Faculdade de Medicina

Programa de Pós-Graduação em Medicina: Ciências Médicas

EFEITO DA ESTIMULAÇÃO TRANSCRANIANA POR CORRENTE CONTÍNUA NA HIPERALGESIA INDUZIDA POR ESTRESSE CRÔNICO EM RATOS.

Lauren Naomi Spezia Adachi

Orientadora: Prof. Dra. Iraci Lucena da Silva Torres

DISSERTAÇÃO DE MESTRADO

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Suplente

"Nunca se afaste de seus sonhos, pois, se eles se forem,

você continuará vivendo, mas terá deixado de existir".

Charles Chaplin

A **Deus** e aos meus **pais**, aos quais dedico esta conquista.

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LISTA DE ABREVIATURAS

ACTH: hormônio adrenocorticotrófico

AIE: analgesia induzida por estresse

BDNF: fator neurotrófico derivado do cérebro

CRH: hormônio liberador de corticotrofina

CRS: Chronic restraint stress

CNS: central nervous system

DC: direct current

EAV: Escala Análogo Visual

EMT: estimulação magnética transcraniana

ETCC: estimulação transcraniana por corrente continua

GCS: glicocorticosteróides

HHA: hipotálamo-hipófise-adrenal

HPA: *hipolamic-pituitary-adrenal*

IASP: International Association for the Study of Pain

IL-1: interleucina 1

IL1β: interleucina 1β

LTP: *Long term potentiation/*potenciaçãode longa duração

NMDA: N-metil D-aspartato

PET: tomografia por emissão de pósitron

SA: simpático-adrenal

PVN: *Paraventricular nucleus/*núcleo para-ventricular hipotalâmico

TFL: tail-flick latency/teste de latência de retirada da cauda

TNF: *tumor necrosis factor*/fator de necrose tumoral

TrKB: tropomyocin receptor kinase B

C: control/controle

E: estresse

EN: estresse + neuromodulação

ES: estresse + Sham ETCC

S: stress

SN: stress + neuromodulation

SS: stress + sham tDCS

tDCS: *Transcranianal direct current stimulation*

RESUMO

Introdução e Objetivo: Sabe-se que o estresse crônico induz hiperalgesia de longa duração. Prévios estudos de nosso grupo demonstraram que o tratamento com a eletroestimulação transcraniana por corrente contínua (ETCC) anódica foi eficaz no tratamento de dor inflamatória crônica, porém pouco se sabe sobre os mecanismos de ação desta técnica. Neste estudo avaliamos o efeito do estresse crônico e do tratamento com ETCC anódica diária por 8 dias na hiperalgesia/alodinia e nos níveis séricos de corticosterona, interleucina 1β, níveis de TNFα em hipocampo e BDNF em medula espinhal e tronco cerebral de ratos.

Métodos e Resultados: Foram utilizados 48 ratos machos Wistar com 60 dias de vida divididos em quatro grupos: controle (C), estresse (E), estresse+ETCC (EN) e estresse+SHAM (ES). O modelo de estresse crônico por restrição foi aplicado por 11 semanas (1h/dia/5dias/sem). Após este tratamento foi avaliada a resposta nociceptiva utilizando os testes de von Frey e da Placa Quente, após verificar o estabelecimento da hiperalgesia/alodinia foi então iniciado o tratamento com ETCC anódica/20 min/dia (500μA)/8 dias. Os animais foram eutanasiados por decapitação, o soro e as estruturas foram coletados para posterior análise por ELISA. Após 11 semanas de estresse diário os animais apresentaram diminuição do limiar de dor em ambos os testes (von Frey- C: 65,71±3,36g; E: 47,88±2,22g, Student's t test, P = 0,000; C=12 e S=29] Placa quente- C: 7,00±0,59; S: 3,15±0,19, Student's t test, P= 0,00; C=13 e E=32). Após o final do tratamento de ETCC anódica – o grupo EN apresentou aumento do limiar de dor imediatamente após a última sessão somente na Placa-quente (C: 5,00±0.49s; E: 2,80±0,24s; ES: 2,75±0,25s; EN: 4,77±0,61s; ANOVA de uma via/Tukey's test, P=0.000, n=9-12/grupo) e 24hs após, a ETCC aumentou o limiar nociceptivo em ambos os testes (von Frey C: 65.9±3.28s; E: 47.04±2.59g; ES: 48.57±3.80g; EN: 63,26±3,62g; Placa quente- C: 4,92±0,51s; E: 2,54±0,15s; ES: 2,41±0,28s; EN: 4,00±0,33s; ANOVA de uma via/Tukey's test, P=0,000; n= 9-12/grupo para ambos os testes). Não foi observada diferença estatisticamente significativa nos níveis séricos de corticosterona (C: 385,90±171,54 nmol/L; E: 295,73±158,72 nmol/L; ES: 418,02 ±89,90 nmol/L; EN: 424,85±102,17 nmol/L; ANOVA de uma via/Tukey's test, P>0,05, n=6-7) e interleucina 1 β (C: 46,76±4,93 pg/L; E: 51,22±11,85 pg/L; ES: 58,38±7,45 pg/L; EN: 42,21±3,90 pg/L; ANOVA de uma via/Tukey's test, P>0,05, n=3-6) entre os grupos, As análises neuroquímicas mostraram diminuição significativa nos níveis de TNFα em hipocampo no grupo EN em relação aos demais grupos (C: 128,76±28,65 pg/L; E:126,77±13,00 pg/L; ES: 123,26±5,22 pg/L; EN:52,50±2,00 pg/L ANOVA de uma via/Tukey's test, $P\leq$0,05, n=3-4), E, diminuição significativa nos níveis de BDNF em medula no grupo E em relação aos grupos C e EN (C:78,9+6,5; E:49,9+4,2; ES: 67,4+3,8; EN:77,4+4,5 - ANOVA de uma via, P<0,05; n=5-7 por grupo) e em tronco cerebral este efeito foi observado em todos os grupos submetidos a estresse crônico (E, ES e EE) em relação ao C (C:108,01+12,8; E:66,4+6,2; ES:62,9+8,8; EN:50,1+4,8. ANOVA de uma via, P<0,05; n=5-7 por grupo).

Conclusão: Os resultados neuroquímicos nos permitem afirmar que o tratamento repetido de ETCC foi capaz de reverter a diminuição nos níveis de BDNF em medula espinhal induzida por estresse crônico, mas não em tronco cerebral. A exposição estresse crônico, além de induzir a hiperalgesia/alodinia, altera os níveis de BDNF em medula espinhal e tronco cerebral, estruturas relacionada à transmissão de dor, sem alterar os níveis séricos de corticosterona e interleucina 1β. Adicionalmente mostram que a ETCC anódica induz à diminuição dos níveis de TNFα em hipocampo e reverteu o efeito do estresse crônico na hiperalgesia e na diminuição nos níveis de BDNF em medula espinhal. Podemos sugerir o envolvimento do TNF e do BDNF no mecanismo de ação da ETCC. Salientando que o efeito da ETCC em medula espinhal pode envolver plasticidade neuronal uma vez que a expressão do BDNF no SNC é modificada por insultos diversos, como estresse, e pode funcionar como um mecanismo adaptativo essencial, como neuromodulador nociceptivo.

Palavras-chave: estimulação transcraniana, estresse crônico, hiperalgesia, BDNF, TNFα.

ABSTRACT

Introduction and objective: It is known that chronic stress induces lon term hyperalgesia. According our previous studies, anodal tDCS treatment is effective in reducing inflammatory pain, however the pathway of this technique is still unknown. In this study we evaluate the effect of chronic restrain stress (CRS) and the treatment with anodal tDCS daily session for eight days on nociceptive response, serum level corticosterone and interleukin 1β , hippocampus level TNFα and BDNF levels in spinal cord and brainstem in rats submitted to chronic stress.

Methods and Results: 48 male Wistar rats 60 days-old were divided into 4 groups: control (C), stress (S), stress+tDCS (SE) and stress+sham tDCS (SS). The stress chronic model was by restraint for 11 weeks (1h per day/5days per wk). After the chronic stress treatment it was verified the nociceptive threshold by von Frey and Hot Plate tests, after hyperalgesia/allodynia was determined , it was initiated the anodal tDCS treatment (20 min per day/8 days at 500μA). The animals were killed by decapitation; the serum and structures were removed, frozen at -80°C and after were analyzed by ELISA assay. The data were analyzed by Student's t test or one-way ANOVA followed by Tukey when necessary, and it was considerable significant when P<0.05After 11 week of restraint stress, the animals presented decrease in the nociceptive threshold in both tests: von Frey (C: 65.71±3.36g; S: 47.88±2.22g, Student's *t* test, *P* = 0.000; C=12 and S=29) Hot Plate (C: 7.00±0.59; S: 3.15±0.19, Student's *t* test, *P*= 0.00; C=13 and S=32). After the end of anodal tDCS treatment per 8 days: immediately after the last session, the SN group presented increase in the nociceptive threshold only in the Hot Plate test (C: 5.00±0.49s; S: 2.80±0.24s; SS: 2.75±0.25s; SN: 4.77±0.61s; one-way ANOVA/Tukey's test, *P*=0.000, n=9-12/group); and 24 hs after the last session, the anodal tDCS there are increase the pain threshold in both tests: von Frey (C: 65.9±3.28s; S: 47.04±2.59g; SS: 48.57±3.80g; SN: 63.26±3.62g) and Hot Plate test (C: 4.92±0.51s; S: 2.54±0.15s; SS: 2.41±0.28s; SN: 4.00±0.33s; one-way ANOVA/Tukey's test, *P*=0.000; n= 9-12/group for both tests). There was no difference in corticosterone(C: 385.90±171.54 nmol/L; S: 295.73±158.72 nmol/L; SS: 418.02 ±89.90 nmol/L; SN: 424.85±102.17 nmol/L; one-way ANOVA /Tukey's test, *P*>0.05, n=6-7) and interleukin 1β levels in serum(C: 46.76±4.93 pg/L; S: 51.22±11.85 pg/L; SS: 58.38±7.45 pg/L; SN: 42.21±3.90 pg/L; one-way ANOVA /Tukey's test, *P*>0.05, n=3-6) between groups. The SN group presented a significant decreased of TNFα in hippocampus in relation to the others groups(C: 128.76±28.65 pg/L; E:126.77±13,00 pg/L; ES: 123.26±5.22 pg/L; EN:52.50±2.00 pg/L one-way ANOVA /Tukey's test, *P*<0.05, n=3-4). The S group presented a significant decreased BDNF levels in spinal cord in relation to other groups (C: 78.9±6.5; S: 49.9±4.2; SE: 77.4±4.5; SS: 67.4±3.8; one-way ANOVA/Tukey, *P*<0,05; n=5-7 per group); in the brainsteim, the S and SEpresented this effect in relation to C group (C: 108.01±12.8; S: 66.4±6.2; SE: 50.1±4.8; SS: 62.9±8.8; one-way ANOVA/Tukey, *P*<0,05; n=5-7 per group).

Conclusion: The neurochemical analysis lead to us to conclude that repeated treatment of anodal tDCS is able to reverse the decrease BDNF levels in spinal cord induced by chronic stress, but this effect was not observed in the brainstem. The chronic stress exposure, besides induces hyperalgesia/allodynia, and alters the BDNF levels in spinal cord and brainstem, both structures related to pain transmission but does not alter corticosterone and interleukin 1β level in serum. Additionally, our results show that anodal tDCS reverses the hyperalgesia and increased BDNF levels in spinal cord and also led to decreased level of TNFα in hippocampus. It is possible to suggest that one probable pathway of tDCS is the decreased level of TNFα in hippocampus and the effect of anodal tDCS in the spinal cord being involved neuroplasticity, and the BDNF expression in CNS is modified by diverse insults, like stress. Our results showed that modulation of tDCS has a significant therapeutic effect in chronic pain.Key-words: trascranial direct current stimulation, chronic stress, hyperalgesia, BDNF, TNFα.

I. INTRODUÇÃO

A dor é um fenômeno multidimensional e de difícil compreensão, referida como uma "experiência sensorial e emocional desagradável associada a um dano real ou descrita em tais termos" (International Association for the Study of Pain - IASP) (Dellaroza et al., 2008). É considerada uma experiência subjetiva que envolve aspectos sensitivos e culturais que podem ser alterados por variáveis socioculturais e psíquicas do indivíduo e do meio (Sá et al., 2009). É o sintoma mais frequente na população sendo classificada temporalmente como aguda ou crônica. Patologias que cursam com dor crônica estão comumente associadas a estresse crônico, dentre estas podemos citar a fibromialgia, síndrome dolorosa miofascial crônica, cefaleia crônica tensional e artrite. Dor crônica é definida como uma dor que persiste de forma contínua e recorrente após a cura da doença ou da lesão. Geralmente não tem caracterização específica e é de difícil definição. É agravada por fatores ambientais, psicopatológicos e estresse, provocando incapacidade funcional e alterando a qualidade de vida de relação e financeira do indivíduo (Castro e Dalto, 2009). Provoca ainda alterações vegetativas, incluindo insônia, falta de apetite e favorece o desenvolvimento de um repertório de respostas de esquiva e fuga relativas a atividades física, social e ocupacional (Margarida et al., 2009).

Estima-se que entre 70 e 85% da população mundial experimentará alguma forma de dor crônica durante a vida (para revisão ver Andresson, 1981). O que determina elevados custos ao sistema de saúde, visto que esta patologia necessita de tratamentos longos e específicos (Almeida et al., 2008). Eventos nocivos, como a dor crônica, provocam ativações dos processos autonômicos, endócrinos e imunológicos. Esses processos interagem entre si, e coletivamente desempenham uma resposta de defesa biológica à lesão (Chapman et al., 2008). Os quadros de dores crônicas são associados a estresse crônico, exemplificado pela desregulação do eixo hipotálamo-hipófise-adrenal (HHA). Indivíduos expostos a condições agudas de estresse mostram um aumento do limiar de dor, conhecido como analgesia induzida por estresse - AIE (*stress-induced analgesia* - SIA), o qual pode apresentar diferentes bases neuroquímicas relacionadas à intensidade de estresse. Por outro lado estudos clínicos têm demonstrado redução nos limiares de dor após longo período de estresse psicoemocional provavelmente decorrente de redução da atividade de sistema opióide central (Ashkinazi & Vershinina, 1999). Consistente com este resultado, prévio estudo demonstrou que ratos machos submetidos a estresse crônico por restrição apresentaram uma diminuição no teste de latência de retirada da cauda

(TFL) (Gamaro et al., 1998, Torres et al., 2001a).

Considerando que o tratamento da dor crônica é relacionado a altos custos direcionados a tratamentos cirúrgicos, farmacêuticos e fisioterapêuticos, como também afastamento do trabalho, deficiência e diminuição da qualidade de vida, a busca por novas terapias que sejam mais eficázes, com menos efeitos adversos e baixo custo são extremamente importantes. Nos últimos anos, métodos relacionados à estimulação do sistema nervoso central têm sido considerados na terapêutica do tratamento da dor crônica. Entre eles o uso de estimulação cortical invasiva e de estimulação cortical não invasiva; esta última dividida em duas modalidades: a estimulação magnética transcraniana (EMT) e a estimulação transcraniana por corrente continua (ETCC). Apesar de a estimulação cortical invasiva ter a vantagem de ser uma estimulação focal e ser usada por um longo período de tempo, esse método é caro e envolve elevados riscos de hemorragia e infecção no campo cirúrgico.

Por outro lado, a estimulação magnética transcraniana é uma técnica segura de estimulação cortical que apresenta efeitos positivos em pacientes com dor neuropática (Lefaucheur, 2006), porém ainda é uma ferramenta de alto custo. Já a ETCC é mais acessível, pois, apesar de oferecer um método menos focal da estimulação do cérebro, a aplicação é simples e oferece pouco ou nenhum risco. Na verdade, pode ser possível projetar dispositivos de ETCC para utilização em casa, de modo que os pacientes possam usar o dispositivo por longos períodos com pouco ou nenhum custo extra (Zaghi et. al., 2010).

A ETCC é aplicada através de um par de eletrodos colocados sobre o crânio nos quais uma corrente contínua de baixa intensidade flui. Dependendo da localização dos eletrodos e da orientação da corrente, sabe-se que esta técnica induz significativas mudanças na excitabilidade cortical. A estimulação anódica leva ao aumento da excitabilidade cortical e a catódica leva a diminuição. Estas mudanças na excitabilidade são explicadas por mudanças sinápticas e efeitos diretos na atividade espontânea neuronal (Riberto et al., 2011).

Nesse contexto, o desenvolvimento de métodos de estimulação cerebral não invasiva pode representar um passo importante no uso clínico da estimulação cortical para o tratamento de dor crônica. Modalidades menos invasivas de estimulação cerebral, como a ETCC, têm demonstrado bons resultados em pacientes com depressão, acidente vascular cerebral, alterações da excitabilidade cortical,

por exemplo, distonia focal, cefaleia, dor crônicae epilepsia (Liebetanz et al., 2006). Essas novas ferramentas de estimulação cortical não invasiva podem ser opções mais seguras com efeitos terapêuticos similares aos da estimulação invasiva.

II. REVISÃO DA LITERATURA

1. ESTRATÉGIAS PARA LOCALIZAR E SELECIONAR INFORMAÇÕES

Na revisão de literatura buscou-se apresentar os principais aspectos do estresse crônico, da indução da hiperalgesia e da dor crônica, assim como as formas de eletroestimulação transcraniana, principalmente, a por Corrente Contínua (ETCC). Abordamos também mediadores de dor e estresse como corticosterona, o fator neurotrófico derivado do cérebro (BDNF), fator de necrose tumoral (TNF) e interleucina 1β (IL1β).

A estratégia de busca envolveu as seguintes bases de dados: MEDLINE (site PubMed), LILACS, SciELO. Foram selecionados artigos publicados entre 1990 e 2012. Os artigos selecionados foram revisados para localizar referências que não haviam sido contempladas na busca. . Também foram utilizados livros-texto e monografias para identificar materiais relevantes.

Nos sites PubMed, LILACS, SciELO e Banco de Teses da CAPES foram realizadas buscas utilizando os termos*: stress, chronic stress, chronic pain, noninvasive brain stimulation pain, TNF, bdnf,IL*1β *and pain.* Em relação ao termo *noninvasive brain stimulation*, foram encontrados 707 artigos no PubMed e 26 artigos no SciELO, já no LILACS foram encontrados apenas 4 artigos. Utilizando-se o termo: *stress* foram encontrados 494471 artigos no PubMed, 11222 artigos no SciELO e 8768 no LILACS. Com o descritor *chronic pain* a busca no PubMed encontrou 61544 artigos, 1745 artigos foram encontrados no SciElo e 1170 no LILACS. Com o descritor: *BDNF* o site PubMed identificou 11138 artigos, no SciElo 32 artigos e no LILACS foram identificados 31 artigos. Ainda, foram encontrados 96233 no PubMed, 469 no Scielo e 571 no LILACS com o termo TNF e 533 no PubMed, 23 no Scielo e 133 no LILACS com o termo IL1β .

Refinando-se a busca, com cruzamentos entre as palavras chave no site da PubMed foram encontrados 25 artigos da combinação entre os termos *noninvasive brain stimulation* e *chronic pain*, com os termos *brain stimulation* e *stress* obteve-se 12 artigos. Enquanto que com os termos *noninvasive brain stimulation* e *BDNF* foram encontrados 3 artigos, *chronic pain* e *tnf* foram encontrados 322 artigos e *stress* e *IL1β* foram encontrados 1316 artigos.

PALAVRAS-CHAVE

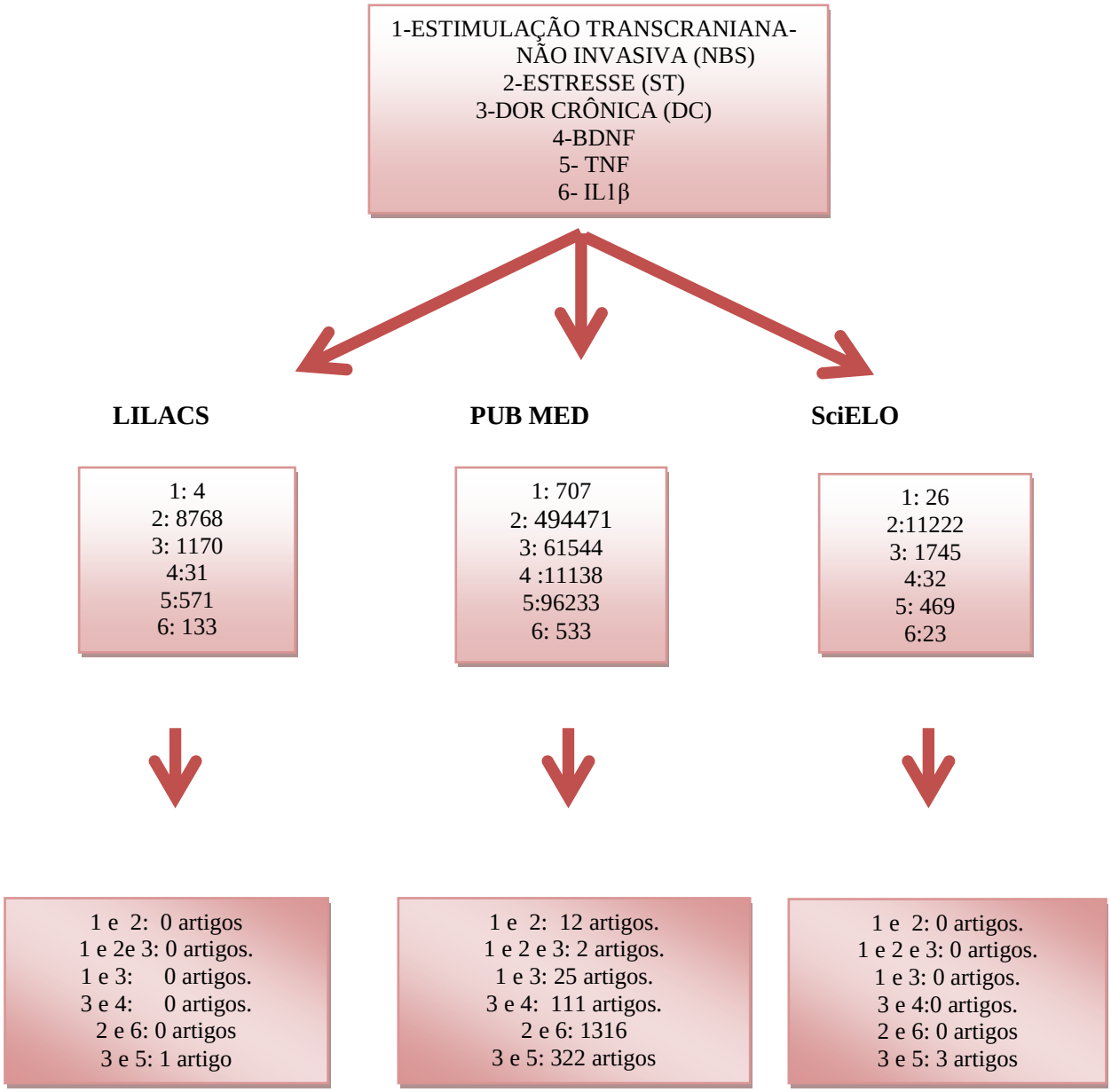

FIGURA 1: Esquematização da pesquisa utilizada para apresentar o tema do projeto.

2. DOR E ESTRESSE

Dor é uma resposta do organismo a qualquer evento prejudicial ao organismo. Segundo Willis (1621 – 1675 d.C), a dor previne a lesão e gera movimentos de proteção ou fuga para proteger o organismo da agressão. Assim, a dor pode ser considerada como o sintoma mais comum de diversas patologias e devido a isso se estima que cerca de 80% da população mundial apresentarão dor em algum período da vida (para revisão ver Andersson, 1981).

A dor pode ser classificada em aguda ou crônica, segundo critério de classificação temporal. A dor aguda tem geralmente uma causa próxima e exerce a função de proteção, associando o estímulo nocivo a uma sensação desagradável (Woolf, 2004). Pode ser causada por doenças subjacentes, traumas ou alterações funcionais musculares ou viscerais. Normalmente cessa em alguns dias ou semanas com o uso de analgésicos clássicos. A dor pode ser considerada crônica quando persiste além do estágio de proteção, muitas vezes não apresentando uma causa específica. A IASP considera três meses como ponto de transição entre dor aguda e dor crônica não oncológica (Merskey e Bogduk, 1994), porém outros autores sugerem que a dor crônica pode variar de um a seis meses (Loeser et al., 2001; Morgan e Mikhail, 1996). A dor crônica não depende da causa original, é de difícil tratamento sendo considerada uma doença (Mainar et al., 2012). Esta patologia atualmente é o terceiro maior problema de saúde pública, perdendo apenas para doenças cardiovasculares e câncer (Mainar et al., 2012). Está ainda relacionada a altos custos direcionados a tratamentos cirúrgicos, farmacêuticos e fisioterapêuticos, como também afastamento do trabalho, deficiência e diminuição da qualidade de vida.

Dor crônica é um problema multidimensional, influenciada por fatores biológicos, psicológicos e sociais. Devido a isso, patologias dolorosas crônicas estão comumente associadas ao estresse crônico, dentre estas podemos citar a fibromialgia, síndrome dolorosa miofascial crônica, cefaleia crônica tensional e artrite. A síndrome dolorosa miofascial é uma das causas mais comuns de dor crônica relacionada ao estresse crônico, e é caracterizada como uma disfunção regional proveniente de pontos de gatilho (pontos hipersensíveis ou *trigger points*) localizados nos músculos, inserções tendinosas e fáscias; pode manifestar efeitos de sensibilização central ou hiperexcitabilidade.

As causas mais comuns da síndrome dolorosa miofascial são traumatismos, microtraumatismos repetitivos, sobrecargas agudas, descondicionamento físico, acidentes automobilísticos e estresse emocional (Simons *et a.l*, 2005).

Sabe-se que a exposição a condições agudas de estresse levam ao aumento do limiar de dor, conhecido como analgesia induzida por estresse agudo (AIE) (Terman et al., 1986). Por outro lado estudos clínicos mostram que há redução no limiar de dor após longo período de estresse psico-emocional, o qual pode ser decorrente da redução da atividade do sistema opióide cerebral (Ashkinazi & Vershinina, 1999). Estudos em animais têm associado este fenômeno ao aumento da sensibilidade dolorosa causando hiperalgesia e alodinia (Bardin et al. 2009, Gamaro et al. 1998, Torres et al. 2001a). Prévio estudo demonstrou que ratos submetidos ao estresse crônico por restrição apresentam resposta hiperalgésica após 6 semanas de estresse diário tanto no estado basal quanto logo após a exposição aguda ao estressor (Torres et al., 2001b). Vinte oito dias após a suspensão do tratamento de estresse o animal recupera a capacidade de responder com AIE, no entanto a hiperalgesia permanece, demonstrando ser de longa duração (Torres et al., 2001b). Paulson e colaboradores (2002) relataram que a dor persistente modifica o sistema reticular ativador ascendente. Este efeito pode ser resultado de adaptação do eixo HHA (Hipotálamo – Hipófise – Adrenal), alteração em receptores opióides e/ou em outros sistemas relacionados com a resposta ao estresse e que envolvam algum tipo de plasticidade neural. Sugere-se que o estresse prolongado induz alterações duradouras no sistema neural envolvido com a modulação nociceptiva e que persistem após suspensão do evento estressante.

A influência do estado psicológico sobre a higidez dos indivíduos bem como sobre sua susceptibilidade e capacidade de recuperação a estados patológicos já é conhecida de longa data. Na Antiguidade, essa interação corpo-mente foi bem evidenciada por Aristóteles (384-322 a.C.) que sugeria que psique e corpo interagiam complementarmente. Assim, alterações da psique eram promotoras de mudanças no corpo, e vice-versa. Já na era Cristã, Galeno (129-200 d.C.) observou que mulheres melancólicas eram mais susceptíveis ao desenvolvimento de tumores de mama que "mulheres de sangue quente" (Dunne, 1988). Na era Moderna, Calzolari em 1898 mostrou que os hormônios gonadais influenciavam a celularidade tímica (1898 apud Lawrence, 2000). A sistematização e definição destes conhecimentos feita por Hans Selye em 1936 permitiram que estas informações

passassem do campo descritivo para o campo conceitual (Neylan, 1998). Selye (1936) definiu estresse como uma resposta estereotipada, não específica do corpo a alguma alteração. Esta resposta foi chamada de síndrome de adaptação geral posteriormente, designada como estresse (Selye, 1974) e caracterizada por aumento de adrenais, sangramento gastrintestinal e diminuição da função de órgãos do sistema imune (Selye, 1974). A partir dos trabalhos de Selye (1936), um maior entendimento do mecanismo de estresse tem sido obtido e tornou-se possível identificar diferentes doenças relacionadas a seus mecanismos de adaptação. A ativação de sistemas envolvidos com estresse leva a mudanças comportamentais e periféricas que buscam manter a homeostase, aumentando a chance de sobrevivência (Tsigos & Chrousos, 2002). Os mecanismos básicos de resposta ao estresse são muito similares e foram bem conservados na escala evolutiva, desde invertebrados a vertebrados superiores (Ottaviani et al., 1994).

O estresse pode ser classificado em duas grandes categorias: (1) estresse físico ou "sistêmico", secundário a estímulos (por exemplo, hemorragia e respostas inflamatórias) que alteram o estado fisiológico e (2) estresse psicológico ou "processivo" causado por estímulos que promovem ameaça imediata ou futura ao organismo (conflito social, estímulo ambiental aversivo, presença ou sinais do predador) (Sawchenkoet al., 1996).

Neste contexto, define-se o estresse como o conjunto de manifestações desencadeadas frente a um evento adverso. Enquanto que os estressores são definidos como estímulos nocivos internos ou externos que produzem múltiplas reações fisiológicas, comportamentais, emocionais e cognitivas (Goshen, et al., 2009). Alterações no meio ambiente levam, em termos neuroendócrinos, a respostas hormonais que têm como objetivo manter a homeostase do organismo. Eixos HHA e simpático-adrenal (SA) são assim ativados pelos estressores (figura 2). Como respostas imediatas, alteram-se a taxa de descarga dos neurônios simpáticos e a secreção hormonal de catecolaminas no sangue. A resposta simpática leva então ao aumento de frequências cardíaca e respiratória e de pressão sangüínea, broncodilatação, dilatação de pupilas, transpiração e palidez. No pico da resposta, fontes fisiológicas de energia são mobilizadas. O estágio mais tardio é caracterizado pela ativação do eixo HHA que induz a secreção de hormônio liberador de corticotrofina (CRH) pelo hipotálamo, de hormônio adrenocorticotrófico (ACTH) pela hipófise anterior e de glicocorticosteróides (GCS) pelo córtex da

adrenal, em humanos, o cortisol e em roedores, a corticosterona. (Kloet et al., 1993) (fig 2). Esta etapa final pode funcionar como mecanismo supressivo, reduzindo as respostas orgânicas e restabelecendo o balanço fisiológico (Ursin & Olff, 1993). Os GCS atuam em todo organismo para mediar modificações nos processos metabólico, imune e inflamatório, por exemplo, requeridos para adaptação e preparação do organismo para lidar com uma situação estressante, incluindo mudanças na forma de obtenção de energia e no metabolismo (Cullinan et al, 1995) (fig 3).

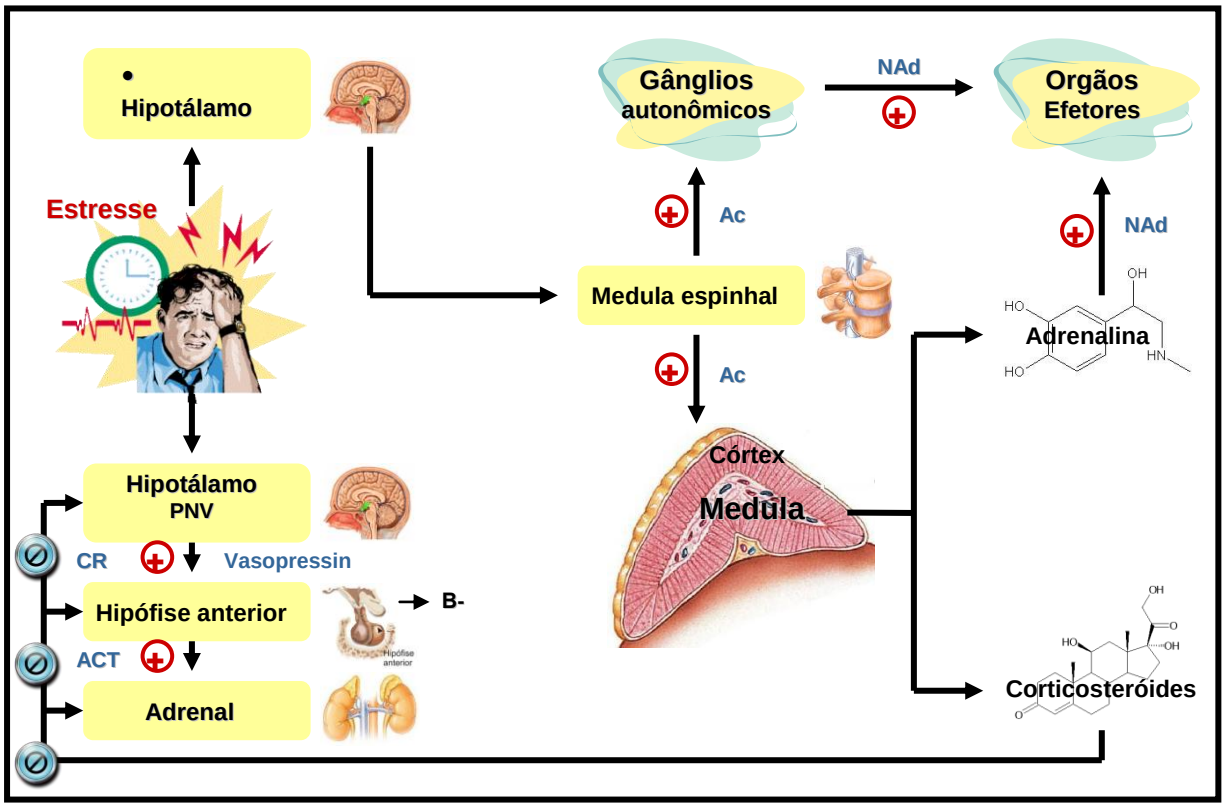

Figura 2: Representação esquemática da ativação de eixos hipotálamo-hipófise-adrenal (HHA) e simpático-adrenal (SA) pelo estresse. PVN= núcleo para-ventricular hipotalâmico (Autor desconhecido).

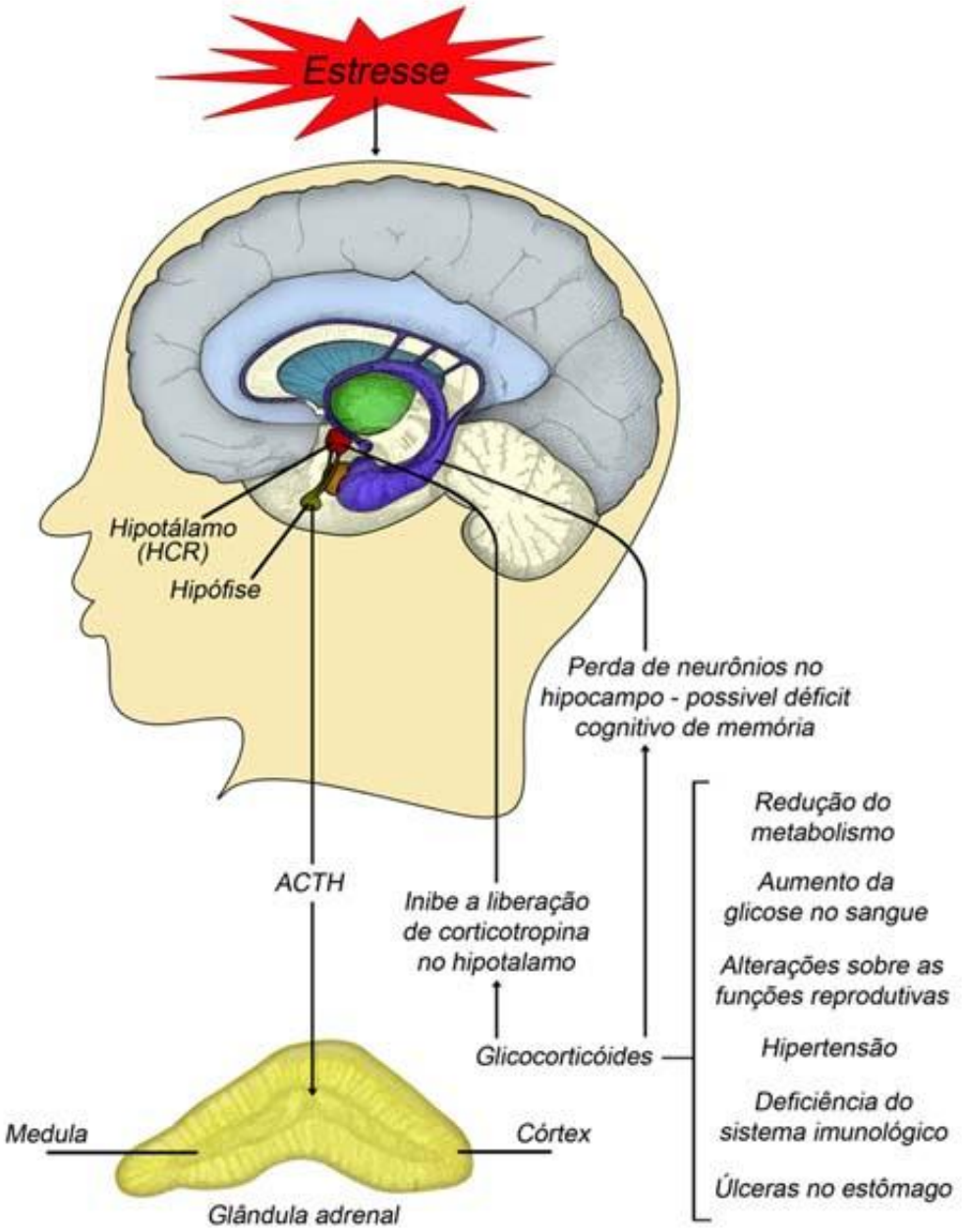

Figura 3: Ações dos Glicocorticóides.

http://www.inec-usp.org/cursos/cursoIV/etiologia_depressao.htm

A ativação do eixo HHA e consequente liberação de GCS visam atenuar o impacto do estressor e frequentemente acontecem de modo organizado e altamente regulado. No entanto, o estresse intenso ou crônico induz uma ruptura nos mecanismos reguladores destas reações de ajustamento. Repercussões patológicas parecem estar associadas a prolongados e sustentados estados de estresse, em

que os mecanismos homeostáticos são mais exigidos (para revisão Goshen & Yirmiya, 2009). Dado a importância do estresse na vida moderna, bem como na psicopatologia, esforços têm sido feitos para identificar estruturas anatômicas, celulares e moleculares que desencadeiam e ou sustentam este processo. Nas últimas duas décadas têm-se evidenciado múltiplas vias de comunicação bidirecional entre o cérebro e os sistemas endócrino e imune que juntos coordenam as repostas ao estresse. Mais recentemente tem sido focado o impacto do estresse na função do sistema imune e na vulnerabilidade ao processo de doença (para revisão Goshen & Yirmiya, 2009). Isto envolve respostas celulares e moleculares. Interessantemente, os processos imunes não têm sido vinculados somente à infecção ou ferimento, mas também aos estressores físicos ou psíquicos. Isto evidencia que citocinas pró-inflamatórias - do sistema de imunidade nata, macrófagos, monócitos, e a micróglia - do sistema nervoso central têm funções nas repostas endócrinas e comportamentais ao estresse (para revisão Goshen & Yirmiya, 2009). Dentre as citocinas inflamatórias relacionadas ao estresse citam-se as interleucinas IL-1, IL-6, associada à modulação do eixo HHA e o fator de necrose tumoral-alfa (TNF-alfa). Frente a situações estressoras a IL-1 ativa o eixo HHA, os processos comportamentais e os mecanismos de plasticidade neural, sendo um mediador crítico da resposta adaptativa ao estresse (para revisão Goshen & Yirmiya, 2009). Várias evidências indicam que a IL-1 induz ativação do eixo HHA, pelo menos em parte, por alterações na neurotransmissão noradrenérgica hipotalâmica. Esta via é mediada pelo IL-1β, que ativa protoncogenes c-fos nas células que produzem o hormônio liberador de corticotrofina (CRH) nos núcleos paraventriculares via projeções noradrenérgicas ascendentes medulares. Além disto, a IL-1 periférica pode estimular o núcleo do trato solitário, medula ventrolateral e locus ceruleus a secretarem noradrenalina, que, consequentemente aumenta a IL-1 hipotalâmica via micróglia (para revisão Goshen & Yirmiya, 2009). Então, esta relação entre a IL-1 cerebral e o sistema noradrenérgico parecem ser bidirecionais, pois os desfechos fisiológicos e comportamentais induzidos pela ativação do sistema imune são similares aos da ativação induzida por estressores psíquicos. Adicionalmente, a IL-1 induz hiperalgesia e sintomas depressivos, mesmo na ausência de inflamação (Johnson et al., 2003). Também medeia um conjunto de sintomas neurocomportamentais e neuropsicológicos que produzem uma síndrome comportamental de doença (sickness behavior syndrome), que é considerada adaptativa em certas condições. Os sintomas desta síndrome incluem

anorexia, perda de peso, má qualidade de sono, retardo psicomotor, fadiga, alterações do comportamento social, mudança na percepção da dor e anedonia (redução na capacidade de experimentar prazer ou gratificação) (Dantzer, 2004).

Adicionalmente, o BDNF, uma neurotrofina envolvida em diversas funções incluindo o processo de plasticidade neuronal tem sua expressão no SNC modificada por insultos diversos, como estresse, isquemia, hipoglicemia e depressão. Como mecanismo adaptativo essencial exerce papel crucial no processo de potenciação sináptica de longa duração (LTP) (Kossel et al., 2001), mecanismo de neuroplasticidade essencial para desencadear e sustentar o processo de memória de dor. O aumento de BDNF incrementa a LTP enquanto a redução de seus níveis a atenua (Figurov et al. 1996; Korte et al. 1995; Patterson et al. 1996). Esta neurotrofina também tem sido descrita como moduladora de dor, perifericamente atuaria sensibilizando neurônios nociceptivos do gânglio da raiz dorsal (para revisão ver Merighi 2008). Shu e colaboradores (1999) demonstraram que animais apresentaram hiperalgesia térmica que perdurou por 5h após a injeção intraplantar de BDNF. Além disto, foi demonstrado aumento na liberação de BDNF em medula espinhal após insultos nervosos, como isquemia do nervo ciático e a transecção do nervo (Walker et al. 2001). Por outro lado, alguns autores sugerem que síndromes dolorosas levariam à modulação supraespinhal dos níveis de BDNF (Pezet e McMahon, 2006). Estudos demonstraram que a dor inflamatória aguda (formalina) ou crônica (adjuvante de Freund) está associada à diminuição dos níveis de BDNF no hipocampo, o que também é observado em ratos estressados cronicamente (Pezet e McMahon, 2006). Concomitante a estes resultados, Chigr e colaboradores (2009) observaram diminuição da neurogênese no Complexo Vagal Dorsal, assim como em hipocampo, em ratos estressados cronicamente por imobilização.

A sensibilização central está frequentemente presente em doenças que cursam com dor crônica envolvendo uma série de adaptações de médio e longo prazo que levam a alterações hormonais e na neurotransmissão. Os dados expostos acima sugerem que os processos de dor crônica e de estresse crônico estão intimamente ligados e dependem de um processo em cadeia de longo prazo, e a mesma via, muito provavelmente é seguida quando tratamentos farmacológicos ou não farmacológicos são utilizados, sendo ncessário a busca de marcadores destes processos. Concomitante a isto, a busca por novas terapias que focalizem a fisiopatologia da dor e do estresse, e que contribuam para o melhor

entendimento dos mecanismos envolvidos na resposta nociceptiva frente à neuromodulação central, são de fundamental importância, uma vez que os resultados obtidos poderão nortear estratégias terapêuticas mais eficazes no tratamento da dor crônica.

3.ELETROESTIMULAÇÃO TRANSCRANIANA POR CORRENTE CONTÍNUA

Desde a Idade Média, existem documentos ilustrados que demonstram o uso dos chamados "peixes elétricos", produtores de descargas elétricas sendo utilizados em crises dolorosas (Young et al., 1994; Jorge et al., 2007). Porém o uso de correntes elétricas foi sempre direcionado para estruturas periféricas (Jorge et al., 2007). Com o desenvolvimento tecnológico, o uso de aparelhos que conduzem correntes elétricas tornou-se mais comum e sua utilização para estimulação de tecidos e órgãos passou a ser foco de atenção (Basford, 1993; Johnson, 2003).

As correntes elétricas são habitualmente utilizadas na reabilitação de lesões de nervos periféricos ou no treinamento funcional de pacientes com lesões medulares ou encefálicas, entretanto o alvo desses tratamentos é quase sempre o nervo periférico ou o próprio músculo (Johnson, 2003).

A estimulação direta do encéfalo vem sendo empregada desde 1930, inicialmente introduzida por Cerletti e Bini na Itália com a eletroconvulsoterapia e, posteriormente, por meio através do uso da estimulação cerebral invasiva e estimulação cerebral por corrente contínua (Delgado, 1963). Porém, devido ao grande avanço da farmacologia entre 1960 e 1970, o uso da estimulação cerebral permaneceu restrito aos relatos de casos esporádicos, apesar da relativa segurança e resultados desta técnica.

As primeiras descrições do uso da estimulação invasiva de estruturas encefálicas para o controle da dor surgiram em 1950. A partir daí a técnica passou a ser utilizada também em pacientes sem transtornos psiquiátricos e em outras estruturas, como a substância cinzenta periaquedutal e periventricular. A estimulação analgésica com corrente elétrica ou campos magnéticos sobre o sistema nervoso central é mais recente (a partir de 1967), e pode ser aplicada sobre a medula e estruturas adjacentes (Young et al., 1994). Estudo de revisão demonstra que o alívio da dor em longo prazo pode

ocorrer em 28,6% a 80% dos pacientes de acordo com a localização dos eletrodos e com sua natureza sindrômica (Bittar et al., 2005).

A estimulação do SNC pode ser realizada de forma não invasiva por meio da estimulação magnética transcraniana de repetição (EMTr) ou da estimulação transcraniana com corrente contínua (ETCC), ou de forma invasiva através do implante de eletrodos diretamente no encéfalo.

A ETCC consiste na aplicação de corrente elétrica contínua sobre o couro cabeludo de forma a produzir alterações da excitabilidade cortical. Tem sido proposto que o efeito modulador desse tipo de estímulo sobre o córtex cerebral ocorre em decorrência da hiperpolarização ou despolarização, consequentemente, alteração da atividade e excitabilidade cortical. Esta mudança na excitabilidade pode ser explicada em função da estimulação catódica reduzir o disparo espontâneo de neurônios corticais, devido a uma hiperpolarização do corpo celular, enquanto que a estimulação anódica tem um efeito inverso (Bindman et. al, 1964).

Estudos em humanos demonstraram que a estimulação do córtex motor muda a excitabilidade cortical de acordo com a polaridade da estimulação. Ou seja, a estimulação anódica, que aumenta a excitabilidade cortical, seria mais efetiva no tratamento de síndromes dolorosas (Nitsche and Paulus 2001; Fregni et al. 2006a). Enquanto que a estimulação catódica, que levaria a uma diminuição da excitabilidade cortical, seria mais efetiva no tratamento de transtornos depressivos.

Trabalhos abordando técnicas de captação de imagem encefálica como a tomografia por emissão de pósitron (PET) demonstram que a estimulação anódica aumenta o fluxo sanguíneo em algumas áreas corticais e subcorticais (Lang et al., 2005). Este método também pode modular a excitabilidade cortical visual e motora (Nitsche et al., 2003a; Antal et al., 2004;). A aplicação de estimulação anódica no córtex motor resulta em melhor desempenho motor (Nitsche et al., 2003b; Antal et al., 2004; Kincses et al., 2004;), aumento do aprendizado motor implícito (Kincses et al., 2004) e da memória operacional em sujeitos saudáveis e pacientes com Doença de Parkinson (Fregni et al., 2005; Boggio et al., 2006).

Uma vez que o dextrometorfano, um antagonista de receptores NMDA (N-metil D-aspartato), bloqueia o efeito em longo prazo da ETCC (Nitsche et al., 2003c; Liebetanz et al. 2002) e que estes receptores estão envolvidos com memória e aprendizado (Collingridge et al. 2012) tem sido proposto

o uso da ETCC em intervenções comportamentais como terapias de reabilitação, considerando o efeito potencializador causado pela estimulação cortical não invasiva. Esta hipótese é suportada por estudo desenvolvido por Flöel e colaboradores (2008) que aplicaram ETCC em um paciente com afasia decorrente de acidente vascular encefálico e observaram aprendizado mais rápido e eficiente com a estimulação anódica.

Assim como a EMT, a ETCC pode ser uma ferramenta útil no tratamento de doenças neuropsiquiátricas e nos processos de reabilitação *(*Fregni & Pascual-Leone, 2007), como depressão (Boggio et al., 2007), epilepsia (Fregni et al., 2006b), acidente vascular encefálico (Fregni et al., 2005), Parkinson (Fregni et al., 2006b) e dor crônica (Fregni et al., 2006a e 2006b).

A segurança da ETCC também tem sido constantemente observada por diversos pontos. A possibilidade de causar lesão cerebral pela formação de produtos tóxicos é remota, pois não há interação dos eletrodos com o córtex cerebral (Nitsche et al., 2003b). Lesões dermatológicas por contato eletrodo/pele são prevenidas com o uso de esponjas embebidas em solução salina. 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 induziu edema cerebral, alterações da barreira hematoencefálica ou do tecido cerebral (Nitsche et al., 2004). Além disso, outro estudo não observou variação na concentração de enolase, proteína é considerada um marcador biológico de morte neuronal, (Nitsche et al., 2003c) fornecendo mais elementos sobre a segurança dessa técnica. Accornero e col (2007) mostraram que durante e após 20 minutos do término da estimulação não são observadas variações em batimento cardíaco, pressão arterial ou temperatura. Adicionalmente Kessler e col. (2011) demonstraram que a ETCC é uma técnica segura, porém efeitos sensoriais são normais e devido a isso o grupo sham pode não ser uma boa forma de controle.

Em relato de caso realizado por Silva e col. (2007), foi proposto o uso específico clínico da ETCC para o controle da dor, no qual um paciente, em uso regular de altas doses de opióides e apresentando grande variabilidade no nível de dor associada a câncer de pâncreas, foi submetido à estimulação com ETCC anódica sobre o córtex motor primário (M1) esquerdo. O paciente foi submetido a duas sessões de estimulação por 20 minutos: a primeira com a corrente ativa ligada e a segunda com o aparelho desligado (*sham*) o avaliador foi cegado quanto ao tipo de estímulo aplicado.

A intensidade da dor após a estimulação *sham* permaneceu inalterada seguida de leve elevação. Contudo, quando a ETCC anódica foi aplicada sobre o couro cabeludo do paciente houve uma redução completa do nível de dor, que persistiu por até quatro horas após o término da sessão. Este efeito foi significativo, uma vez que anteriormente o paciente necessitava estar permanentemente em uso de analgésico.

Outro estudo utilizou pacientes com dor neuropática, decorrente de lesão medular traumática, submetidos ao tratamento da dor com ETCC. Onze pacientes receberam a estimulação anódica ativa sobre M1 por 20 minutos por 5 dias consecutivos, enquanto outros 6 pacientes foram aleatoriamente alocados para receber a estimulação *sham*. Houve redução progressiva da dor ao longo dos 5 dias somente nos participantes do grupo sob estimulação ativa. Após 2 semanas, os pacientes foram reavaliados, e dentre os pacientes sob estimulação ativa ainda havia quatro que relatavam aumento do limiar de dor (Fregni et al., 2006).

Fregni e colaboradores (2006) sugeriram eficácia da ETCC no controle da dor utilizando 32 pacientes com fibromialgia aleatoriamente alocadas em três grupos: 1)estimulação ativa sobre o córtex motor primário; 2) sobre o córtex prefrontal dorsolateral; 3) estimulação *sham*. O nível de dor foi avaliado pela Escala Análogo Visual (EAV), que mostrou redução significantemente mais pronunciada nas pacientes com estímulo ativo sobre M1. Apesar de diversos estudos sugerirem a eficácia clínica da ETCC no tratamento de patologias dolorosas, não há rigor suficiente na maioria dos trabalhos para a ilustração da aplicabilidade clínica da neuromodulação cerebral. As principais limitações são grupos heterogêneos, pacientes com diferentes etiologias e a metodologia aplicada (Plow et al. 2012)

Estudos em animais mostraram que o efeito da ETCC sobre a atividade cortical pode permanecer por até uma hora após o término da estimulação, porém em estudos clínicos foram observados efeitos somatórios de aplicações diárias repetidas e persistência destes efeitos por até 2 semanas (Fregni et al., 2006a). Outros estudos demonstraram bons resultados da ETCC em modelos animais de epilepsia focal (Liebetanz et al. 2006), memória (Dockery et al. 2011), Parkinson (Li et al. 2011) e acidente vascular cerebral (Watcher et al. 2011). Nosso grupo de pesquisa demonstrou eficácia

da ETCC em modelo animal de dor inflamatória crônica por pelo menos até 24 horas após o final do tratamento (Laste et al., *2012*).

Concluindo, a ETCC de fraca intensidade é uma modalidade de estimulação cerebral não invasiva, indolor, que é bem tolerada e não apresenta nenhuma sensação auditiva ou sensitiva desagradável como outras técnicas de estimulação cerebral (Nitsche et al., 2003b). Além disso, trata-se de um método seguro e de baixo custo para ser empregado em seres humanos. Com isso, a ETCC pode ser uma opção não farmacológica no tratamento da dor em pacientes com doenças que cursam com dor crônica.

III. JUSTIFICATIVA E OBJETIVOS

3.1.JUSTIFICATIVA E OBJETIVO GERAL

Neste contexto, torna-se premente investigar o efeito da ETCC na função do eixo HHA, buscando possíveis marcadores associados aos mecanismos implicados no processo fisiopatogênico que envolve estresse e hiperalgesia e contribuindo para o melhor entendimento da resposta antinociceptiva frente à eletroestimulação transcraniana. Sendo assim, o presente estudo teve como objetivo avaliar o efeito de uma sessão diária de eletroestimulação transcraniana durante 8 dias na hiperalgesia de ratos estressados cronicamente. Para tanto foi avaliada a resposta nociceptiva e parâmetros neuroquímicos.

3.2. OBJETIVOS ESPECÍFICOS

a. Avaliação da hiperalgesia, através da resposta nociceptiva térmica.

b. Avaliação da alodinia mecânica.

c. Quantificação de IL1β e corticosterona em soro, sendo estes mediadores relacionados à dor e ao estresse, respectivamente.

d. Quantificação de TNFα em hipocampo, já que esta citocina está relacionada a hiperalgesia e a inflamação e o hipocampo é uma das estruturas mais relacionadas a resposta ao estresse.

e. Quantificação de BDNF em tronco cerebral e medula espinhal dos animais, visto que o BDNF é considerado um modulador da dor e está relacionado à plasticidade neuronal e estas estruturas estão diretamente relacionadas a transmissão da dor.

IV. REFERÊNCIAS DA REVISÃO DA LITERATURA

Acconero N, Li Voti P, La Riccia M, Gregori B,.Visual evoked potentials modulation during direct current cortical polarization. Exp. Brain Res. 2007;178(2):261-266.

Almeida I, Sá K, Silva M, Baptista A, Matos M, Lessa I,. Chronic low back pain prevalence in the population of the city of Salvador. Rev Bras Ortop. 2008;43(3):96-102.

Andersson GB. Epidemiologic aspects on low-back pain in industry. Spine. 1981;6(1):53-60. Review.

Antal A, Kincses TZ, Nitsche MA, Bartfai O, Paulus W. Excitability changes induced in the human primary visual cortex by transcranial direct current stimulation: direct electrophysiological evidence. Invest. Ophthalmol. Vis. Sci. 2004; 45(2):702-707.

Ashkinazi IYa, Vershinina EA. Pain sensitivity in chronic psychoemotional stress in humans. Neurosci Behav Physiol. 1999;29(3):333-7.

Bardin L, Malfetes N, Newman-Tancredi A, Depoortère R. Chronic restraint stress induces mechanical and cold allodynia, and enhances inflammatory pain in rat: Relevance to human stress-associated painful pathologies. Behavioural Brain Research 2009; 205:360–366

Basford JR. Physical agents. In: DeLisa JA, Gans BM. Rehabilitation medicine: principles and practice. 2nd ed. Philadelphia: J. B. Lippincott Company; 1993:404-24.

Bindman LJ, Lippold OC, Redfearn JW. The action of brief polarizing currents on the cerebral cortex of the rat (1) during current flow and (2) in the production of long-lasting after effects. J Physiol. 1964;172:369-82

Bittar GR, Kar-Purkayastha I, Owen SL, Bear RE, Green A, Wang SY, Aziz TZ. Deep brain stimulation for pain relief: a meta-analysis. J Clin Neurosci 2005;12(5):515-9.

Boggio PS, Ferrucci R, Rigonatti SP, Covre P, Nitsche M, Pascual-Leone A, Fregni F. Effects of transcranial direct current stimulation on working memory in patients with Parkinson's disease. J. Neurol. Sci., v. 2006;249(1):31-38.

Boggio PS, Rigonatti SP, Ribeiro RB, Myczkowski ML, Nitsche MA, Pascual-Leone A, Fregni F. A randomized, double-blind clinical trial on the efficacy of cortical direct current stimulation for the treatment of major depression. Int. J. Neuropsychopharmacol., 2007; 11(2):249-54.

Castro M, Daltro C. Sleep patterns and symptoms of anxiety and depression in patients with chronic pain. Arq. Neuro-Psiquiatr. 2009;67(1):25-8.

Chapman CA, Jones RS, Jung M. Neuronal plasticity in the entorhinal cortex. Neural Plast. 2008;2008:314785.

Chigr F, Rachidi F, Segura S, Mahaut S, Tardivel C, Jean A, Najimi M, Moyse E. Neurogenesis inhibition in the dorsal vagal complex by chronic immobilization stress in the adult rat. Neuroscience. 2009;158(2):524-36.

Cullinan WE, Herman JP, Battaglia DF, Akil H, Watson SJ. Pattern and time course of immediate early gene expression in rat brain following acute stress. Neuroscience. 1995;64(2):477-505.

Dantzer R. Cytokine-induced sickness behaviour: a neuroimmune response
to activation of innate immunity, Eur. J. Pharmacol. 2004;399–411.

Delgado, JM. Free behavior and brain stimulation. Int Rev Neurobiol 1964;33:349-449.

Dellaroza M, Furuya R, Cabrara M, Matsuo T, Trelha C, Yamada K et al. Caracterização da dor crônica e métodos analgésicos utilizados por idosos da comunidade. Rev. Assoc. Med. Bras. 2008; 54(1): 36-41.

Dockery CA, Liebetanz D, Birbaumer N, Malinowska M, Wesierska MJ. Cumulative benefits of frontal transcranial direct current stimulation on visuospatial working memory training and skill learning in rats. Neurobiology of Learning and Memory 2011; 96:452–460.

Dunne CF, Hormonal Therapy for breast cancer. Cancer Nurs. 1988;11(5):288-94.

Figurov A, Pozzo-Miller LD, Olafsson P, Wang T, Lu B. Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. Nature. 1996;381(6584):706-9.

Flöel A, Rösser N, Michka O, Knecht S, Breitenstein C. Noninvasive brain stimulation improves language learning. J Cogn Neurosci. 2008;20(8):1415-22.

Fregni F, DaSilva D, Potvin K, Ramos-Estebanez C, Cohen D, Pascual AP, et al. Treatment of Chronic Visceral Pain with Brain Stimulation. Ann Neurol 2005;58(6):971-2.

Fregni F, Boggio PS, Lima MC, Ferreira MJ, Wagner T, Rigonatti SP, Castro AW, Souza DR, Riberto M, Freedman SD, Nitsche MA, Pascual-Leone A. A sham-controlled, phase II trial of transcranial direct current stimulation for the treatment of central pain in traumatic spinal cord injury. Pain. 2006a;122(1-2):197-209.

Fregni F, Gimenes R, Valle AC, Ferreita MJ, Rocha RR, Natalle L et al. A randomized, sham-controlled, proof-of-principle study of transcranial direct current stimulation for the treatment of pain in fibromyalgia. Arthritis Rheum 2006b;54(12):3988-98.

Fregni F, Pascual-Leone A. Technology insight: noninvasive brain stimulation in neurology-perspectives on the therapeutic potential of rTMS and tDCS. Nat Clin Pract Neurol. 2007;3(7):383-93.

Gamaro GD, Xavier MH, Denardin JD, Pilger JA, Ely DR, Ferreira MB, Dalmaz C. The effects of acute and repeated restraint stress on the nociceptive response in rats. Physiol Behav.1998;63(4):693-7

Goshen I, Yirmiya R. Interleukin-1 (IL-1): a central regulator of stress responses. Front Neuroendocrinol. 2009;30(1):30-45. Review.

Jorge LL, Hsing WT. Meios físicos em reabilitação. In: Greve JMD. Tratado de Medicina de Reabilitação. São Paulo: Roca; 2007;238-56.

Johnson M. Estimulação elétrica nervosa transcutânea. In: Kitchen S. Eletroterapia: prática baseada em evidências. 2a edição. Barueri: Manole;2003;59-286.

Kessler SK, Turkeltaub PE, Benson JG, Hamilton RH. Differences in the experience of active and sham transcranial direct current stimulation. Brain Stimul. 2012;5(2):155-62.

Kincses TZ, Antal A, Nitsche MA, Bártfai O, Paulus W. Facilitation of probabilistic classification learning by transcranial direct current stimulation of the prefrontal cortex in the human. Neuropsychologia 2004;42(1):113-117.

Kloet ER, Oitzl MS, Joëls M. Functional implications of brain corticosteroid receptor diversity. Cell Mol Neurobiol. 1993;13(4):433-55. Review

Kossel Ah, Cambridge Sb, Wagner U, Bonhoeffer T. A caged Ab reveals an immediate/instructive effect of BDNF during hippocampal synaptic potentiation. Proc Natl Acad Sci U S A. 2001;98(25):14702-7.

Lang N, Siebner HR, Ward NS, et al. How does transcranial DC stimulation of the primary motor cortex alter regional neuronal activity in the human brain? Eur J Neurosci 2005;22(2):495-504.

Laste G, Caumo W, Adachi LNS, Rozisky JR, Macedo IC, Filho PM, Partata WA, Fregni F, Torres ILS. Effect of trancranial direct current stimulation in hyperalgesia for chronic inflammation in rats. Experimental Brain Research, 2012;221(1):75-83.

Lavelle ED, Lavelle W, Smith HS. Myofascial Trigger Points. Anesthesiology Clin 2007;25:841–851.

Lawrence H. Keloid (Alibert)and Intractable Patches of Chronic Inflammantion of The SkinTreated by Scarification. Br. Med. J 2000;2(1959):151.

Liebetanz D, Nitsche MA, Tergau F, Paulus W. Pharmacological approach to the mechanisms of transcranial DC-stimulation- induced after-effects of human motor cortex excitability. Brain 2002;125(10):2238-2247.

Liebetanz D, Klinker F, Hering D, Koch R, Nitsche MA, Potschka H, Löscher W, Paulus W, Tergau. Anticonvulsant Effects of Transcranial Direct-current Stimulation (tDCS) in the Rat Cortical Ramp Model of Focal Epilepsy. Epilepsia 2006;47(7):1216–1224.

Lefaucheur JP, Boërio D, Créange A. Nerve conduction blocks and peripheral neuropathies. J Soc Biol. 2006;200(4):307-21. Review.

Loeser JD; Butter SH; Chapman CR, Turk DC. Bonica`s Management os Pain. 3ed. Philadelphia: Lippincott Williams &Wilkins, 2001.

Li Y, Tian X, Qian L, Yu X, Jiang W. Anodal Transcranial Direct Current Stimulation Relieves the Unilateral Bias of a Rat Model of Parkinson's Disease. 33rd Annual International Conference of the IEEE EMBS Boston, Massachusetts USA, 2011.

Mainar AS, Artieda RN, Morillo JV, Escobar AE. Treatment profiles and costs of patients with chronic pain in the population setting. Clinicoecon Outcomes Res. 2012;4:39-47.
Margarida M et al. Dor um estudo multidisciplinar. 2ª ed. LOCAL: Sumus Editorial; 1999;174.

Martenson M E, Cetas J S, Heinricher M M. A possible neural basis for stress-induced hyperalgesia. PAIN 2009;142:236–244

Merighi A, Salio C, Ghirri A, Lossi L, Ferrini F, Betelli C, Bardoni R. BDNF as a pain modulator. Prog Neurobiol. 2008;5:297-317. Review.

Merskey H, Bogduk N. Classification of chronic pain descriptions of chronic pain syndromes and definitions of pain terms. IASP press Seattle WA. 1994;1-215.

Morgan GE, Mikhail MS. Pain Management. In: Morgan GE, Mikhail MS (eds). Clinical Anesthesiology. 2ed. Stamford: Appleton & Lange, 1996;274-316.

Nitsche MA, Paulus W. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J Physiol 2000;527(3):633-9.

Nitsche MA, Schauenburg A, Lang N, Liebetanz D, Exner C, Paulus W, Tergau F. Facilitation of implicit motor learning by weak transcranial direct current stimulation of the primary motor cortex in the human. J. Cogn. Neurosci. 2003a;15(4):619-626.

Nitsche MA, Liebetanz D, Lang N, Antal A, Tergau F, Paulus W. Safety criteria for transcranial direct current stimulation (tDCS) in humans. Clin. Neurophysiol. 2003b;114(11):2220-2222.

Nitsche MA, Friecke K, Henschke U, Schlitterlau A, Liebetanz D, Lang N et al. Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation. J Physiol 2003c;553(1):293-301.

Nitsche MA, Niehaus L, Hoffmann KT, Hengst S, Liebetanz D, Paulus W, Meyer BU. MRI study of human brain exposed to weak direct current stimulation of the frontal cortex. Clin. Neurophysiol. 2004;115(10):2419-2423.

Neylan TC, Marmar CR, Metzler TJ, Weiss DS, Zatzick DF, Delucchi KL, Wu RM, Schoenfeld FB. Sleep disturbances in the Vietnam generation: findings from a nationally representative sample of male Vietnam veterans. Am J Psychiatry. 1998;155(7):929-33.

Ottaviani E, Franchini A, Caselgrandi E, Cossarizza A, Franceschi C. Relationship between corticotropin-releasing factor and interleukin-2: evolutionary evidence. FEBS Lett. 1994;351(1):19-21.

Paulson PE, Casey KL, Morrow TJ.Long-term changes in behavior and regional cerebral blood flow associated with painful peripheral mononeuropathy in the rat. Pain. 2002;95(1-2):31-40.

Patterson SL, Abel T, Duel TA, Martin KC, Rose JC, Kandel ER.Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. Neuron. 1996; 6(6): 1137-45.

Pezet S, McMahon SB.Neurotrophins: mediators and modulators of pain. Annu Rev Neurosci. 2006;29:507-38. Review

Plow EB, Pascual-Leone A, Machado A. Brain stimulation in the treatment of chronic neuropathic and non-cancerous pain. J Pain. 2012;13(5):411-24.

Riberto M, Marcon Alfieri F, Monteiro de Benedetto Pacheco K, Dini Leite V, Nemoto Kaihami H, Fregni F, Rizzo Battistella L.Efficacy of transcranial direct current stimulation coupled with a multidisciplinary rehabilitation program for the treatment of fibromyalgia. Open Rheumatol J. 2011;5:45-50.

Seyle H. Stress Without Distress. New York: New York American Library. 1974

Sá K , Baptista A Matos M - Lessa I. Prevalência de dor crônica e fatores associados na população de Salvador, Bahia. Rev. de Saúde Pública 2009;43.

Sawchenko PE, Brown ER, Chan RK, Ericsson A, Li HY, Roland BL, Kovacs KJ. The paraventricular nucleus of the hypothalamus and the functional neuroanatomy of visceromotor responses to stress. Progress in Brain Research 1996;107:201-222.

Shu XQ, Llinas A, Mendell LM. Effects of trkB and trkC neurotrophin receptor agonists on thermal nociception: a behavioral and electrophysiological study. Pain 1999;80:463–470.

Silva G, Miksad R, Freedman SD, Pascual-Leone A, Jain S, Gomes DL, Amancio EJ, Boggio PS, Correa CF, Fregni F. Treatment of cancer pain with noninvasive brain stimulation. J Pain Symptom Manage. 2007;34(4):342-5

Simons DG, Travell JG, Simons LS. Dor e disfunção miofascial. Manual dos pontos gatilhos. Volume I – Parte superior do corpo. 2. ed. São Paulo: Artes Medicas; 2005; 800.

Torres ILS, Vasconcellos AP, Silveira SN, Dalmaz C. Effect of repeated stress on novelty-induced antinociception in rats. Brazilian Journal of Medical and Biological Research 2001;34:241-244.

Torres ILS, Cucco SN, Vasconcellos, AP, Dalmaz, C. Effect of repeated stress on novelty-induced antinociception in rats. Braz. J. Med. Biol. Res. 2001b;34:241-244.

.

Tsigos C, Chrousos GP. Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. J Psychosom Res. 2002;53(4):865-71.

Terman GW, Lewis JW, Liebeskind JC. Two opioid forms of stress analgesia: studies of tolerance and cross-tolerance. Brain Res. 1986;368(1):101-6.

Ursin H, Olff M. The stress response. Stress: from synapse to syndrome. Academic Press. 1993.

Young FR, Rinaldi P. Brain stimulation for relief of chronic pain. In: Wall PD, Melzack R. Textbook of pain. London: Churchill Livingstone;1994;1225-33.

Wachter D, Wrede A, Schulz-Schaeffer W, Taghizadeh-Waghefi A, Nitsche MA, Kutschenko A, Rohde V, Liebetanz D. Transcranial direct current stimulation induces polarity-specific changes of cortical blood perfusion in the rat. Experimental Neurology 2011; 227:322–327.

Walker SM, Mitchell VA, White DM, Rush RA, Duggan AW. Release of immunoreactive brain-derived neurotrophic factor in the spinal cord of the rat following sciatic nerve transection. Brain Res. 2001;899:240.

Woolf CJ. Pain: Moving from symptom control toward mechanism specific pharmacologic management. Ann. Intern. Med. 2004; 140:441-451.

Zaghi S, Acar M, Hultgren B, Boggio PS, Fregni F. Noninvasive brainstimulation with low intensity electrical currents: putative mechanisms of action for direct and alternating current stimulation. Neuroscientist. 2010; 16(3):285-307.

V. MANUSCRITO I:

Reversal of Chronic Stress-Induced Pain by Transcranial Direct Current Stimulation (tDCS) in an Animal Model

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Reversal of Chronic Stress-Induced Pain by Transcranial Direct Current Stimulation (tDCS) in an Animal Model

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Footnote

tDCS = Transcranial direct current stimulation

CRS = chronic restraint stress

HPA = hypothalamic-pituitary-adrenal axis

HD-tDCS= High-Definition tDCS

DC = direct current

IL1β = Interleukin-1β

TNFα = Tumor necrosis factor-α

BDNF = brain-derived neurotrophic factor

LTP = long-term potentiation

ABSTRACT

Transcranial direct current stimulation (tDCS) has been suggested as a therapeutic tool for pain syndromes. Although initial results in human subjects are encouraging, it is still unclear whether the effects of tDCS can reverse maladaptive plasticity associated with chronic pain. To investigate this question, we tested whether tDCS can reverse the specific behavioral effects of chronic stress in the pain system, and also those indexed by corticosterone and interleukin 1β levels in serum and TNFα levels in the hippocampus, in a well-controlled rat model of chronic restraint stress (CRS). Forty-one adult male Wistar rats were divided into two groups: *control* and *stress*. The stress group was exposed to CRS for 11 weeks for the establishment of hyperalgesia and mechanical allodynia as shown by the hot plate and von Frey tests respectively. Rats were then divided into 4 groups: *control*, *stress*, *stress+sham tDCS* and *stress+tDCS*. Anodal or sham tDCS was applied for 20 minutes/day over 8 days and the tests were repeated. Then, the animals were killed and blood collected and hippocampus removed for ELISA testing. This model of CRS proved effective to induce chronic pain, as the animals exhibited hyperalgesia and mechanical allodynia. The hot plate test showed an analgesic effect, and the von Frey test, an anti-allodynic effect after the last tDCS session, and there was a significant decrease in hippocampal TNFα levels. These results support the notion that tDCS reverses the detrimental effects of chronic stress on the pain system and decreases TNFα levels in the hippocampus.

Keywords: Chronic restraint stress; hyperalgesia; allodynia; transcranial direct current stimulation (tDCS); neuromodulation, TNFα.

1. Introduction

Several pain syndromes, such as fibromyalgia, chronic back pain, and neuropathic pain, are associated with significant effects on neuroplasticity in pain-related neural circuits, which, in turn, lead to significant effects on the sensory and affective-emotional domains, such as hyperalgesia, allodynia, anxiety and depression (Staud, 2006a, 2006b). In most cases, these conditions are associated with psychiatric disorders, absenteeism, and high costs of chronic treatment or poor outcomes despite treatment (Jensen et al. 2007; Van Hanswijck de Jonge et al. 2008). Pain syndromes are associated with chronic stress, as chronic exposure to pain produces suffering, which activates the hypothalamic-pituitary-adrenal (HPA) axis, thus stimulating the production of corticosterone, the hormone released in stress conditions (for a review, see Martenson et al. 2009). It is known that serum corticosterone levels in rats subjected to chronic stress do not show a significant increase in comparison to control animals; however, this increase is statistically significant when rats are subjected to acute stress (Park et al. 2012; Torres et al. 2001a).

Unlike acute stress, which has been associated with a reduction in pain sensitivity, probably mediated by brain stem pain modulation (for a review, see Martenson et al. 2009), chronic stress has been associated with decreased pain thresholds. Indeed, chronic stress is associated with hyperalgesia (enhanced response to noxious stimuli) (Gamaro et al. 1998; Torres et al. 2001a; Bardin et al. 2009) and allodynia (pain induced by non-noxious stimuli) (Bardin et al. 2009). In a previous study, we demonstrated that chronic stress-induced hyperalgesia remained for 28 days after discontinuation of treatment (Torres et al. 2003). Interestingly, the analgesic response to acute restraint stress (i.e., inhibition of pain) was re-established only after 14 days of discontinuation of chronic stress (Torres et al. 2003).

Although the underlying mechanisms of long-lasting hyperalgesia after chronic stress are still elusive, some studies have advanced understanding of this topic. Human studies have shown that a reduction in pain threshold after long-term psychoemotional stress probably occurs due to a reduction in the activity of the brain's opioid system (Ashkinazi and Vershinina, 1999). Previous data from our group also suggest involvement of the opioid system in the hyperalgesic response induced by prolonged restraint stress (Torres et al. 2001b; Torres et al. 2003; Dantas et al. 2005) Furthermore, activation of stress-related circuitry in the hypothalamus activates pain-facilitating neurons in the rostral ventromedial

medulla to produce hyperalgesia (for a review, see Martenson et al. 2009), suggesting possible changes in brain activity. Another possibility is increased expression of pro-inflammatory cytokines, such as interleukin-1β and tumor necrosis factor (TNFα), in brain tissue and blood due to stress conditions. These cytokines are closely related to painful and inflammatory diseases, and their release is increased under stressful conditions (for a review, see Goshen and Yirmiya 2009).

In view of the neuroplastic effects of chronic stress on pain-related neural circuitry, deactivation of the stress-induced pain-related neural changes would be best achieved with techniques to induce neuroplasticity (Brunoni et al. 2011). One simple but powerful technique is transcranial direct current stimulation (tDCS). This technique produces modulation of neural activity via small electrical currents that, when applied as a direct current (DC) component, polarize neural tissue, inducing significant changes in the resting membrane threshold (Zaghi 2009) and subsequent changes in synaptic plasticity, as recently shown in an elegant animal model in mice brain slices DC stimulation (Fristch and Cohen 2010). In addition, it carries little risk and produces little discomfort, and, with repeated sessions, may produce enduring effects (Poreisz et al. 2007). Previous studies have shown that excitability-enhancing anodal tDCS is effective in reducing pain in patients with fibromyalgia (Fregni et al. 2006a) and spinal cord injury (Fregni et al. 2006b). In addition, anodal and cathodal tDCS of the primary motor cortex and dorsolateral prefrontal cortex have been associated with significant changes in experimental pain in healthy subjects (Reidler et al. 2012; Grundman et al. 2012). Finally, the neuromodulatory effects of tDCS have also been consistently demonstrated in animals, such as in rat models of focal epilepsy (Liebetanz et al. 2006), memory (Dockery et al. 2011), Parkinson's disease (Li et al. 2011), and acute stroke (Watcher et al. 2011)

Given the importance of chronic pain and the variability in its pathophysiology, investigation of techniques that can modulate neural mechanisms is relevant to the development of more rational therapies. Noninvasive stimulation techniques, such as tDCS, may be suitable for treatment of chronic pain. Thus, we investigated whether tDCS reverses the hyperalgesia and allodynia induced by chronic restraint stress. We also measured its effect on serum levels of corticosterone and interleukin 1β, as well as TNFα levels in the hippocampus. The importance of this study lies in the fact that it provides, for the first time, evidence that tDCS can reverse the detrimental effects of a specific causal factor of pain on the pain system. Because such a controlled study (i.e. one including control of level of exposure, timing of application of

intervention in relation to exposure, and certain measures in the hippocampus) would not be possible in humans due to ethical issues, this study provides invaluable data for the development of tDCS as a therapeutic tool in chronic pain.

2. Results

2.1 Basal measure after chronic stress and effects of tDCS on allodynia after the end of tDCS treatment as measured by the von Frey test

When the stress group was divided into the stress, stress + sham tDCS, and stress + active tDCS groups, we again observed a significant difference between baseline measurements in the control group and the other groups (**C**: 65.71±3.39g; **S**: 49.07±2.63g; **SS**: 45.36±3.34g; **SN**: 53.10±2.23g; one-way ANOVA/Tukey's test, P=0.001, n=9-12/group. Figure 1 – basal measure). We tested whether tDCS treatment was associated with a significant change in allodynia as compared with the other no-tDCS groups. We conducted an ANOVA testing group differences immediately and 24-h after treatment adjusting for baseline values (including pre-tDCS as the covariate in this ANOVA model). We did not find a significant effect of time ($F(1,44)=0.05$, P=0.82), neither in the interaction time*group ($F(3,44)=1.89$, P=0.14), suggesting that after treatment, there was not differences in group behavior over time. But, we found a significant effect of group ($F(3,44)=3.87$, P=0.015) considering results after treatment. Post-hoc analysis confirmed that **SN** group showed significant differences as compared with **SS** group (P=0.028). Interestingly, the difference between SN and C that we observed at baseline disappeared after tDCS treatment, confirming that after tDCS, animals' behavior was similar to the non-stress control group. Although there was also a difference between **S** and **C** (P=0.012), there was no difference between **S** and **SN** (P=0.28), suggesting likely a lack of power for this later analysis.

Insert Figure 1

2.2 Basal measure after chronic stress and effects of tDCS on nociception immediately and 24 hours after the end of tDCS as measured by the hot plate test

We then performed similar analysis for the hot plate test. We initially tested whether tDCS treatment was associated with a significant change in hyperalgesia as compared with the other no-tDCS groups (C: 5.75+0.41s; S: 2.70±0.15s; SS: 3.08±0.90s; SN: 3.62±0.59s; one-way ANOVA/Tukey's test, F(P=0.000, n=9-12/group, Figure 2). Same ANOVA controlled for baseline differences disclosed similar findings: no significant effect of time (F(3,90), *P*=0.0525) and no significant interaction time*group (F(3,44)=0.31, p=0.7320, suggesting that after treatment, there was no differences in group behavior over time. But, we found a significant effect of group (F(9,42)=7.08, p=0.0000) considering results after treatment. Here post-hoc analysis confirmed that **SN** group showed significant differences as compared with **SS** group (*P*=0.000) and S group (*P*=0.002). Similarly the difference between SN and C that we observed at baseline also disappeared after tDCS treatment (*P*=1.000), confirming that after tDCS, animals behavior was similar to the non-stress control group.

Insert Figure 2

2.3 Effects of tDCS on serum corticosterone and interleukin-1beta levels after the end of tDCS treatment

No effect of stress or tDCS treatment was observed in serum levels of corticosterone (**C**: 385.90±171.54 nmol/L; **S**: 295.73±158.72 nmol/L; **SS**: 418.02 ±89.90 nmol/L; **SN**: 424.85±102.17 nmol/L; one-way ANOVA/Tukey's test, *P*>0.05, n=6-7, Figure 3A) or interleukin-1beta (**C**: 46.76±4.93 pg/L; S: 51.22±11.85 pg/L; **SS**: 58.38±7.45 pg/L; **SN**: 42.21±3.90 pg/L; one-way ANOVA/Tukey's test, *P*>0.05, n=3-6, Figure 3B).

Insert Figure 3A

Insert Figure 3B

2.4 Effects of tDCS on hippocampal TNFα levels after the end of tDCS treatment

We observed a significant between-group difference in TNFα levels in the hippocampus. The active tDCS group showed decreased levels of TNFα in hippocampus in comparison to the other groups (**C**: 128.76±28.65 pg/L; **S**:126.77±13,00 pg/L; **SS**: 123.26±5.22 pg/L; **SN**:52.50±2.00 pg/L one-way ANOVA/Tukey's test, $P \leq 0.05$, n=3-4, Figure 4).

Insert Figure 4

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3. Discussion

In this study, we demonstrated that tDCS stimulation effectively reversed the hyperalgesia and allodynia induced by the chronic restraint stress rat model. This result persisted for at least 24 h, which demonstrates the cumulative effects of repetitive tDCS treatment, as, in a previous study, the antinociceptive effect of one session of transcranial eletrostimulation in rats disappeared within 15 min after cessation of electrical stimulation (Nekhendzy et al. 2004). The hyperalgesic effect was assessed by two behavioral components on hot plate (paw licking and jumping), both considered supraspinally integrated responses. This constitutes, at least in part, the rationale for testing of the antihyperalgesic effect of tDCS. Given our electrode montage, it is conceivable that most of the effects found in this

study were due to cortical modulation. In this scenario, it is likely that effects of transcranial stimulation on pain relief depend on the projection of fibers from cortical structures to other neural areas involved in pain processing, such as the thalamus and brainstem nuclei, which could activate non-nociceptive neurons (Drouot et al. 2002; Lefaucheur et al. 2006). Thus, we can suggest that stimulation activates descending inhibitory pathways, suppressing pain through a top-down modulation mechanism (Lima and Fregni, 2008).

Although anodal tDCS has been shown to induce pain relief in human studies (for a review, see Myllius et al. 2012), this study fills a critical gap in the knowledge of the field, as we show that consecutive sessions of tDCS can reverse chronic stress-induced pain. In our study, we were able to control the source of pain, thus providing a homogeneous sample in terms of chronic pain mechanisms and demonstrating the effects of tDCS in this condition. In this context, we will briefly review the putative mechanisms involved in the development of hyperalgesia after repeated restraint stress. Previous studies have suggested that this phenomenon could be related to changes in central or peripheral opioid activity (Torres et al. 2001b; Torres et al. 2003; Dantas et al. 2005). The absence of novelty-induced antinociception in these animals supports this theory (Torres et al. 2001b). Exposure of rats to a novel environment is known to be followed by mild, naloxone-reversible antinociception (Siegfried et al. 1987). Opioid receptors can be highly plastic, as reflected by their susceptibility to modifications by various pharmacological and behavioral manipulations (for a review, see Drolet et al. 2001). Dantas and colleagues (2005) showed decreases in binding of opioid receptors in the hippocampus and cerebral cortex. Additionally, Torres and colleagues (2003) demonstrated that animals subjected to chronic restraint stress for 6 weeks needed high doses of morphine to exhibit an analgesic response, suggesting that prolonged stress could lead to longer-lasting changes in the neural systems involved in nociceptive modulation. On the other hand, in acute stress, the opiate system seems to be modulated in the opposite direction. In fact, a previous study has demonstrated that animals subjected to acute stress show an increase in the magnitude and duration of the analgesic effect to some opiate agonists (Calcagnetti and Holtzman 1992).

Other important finding of this study was that corticosterone and interleukin 1β levels in serum did not present statistically significant changes by the tDCS sessions and/or chronic restraint

stress. These results are consistent with the literature, which has shown that chronic restraint stress leads to disorganization and deregulation of HPA axis stress responses (for a review, see Goshen and Yirmiya 2009). In addition, we showed that hippocampal TNFα levels were not increased by chronic restraint stress, unlike a previous study, which reported increased TNFα level in the hippocampus after 40 days of variable stress (Tagliari et al. 2011). This result was due to the long period of stress used in this study - almost twice which cited in the Tagliari paper. Therefore, this reaction was probably reestablished by an adaptive response. On the other hand, hippocampal TNFα levels were significantly decreased in the group that received tDCS as compared with other groups. As TNFα is a proinflammatory cytokine, this could be related to the effects of tDCS on reversal of maladaptative changes in the pain system induced by chronic restraint stress. Hence, one possible mode of action of anodal tDCS is by decreasing hippocampal TNFα levels, causing an anti-inflammatory and anti-hyperalgesic response, even considering normal baseline (pre-stimulation) TNFα levels in the hippocampus.

Although the mechanisms underlying tDCS-mediated pain regulation have yet to be elucidated, its mechanisms of action involve changes in the neuronal electrical membrane potential and modifications in the synaptic microenvironment. Changes in synaptic strength are NMDA receptor-dependent or can alter GABAergic activity (Liebetanz et al. 2002; Nitsche et al. 2003a; Stagg et al. 2009). The tDCS also interferes with brain excitability through modulation of intracortical and corticospinal neurons (Nitsche et al. 2005; Ardolino et al. 2005). The effects of tDCS might be similar to those observed in long-term potentiation (LTP), as demonstrated in an animal study that used anodal motor cortex stimulation (Fritsch et al. 2010). Experiments with spinal cord stimulation have shown that the effects of tDCS are also non-synaptic, possibly involving transient changes in the density of protein channels located below the stimulating electrode (Cogiamanian et al. 2008) or due to glial changes (Radman et al. 2009). Given that a constant electric field displaces all polar molecules and that most neurotransmitters and receptors in the brain have electrical properties, tDCS might also influence neuronal function by inducing prolonged neurochemical changes (Stagg et al. 2009; Cogiamanian et al. 2008).

In addition to neurochemical changes, it is known that tDCS also has a significant effect on current blood flow. Some experiments combining tDCS and transcranial laser Doppler flowmetry (LDF)

in a rat model demonstrated that tDCS induces sustained changes on current blood flow. These changes were polarity-specific; anodal tDCS leads to an increase, whereas cathodal tDCS leads to a decrease in current blood flow (Watcher et al. 2011). Whether increased metabolic activity in the experimental model of chronic pain is involved in the reversal of hyperalgesia has yet to be determined.

According to Fertonani and colleagues (2010), the long-term effects of tDCS also involve glutamatergic NMDA receptors, and synaptic plasticity is also dependent on NMDA receptors. D-cycloserine, a partial NMDA agonist, has been shown to selectively potentiate the duration of motor cortical excitability enhancements induced by anodal tDCS, but not the decrease in excitability induced by cathodal stimulation. A patient with chronic pain was successfully treated with repeated applications of tDCS over the motor cortex combined with D-cycloserine and dextromethorphan administration to prevent recurrence of pain (Antal et al. 2011). The analgesic effect of tDCS could be mediated by modulatory effects in pain sensation in several neurotransmitter systems, including opioid, adrenergic, substance P, glutamate and neurokinin receptors (Morgan et al. 1994; Wu et al. 2000). It leads to a cascade of events resulting in the modulation of synaptic neural chains that include several thalamic nuclei, the limbic system, brainstem nuclei, and the spinal cord (Lima and Fregni, 2008).

It has been demonstrated that pain relief induced by invasive cortical stimulation is also mediated by activation of the endogenous opioid system. In fact, motor cortex stimulation produces activation of the cortical segment and acts on intracortical interneurons. Stimulation of these fibers spreads to different areas: thalamic cortical projections, cortical-cortical lateral projections and local cortical connections (Lima and Fregni 2008). We can hypothesize that the results obtained might depend on the aforementioned mechanisms. However, we did not measure the duration of the antihyperalgesic effect observed.

Viewed as a whole, our findings support the hypothesis of an antihyperalgesic and antiallodynic effect of tDCS. Although the mechanisms underlying this effect remain unclear, the evidence suggests that they include non-synaptic and synaptic mechanisms alike. The non-synaptic mechanism would include changes which, apart from reflecting local changes in ionic concentrations, could arise from alterations in transmembrane proteins and from electrolysis-related changes in H(+), induced by exposure to a constant electric field (Ardolino et al. 2005). The synaptic mechanisms

would involve neuroplastic alterations, such as changes in the strength of connections, representational patterns, or neuronal properties, either morphological or functional (Antal et al. 2006). tDCS induces prolonged neuronal excitability and activity changes in the human brain via alterations in neuronal membrane potential, resulting in prolonged synaptic efficacy changes.

One important question that has yet to be fully elucidated is optimal electrode placement for induction of analgesic effects (Fregni 2010). It is not clear whether the effects are mainly due to anodal stimulation of frontal areas (including M1) or are also associated with cathodal stimulation of the contralateral area, although there is extensive evidence showing that modulation of M1 is critically involved with pain modulation, as shown by modeling studies (Mendonca et al. 2011 and DaSilva et al. 2012) and High-Definition-tDCS(HD-tDCS) (Borckardt et al. 2012). Finally, another important issue is the association between electrode montage and shunting. Although our montage may be associated with shunting, it has previously proved effective, such as in the Takano et al. (2011) study. These authors examined the effectiveness of tDCS using functional magnetic resonance imaging (fMRI) and the signal intensities of fMRI in the frontal cortex and nucleus accumbens, and found significant increases in activity after anodal tDCS exposure in rats. In addition, in silicon finite element model studies have shown that even with close electrodes, such as those used in HD-tDCS, a significant amount of current is injected and reaches cortical areas (Minhas 2010; Datta 2009). On the basis of these considerations, we decided to use a cephalic montage as this has been the most widely used method in humans. In fact, a recent study in humans showed that extra-cephalic montages were less effective to provide pain relief (Mendonca et al. 2011). Another important limitation, also discussed in a recent review, is extrapolation of these results to humans (Volz et al. 2012). In this context, this study, to the best of our knowledge, was the first to show that tDCS can reverse the effects of maladaptive plasticity as expressed by behavioral changes and measured by TNFα levels. On the other hand, one limitation of the study was the lack of difference between one of the analysis for von Frey test – S vs. SN – probably because of less sensitivity of this measurement as compared to hot plate test and also because of differences what these measurements index such as hot plate related to hyperalgesia and von Frey related to allodynia.

In summary, we showed that tDCS was able to reverse completely the detrimental effects of chronic stress on the pain system, as expressed by hyperalgesia and allodynia, and that this effect continued for 24h. Serum levels of corticosterone and interleukin-1β were not changed by tDCS sessions or chronic restraint stress, but hippocampal TNFα levels decreased. Given that, in this study, animals were exposed to the same level of stress under the same conditions, our findings support further exploration of tDCS as a therapeutic tool early in the exposure to stressful situations that may lead to chronic pain, such as post-traumatic stress disorder, and demonstrate one possible pathway of anodal tDCS treatment. Future studies should also consider assessing other outcomes of stress response, including other behavioral outcomes, as well as measurement of other biochemical variables, such as PCPA (inhibitor of serotonin synthesis), AMPT (inhibitor of tyrosine hydroxylase) and naloxone, to provide a better understanding of the effects of chronic restraint stress on mood and anxiety and further elucidate and optimize this intervention into a potential clinical tool for stress-related conditions.

4. Experimental Procedure

4.1 Animals

Sixty-day-old male Wistar rats weighing 180–230 g were used. Experimentally naive animals were housed in groups of five in 49x34x16 cm polypropylene home cages. All animals were kept on a standard 12-hour light/dark cycle (lights on at 07:00 a.m. and lights off at 07:00 p.m.) in a temperature-controlled environment (22±2°C). Animals had access to water and chow *ad libitum*. All experiments and procedures were approved by the Institutional Committee for Animal Care and Use (GPPG-HCPA protocol No. 100.381) and performed in accordance with the Guide for the Care and Use of Laboratory Animals 8th edition (2011). Animal handling and all experiments were performed in accordance with international guidelines for animal welfare and measures were taken to minimize animal pain and discomfort. The experiment used the minimum number of animals required to produce reliable scientific data.

To control the possible effect of outliers, we excluded rats which did not present any response on behavioral testing. All the experimenters were blinded to condition (active or sham tDCS) during post-treatment behavioral testing.

4.2 Chronic restraint stress

The animals were subjected to 1 h of restraint daily, 5 days a week for 11 weeks. Restraint was applied by placing the animal in a 25 x 7 cm plastic bottle with a 1-cm hole at the far end for breathing (Ely et al. 1998 with modifications). The animal was unable to move. The control group was not subjected to restraint. These procedures were always performed between 0800h and 0900h. Restraint sessions continued during the behavioral test period and during tDCS sessions, which were carried out in the afternoon. The animals were divided into 4 groups (n=12-13): control (C), stress (S), stress + sham tDCS (SS) and stress + tDCS (SN). After 11 weeks of chronic stress exposure, behavioral tests were performed in the afternoon.

4.3 Pain outcome I: von Frey test

Mechanical allodynia was assessed before, immediately and 24 hours after the end of tDCS treatment using an automatic von Frey esthesiometer (Insight, São Paulo, Brazil). This is an adaptation of the classical von Frey filaments test in which pressure intensity is recorded automatically after paw removal (Vivancos et al. 2004). It has been proposed that tactile hypersensitivity is likely to be the consequence of a change in function and a phenotypic switch in primary afferent neurons innervating the inflamed tissue and the pattern of excitation they produce in spinal neurons. This assumption was partially confirmed by the finding that a subpopulation of A beta primary afferent neurons came to express substance P after conditioning inflammation, thereby enhancing synaptic transmission in the spinal cord and exaggerating the central response to innocuous stimuli (Ma and Woolf 1996; Neumann et al. 1996).

Rats were placed in 12 x 20 x 17 cm polypropylene cages with wire grid floors and acclimatized for 15 minutes, 24 hours prior to the test, as the novelty of the apparatus itself can induce antinociception (Netto et al. 2004). For testing, a polypropylene tip was placed perpendicularly underneath the mesh floor

and applied to one of the five distal footpads with a gradual increase in pressure. A tilted mirror below the grid provided a clear view of the animal's hind paw. The test consisted of poking the hind paw to provoke a flexion reflex followed by a clear flinch response after paw withdrawal. The intensity of the stimulus was automatically recorded when the paw was withdrawn. Three successive von Frey readings were averaged, and these averages were used as the final measurements. The paw withdrawal threshold was expressed in grams (g) (Amaral et al. 2008; Vivancos et al. 2004).

4.4 Pain outcome II: hot plate

The hot plate test was carried out to assess the effects of tDCS on the thermal nociceptive threshold (Woolfe and Macdonald 1944). This test was assessed before, immediately and 24 hours after the end of tDCS treatment. We used the hot-plate test to determine changes in latency as an indicator of modifications of the supraspinal pain process (Ossipov et al. 1995), as licking or jumping responses during this test are considered to be the result of supraspinal sensory integration (Caggiula et al. 1995; Rubinstein et al. 1996).

The hot plate was pre-heated and kept at a temperature of 55±0.5°C. All rats were acclimated to the hot plate for five minutes, 24 hours prior to testing, as, again, the novelty of the apparatus itself can induce antinociception (Netto et al. 2004). Rats were placed in glass funnels on the heated surface and the nociceptive threshold was assessed recording to the time taken to first response (foot licking, jumping, or rapidly removing paws), as described by Minami and colleagues (Minami et al. 1994). Response was recorded in seconds (s) and a cutoff time of 20 s was used.

4.5 Transcranial direct current stimulation (tDCS)

After 11 weeks of chronic stress exposure, the rats of SN were subjected to a 20-minute session of anodal tDCS every afternoon for 8 days. This period was established because tDCS has been shown to modify cortical excitability for up 1 h after one session of stimulation (Nitsche and Paulus 2001; Nitsche et al. 2003b). However, repetitive tDCS application has demonstrated better and longer-lasting effects on pain relief, and in recent study our group showed antihyperalgesic response in paw inflamed rats with this treatment period (Laste et al. 2012). 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 better fit. After placement, electrodes were fixed onto the head with adhesive tape (Micropore™) and covered with a protective mesh to prevent removal (fig. 5A).

The anodal electrode was positioned between the ears, from the neck of the rat (parietal cortex) (fig.5B) (Takano, 2011 with modifications), so as to mimic anodal placement in human pain studies (Mendonca et al. 2011; Dasilva et al. 2012).The cathodal electrode was positioned at the midpoint of the lateral angle of the eyes (supraorbital area). The electrodes were placed on the skin in a similar manner to that used in human studies of tDCS for pain (Nitsche et al. 2008; Antal and Paulus 2011; Rosen et al. 2009; Fregni et al. 2006c).

A constant current of 0.5 mA intensity was applied for 20 min (Fregni et al. 2006b; Dockery et al. 2011; Wachter et al. 2011; Liebetanz et al. 2006). According to an earlier study (Liebetanz et al. 2009), a constant current of 1 mA intensity causes skin lesions, as current density is comparatively much higher than the traditional 1mA tDCS using large pads in humans. We therefore chose to use 0.5 mA, an intensity that has also been used in other animal studies. In addition, in our study, electrodes were fixed onto the skin. We did not observe any lesions with montage and current intensity.

An important point to consider was that this model required neither anesthesia nor surgery, unlike models used in previous tDCS studies in rats (Dockery et al. 2011; Wachter et al. 2011; Liebetanz et al. 2006). In fact, this represents a strength in this study, as volatile anesthesia (such as isoflurane) has been shown to decrease excitatory and increase inhibitory transmission (Gomez and Guatimosim, 2003; Ouyang and Hemmings, 2005), altering BDNF expression and thus neuroplasticity (Lu et al. 2006; Head et al. 2009). We were thus able to remove this confounding factor in our study by adapting a human model using ECG electrodes (Fregni et al. 2006c).

For sham stimulation, the electrodes were placed in the same positions as for real stimulation; however, the stimulator was turned off after 30 s of stimulation so the animals could maintain continuity of the physical sensation of real tDCS conditions (Gandiga et al. 2006).

Insert Figure 5 A

Insert Figure 5 B

4.6 *Blood sampling and tissue collection*

Forty-eight hours after tDCS treatment, the animals were killed by decapitation. Trunk blood was collected and centrifuged for five minutes at 5000*g* at room temperature. Animals were killed by an experienced investigator. Serum and hippocampus were frozen at -70°C for subsequent analysis.

4.7 Analyses of corticosterone and interleukin-1β serum levels

Serum interleukin-1 and corticosterone levels were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits for rat interleukin-1 (Uscn Life Science Inc.) or corticosterone (IBL Corticosterone Kit), according to manufacturer instructions. The results are expressed in pg/mL and nmol/L respectively.

4.8 Analysis of TNF-α Immunocontent

TNF analysis was performed on hippocampus homogenates. TNF levels were measured by a commercially available enzyme-linked immunosorbent assay (ELISA) kit for rat tumor necrosis factor-alpha (Uscn Life Science Inc.), according to manufacturer protocols. The results are expressed in pg/mL.

4.9 Statistical analysis

The results are presented as the mean ± standard error of the mean (SEM). As data were normally distributed, we assessed the difference between groups using one-way ANOVA with Tukey's test when necessary. *P*-values less than 0.05 were considered significant.

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References

Antal, A., Nitsche, M.A., Paulus, W., 2006. Transcranial direct current stimulation and the visual cortex. Brain Research Bulletin. 459, 463-68.

Antal, P., Paulus, W., 2011. A case of refractory orofacial pain treated by transcranial direct current stimulation applied over hand motor area in combination with NDA agonist drug intake. Brain Stimulation 4, 117-21.

Ardolino, G., Bossi, B., Barbieri, S., Priori, A., 2005. Non-synaptic mechanisms underlie the after-effects of cathodal transcutaneous direct current stimulation of the human brain. The Journal of Physiology 653, 663-568.

Ashkinazi, I.Y., Vershinina, E.A., 1999. Pain sensitivity in chronic psychoemotional stress in humans. Neurosci Behav Physiol. 29(3), 333-7.

Bardin, L., Malfetes, N., Newman-Tancredi, A., Depoortère, R., 2009. Chronic restraint stress induces mechanical and cold allodynia, and enhances inflammatory pain in rat: Relevance to human stress-associated painful pathologies. Behavioural Brain Research, 360–366.

Borckardt, J.J., Bikson, M., Frohman, H., Reeves, S.T., Datta, A., Bansal, V., Madan, A., Barth, K., George, M.S., 2012. A pilot study of the tolerability and effects of high-definition transcranial direct current stimulation (HD-tDCS) on pain perception. J Pain.13(2), 112-20.

Brunoni, A.R., Nitsche, M.A., Bolognini, N., Bikson, M., Wagner, T., Merabet, L., Edwards,

D.J., Valero-Cabre, A., Rotenberg, A., Pascual-Leone, A., Ferrucci, R., Priori, A., Boggio, P.S., Fregni, F., 2011. Clinical research with transcranial direct current stimulation (tDCS): Challenges and future directions. Brain Stimul. 5(3), 175-95.

Brown, J.A., Barbaro, N.M., 2003. Motor cortex stimulation for central and neuropathic pain: current status. Pain 104, 431-435.

Caggiula, A.R., Epstein, L.H., Perkins K.A., Saylor, S., 1995. Different methods of assessing nicotine-induced antinociception may engage different neuronal mechanisms. Psychopharmacology 122, 301–306.

Calcagnetti, D.J., Holtzman, S.G.,1992. Potentiation of morphine analgesia in rats given a single exposure to restraint stress immobilization. Pharmacol. Biochem. Behav. 41, 449-453.

Carroll, D., Joint, C., Maartens, N., Shlugman, D., Stein, J., Aziz, T.Z., 2000. Motor cortex stimulation for chronic neuropathic pain: a preliminary study of 10 cases. Pain 84, 431–7.

Cogiamanian, F., Vergari, M., Pulecchi, F., Marceglia, S., Priori, A., 2008. Effect of spinal transcutaneous direct current stimulation on somatosensory evoked potentials in humans. Clin Neurophysiology 119(11), 2636-2640.

Dantas, G., Torres, I.L.S., Crema, L. M., Lara, D., Dalmaz, C., 2005. Repeated Restraint Stress Reduces Opioid Receptor Binding in Different Rat CNS Structures. Neurochemical Research, 30(1), 1-7.

Dasilva, A.F., Mendonca, M.E., Zaghi, S., Lopes, M., Dossantos, M.F., Spierings, E.L., Bajwa, Z., Datta, A., Bikson, M., Fregni, F., 2012. tDCS-Induced Analgesia and Electrical Fields in Pain-Related Neural Networks in Chronic Migraine. Headache.

Datta, A., Bansal, V., Diaz, J., Patel, J., Reato, D., Bikson, M., 2009. Gyri -precise head model of transcranial DC stimulation: Improved spatial focality using a ring electrode versus conventional rectangular pad. Brain Stimul. 4, 201–207.

Dansereau, M.A., Gosselin, R.D., Pohl, M., Pommier, B., Mechighel, P., Mauborgne, A., Rostene, W., Kitabgi, P., Beaudet, N., Sarret, P., Melik-Parsadaniantz, S., 2008. Spinal CCL2 pronociceptive action is no longer effective in CCR2 receptor antagonist-treated rats. J Neurochem. 106(2), 757-69.

Dockery, C.A., Liebetanz, D., Birbaumer, N., Malinowska, M., Wesierska, M.J., 2011. Cumulative benefits of frontal transcranial direct current stimulation on visuospatial working memory training and skill learning in rats. Neurobiology of Learning and Memory 96, 452–460.

Drolet, G., Dumont, E.C., Gosselin, I., Kinkead, R., Laforest, S., Trottier, J.F., 2001. Role of endogenous opioid system in the regulation of stress response. Prog. Neuro-psychopharmacol. Biol. Psychiatry 25, 729-741.

Drouot, X., Nguyen, J.P., Peschanski, M., Lefaucheur, J.P., 2002. The antalgic efficacy of chronic motor cortex stimulation is related to sensory changes in the painful zone. Brain 1660, 1664-125.

Ely, D.R., Dapper, V., Marasca, J., Corrêa, J.B., Gamaro, G.D., Xavier, M.H., Michalowski, M.B., Catelli, D., Rosat, R., Ferreira, M.B., Dalmaz, C., 1997. Effect of restraint stress on feeding behavior of rats. Physiol Behav. 61(3), 395-8.

Fertonani, A., Rosini, S., Cotelli, M., Rossini, P. M., Miniussi, C., 2010. Naming facilitation induced by transcranial direct current stimulation. Behavior Brain Research 311, 318-208.Fregni, F., Boggio, P.S., Lima, M.C., Ferreira, M.J., Wagner, T., Rigonatti, S.P., Castro, A.W., Souza, D.R., Riberto, M., Freedman, S.D., Nitsche, M.A., Pascual-Leone, A., 2006b. A sham-controlled, phase II trial of transcranial direct current stimulation for the treatment of central pain in traumatic spinal cord injury. Pain.122(1-2), 197-209.

Fregni, F., Gimenes, R., Valle, A.C., Ferreira, M.J.L., Rocha, R.R., Natalle, L., Bravo R, Rigonatti, S.P., Freedman, S.D., 2006c. A Randomized, Sham-Controlled, Proof of Principle Study of Transcranial Direct Current Stimulation for the Treatment of Pain in Fibromyalgia. Arthritis & Rheumatism; 54, 3988–3998.

Fregni, F., Pascual-Leone, A., Freedman, S.D., 2007. Pain in chronic pancreatitis: a salutogenic mechanism or a maladaptive brain response? Pancreatology. 7(5-6), 411-22.

Fregni, F., 2010. Analgesia with noninvasive electrical cortical stimulation: challenges to find optimal parameters of stimulation. Anesth Analg. 111(5), 1083-5.

Fritsch, B., Reis, J., Martinowich, K., Schambra, H.M., Ji, Y., Cohen, L.G., Lu, B., 2010. Direct current stimulation promotes BDNF-dependent synaptic plasticity: potential implications for motor learning. Neuron. 66(2), 198-204.

Gamaro, G.D., Xavier, M.H., Denardin, J.D.,Pilger, J.A., Ely, D.R., Ferreira, M.B.C, Dalmaz, C., 1998. The effects of acute and repeated restraint stress on the nociceptive response in rats. Physiology and Behavior 63, 693-697.

Gandiga, P.C., Hummel, F.C, Cohen, L.G., 2006. Transcranial DC stimulation (tDCS): a tool for the double-blind sham-controlled clinical studies in brain stimulation. Neurophysiol 117, 845-50.

Gomez, R.S., Guatimosim, C., 2003. Mechanism of action of volatile anesthetics: involvement of intracellular calcium signaling. Curr Drug Targets CNS Neurol Disord 2, 123–129.

Grundmann, L., Rolke, R., Nitsche, M.A., Pavlakovic, G., Happe, S., Treede, R.D., Paulus, W., Bachmann, C.G., 2011. Effects of transcranial direct current stimulation of the primary sensory cortex on somatosensory perception. Brain Stimul.4(4), 253-60.

Head, B.P., Patel, H.H., Niesman, I.R., Drummond, J.C., Roth, D.M., Patel, P.M., 2009. Inhibition of p75 neurotrophin receptor attenuates isoflurane-mediated neuronal apoptosis in the neonatal central nervous system. Anesthesiology 110, 813– 825.

Jensen, M.P., Grierson, C., Tracy-Smith, V., Bacigalupi, S.C., Othmer, S., 2007. Neurofeedback treatment for pain associated with complex regional pain syndrome type I: A case series. J Neurother. 11, 45-53.

Laste, G., Caumo, W., Adachi, L.N.S., Rozisky, J.R., Macedo, I.C., Filho, P.M., Partata, W.A., Fregni, F., Torres, I.L.S., 2012. Effect of trancranial direct current stimulation in hyperalgesia for chronic inflammation in rats. Experimental Brain Research 221(1), 75-83.

Lefaucheur, J.P., Hatem, S., Nineb, A., Menard-Lefaucheur, I., Wendling, S., Keravel, Y., Nguyen, J.P., 2006. Somatotopic organization of the analgesic effects of motor cortex rTMS in neuropathic pain. Neurology; 67, 1998-2004.

Li, Y., Tian, X., Qian, L., Yu, X., Jiang, W,. 2011. Anodal Transcranial Direct Current Stimulation Relieves the Unilateral Bias of a Rat Model of Parkinson's Disease. 33rd Annual International Conference of the IEEE EMBS Boston, Massachusetts USA.

Liebetanz, D., Nitsche, M.A., Tergau, F., Paulus, W., 2002. Pharmacological approach to the mechanisms of transcranial DC-stimulation- induced after-effects of human motor cortex excitability. Brain 125, 2238-2247.

Liebetanz, D., Klinker, F., Hering, D., Koch, R., Nitsche, M.A., Potschka, H., Löscher, W., Paulus, W., 2006. Anticonvulsant Effects of Transcranial Direct-current Stimulation (tDCS) in the Rat Cortical Ramp Model of Focal Epilepsy. Epilepsia; 47(7), 1216–1224.

Liebetanz, D., Koch, R., Mayenfels, S., König, F., Paulus, W., Nitsche, M.A., 2009. Safety limits of cathodal transcranial direct current stimulation in rats. Clinical Neurophysiology; 120, 1161–1167

Lima, M.C., Fregni, F., 2008. Motor cortex stimulation for chronic pain: systematic review and meta-analysis of the literature. Neurology.; 2329, 37-70.

Lu, L.X., Yon, J.H., Carter, L.B., Jevtovic-Todorovic, V., 2006.General anesthesia activates BDNF-dependent neuroapoptosis in the developing rat brain. Apoptosis; 11, 1603–1615.

Ma, Q.P., Woolf, C.J. 1996. Progressive tactile hypersensitivity: an inflammation induced incremental increase in the excitability of the spinal cord. Pain; 67, 97-106.

Martenson, M. E., Cetas, J.S., Heinricher, M. M., 2009. A possible neural basis for stress-induced hyperalgesia. Pain 142, 236–244.

Minami, T., Uda, R., Horiguchi, S., Ito, S., Hyodo, M., Hayaishi, O., 1994. Allodynia evoked by intrathecal administration of prostaglandin E2 to conscious mice. Pain. 57, 217–223

Mendonca, M.E., Santana, M.B., Baptista, A.F., Datta, A., Bikson, M., Fregni, F., Araujo, C.P., 2011. Transcranial DC stimulation in fibromyalgia: optimized cortical target supported by high-resolution computational models. J Pain 5, 610-7.

Minhas, P., Bansal, V., Patel, J., Ho, J.S., Diaz, J., Datta, A., Bikson, M., 2010. Electrodes for high-definition transcutaneous DC stimulation for applications in drug delivery and electrotherapy, including tDCS. J Neurosci Methods 2, 188-97.

Morgan, P.J., Barret, P., Howel, H.E., Holliwell, R., 1994. Melatonin receptors: localization, molecular pharmacology and physiological significance. Neurochem Int 101, 146-24.

Mylius, V., Borckardt, J.J., Lefaucheur, J.P., 2012. Noninvasive cortical modulation of experimental pain. Pain in press.

Neumann, S., Doubell, T.P., Leslie, T., Woolf, C.J., 1996. In ammatory pain hypersen sitivity mediated by phenotypic switch in myelinated primary sensory neurons. Nature 384, 360-364.

Netto, C.A., Siegfried, B., Izquierdo, I., 2004. Analgesia induced by exposure to a novel environment in rats: effect of a concurrent and post-training stressful stimulation. Behav. Neural Biol. 48, 304–309.

Nekhendzy, V., Fender, C.P., Davies, M.F., Lemmens, H.J.M., Kim, M.S., Bouley, D.M., Maze, M., 2004. The Antinociceptive Effect of Transcranial Electrostimulation with Combined Direct and Alternating Current in Freely Moving Rats. Anesth Analg 98, 730 –7.

Nitsche, M.A., Liebetanz, D., Antal, A., Lang, N., Tergau, F., Paulus, W., 2003a. Modulation of cortical excitability by weak direct current stimulation–technical, safety and functional aspects. Suppl Clin Neurophysiol 56, 255-276.

Nitsche, M.A., Fricke, K., Henschke, U., Schlitterlau, A., Liebetanz, D., Lang, N., Henning, S., Tergau, F., Paulus, W. Pharmacological modulation of cortical excitability shifts induced by transcranial direct current. The Journal of Physiology 2003b; 293:301-553.

Nitsche, M.A., Seeber, A., Frommann, K. 2005. Modulating parameters of excitability during and after transcranial direct current stimulation of the human motor cortex. J Physiol 568(1), 291-303.

Nitsche, M.A., Cohen, L.G., Wassermann, E.M., Priori, A., Lang, N., Antal, A., Paulus, W., Hummel, F., Boggio, P.S., Fregni, F., Pascual-Leone, A., 2008. Transcranial direct current stimulation: state of the art. Brain Stimul; 1, 206-23.

Ossipov, M.H., Kovelowski, C.J., Nichols, M.L., Hruby, V.J., Porreca, F., 1995. Characterization of spuraspinal antinociceptive actions of opioid delta agonists in the rat. Pain; 62, 287–293.

Ouyang, W., Hemmings, H.C. Jr., 2005. Depression by isoflurane of the action potential and underlying voltage-gated ion currents in isolated rat neurohypophysial nerve terminals. J Pharmacol Exp Ther 312, 801– 808.

Park, J.H., Lee, C.H., Yan, B.C., Ahn, J.H., Lee, Y.J., Park, C.W., Cho, J.H., Choi, S.Y., Lee, Y.L., Won, M.H., Lee, H.Y., 2012. Changes in Ribosomal Protein S3 Immunoreactivity and its Protein Levels in the Gerbil Hippocampus Following Subacute and Chronic Restraint Stress. Neurochem Res 37, 1428–1435.

Poreisz, C., Boros, K., Antal, A., Paulus, W., 2007. Safety aspects of transcranial direct current stimulation concerning healthy subjects and patients. Brain Res Bull 72, 208–214.

Radman, T., Ramos, R.L., Brumberg, J.C., Bikson, M., 2009.Role of cortical cell type and morphology in subthreshold and suprathreshold uniform electric field stimulation in vitro. Brain Stimul; 2, 215-228, 228 e211-213.

Reidler, J.S., Mendonca, M.E., Santana, M.B., Wang, X., Lenkinski, R., Motta, A.F., Marchand,

S., Latif, L., Fregni, F., 2012. Effects of Motor Cortex Modulation and Descending Inhibitory Systems on Pain Thresholds in Healthy Subjects. J Pain.

Rosen, A.C., Ramkumar, M., Nguyen, T., Hoeft, F., 2009. Noinvasive Transcranial Brain Stimulation and Pain. Curr Pain Headache Rep. 13(1), 12-17.

Rubinstein, M., Mogil, J.S., Japón, M., Chan, E.C., Allen, R.G., Low, M.J., 1996. Absence of opioid stress-induced analgesia in mice lacking beta-endorphin by site-directed mutagenesis. Proc Natl Acad Sci; 93, 3995–4000.

Siegfried, B., Netto, C.A., Izquierdo., 1987. Exposure to novelty induces naltrexone reversible analgesia in rats. Behavioral Neuroscience, 101, 436-438.

Stagg, C.J., Best, J.G., Stephenson, M.C., 2009. Polarity-sensitive modulation of cortical neurotransmitters by transcranial stimulation. J Neurosci 29(16), 5202-5206.

Staud, R., 2006a. Biology and therapy of fibromyalgia: pain in fibromyalgia syndrome. Arthritis Res Ther 8, 208–14.

Staud, R., Rodriguez, M.E., 2006b. Mechanisms of disease: pain in fibromyalgia syndrome. Nat Clin Pract Rheumatol 2, 90–8.

Takano, Y., Yokawac, T., Masudac, A., Niimic, J., Tanakad, S., Hironakaa, N., 2011.A rat model for measuring the effectiveness of transcranial direct current stimulation using fMRI. Neuroscience Letters 491, 40–43.

Torres, I.L.S., Vasconcellos, A.P., Silveira, S.N., Dalmaz, C., 2001a. Effect of repeated stress on novelty-induced antinociception in rats. Brazilian Journal of Medical and Biological Research 34, 241-244.

Torres, I.L.S., Cucco, S.N.S., Bassani, M., Duarte, M.S., Silveira, P.P., Vasconcellos, A.P., Tabajara, A.S., Dantas, G., Fontella, F.U., Dalmaz, C., Ferreira, M.B.C., 2003. Long-lasting delayed hyperalgesia after chronic restraint stress in rats: effect of morphine administration. Neurosci Res. 45, 277-283.

Torres, I.L.S., Cucco, S.N., Vasconcellos, A.P., Dalmaz, C., 2001b. Effect of repeated stress on novelty-induced antinociception in rats. Braz. J. Med. Biol. Res. 34, 241-244.

van Hanswijck de Jonge, Lloyd, A., Horsfall, L., Tan, R., O'Dwyer, P.J., 2008. The measurement of chronic pain and health-related quality of life following inguinal hernia repair: a review of the literature. 561, 569-12.

Volz, M.S., Volz, T.S., Brunoni, A.R., de Oliveira, J.P., Fregni, F. 2012. Analgesic Effects of Noninvasive Brain Stimulation in Rodent Animal Models: A Systematic Review of Translational Findings. Neuromodulation.

Wachter, D., Wrede, A., Schulz-Schaeffer, W., Taghizadeh-Waghefi, A., Nitsche, M.A., Kutschenko, A., Rohde, V., Liebetanz, D., 2011. Transcranial direct current stimulation induces polarity-specific changes of cortical blood perfusion in the rat. Experimental Neurology 227, 322–327.

Vivancos, G.G., Verri, Jr. W.A, Cunha, T.M., Schivo, I.R., Parada, C.A., Cunha, F.Q., Ferreira, S.H., 2004. An electronic pressure-meter nociception paw test for rats. Braz J Med Biol Res 37, 391-399.

Wegert, S., Ossipov, M.H., Nichols, M.L., Bian, D., Vanderah, T.W., Malan, Jr. T.P., Porreca, F., 1997. Differential activities of intrathecal MK-801 or morphine to alter responses to thermal and mechanical stimuli in normal or nerve-injured rats. Pain 71, 57–64.

Woolfe, G., Macdonald, A.D., 1944.The evaluation of the analgesic action of pethidine hydrochloride. J Pharmacol Exp Ther; 80, 300-307.

Wu, F.S., Yang, Y.C., Tsai, J.J., 2000. Noncompetitive inhibition of the glycine receptor-mediated current by melatonin in cultured neurons. Brain Res 881, 208–11.

Zaghi, S., Acar, M., Hultgren, B., Boggio, P.S., Fregni, F., 2010.Noninvasive brainstimulation with low-intensity electrical currents: putative mechanisms of action for direct and alternating current stimulation. Neuroscientist. 16(3), 285-307.

Zaghi, S., Thiele, B., Pimentel, D., Pimentel, T., Fregni, F., 2011. Assessment and treatment of pain with non-invasive cortical stimulation. Restor Neurol Neurosci. 29(6), 439-51.

LEGENDS

Figure 1. Basal measure, immediately and 24 hours after the end of tDCS treatment on alodynia induced by chronic stress evaluated with the von Frey test

Data presented as mean ± SEM of withdrawal response in grams (g). Groups: C- control; S – stress; SS- stress+sham; SN- stress+neuromodulation.

*Significant difference versus control group (C) (one-way ANOVA/Tukey, $P<0.05$, n=9-13)

[#]Significant difference versus the control (C) and stress + tDCS (SN) groups (one-way ANOVA/Tukey, $P<0.05$, n=9-13)

Figure 2. Basal measure, immediately and 24 hours after the end of tDCS treatment on hyperalgesia induced by chronic rats evaluated with the hot-plate test

Data presented as mean ± SEM of response latency (time to onset of paw-licking or jumping) in seconds (s). Groups: C- control; S – stress; SS- stress+sham; SN- stress+neuromodulation.

*Significant difference versus control group (C) (one-way ANOVA/Tukey, $P<0.05$, n=9-13)

Significant difference versus the control (C) and stress + tDCS (SN) groups (one-way ANOVA/Tukey, $P<0.05$, n= 9-13)

Figure 3.

Panel A. Evaluation of serum corticosterone levels of chronic stressed rats 48h after the end of tDCS treatment. Data presented as mean ± SEM of serum corticosterone level in nmol/L. Groups: C- control; S – stress; SS- stress+sham; SN- stress+neuromodulation.

There were no significant between-group differences (one-way ANOVA, $P>0.05$, n= 3-4)

Panel B. Evaluation of serum interleukin-1beta levels of chronic stressed rats 48h after the end of tDCS treatment

Data presented as mean ± SEM of serum interleukin-1beta level in pg/mL. Groups: C- control; S – stress; SS- stress+sham; SN- stress+neuromodulation.

There were no significant between-group differences (one-way ANOVA, *P>0.05*, n= 6-3)

Figure 4. Evaluation of hippocampal TNFα levels of chronic stressed rats 48h after the end of tDCS treatment

Data presented as mean ± SEM of hippocampal TNFα levels in pg/mL. Groups: C- control; S – stress; SS- stress+sham; SN- stress+neuromodulation.

*****Significant difference in relation to other groups (one-way ANOVA/Tukey, $P<0.05$, n=3-4)

Figure 5.

Panel A: tDCS electrode placement

The cathodal stimulus electrode was positioned at the midpoint of the lateral angle of the eyes, and the anodal electrode is positioned over the neck and shoulder areas.

Panel B: tDCS stimulation procedure

The stimulator was placed onto the thorax with a corset and the electrodes were fixed onto the rat's head.

Figure 1

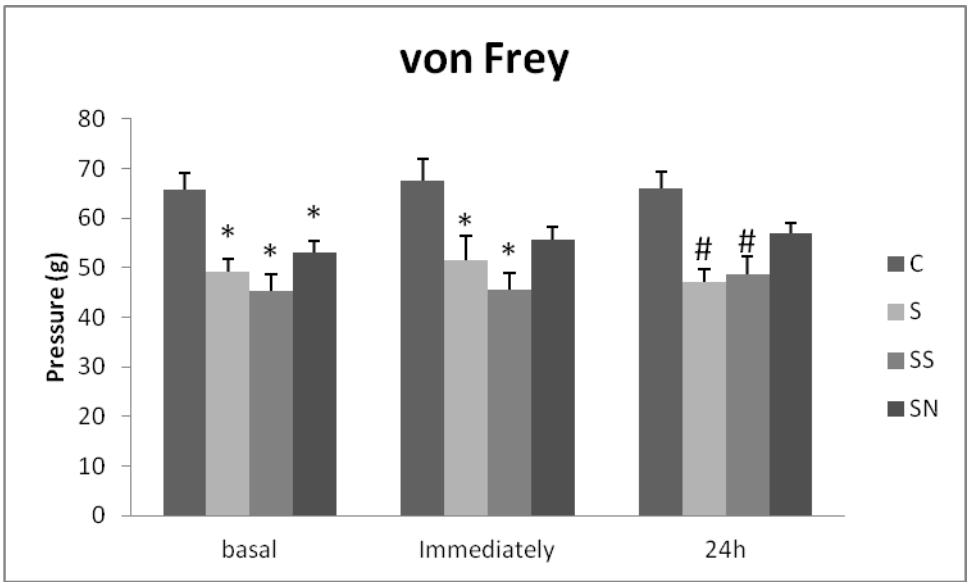


Figure 2

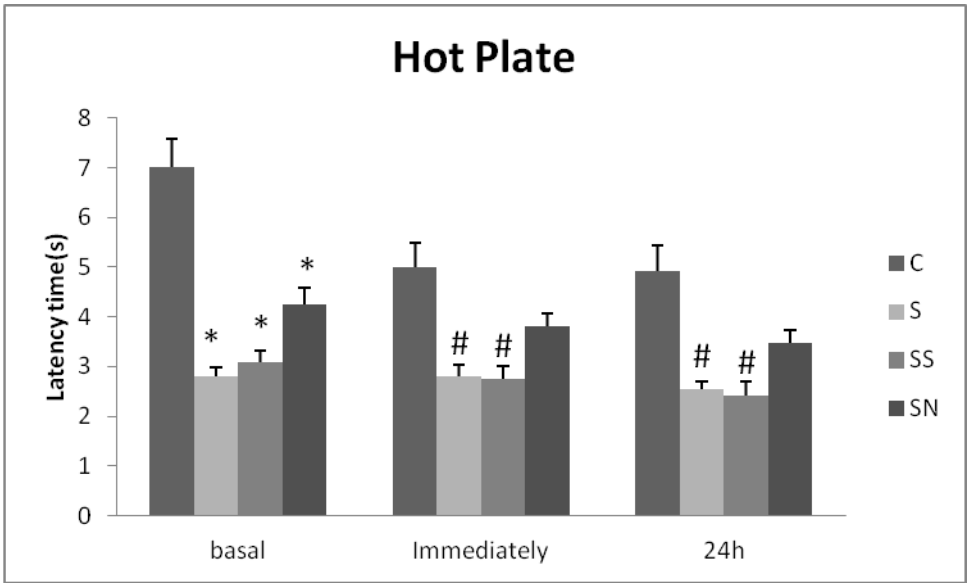
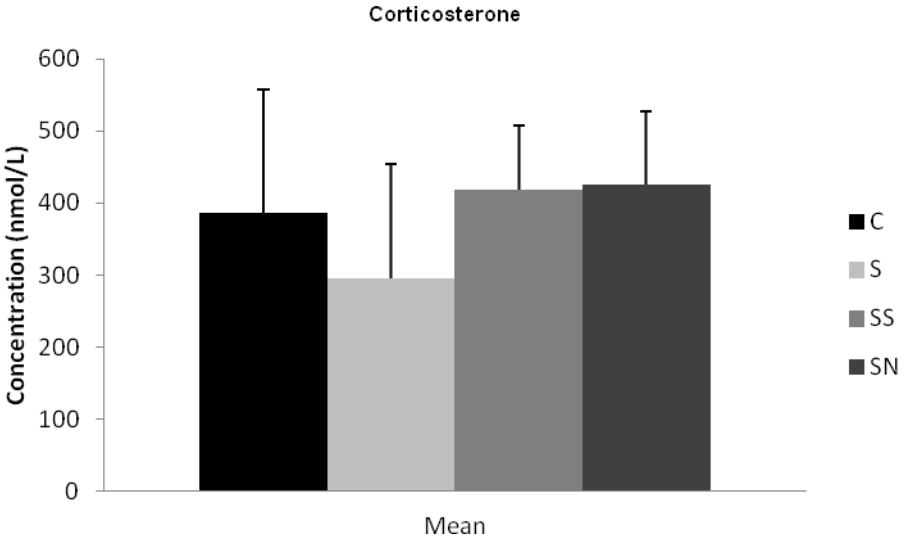


Figure 3
Panel A



Panel B

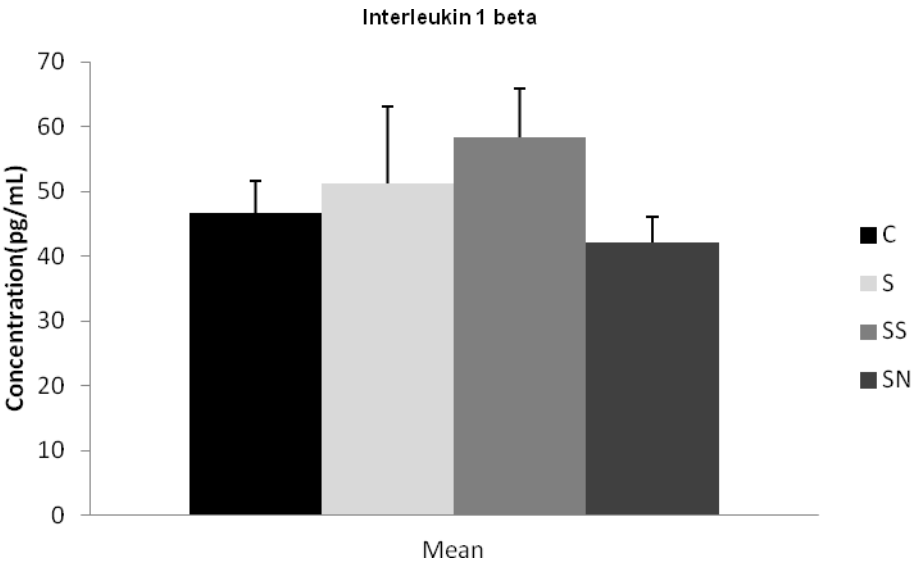


Figure 4

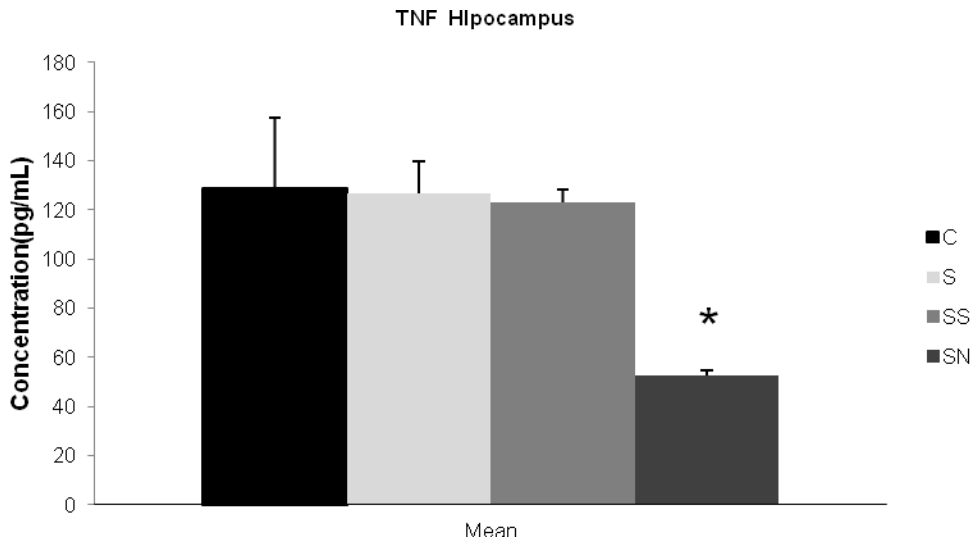

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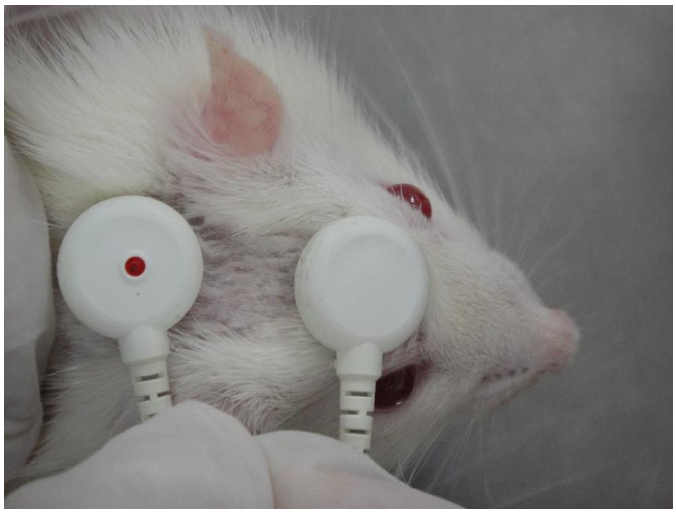
Figure 5
Panel A



Panel B

IV. MANUSCRITO II:

Transcranianal direct current stimulation (tDCS) reverses chronic stress effect in spinal cord BDNF levels of rats.

Periódico: Brain Stimulation

Status: a ser submetido

Transcranianal direct current stimulation (tDCS) reverses chronic stress effect in spinal cord BDNF levels of rats.

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ABSTRACT

It is known that chronic restraint stress (CRS) induces hyperalgesia/allodynia, and our previous study showed that this is reverted by anodal tDCS treatment. BDNF is a neurotrophin related to pain memory, and it can be related to efficacy of this treatment. In this study we evaluate the effect of anodal tDCS treatment on the BDNF levels in spinal cord and brainstem of rats submitted to CRS. 24 male Male Wistar rats 60 days-old were divided into 4 groups: control (C), stress (S), stress+sham tDCS (SS) and stress+tDCS (SN). The CRS was applied for 11 weeks (1h/ day/5days/wk). Then, it was initiated the anodal tDCS treatment (20 min/day/8 days at 500μA). After tDCS treatment the animals were killed by decapitation; the central nervous system structures were removed, frozen at -80°C and analyzed by ELISA assay. One-way ANOVA was used followed by Tukey when necessary, and $P<0.05$ was considerable significant. After 11 week of CRS, the stressed animals presented hyperalgesia and allodynia (data not show). The S group showed a significant decrease in BDNF levels in spinal cord compared to other groups. The stressed groups S and SN showed this effect in the brainstem compared to C group. In this study, we demonstrated that the CRS, besides induces hyperalgesia, alters BDNF levels in spinal cord and brainstem, it is a modulator of neural activity and neuronal plasticity. Additionally, tDCS stimulation was effective in reversing this effect in the spinal cord. We suggested that the effect of anodal tDCS in the spinal cord is involved with neuroplasticity and pain modulation.
Keywords: transcranial direct current stimulation, chronic stress, BDNF, rats, pain

Introduction

The transcranial direct current stimulation (tDCs) is a well known technique effective in reducing pain in patients with fibromyalgia (1), spinal cord injury (2), epilepsy (3), and others. The tDCs is based on modulation of neural activity via small electrical currents that when applied as a direct current (DC) component lead to a polarization of the neural tissue inducing significant changes in the resting membrane threshold (4) and subsequent changes in synaptic plasticity as shown in an elegant animal model (5). In previous study of our group we demonstrated that the treatment with repetitive anodal tDCS was effective in reduce pain in a rat model of chronic inflammation induced by complete Freund's adjuvant (6). In other study we showed that tDCS could fully revert the detrimental effects of chronic stress in the pain system as indexed by hyperalgesia and allodynia, and this effect is persistent for 24h. This result is concomitant with a decreased in hippocampus TNFα level but without effect in serum corticosterone and interleukin 1β levels (Adachi et al, personal communication). This modification on central nervous system (CNS) TNFα levels probably is one of the pathways of tDCS effect, but many others possibilities can to be considered. Although this technique has demonstrated good results in treatment of pain syndromes (1,2); the pathway whereby it acts is still unknown.

Pain syndromes are associated with chronic stress, since chronic exposure to pain produces suffering, which activates the hypothalamic-pituitary-adrenal (HPA) axis (7). Unlike acute stress, which has been associated with a reduction in pain sensitivity, probably mediated by brainstem pain modulation (7), chronic stress has been associated with increased sensitivity to pain, producing hyperalgesia (decrease pain threshold) (8, 9, 10) and allodynia (pain induced by no noxious stimuli) (10). In this context, we briefly review the putative mechanisms involved in the development of hyperalgesia after repeated restraint stress. Previous studies have suggested that it could be related to changes in central or peripheral opioid activity (11; 12; 13). The absence of novelty-induced antinociception in these animals supports this theory (11). Exposure of rats to a novel environment is known to be followed by mild, naloxone-reversible antinociception (14). Opioid receptors can be highly plastic, as reflected by their susceptibility to modifications by various pharmacological and behavioral manipulations (15). Dantas and colleagues (13) showed decreases in binding of opioid receptors in the hippocampus and cerebral cortex. Additionally, Torres and colleagues (12) demonstrated that animals subjected to

chronic restraint stress for 6 weeks needed high doses of morphine to exhibit an analgesic response, suggesting that prolonged stress could lead to longer-lasting changes in the neural systems involved in nociceptive modulation. On the other hand, in acute stress, the opiate system seems to be modulated in the opposite direction. In fact, a previous study has demonstrated that animals subjected to acute stress show an increase in the magnitude and duration of the analgesic effect to some opiate agonists (16).

In previous study, we demonstrated that the chronic stress-induced hyperalgesia remained for 28 days after discontinuation of treatment (12). It is interesting the analgesic response to acute restraint stress (i.e., inhibition of pain) was re-established after 14 days of discontinuation of chronic stress (12). However the underlying mechanisms of long-lasting hyperalgesia induced by chronic stress are still unknown.

Studies have shown that acute and chronic inflammatory pain were associated with decreased levels of brain-derived neurotrophic factor (BDNF) in the hippocampus, which is also observed in chronically stressed rats (17). The BDNF is abundantly expressed in the adult brain and spinal cord, and this expression is modified by various insults such as stress, ischemia, hypoglycemia, depression, pain and others (18). After binding to the TrkB (tropomyocin receptor kinase B) on the cell surface of neurons, BDNF regulates neuronal survival, promotes neurite outgrowth, and maintains synaptic connectivity in the adult nervous system (19). It has neurotrophic and neuroprotective effects (18), and can act as an essential adaptive mechanism in the long-term potentiation (LTP) (20) process that is crucial to initiate and sustain the process memory pain, and it is directly proportional to BDNF levels (21; 22). This neurotrophin has also been described as a pain modulator, peripherally acts sensitizing nociceptive neurons of the dorsal root ganglion (23). Furthermore, some authors suggest that pain syndromes lead to supraspinal modulation of BDNF levels (17).

Chronic stress and depression are related to the hyperactivity of HPA axis (24; 25) and suppressed levels of BDNF in brain (26). In addition it was observed a decrease of neurogenesis in dorsal vagal complex and hippocampus of rats chronically stressed by immobilization (27).

The BDNF level is also related to the treatment response with antidepressant and electroconvulsive therapy, since they lead to increased expression of this neurotrophin on specific areas of the brain rats. Furthermore, a neuroprotective effect was shown in patients with major depression that underwent a

treatment with repetitive transcranial magnetic stimulation represented by an increase of BDNF levels (18).

Despite the progress in animal studies is important to know the real relevance of this neurotrophin in chronic pain and the effect of the restraint stress and the tDCs treatment in its levels in brainstem and spinal cord, two structures involved in pain pathway.

1. Methods

2.1 Animals

Sixty-day-old male Wistar rats weighing 180–230 g were used. Experimentally naive animals were housed in groups of five in home cages made of Polypropylene material (49 x34x16cm). All animals were maintained in a standard 12:12 light-dark cycle (lights on at 07:00 a.m. and lights off at 07:00 p.m.) in a controlled environment (22±2°C). Animals had *ad libitum* access to water and chow. All experiments and procedures were approved by the Institutional Committee for Animal Care and Use (GPPG-HCPA protocol No. 100.381) and conformed to the Guide Laboratory for the care and use of animals 8thed 2011. Animal handling and all experiments were performed in accordance with international guidelines for animal welfare and measures were taken to minimize animal pain and discomfort. The experiment used the number of animals necessary to produce reliable scientific data

2.2 Chronic Restraint Stress

The animals were stressed by 1h of restraint daily, 5 days a week for 11 weeks. Restraint was applied by placing the animal in a 25 x 7 cm plastic bottle with a 1-cm hole at the far end for breathing. The animal was unable to move. The control group was not subjected to restraint. These procedures were always performed between 08:00h a.m. and 09:00h a.m. The animals were divided into 4 groups (n=12-13): control (C), stress (S), stress + sham tDCS (SS) and stress + tDCS (SN). After 11 weeks of chronic stress exposure, behavioral tests were performed in the afternoon.

2.3 Transcranial Direct Current Stimulation (tDCS)

After 11 weeks of chronic stress exposure, the rats were subjected to a 20-minute daily session of anodal tDCS every afternoon for 8 days. This period was established because tDCS has been shown to modify cortical excitability for up 1h after 1 session of stimulation (28, 29). However, the repetitive tDCS application has demonstrated better and long lasting effect on relief pain and our group has good results with this treatment period (6). The direct current was delivered from a battery-driven, constant current stimulator using ECG electrodes with conductive adhesive hydrogel. We made a trichotomy of the animal head to have a better adherence and the size of the electrodes was reduced to $1.5cm^2$ to fit the rat's head. After the electrodes were positioned, they were fixed on the head with adhesive tape (Micropore™) and with a protective mesh to prevent removal (fig. 1A).

The anodal electrode was positioned between the ears from the neck of the rat (parietal cortex) (fig.1B) (30 with modifications) as to mimic the anodal placement in human pain studies (31; 32).The cathodal electrode was positioned at the midpoint of the lateral angle of the eyes (supraorbital area). The electrodes were placed on the skin, in a similar manner to human pain tDCS studies (33; 34; 1; 35).

A constant current of 0.5mA intensity was applied for 20 min (2; 36; 37; 3). According to the earlier study (38) we observed that a constant current of 1 mA intensity causes skin lesions as current density is comparatively much higher than compared to the traditional 1mA tDCS using large pads in humans. We therefore choose to use 0.5mA, as has been used also in other animal studies (6), and in addition in our study the electrodes were fixed in the skin. Using this montage we did not observe lesions with this current intensity.

An important point to consider was that in our model it was not necessary to use anesthesia nor surgery according to other models used in previous tDCS studies in rats (36; 37; 3). In fact, this represents strength in this study as it has been shown that volatile anesthesia (such as isoflurane), decreases excitatory and increases inhibitory transmission (39; 40), altering BDNF expression and thus neuroplasticity (41; 42). We were able thus to remove this confounding factor in our study by adapting a human model using ECG electrodes (35).

For sham stimulation, the electrodes were placed in the same positions as for real stimulation; however, the stimulator was turned off after 30 s of stimulation so that the animals maintain continuity of the physical sensation of real tDCS conditions (43).

Insert Figure 1A

Insert Figure 1B

48h after the treatment the rats were decapitated and their spinal cord and brainstem was quickly dissected out and stored at –70^{o} C for subsequent analysis.

2.4 Analysis of BDNF Immunocontent

BDNF levels were determined by Enzyme-Linked Immunoabsorbent Assay (ELISA) using the ChemiKine kit (Millipore) according to the manufacturer's recommendations. The results are expressed in pg/mL.

2.5 Statistical analysis

The results were presented as the mean ± standard error of the mean (S.E.M.). For analysis comparing the BDNF measures of all the groups, we used one-way ANOVA with Tukey's test when necessary. *P*-values less than 0.05 were considered significant.

2. Results

3.1 Effects of tDCS on BDNF levels in brainstem after the end of treatment with tDCS

We observed a decreased on BDNF levels in brainstem induced by the chronic stress exposure (S, SS, SE groups) compared to control group, on the other hand, no effect was observed after the end of treatment with tDCS (C:108.01+12.88pg/mL; S: 66.40+6.28 pg/mL; SS: 62.94+8.88 pg/mL; SN: 50.16+4.89 pg/mL; one-way ANOVA/Tukey's test, P>0.05, n=6-7, Figure 2).

Insert Figure 2

3.2 Effects of tDCS on BDNF levels in spinal cord after the end of treatment with tDCS

We observed a decreased on BDNF levels in spinal cord induced by the chronic stress exposure (S, SS, SE groups) compared to control group. In addition, the treatment with tDCS was effective in revert this effect (C:78.96±6.51 pg/mL; S: 49.95±4.24 pg/mL; SS: 67.41±3.88 pg/mL; SN: 77.45±4.55 pg/mL; one-way ANOVA/Tukey's test, $P<0.05$, n=6-7, Figure 3).

Insert Figure 3

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3. Discussion

In this study we confirmed chronic stress-induced hyperalgesia/allodynia, according to previous studies (8; 9; 10). In addition, we demonstrated that the chronic stress induces decreased in BDNF level in spinal cord and brainstem. Furthermore we showed that tDCS stimulation was effective in reversing this effect in the spinal cord but no in the brainstem. These findings about chronic stress exposure are in accordance with previous studies that suggest the hyperactivity of HPA axis is related to suppression of central BDNF levels (26) and that rats chronically stressed by immobilization present neurogenesis decrease in dorsal vagal complex and hippocampus (27). Taking into account that BDNF has an important role in neuronal plasticity; these results suggest that chronic restraint stress exposure can lead to neuronal plasticity decrease.

BDNF is also considered a pain modulator and some authors suggest that peripherally this neurotrophin acts sensitizing nociceptive neurons of the dorsal root ganglion (23). Although BDNF regulates neuronal survival, promotes neurite outgrowth, and maintains synaptic connectivity in the adult CNS (19). It exerts effects on nociception by binding to trkB receptor, and initiating intracellular

signaling cascades that lead to transcriptional changes. In addition Shu and colleagues (44) demonstrated that animals showed thermal hyperagesia that lasted for 5h after intraplantar injection of BDNF. It has been demonstrated increased released of BDNF in the spinal cord after nervous insult, such as ischemia and sciatic nerve transaction (45). Furthermore some authors suggest that pain syndromes lead to BDNF supraspinal modulation for example, there are reports that noxious stimulation increases BDNF production in the spinal dorsal horn (46; 47; 48) and brainstem (49; 50) leading to hyperalgesia and the formation of nocifensive behaviors. In addition, wind-up (a form of central sensitization) of spinal nociceptive reflexes is significantly reduced in BDNF-deficient mice (51), and BDNF superfusion significantly increases C-fiber mediated reflexes in an isolated spinal cord preparation (52). On the other hand, another study has shown that acute inflammatory pain (formalin) or chronic (Freud`s adjuvant) are associated with decreased hippocampus BDNF levels in (17), suggesting that the pain process is related to decrease levels of BDNF in CNS. Then, we can suggest that the hyperalgesia and allodynia induced by CRS (Adachi et al., personal communication) can be related to decrease brainstem and spinal cord BDNF level in these animals. This suggestion corroborated the fact of the tDCS treatment to be effective to reverse both effects induced by chronic stress: the hyperalgesia/allodynia and the decrease of spinal cord BDNF levels. .

The reversion of the decrease spinal cord BDNF levels of rats chronically stressed by the tDCS treatment could be considered one possible pathway of this treatment to revert hyperalgesia pattern in the same animals, as seen in the hot plate (hyperalgesia) and von Frey Tests (allodynia) (Adachi et al. personal communication). Is important to note that the stress sham tDCS group (SS group) was not different of the others groups. It is possible that this result could be due to 30s period of stimulation or to immobilization for 20 minutes per 8 days in the same position used in the stress neuromodulation group.

Although anodal tDCS has been demonstrated to induce pain relief in human studies (53), and this study fills a critical gap in the knowledge of this field as we show that consecutive sessions of tDCS can reverse chronic stress induced pain. In our previous study we were able to control the source of pain; thus having a homogeneous sample in terms of mechanisms of chronic pain and demonstrating the effects of tDCS in this condition. Other important findings were that corticosterone and interleukin

1β serum levels did not changed by the tDCS sessions and/or chronic restraint stress exposures. These results are in accordance with the literature that demonstrated that chronic restraint stress lead to disorganization and deregulation of stress responses of HPA axis (54). In addition, in previous study we showed that the TNFα level in hippocampus was statistically significant decreased in the group that received the tDCS in relation of other groups (Adachi et al., personal communication). As it is a pro-inflammatory cytokine, this also could be related to effects of tDCS on reversing the maladaptative changes in the pain system induced by CRS.

Even though the mechanisms underlying tDCS-mediated pain regulation have yet to be elucidated, its mechanisms of action involve changes in the neuronal electrical membrane potential and modifications in the synaptic microenvironment. Besides, it is known that noninvasive brain stimulation provided a neuroprotective effect both *in vitro* and *in vivo* (56). Muller and colleagues (18) demonstrated that long-term rTMS increases the expression of BDNF in specific areas of the hippocampus and cerebral cortex. BDNF is characterized by their ability to regulate diverse neuronal responses, including the type and number of afferent synapses, by promoting the survival of neuronal subpopulations (57). Furthermore, a significant reduction in BNDF expression was found in the hippocampus of patients with Alzheimer's disease when compared to controls, this decrease probably contribute to the neuronal degeneration (58). It therefore seems possible that BDNF acts as a mediator of the neuroprotective effects of tDCS.

Taken together, our findings support the hypothesis of an antihyperalgesic/ antiallodynic effect of tDCS. Although the mechanisms underlying this effect remain unclear, the evidence suggests that they include non-synaptic and synaptic mechanisms. The non-synaptic mechanism includes changes which, apart from reflecting local changes in ionic concentrations, could arise from alterations in transmembrane proteins and from electrolysis-related changes in H(+) induced by exposure to constant electric field (59). The synaptic mechanisms involve neuroplastic alterations, such as changes in the strength of connections, representational patterns, or neuronal properties, either morphological or functional (60). tDCS induces prolonged neuronal excitability and activity changes in the human brain via alterations in neuronal membrane potential, resulting in prolonged synaptic efficacy changes.

One important question that is not fully elucidated is the optimal electrode position to induce analgesic effects (61). It is not clear whether the effects are mainly due to anodal stimulation of frontal areas (including M1) or is also associated with cathodal stimulation of the contralateral area. Though there is extensive evidence showing that modulation of M1 is critically involved with pain modulation as also it showed by modeling studies (31;32) and HD-tDCS (62). Finally another important issue is electrode montage and shunting. Although our montage may be associated with shunting, it should be considered that this montage was previously demonstrated as effective in Takano and colleagues (30) study. They examined the effectiveness of tDCS using functional magnetic resonance imaging (fMRI) and the signal intensities of fMRI in the frontal cortex and the *nucleus accumbens*; showing significant activity increase by anodal tDCS in rats. In addition, computer finite element model studies showed that even with close electrodes such as used in HD-tDCS there is a significant amount of current being injected and reaching cortical areas (63; 64). Therefore based on these considerations we decided to use a cephalic montage as this has been the most used methods in humans. In fact a recent study in humans showed that extra-cephalic montage was less effective to induce pain improvements in humans (31). Another important limitation as also discussed in a recent review is the translation of these results to humans (66). In our study all experiments were conducted in male rats, complicating the translation of the results to both genders in humans, particularly because the effects of nociceptive response are altered by modulations in hormone state (67).

In this context, this study shows for the first time to the best of our knowledge that tDCS could revert the effects of maladaptive plasticity as indexed by behavioral changes and also as indexed by BDNF levels. The evaluation of binding and expression of TrkB receptors will be the next step of study for better understand the tDCS mechanisms in the BDNF levels.

Given that in this study, animals were exposed to the same level of stress in the same conditions, our findings support further exploration of tDCS as a therapeutic tool early in the exposure to stressful situations that may lead to chronic pain such as in post-traumatic stress disorder and demonstrated one possible pathway of anodal tDCS treatment.

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References

1. Fregni F, Gimenes R, Valle A, et al. A Randomized, Sham- Controlled, Proof of Principle Study of Transcranial Direct Current Stimulation for the Treatment of Pain in Fibromyalgia. Arthritis & Rheumatism. 2006a;54(12):3988–3998.
2. Fregni F, Boggio PS, Lima MC, et al. A sham-controlled, phase II trial of transcranial direct current stimulation for the treatment of central pain in traumatic spinal cord injury. Pain. 2006b;122(1-2):197-209.
3. Liebetanz D, Klinker F, Hering D, et al. Anticonvulsant Effects of Transcranial Direct-current Stimulation (tDCS) in the Rat Cortical Ramp Model of Focal Epilepsy. Epilepsia 2006; 47(7):1216–1224.
4. Zaghi S, Acar M, Hultgren B, Boggio PS, Fregni F. Noninvasive brain stimulation with low-intensity electrical currents: putative mechanisms of action for direct and alternating current stimulation. Neuroscientist. 2010;16(3):285-307.
5. Fritsch B, Reis J, Martinowich K, Schambra HM, Ji Y, Cohen LG, Lu B. Direct current stimulation promotes BDNF-dependent synaptic plasticity: potential implications for motor learning. Neuron. 2010;66(2):198-204.
6. Laste G, Caumo W, Adachi LNS., et al. Effect of trancranial direct current stimulation in hyperalgesia for chronic inflammation in rats. Experimental Brain Research, 2012; 221(1), 75-83.

7. Martenson M E, Cetas J S., Heinricher M M.. A possible neural basis for stress-induced hyperalgesia. PAIN 2009;142:236–244.
8. Gamaro GD, Xavier MH, Denardin JD,et al. The effects of acute and repeated restraint stress on the nociceptive response in rats. Physiology and Behavior 1998;63:693-697.
9. Torres ILS, Vasconcellos AP, Silveira SN, Dalmaz C. Effect of repeated stress on novelty-induced antinociception in rats. Brazilian Journal of Medical and Biological Research 2001a;34: 241-244.
10. Bardin L, Malfetes N, Newman-Tancredi A, Depoortère R. Chronic restraint stress induces mechanical and cold allodynia, and enhances inflammatory pain in rat: Relevance to human stress-associated painful pathologies. Behavioural Brain Research 2009;360–366.
11. Torres ILS, Cucco SN, Vasconcellos AP, Dalmaz C. Effect of repeated stress on novelty-induced antinociception in rats. Braz. J. Med. Biol. Res. 2001b;34:241-244.
12. Torres ILS, Cucco SNS, Bassani M, et al. Long-lasting delayed hyperalgesia after chronic restraint stress in rats: effect of morphine administration. Neurosci Res. 2003; 45:277-283.
13. Dantas G, Torres ILS, Crema L M, Lara D, Dalmaz C. Repeated Restraint Stress Reduces Opioid Receptor Binding in Different Rat CNS Structures. Neurochemical Research 2005;30(1):1-7.
14. Siegfried B, Netto CA, Izquierdo. Exposure to novelty induces naltrexone reversible analgesia in rats. Behavioral Neuroscience 1987;101:436-438.
15. Drolet G, Dumont EC, Gosselin I, et al. Role of endogenous opioid system in the regulation of stress response. Prog. Neuro-psychopharmacol. Biol. Psychiatry 2001;25:729-741.
16. Calcagnetti DJ, Holtzman SG. Intake of sweet water attenuates restraint-stress-induced potentiation of morphine analgesia in rats. Brain Res Bull. 1992;29(6):859-64.
17. Pezet S, McMahon SB. Neurotrophins: mediators and modulators of pain. Annu Rev Neurosci. 2006;29:507-38. Review.
18. Müller M B., Toschi N, Kresse A E., Post A, Keck M E. Long-Term Repetitive Transcranial Magnetic Stimulation Increases the Expression of Brain-Derived Neurotrophic Factor and

Cholecystokinin mRNA, but not Neuropeptide Tyrosine mRNA in Specific Areas of Rat Brain. Neuropsychopharmacology 2000;23(2).

19. Zhang HN, Ko MC. Seizure activity involved in the up-regulation of BDNF mRNA expression by activation of central mu opioid receptors. Neuroscience 2009;161:301-10.

20. Kossel AH, Cambridge SB, Wagner U, Bonhoeffer T. A caged Ab reveals an immediate/instructive effect of BDNF during hippocampal synaptic potentiation. Proc Natl Acad Sci. 2001;98(25):14702-7.

21. Korte M, Carroll P, Wolf E, et al. Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor.Proc Natl Acad Sci. 1995;92(19):8856-60.

22. Figurov A, Pozzo-Miller LD, Olafsson P, Wang T, Lu B. Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. Nature. 1996;381(6584):706-9.

23. Merighi A, Salio C, Ghirri A, et al. BDNF as a pain modulator. Prog Neurobiol. 2008;85(3):297-317.Review

24. Holsboer F, von Bardeleben U, Wiedemann K, Müller OA, Stalla GK. Serial assessment of corticotropin-releasing hormone response after dexamethasone in depression. Implications for pathophysiology of DST nonsuppression. Biol Psychiatry. 1987;22(2):228-34.

25. Kunugi H, Ida I, Owashi T, et al. Assessment of the dexamethasone/CRH test as a state-dependent marker for hypothalamic-pituitary-adrenal (HPA) axis abnormalities in major depressive episode: a Multicenter Study. Neuropsychopharmacology. 2006;31(1):212-20.

26. Dunham JS, Deakin JF, Miyajima F, Payton A, Toro CT. Expression of hippocampal brain-derived neurotrophic factor and its receptors in Stanley consortium brains. J Psychiatr Res. 2009;43(14):1175-84.

27. Chigr F, Rachidi F, Segura S, et al. Neurogenesis inhibition in the dorsal vagal complex by chronic immobilization stress in the adult rat. Neuroscience. 2009; 23;158(2):524-36.

28. Nitsche MA, Paulus W. Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. Neurology. 2001;57(10):1899-901.
29. Nitsche M A, Fricke K, Henschke U, et al. Pharmacological modulation of cortical excitability shifts induced by transcranial direct current. The Journal of Physiology 2003;293:301-553.
30. Takano Y, Yokawac T, Masudac A, et al. A rat model for measuring the effectiveness of transcranial direct current stimulation using fMRI. Neuroscience Letters 2011;491:40–43.
31. Mendonca M E, Santana M B, Baptista A F,et al. Transcranial DC stimulation in fibromyalgia: optimized cortical target supported by high-resolution computational models. J Pain 2011;5:610-7.
32. Dasilva A F, Mendonca M E, Zaghi S, et al. tDCS-Induced Analgesia and Electrical Fields in Pain-Related Neural Networks in Chronic Migraine. Headache. 2012
33. Nitsche M A, Cohen L G, Wassermann E M., et al. Transcranial direct current stimulation: state of the art. Brain Stimul 2008;1:206-23.
34. Antal P, Paulus W. A case of refractory orofacial pain treated by transcranial direct current stimulation applied over hand motor area in combination with NDA agonist drug intake. Brain Stimulation 2011;4:117-21.
35. Rosen A C, Ramkumar M, Nguyen T, Hoeft F. Noinvasive Transcranial Brain Stimulation and Pain. Curr Pain Headache Rep. 2009;13(1):12-17.
36. Dockery C A, Liebetanz D, Birbaumer N, Malinowska M, Wesierska M J Cumulative benefits of frontal transcranial direct current stimulation on visuospatial working memory training and skill learning in rats. Neurobiology of Learning and Memory 2011; 96:452–460.
37. Wachter D, Wrede A, Schulz-Schaeffer W, et al. Transcranial direct current stimulation induces polarity-specific changes of cortical blood perfusion in the rat. Experimental Neurology 2011;227:322–327.
38. Liebetanz D, Koch R, Mayenfels S, et al. Safety limits of cathodal transcranial direct current stimulation in rats. Clinical Neurophysiology 2009;120:1161–1167.

39. Gomez RS, Guatimosim C. Mechanism of action of volatile anesthetics: involvement of intracellular calcium signaling. Curr Drug Targets CNS Neurol Disord. 2003;2(2):123-9. Review

40. Ouyang W, Hemmings HC Jr. Depression by isoflurane of the action potential and underlying voltage-gated ion currents in isolated rat neurohypophysial nerve terminals. J Pharmacol Exp Ther. 2005;312(2):801-8.

41. Lu LX, Yon JH, Carter LB, Jevtovic-Todorovic V. General anesthesia activates BDNF-dependent neuroapoptosis in the developing rat brain. Apoptosis 2006; 11:1603–1615.

42. Head B P, Patel H H, Niesman I R, Drummond J C, Roth D M., Patel P M. Inhibition of p75 neurotrophin receptor attenuates isoflurane-mediated neuronal apoptosis in the neonatal central nervous system. Anesthesiology 2009;110:813– 825.

43. Gandiga P C, Hummel F C, Cohen L G. Transcranial DC stimulation (tDCS): a tool for the double-blind sham-controlled clinical studies in brain stimulation. Neurophysiol 2006;117:845-50.

44. Shu XQ, Llinas A, Mendell LM. Effects of trkB and trkC neurotrophin receptor agonists on thermal nociception: a behavioral and electrophysiological study. Pain 1999;80, 463–470.

45. Walker SM, Mitchell VA, White DM, Rush RA, Duggan AW. Release of immunoreactive brain-derived neurotrophic factor in the spinal cord of the rat following sciatic nerve transection. Brain Res. 2001;899:240.

46. Michael GJ, Averill S, Shortland PJ, Yan Q, Priestley JV. Axotomy results in major changes in BDNF expression by dorsal root ganglion cells: BDNF expression in large trkB and trkC cells, in pericellular baskets, and in projections to deep dorsal horn and dorsal column nuclei. Eur J Neurosci. 1999;11:3539-3551.

47. Ha SO, Kim JK, Hong HS, Kim DS, Cho HJ. Expression of brain derived neurotrophic factor in rat dorsal root ganglia, spinal cord and gracile nuclei in experimental models of neuropathic pain. Neuroscience 2001;107:301-309.

48. Pezet S, Malcangio M, Lever IJ, Perkinton MS, Thompson SW, Williams RJ, McMahon SB. Noxious stimulation induces trk receptor and downstream ERK phosphorylation in spinal dorsal horn. Mol Cell Neurosci 2002;21:684-695.

49.Guo W, Robbins MT, Wei F, Zhou S, Dubner R, Ren K. Supraspinal brain-derived neurotrophic factor signaling: A novel mechanism for descending pain faciliatation. J Neurosci 2006;26:126-137.

50.Renn CL, Lin L, Thomas S, Dorsey SG. Full-length tropomyosin related kinase B expression in the brainstem in response to persistent inflammatory pain. Neuro Report 2006;17:1175-1179.

51.Heppenstall PA, Lewin GR. BDNF but not NT-4 is required for normal flexion reflex plasticity and function.

52.Kerr BJ, Bradbury EJ, Bennett DL, Trivedi PM, et al. Brain-derived neurotrophic factor modulates nociceptive sensory inputs and NMDA-evoked responses in the rat spinal cord.J Neurosci. 1999;19(12):5138-48.

53.Mylius, V., Borckardt, J.J., Lefaucheur, J.P. Noninvasive cortical modulation of experimental pain. Pain 2012 in press.

54.Goshen I, Yirmiya R. Interleukin-1 (IL-1): a central regulator of stress responses. Front Neuroendocrinol. 2009;30(1):30-45. Review.

55.Tagliari, B., Tagliari, A.P., Schmitz, F., Cunha, A.A., Dalmaz, C., Wyse, A.T.S. Chronic Variable Stress Alters Inflammatory and Cholinergic Parameters in Hippocampus of Rats. Neurochem Res. 2011; 36:487–493.

56.Post A, Müller MB, Engelmann M, Keck ME. Repetitive transcranial magnetic stimulation in rats—evidence for a neuroprotective effect in vitro and in vivo. Eur J Neurosci 1999;11:3247–3254.

57.Connor B, Dragunow M. The role of neuronal growth factors in neurodegenerative disorders of the human brain. Brain Res Brain Res Rev. 1998;27(1):1-39. Review.

58. Phillips, H.S., Hains, J.M., Armanini, M., Laramee, G.R., Johnson, S.A., Winslow, J.W., 1991. BDNF mRNA is decreased in the hippocampus of individuals with Alzheimer's disease. Neuron 2007:695–702.

59. Ardolino, G., Bossi, B., Barbieri, S., Priori, A. Non-synaptic mechanisms underlie the after-effects of cathodal transcutaneous direct current stimulation of the human brain. The Journal of Physiology 2005;653:663-568.

60. Antal, A., Nitsche, M.A., Paulus, W. Transcranial direct current stimulation and the visual cortex. Brain Research Bulletin 2006;459:463-68.

61. Fregni, F. Analgesia with noninvasive electrical cortical stimulation: challenges to find optimal parameters of stimulation. Anesth Analg. 2010;111(5):1083-5.

62. Borckardt, J.J., Bikson, M., Frohman, H., et al. A pilot study of the tolerability and effects of high-definition transcranial direct current stimulation (HD-tDCS) on pain perception. J Pain. 2012;13(2):112-20.

63. Minhas, P., Bansal, V., Patel, J., et al. Electrodes for high-definition transcutaneous DC stimulation for applications in drug delivery and electrotherapy, including tDCS. J Neurosci Methods 2010; 2:188-97.

64. Datta, A., Bansal, V., Diaz, J., Patel, J., Reato, D., Bikson, M. Gyri -precise head model of transcranial DC stimulation: Improved spatial focality using a ring electrode versus conventional rectangular pad. Brain Stimul. 2009; 4:201–207.

65. Volz, M.S., Volz, T.S., Brunoni, A.R., de Oliveira, J.P., Fregni, F. Analgesic Effects of Noninvasive Brain Stimulation in Rodent Animal Models: A Systematic Review of Translational Findings. Neuromodulation. 2012

66. Ribeiro S, Yang P, Reyes-Vazquez C, Swann A, Dafny N. Sex differences in tail-flick latency of non-stressed and stressed rats. Int J Neurosci. 2005;115(10):1383-95

Legends
Figure 1.

A: Positioning of the rats tDCS electrodes

The cathodal stimulus electrode was positioned at the midpoint of the lateral angle of the eyes and the anodal electrode is positioned from the neck to shoulder areas

B: The tDCS rats stimulation

The stimulator was placed onto the thorax with a corset and the electrodes were fixed onto rat's head

Figure 2.

Effects of tDCS on BDNF level in brainstem after the end of treatment with tDCS

Data presented as mean ± S.E.M. of BDNF level in brainstem in pg/mL

There was significant difference between the groups stress, stress sham and stress neuromodulation in comparison to control group (one-way ANOVA, $P>0.05$, n= 4-6)

Figure 3.

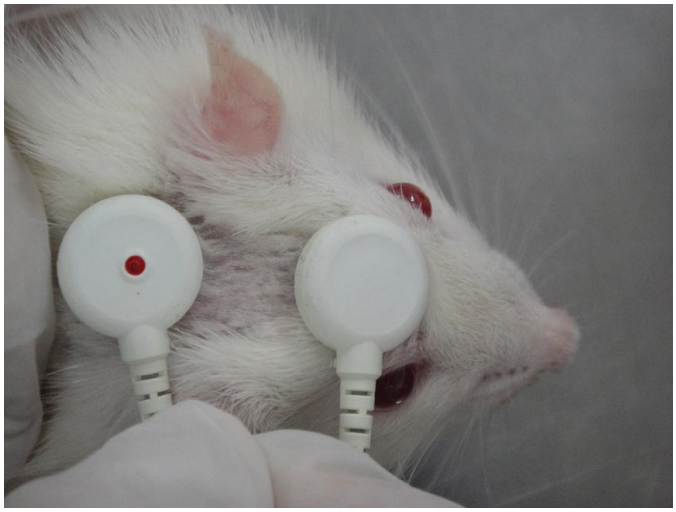
Effects of tDCS on BDNF level in spinal cord after the end of treatment with tDCS

Data presented as mean ± S.E.M. of BDNF level in spinal cord in pg/mL

There was significant difference between the stress groups in comparison to control and stress neuromodulation (one-way ANOVA, $P<0.05$, n= 4-6)

Figures

Figure 1
Panel A



Panel B

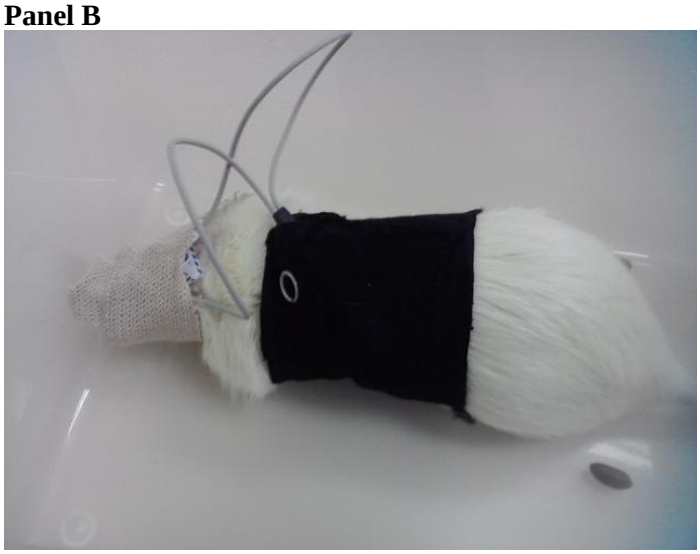


Figure 2.

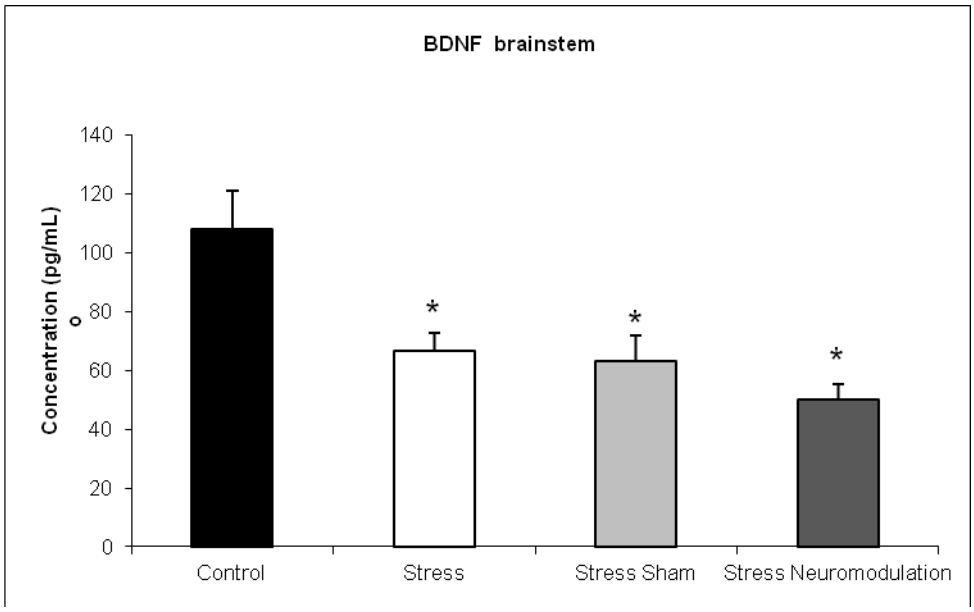
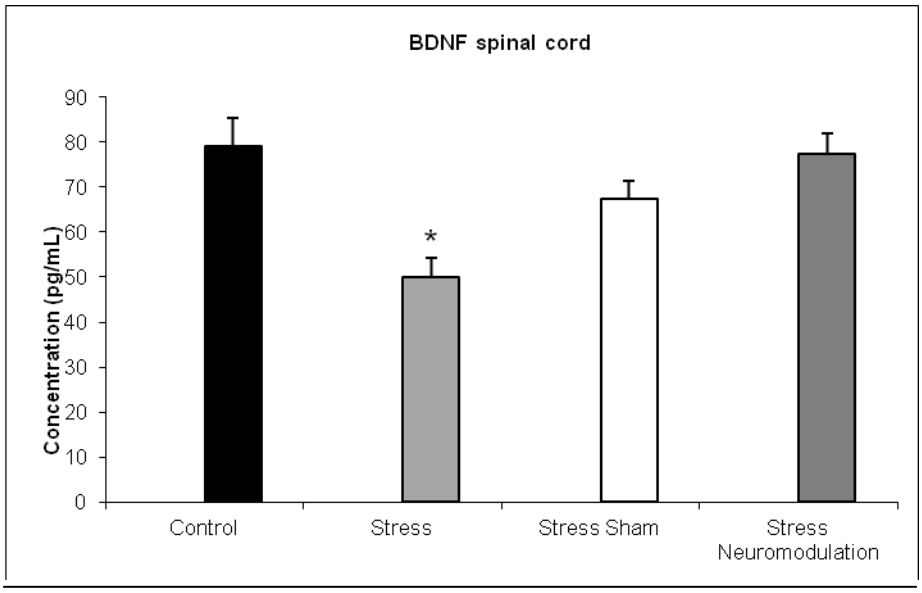


Figure 3.



VII. CONSIDERAÇÕES GERAIS

Os resultados obtidos com esta dissertação de mestrado permitem emitir as seguintes conclusões:

- ✓ O modelo de estresse crônico diário por restrição durante 11 semanas foi capaz de produzir hiperalgesia e alodinia nos animais submetidos, demosntrando ser um bom modelo para o estudo da dor crônica.
- ✓ O tratamento com ETCC anódica durante 8 dias por 20 minutos foi eficaz em reverter a hiperalgesia e a alodinia causadas pelo estresse crônico, demonstrando ter potencial na terapêutica de quadros que cursam com dor crônica;
- ✓ O modelo de estresse crônico por restrição e tratamento com ETCC não alteraram os níveis de interleucina 1β e corticosterona em soro;
- ✓ O tratamento com ETCC diminuiu os níveis de TNF α em hipocampo;
- ✓ A exposição ao estresse crônico por restrição induziu uma diminuição nos níveis de BDNF em tronco cerebral e medula espinhal;
- ✓ A exposição à ETCC foi efetiva em reverter a diminuição dos níveis de BDNF em medula induzida pelo estresse crônico, porém este efeito não foi observado em tronco cerebral;
- ✓ Nossos resultados sugerem possíveis mecanismos de ação da tDCS anódica para o tratamento de patologias dolorosas, como diminuição dos níveis de TNF α em hipocampo e re-estabelecimento dos níveis de BDNF em medula espinhal.
- ✓ Concomitante a isso, nossos resultados corroboram a literatura e sugerem que a estimulação ativa vias descendentes inibitórias a suprimir a dor por meio de um mecanismo de ação central.

VIII. DIVULGAÇÕES

DIVULGAÇÕES

2011:

a) Salão de Iniciação Científica UFCSPA 2011, Porto Alegre-RS.

Título: Efeito do Estresse crônico por restrição na resposta nociceptiva de ratos: von Frey e Placa quente.

Apresentadora: Yasmine Nonose

b) Simpósio Internacional de Neuromodulação, São Paulo-SP.

Título: Impact of transcranial direct current stimulation in animal model of hyperalgesia induced by chronic stress

Apresentadora: Lauren Naomi S. adachi

2012 (trabalhos já submetidos que serão apresentados):

a) Salão de Iniciação Científica UFRGS 2012, Porto Alegre-RS.

Título: Modelo de eletroestimulação trancraniana por corrente contínua em ratos Wistar.
Apresentador: Ivan Gluz

b) Fesbe – Federação de Sociedades de Biologia Experimental, Águas de Lindóia – SP.

Título: Avaliação dos níveis de corticoesterona e interleucina 1β séricos e níveis de fator de necrose tumoral-alfa (TNF-α) hipocampal em ratos hiperalgésicos tratados com eletroestimulação trancraniana por corrente contínua (ETCC).
Apresentador: Fernanda Ribeiro

c) Fesbe – Federação de Sociedades de Biologia Experimental, Águas de Lindóia – SP.

Título: Eletroestimulação trancraniana por corrente contínua (ETCC) reverte hiperalgesia e diminuição de níveis de BDNF em medula espinhal induzidos por estresse crônico.
Apresentador: Profa. Dra. Iraci Torres
d) Semana Científica do HCPA, Porto Alegre – RS.
Título: Impacto da eletroestimulação transcraniana (etcc) em modelo animal de alodinia nduzida pelo estresse crônico
Apresentador: Paulo Marques Filho
e) Semana Científica do HCPA, Porto Alegre – RS.
Título: Estimulador transcraniano por corrente contínua reverte hiperalgesia induzida pelo estresse crônico em animais
Apresentador: Andressa de Souza

VII. ANEXOS

A) Aprovação do Comitê de Ética

B) Outros Artigos Científicos realizados em co-autoria durante o período de mestrado

1. Rozisky JR, da Silva RS, Adachi LS, Capiotti KM, Ramos DB, Bogo MR, Bonan CD, Sarkis JJ, Torres IL. Neonatal morphine exposure alters E-NTPDase activity and gene expression pattern in spinal cord and cerebral cortex of rats. European Journal of Pharmacology 2010;(642):72–76.

2. Rozisky JR, Medeiros LF, Adachi LS, Espinosa J, de Souza A, Neto AS, Bonan CD, Caumo W, Torres IL. Morphine exposure in early life increases nociceptive behavior in a rat formalin tonic pain model in adult life.Brain Research 2011; 1367:122 – 129.

3. Laste G, Caumo W, Adachi LN, Rozisky JR, de Macedo IC, Filho PR, Partata WA, Fregni F, Torres IL.After-effects of consecutive sessions of transcranial direct current stimulation (tDCS) in a rat model of chronic inflammation. Exp Brain Res. 2012;221(1):75-83

4. JR Rozisky, L. S. Adachi, G. Laste, A. Souza, W. Caumo, I. L. S. Torres. Involvement of the dopamine D2 receptor in sensitization-like behaviours after morphine exposure in early life. Submetido.