkB (tirosina-quinase B) (117). Dependent activation of TrkB-BDNF can enhance the release of glutamate (118,119) through activation of NDMA receptor (120,121). BDNF acts on presynaptic and postsynaptic terminals (122). Moreover, the increased expression of BDNF regulates NMDA and AMPA receptors and facilitate induction and maintenance phase of long-term potential (LTP) (123) . Alternatively, BDNF is also responsible for regulating of the organization of inhibitory synapses (124). It has been demonstrated that BDNF also acts on the presynaptic terminals to increase the release of inibitory neurotransmitter gamma-aminobutyric acid (GABA), and this effect was suggested to suppress indirectly through excitation by GABAergic signaling (125).

The evaluation of serum and cortex BDNF levels showed that there was decreased in phase I and II, respectively, in NpT group. We suggested that tDCS treatment may exert its therapeutic effects via depolarization induced by anodal stimulation cortical leading to a decreased cortex BDNF level (phase I), consequently, decreasing the serum BDNF level, in long-term (phase II). About this result, we can hypothesize that during tDCS treatment there was increased of central BDNF levels, and 48 hours after, possibly a down-regulation occurred through negative feedback, leading a reduction of these levels. However, 7 days after last session tDCS treatment, this same effect was observed only in peripheral BDNF levels, demonstrating a possible adaptive mechanism of system.

We show in this study that BDNF is increased in brainstem of Np group 23 days after surgery. This result could be associated to brainstem role in ascending pathways. This pathway includes the spinothalamic tract for pain and temperature sensation (126). We believe that the BDNF increase was induced by chronic neuropathic pain. Additionally, the chronic neuropathic pain can be associated directly with high levels of anxiety symptoms (127). Beyond pain management, the tDCS can be a tool to control the anxiety symptoms. The treatment with tDCS was able to alter brainstem BDNF levels in both phases (NpT group), and reduces the anxiety-like behavior, only phase II . Thus these effects can be associated to decreased brainstem BDNF levels in phase II. It is possible that the BDNF plays an important role in anxiety-like behavior in neuropathic pain model. Interestingly, NpS group showed a modulatory effect of brainstem BDNF levels in two phases. In phase I was observed an increase in levels of the NpS group compared to Np. This result could be due to sinergic effect of pain and stress caused by movement restraint. Moreover, in phase II, NpS group decreased BDNF levels when compared to Np group. We suggest that this effect occurs due the subacute stress induced by movement restraint modulating the expression of BDNF, and indicating a functional impairment in their regulation (128). In contrast, the present study

demonstrates an increase in BDNF levels when the animals were exposed to the stressor caused by sham treatment (phase I) and phase II the effect did not happen. Therefore, this can be an acute effect de short term due restraint. Marmigère et al. (2003) observed in rats, acute restraint led to rapid increase in BDNF levels in CNS. As tDCS treatment induces neuronal changes activating various neurotransmitters we believe that this variability in BDNF levels in different structures CNS could be due to its wide action in the neural network circuits that can activate different signaling pathways.

Taken together, the results of this study corroborate previous investigations that showed that tDCS induces therapeutic effects on several neurological and psychiatric disorders, including anxiety and pain symptoms (63,130–133).

5. CONCLUSION

The present data indicate that tDCS could reverte the behavioral alterations caused by chronic neuropathic pain, indexed by changes in the locomotor and exploratory activities, and anxiety-like behavior. Interestingly, tDCS treatment also reverted increased in BDNF brainsteim levels induced by CCI model. In addition the tDCS treatment alters BDNF levels in serum and different CNS regions independently of CCI presence. The variability of the tDCS effects in the levels of BDNF is probability due to activation of different signaling pathways. Future studies should consider neurotransmitter mechanism (opioid system, dopaminergic, glutamatergic and GABAergic system) to provide a better understanding of the effects of tDCS in the neuropathic pain treatment, indexed by locomotor and exploratory activities, anxiety disorders. In addition, it is possible to optimize treatment effects by evaluation of levels BDNF control.

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LEGENDS

Figure 1 .Open field Test. Data expressed as mean ± SEM, n=21-24 animals/group. Ss (sham surgery); SsS (sham surgery+sham tDCS); SsT (sham surgery+tDCS); Np (Neuropathic pain); NpS (neuropathic pain+sham tDCS); NpT (neuropathic pain+tDCS). Group analysis was performed by one-way ANOVA followed by SNK.

Panel A: Number of line crossings.

Phase I: $^{\#}$significant difference in relation to Np groups $P< 0.05$.

Phase II: * significant difference in relation to Ss, SsS, SsT and NpT groups $P<0.05$.

Panel B: Latency to leave first quadrant (seconds).

Phase I there is no significant difference $P> 0.05$.

Phase II: *significant difference in relation to Ss, SsS, SsT, NpS and NpT groups $P<0.05$.

§ significant difference in relation to SsS, SsT, Np and NpT groups $P<0.05$.

Panel C: Number of rearing behaviors (exploratory activity).

Phase I: there was no significant difference $P> 0.05$.

Phase II: *significant difference in relation to Ss, SsS, SsT and NpT groups $P<0.05$.

Figure 2. Plus Maze Test. Data expressed as mean ± SEM, n = 19-22 animals/ group. Ss sham surgery; Ss (sham surgery); SsS (sham surgery+sham tDCS); SsT (sham surgery+tDCS); Np (Neuropathic pain); NpS (neuropathic pain+sham tDCS); NpT (neuropathic pain+tDCS). Group analysis was performed by one-way ANOVA followed by SNK.

Panel A: Total time spent on the open arms (TOA).

Phase I $^{\#}$ significant difference of Ss, SsS, SsT, NpS and NpT groups, $P< 0.05$.

Phase II: * significant difference of Ss, SsS, SsT and NpT groups, $P<0.05$.

Panel B: Total time spent on the closed arms (s) (TCA).

Phase I: $^{\#}$ significant difference of Ss, SsS, SsT, NpS and NpT groups, $P< 0.05$.

Phase II: * significant difference of Ss, SsS, SsT and NpT groups $P<0.05$.

Panel C: Number NDPH.

Phase I: *significant difference of Ss, SsS, SsT, NpS and NpT groups $P< 0.05$.

Phase II: *significant difference of Ss, SsS, SsT and NpT groups $P<0.05$.

Figure 3. BDNF levels. Data expressed as mean ± SEM, n = 5-6 animals/group. Ss (sham surgery); SsS (sham surgery+sham tDCS); SsT (sham surgery+tDCS); Np (Neuropathic pain); NpS (neuropathic pain+sham tDCS); NpT (neuropathic pain+tDCS). Group analysis was performed by one-way ANOVA followed by SNK.

Panel A: BDNF serum levels.

Phase I there was no significant difference, P> 0.05.

Phase II: * significant difference of Ss, SsS SsT, Np and NpS groups, P<0.05.

Panel B: BDNF spinal cord levels .

Phase I there was no significant differenc, P> 0.05.

Phase II: * significant difference of Ss, SsT, Np and NpS groups, P<0.05.

Panel C: BDNF cortex cerebral levels.

Phase I: there was no significant difference, P> 0.05.

Phase II: * significant difference of Ss, SsT, Np and NpS groups, P<0.05.

Panel D: BDNF brainstem levels.

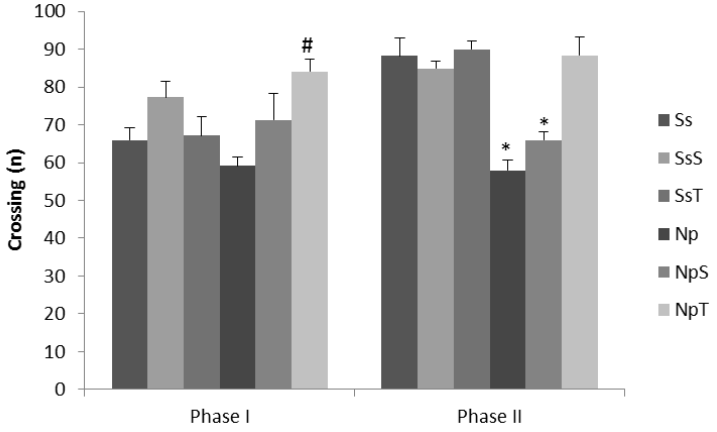
Phase I: *s significant difference of Ss, SsT, Np and NpS groups, P<0.05. # significant difference of. NpS group, P<0.05.

Phase II: * significant difference of Ss, SsT, Np and NpS groups P<0.05. § significant difference of. Ss, SsT and NpS groups, P<0.05. ,

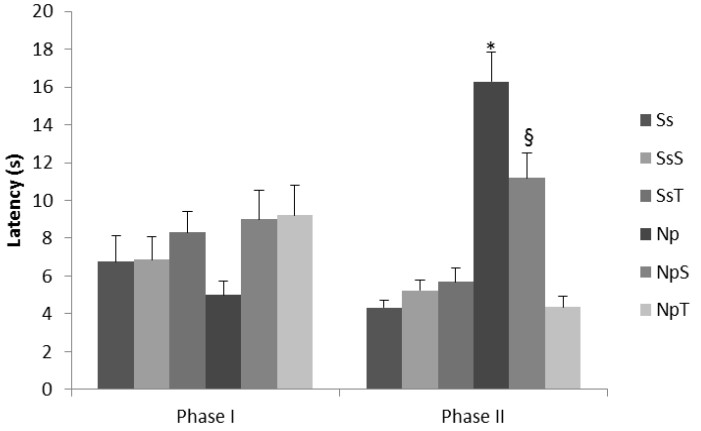
FIGURES

Figure 1

PANEL A



PANEL B



PAINEL C

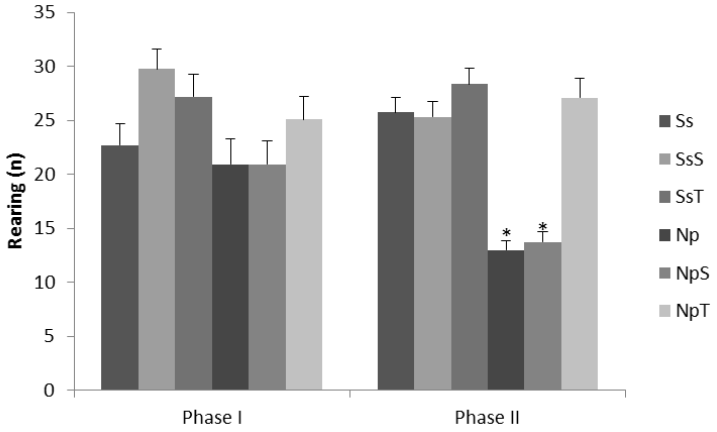
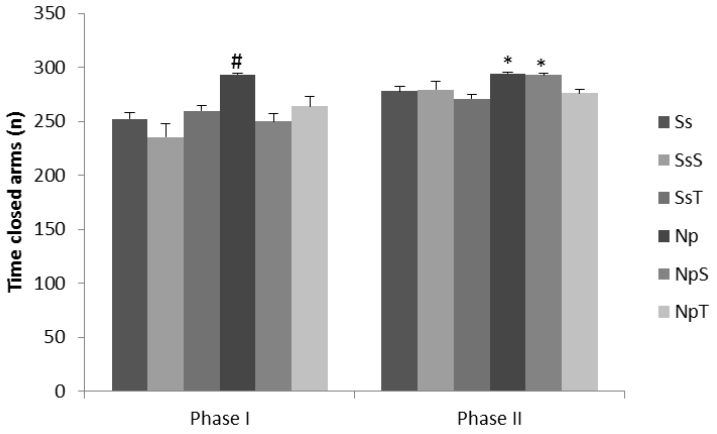
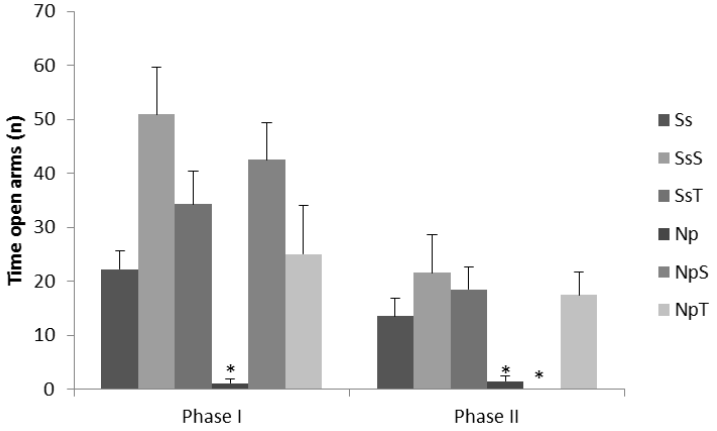


Figure 2

PANEL A



PANEL B



PANEL C

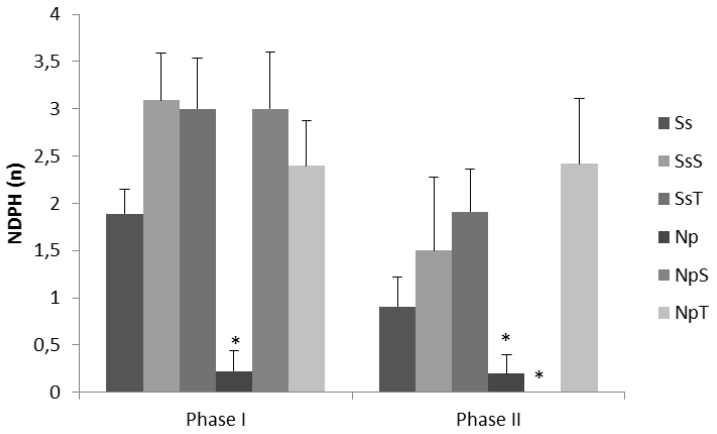
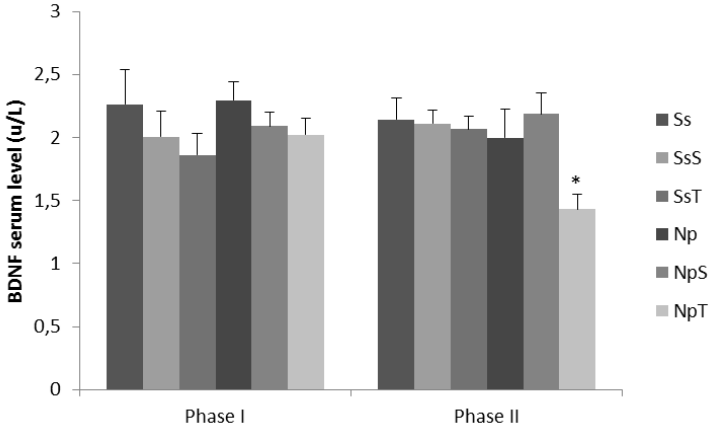
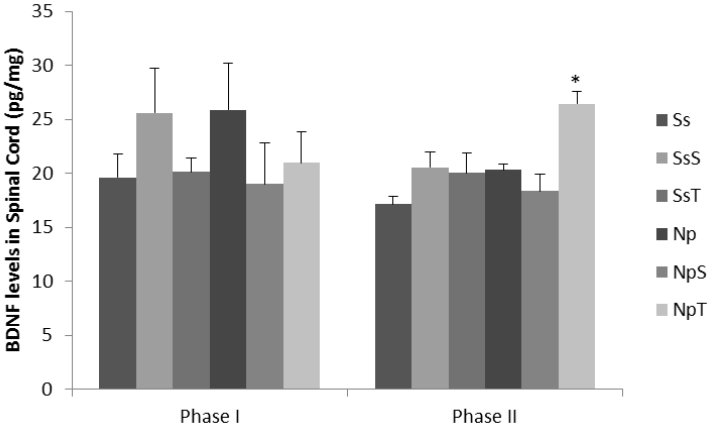


Figure 3

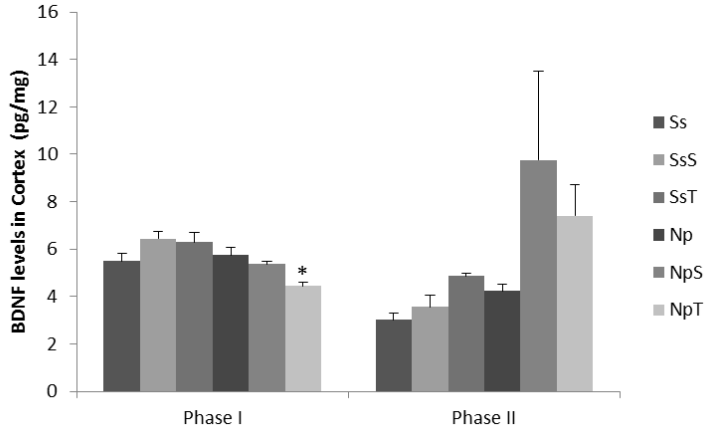
PANEL A



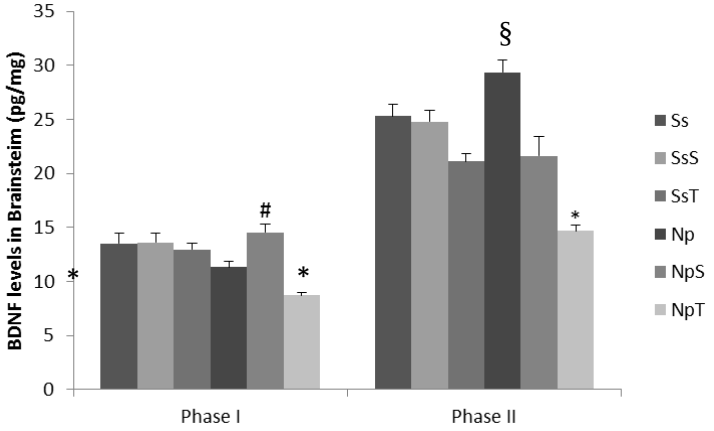
PANEL B



PANEL C



PANEL D


BDNF levels in Brainsteim (pg/mg)
35
30
25
20
15
10
5
0
*
#
*
§
*
Phase I
Phase II
Ss
SsS
SsT
Np
NpS
NpT

VIII. CONSIDERAÇÕES GERAIS

8 CONSIDERAÇÕES GERAIS

O presente estudo demonstrou que a dor neuropática provoca uma diminuição da atividade locomotora e exploratória associada a um efeito ansiogênico, concomitantemente induziu alterações a nível central de BDNF. Salientando, a indução de redução dos níveis de BDNF em tronco cerebral. Estes resultados demonstram que dor crônica pode causar alterações comportamentais, como alterações locomotoras e sintomas ansiedade. Alem de alterações neuroquímicas a nível central.

De acordo com os nossos resultados, o tratamento com ETCC foi efetivo em reduzir estas alterações comportamentais induzidas pelo modelo experimental de dor neuropática, demonstrando ter potencial terapêutico em quadros que cursam com dor crônica. Além disso, a ETCC levou a alterações nos níveis de BDNF independente da presença de dor neuropática, demonstrando o seu potencial em provocar mudanças neuroplásticas em estrututras do sistema nervoso periférico e central.

Concluindo, esta dissertação produziu importantes resultados que podem colaborar no desenvolvimento de terapias não-farmacologicas e não invasivas para o tratamento da dor neuropática. É importante salientar que novos estudos são necessários para o entendimento dos mecanismos da ETCC, acrescentando novos conhecimentos sobre seus efeitos neuroplásticos.

IX. PERSPECTIVAS

9 PERSPECTIVAS

Para melhor entendimento dos efeitos da ETCC nas comorbidades associadas a dor neuropática e seus mecanismos neuroplásticos, torna-se necessário avaliar a medida da expressão do receptor TrkB (tirosina quinase), pelo qual o BDNF tem alta afinidade, e que esta envolvido em mecanismos sinápticos subjacentes de memória e dor. Também, é importante avaliar a funcionalidade de sistemas opióide e dopaminergico, que sao relacionados a atividade exploratoria e comportamentos do tipo ansioso. Além disto, se faz necessario quantificar o receptor NMDA devido ao seu significativo papel no processo de neuroplasticidade.

X. DIVULGAÇÕES

10 DIVULGAÇÕES

2013:

1 Paulo Ricardo Marques Filho; Gabriela Laste; Lauren Naomi Spezia Adachi; Isabel Cristina de Macedo; Joanna Ripoll Rozisky; Izabel Cristina de Souza; Wolnei Caumo; Iraci Lucena da Silva. **Estimulação Transcraniana por Corrente Contínua (ETCC) altera níveis de BDNF no sistema nervoso em modelo animal**. Apresentado sob a forma de Pôster**.** Em: 33ª Semana Cientifica do HCPA, 2013, Porto Alegre, RS Anais da 33ª Semana Cientifica do HCPA, 2013. v. 33. p. 219-219.

2 Stefania Giotti Cioato; **Paulo Ricardo Marques Filho**; Carla de Oliveira; Vanessa Leal Scarabelot; Éllen Nunes; Lauren Spezia Adachi; Alexandre Quevedo; Iraci Lucena da Silva. Efeito da Eletroe**stimulação Transcraniana por Corrente Contínua (ETCC) sobre a resposta nociceptiva de ratos Wistar submetidos a modelo de dor neuropática.** Apresentado sob a forma de Pôster**.** Em: 33ª Semana Cientifica do HCPA, 2013, Porto Alegre, RS Anais da 33ª Semana Cientifica do HCPA, 2013. v. 33. p. 218-218.

3 Ana Claudia de Souza; Liciane Fernandes Medeiros; Andressa de Souza; Carla de Oliveira; Lauren Naomi Spezia Adachi; Rosane Souza da Silva; **Paulo Ricardo Marques Filho**; Vanessa Leal Scarabelot; Joanna Ripoll Rozisky; Wolnei Caumo; Iraci Lucena da Silva. **Exposição gestacional e neonatal a cafeína altera a resposta farmacológica de ratos a agonista adenosinérgico.** Apresentado sob a forma de Pôster**.** In: 33ª Semana Científica do Hospital de Clínicas de Porto Alegre, 2012, Porto Alegre. Anais da 33ª Semana Científica do Hospital de Clínicas de Porto Alegre. HCPA, 2013. v. 33. p. 225-225.

4 Vanessa Leal Scarabelot; Carla de Oliveira; **Paulo Ricardo Marques Filho**; Stefânia Giotti Cioato; Liciane Fernandes Medeiros; Lauren Naomi Spezia Adachi; Ellen Nunes; Andressa de Souza; Wolnei Caumo; Iraci Lucena da Silva Torres**. Reversão da alodinia mecânica por Estimulação Transcraniana por Corrente Contínua (ETCC) em ratos submetidos a um modelo de dor crônica orofacial.** Apresentado sob a forma de **pôster.** In: 33ª Semana Científica do Hospital de Clínicas de Porto Alegre, 2012, Porto Alegre. Anais da 33ª Semana Científica do Hospital de Clínicas de Porto Alegre. HCPA, 2013. v. 33. p. 222-222.

5 Ivan Cirilo Gluz; Vanessa Leal Scarabelot; Carla de Oliveira; Liciane Fernandes Medeiros **Paulo Ricardo Marques Filho**; Stefânia Giotti Cioato; Lauren Naomi Spezia Adachi; Andressa de Souza; Wolnei Caumo; Iraci Lucena da Silva Torres**. Administração aguda de melatonina em ratos Sprague Dawley submetidos a um modelo de dor crônica orofacial reverte alodinia mecânica.** Apresentado sob a forma de **pôster.** In: 33ª Semana Científica do Hospital de Clínicas de Porto Alegre, 2013, Porto Alegre. Anais da 33ª Semana Científica do Hospital de Clínicas de Porto Alegre. HCPA, 2013. v. 33. p. 234-234.

6 Paulo Ricardo Marques Filho; Stefania Giotti Cioato; Carla de Oliveira; Vanessa Leal Scarabelot; Lauren Spezia Adachi; Liciane Fernande Medeiros; Wolnei Caumo; Iraci Lucena da Silva Torres. **Efeitos da Estimulação Transcranian por Corrente Contínua (tDCS) na alodinia mecânica de ratos submetidos a modelo de dor neuropática.** Apresentado sob a forma de pôster. In: V Simpósio Internacional de Neuromodulação, 2013, São Paulo, SP. Anais do V Simpósio Internacional de Neuromodulação, 2013 v. 01, p 1-34

7 Stefania Giotti Cioato; **Paulo Ricardo Marques Filho**; Vanessa Leal Scarabelot; Carla de Oliveira; Liciane Fernandes Medeiros; Lauren Spezia Adachi; Wolnei Caumo; Iraci Lucena

da Silva. **Estimulação Transcraniana por Corrente Contínua (ETCC) reverte hiperalgesia em ratos submetidos a modelo de dor neuropática.** Apresentado sob a forma de pôster. In: V Simpósio Internacional de Neuromodulação, 2013, São Paulo, SP. Anais do V Simpósio Internacional de Neuromodulação, 2013 v. 01, p 1-34

8 Vanessa Leal Scarabelot; Carla de Oliveira; Stefania Giotti Cioato; **Paulo Ricardo Marques Filho;** Lauren Spezia Adachi; Liciane Fernandes Medeiros; Andressa de Souza; Isabel Cristina de Macedo; Wolnei Caumo; Iraci Lucena da Silva. **Eletroestimulação transcraniana por corrente direta (tDCS) reverte alodinia mecânica em ratos expostos a um modelo de dor crônica orofacial.** Apresentado sob a forma de pôster. In: V Simpósio Internacional de Neuromodulação, 2013, São Paulo, SP. Anais do V Simpósio Internacional de Neuromodulação, 2013 v. 01, p 1-34.

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10 Vanessa Leal Scarabelot; Carla de Oliveira; Liciane Fernandes Medeiros **Paulo Ricardo Marques Filho**; Stefânia Giotti Cioato; Andressa de Souza; Ellen Nunes; Iraci Lucena da S'ilva Torres. **Eletroestimulação Transcraniana por Corrente Contínua (ETCC) reverte a alodinia mecânica de ratos submetidos a um modelo de dor crônica orofacial.** Apresentado sob a forma de pôster. In: XLVIII Congresso Anual da Sociedade Brasileira de

Fisiologia, 2013, Ribeirão Preto, SP. Anais da XLVIII Congresso Anual da Sociedade Brasileira de Fisiologia, 2013, p 245-246

11 Liciane Fernandes Medeiros; Vanessa Leal Scarabelot; Carla de Oliveira; **Paulo Ricardo Marques Filho**; Stefania Giotti Cioato; Andressa Souza; Iraci Lucena da Silva Torres. **Administração aguda de melatonina reverte alodinia mecânica em ratos Sprague-Dawley submetidos a um modelo de dor crônica orofacial.** Apresentado sob a forma de pôster. In: XLVIII Congresso Anual da Sociedade Brasileira de Fisiologia, 2013, Ribeirão Preto, SP. Anais da XLVIII Congresso Anual da Sociedade Brasileira de Fisiologia, 2013, p 247-248.

2014

1 Paulo Ricardo Marques Filho; Stefania Giotti Cioato; Carla de Oliveira; Vanessa Leal Scarabelot; Lauren Naomi Adachi; Joanna Ripoll Rozisky; Rafael Vercelino; Alexandre Quevedo; Wolnei Caumo; Iraci Lucena da Silva Torres. **Estimulação Transcraniana por Corrente Contínua (ETCC) aumenta a atividade locomotora e exploratória em ratos com dor neuropática.** Apresentado sob a forma de **pôster.** In: 34ª Semana Científica do Hospital de Clínicas de Porto Alegre, 2014, Porto Alegre. Anais da 34ª Semana Científica do Hospital de Clínicas de Porto Alegre. Clinical Biomedical Research 2014. v. 34. p. 46-47.

2 Carla de Oliveira; Sônia Fátima da Silva Moreira; Vanessa Leal Scarabelot; **Paulo Ricardo Marques Filho**; Liciane Fernandes Medeiros; Éllen Nunes; Jonnsin Kuo; Álexi Vargas Muchale; Wolnei Caumo; Iraci Lucena da Silva Torres. **Depressive-like behavior is reduced by administration of ketamine in ovariectomized rats.** Apresentado sob a forma de **pôster.** In: 34ª Semana Científica do Hospital de Clínicas de Porto Alegre, 2014, Porto Alegre. Anais

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3 Lauren Naomi Spezia Adachi; Rafael Vercelino; Vanessa Leal Scarabelot; **Paulo Ricardo Marques Filho;** Carla de Oliveira; Liciane Fernandes Medeiros; Stefania Giotti Cioato; Alexandre Quevedo; Wolnei Caumo; Iraci Lucena da Silva Torres. **Avaliação do Efeito da Acupuntura e Eletroacupuntura em modelo animal de dor neuropática- Estudo Piloto.** Apresentado sob a forma de pôster. In: 34ª Semana Científica do Hospital de Clínicas de Porto Alegre, 2014, Porto Alegre. Anais da 34ª Semana Científica do Hospital de Clínicas de Porto Alegre. Clinical Biomedical Research 2014. v. 34. p. 190-190.

4 Vanessa Leal Scarabelot; Carla de Oliveira; **Paulo Ricardo Marques Filho**; Stefania Giotti Cioato; Lauren Naomi Spezia Adachi; Liciane Fernandes Medeiros; Andressa de Souza; Alexandre Quevedo; Wolnei Caumo; Iraci Lucena da Silva de Torres. **Estimulação Transcraniana por Corrente Contínua (ETCC) reverte o aumento nos níveis de BDNF em tronco encefálico de ratos submetidos a um modelo de dor crônica orofacial**. Apresentado sob a forma de pôster. In: 34ª Semana Científica do Hospital de Clínicas de Porto Alegre, 2014, Porto Alegre. Anais da 34ª Semana Científica do Hospital de Clínicas de Porto Alegre. Clinical Biomedical Research 2014. v. 34. p. 283-284.

5 Éllen Nunes; Sônia Fátima da Silva Moreira; Isabel Cristina de Macedo; Carla de Oliveira; **Paulo Ricardo Marques Filho**; Jonnsin Kuo; Alexi Vargas Muchale; Iraci Lucena da Silva Torres. **Depressive-like behavior caused by ovariectomy can be reversed with ketamine exposure in Wistar rats.** Apresentado sob forma de poster. In: 1st PanAmerican Congress of Physiological Science, 2014, Paraná.

6 Carla de Oliveira; Sônia Fátima da Silva Moreira; Vanessa Leal Scarabelot; **Paulo Ricardo Marques Filho;** Liciane Fernandes Medeiros; Éllen Nunes; Jonnsin Kuo; Isabel Cristina de Macedo; Alexi Vargas Muchale; Wolnei Caumo; Iraci Lucena da Silva de Torres. **Administration of ketamine reduced depressive-like behavior induced by ovariectomy in rats.** Apresetado sob a forma de poster. In 46th Brazilian Congress on Pharmacology and Experimental Therapeutics, 2014, Fortaleza, Ceára.

7 Vanessa Leal Scarabelot; Carla de Oliveira; Liciane Fernandes Medeiros; **Paulo Ricardo Marques Filho**; Stefania Giotti Cioato; Lauren Naomi Spezia Adachi; Andressa de Souza; Alexandre Quevedo; Wolnei Caumo; Iraci Lucena da Silva de Torres. **Effect of acute melatonin administration in a model of chronic orofacial pain**. Apresentado sob a forma de poster. In 46th Brazilian Congress on Pharmacology and Experimental Therapeutics, 2014, Fortaleza, Ceára.

8 Stefania Giotti Cioato; **Paulo Ricardo Marques Filho**; Vanessa Leal Scarabelot; Carla de Oliveira; Lauren Spezia Adachi; Liciane Fernandes Medeiros; Joanna Ripoll Rozisky; Alexandre Quevedo; Rafael Vercelino; Iraci Lucena da Silva. **Effects of Transcranial Direct Stimulation (tDCS) on mechanical allodynia and hyperalgesia in rats submitted to neuropathic pain model.** Apresentado sob a forma de poster. In 15th World Congress on Pain, 2014, Buenos Aires, Argentina.

9 Vanessa Leal Scarabelot; Carla de Oliveira; **Paulo Ricardo Marques Filho**; Stefania Giotti Cioato; Lauren Spezia Adachi; Liciane Fernandes Medeiros; Andressa de Souza; Alexandre Quevedo; Iraci Lucena da Silva. **Transcranial Direct Current Stimulation (tDCS) reverses the increase in BDNF levels in the brainstem of rats submitted to a model of orofacial**

chronic pain. Apresentado sob a forma de poster. In 15th World Congress on Pain, 2014, Buenos Aires, Argentina.

XI. 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: 120514
Data da Versão do Projeto: 18/01/2013

Pesquisadores:
IRACI LUCENA DA SILVA TORRES
STEFANIA GIOTTI CIOATO
PAULO RICARDO MARQUES FILHO

Título: IMPACTO DA ESTIMULAÇÃO TRANSCRANIANA POR CORRENTE CONTÍNUA (ETCC) NA RESPOSTA COMPORTAMENTAL E NEUROQUÍMICA DE RATOS SUBMETIDOS A UM MODELO DE DOR NEUROPÁTICA

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, 14 de fevereiro de 2013.

Dr. Alessandro Osvaldt
Coordenador CEUA/HCPA

B) Co-autorias em artigos científicos

1. Gabriela Laste; Wolnei Caumo; Lauren Naomi Spezia Adachi; Joanna Ripoll Rozisky; **Paulo Ricardo Marques Filho;** Wanda Parttatta; Felipe Fregni; Iraci Lucena da Silva Torres. **After-effects of consecutive sessions of transcranial direct current stimulation (tDCS) in a rat model of chronic inflammation.** Experimental Brain Research August 2012, Volume 221, Issue 1, pp 75-83

2. Carla de Oliveira; Vanessa Leal Scarabelot; Andressa de Souza; Cleverson Moraes de Oliveira; Liciane Fernandes Medeiros; Isabel Cristina de Macedo; **Paulo Ricardo Marques Filho**; Stefania Giotti Cioto; Wolnei Caumo; Iraci Lucena da Silva Torres. **Obesity and chronic stress are able to desynchronize the temporal pattern of serum levels of leptin and triglycerides.** Peptides 51 (2014) p. 46-53