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# SÍNDROME DOLOROSA MIOFASCIAL, FUNÇÃO DO SISTEMA DESCENDENTE DA DOR E EFICÁCIA DA ESTIMULAÇÃO ELÉTRICA INTRAMUSCULAR: ENSAIO CLÍNICO RANDOMIZADO DUPLO-CEGO *SHAM* CONTROLADO EXPLORATÓRIO

Porto Alegre

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“Aquele que não sabe, e pensa que sabe. Ele é tolo. Evite-o.
Aquele que sabe e não sabe o que sabe. Ele está adormecido. Desperte-o.
Aquele que não sabe e admite que não sabe. Ele é humilde. Guie-o.
Aquele que sabe e sabe o que sabe. Ele é sábio. Siga-o.”

Provérbio Árabe

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## RESUMO

A síndrome dolorosa miofascial (MPS) é uma das causas mais prevalentes de dor crônica na população, provocando altos graus de incapacidade, sem normalmente responder ao tratamento analgésico conservador. Embora sua fisiopatologia não esteja completamente esclarecida, vários processos ocorrem tanto no tecido periférico quanto no nível do sistema nervoso periferico e central, levando a um processamento de neuroplasticidade mal adaptativa nas redes neurais da neuromatriz da dor, o que resulta na amplificação de sua percepção e na alteração comportamental observada em seus portadores.

Evidências sugerem que existem três sistemas neurais primariamente envolvidos na dor da MPS: (i) o sistema corticoespinal; (ii) o sistema modulador descendente da dor: e (iii) o sistema regulador da neuroplasticidade. A estimulação elétrica intramuscular (IMES) é uma forma de estimulação neuromuscular periférica que promove aumento do fluxo sanguíneo regional, ativa centros relacionados ao processamento da dor e, de forma ascendente, também ativa as vias modulatórias da dor (efeito *bottom-up*).

Nesta tese buscamos avaliar duas perguntas que deram origem a dois artigos: (1) se a disrupção do sistema modulador descendente da dor, aferida pelo *CPM-task*, correlaciona-se com disfunção na condução corticoespinal e desinibição no nível cortical, acessados pela Estimulação Magnética Transcraniana (TMS), e nível sérico do BDNF; e (2) avaliar os efeitos neuromodulatórios de 10 sessões de IMES comparados a intervenção *sham* em 24 pacientes do sexo feminino com idade de 18 a 65 anos, com dor crônica de origem miofascial do complexo craniocervicomandibular. Nossa hipótese era de que: (i) a disrupção do sistema modulador descendente da dor correlacionava-se com disfunção na condução corticoespinal, desinibição no nível cortical, e nível sérico do BDNF; (ii) que os efeitos neuromodulatórios da IMES se manifestariam como melhora clínica nos escores de dor e funcionalidade; e (iii) seus efeitos terapêuticos envolvessem mecanismos neuroplásticos implicados na secreção de BDNF, alteração da excitabilidade cortical e corticoespinal e potencialização do sistema modulador descendente da dor. RESULTADOS: (1) A análise MANCOVA, após ajuste para comparações múltiplas por meio do Teste de Bonferroni, revelou que os pacientes com disrupção do sistema modulador descendente da dor apresentaram maior Facilitação Intracortical (ICF; média ± *DP*) 1,43 (0,3) vs. 1,11 (0,12), maior excitabilidade do sistema corticoespinal (MEP; μV) 1.93 (0,54) vs. 1,40 (0,27), e níveis séricos de BDNF mais elevados (pg/Ml) 32,56 (9,95) vs. 25,59 (10,24), ($p < 0,05$ para todos), e (2) a análise de variância num modelo de efeito misto demonstrou redução nos escores de dor de -73,02% (IC95% = -95,28 to -52,30), e diminuição da disfunção em -43,19% (IC95%, -57,23 a -29,39), diminuição da excitabilidade corticoespinal ($p=0,02$), potencialização do sistema modulador

descendente da dor ( p=0,01) e aumento dos níveis séricos de BDNF (p<0,01). Além disso, a magnitude do aumento do BDNF foi preditora dos efeitos nos níveis de dor e disfunção a longo prazo (Beta = 0,67; IC95% = 0,07 a 1,26). Esses achados sugerem que o enfraquecimento do sistema descendente inibitório da dor está associado ao aumento da ICF, MEP e níveis séricos do BDNF e o efeito bottom–up induzido pela IMES diminuiu a dor e reduziu a disfunção nessa amostra de pacientes. Esses efeitos podem ser mediados por melhora de mecanismos inibitórios corticoespinais. Outros achados sugerem que a magnitude do aumento do BDNF gerado pela IMES predizeram o impacto nos efeitos clínicos, ao longo de doze semanas, nessa população de pacientes.

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**Palavras-Chave**: Síndrome dolorosa; Estimulação elétrica intramuscular miofascial; Modulação condicionada da dor; Estimulação magnética transcraniana; BDNF.

## ABSTRACT

Myofascial pain syndrome (MPS) is one of the most prevalent causes of chronic pain in the population, causing high degrees of disability without normally responding to conservative analgesic treatment. Although its pathophysiology is not completely understood, several processes occur at both peripheral tisue and central and peripheral nervous system levels, leading to a maladaptive neuroplasticity processing in the pain neuromatrix networks, which results in the amplification of their perception and in the behavioral change observed in its carriers.

Evidence suggests that there are three neural systems primarily involved in MPS pain: (i) the corticospinal system; (ii) the descending pain modulating system; and (iii) the neuroplasticity regulating system. Intramuscular electrical stimulation (IMES) is a form of peripheral neuromuscular stimulation that promotes increased regional blood flow, activates centers related to pain processing, and upwardly activates pain modulatory pathways (botton-up effect).

In this thesis we aimed to evaluate two questions that gave rise to two articles: (1) if the disruption of the descending pain modulator system, measured by the CPM-task, correlates with corticospinal conduction dysfunction and cortical disinhibition, accessed by Magnetic Stimulation. Transcranial (TMS), and BDNF serum level; and (2) to evaluate the neuromodulatory effects of 10 IMES sessions compared to sham intervention in 24 female patients aged 18 to 65 years with chronic myofascial pain of the craniocervicomandibular complex. Our hypothesis was that: (i) disruption of the descending pain modulatory system was correlated with dysfunction in corticospinal conduction and disinhibition at cortical level and serum BDNF level; (ii) that the neuromodulatory effects of IMES would manifest as clinical improvement in pain scores and functionality; and (iii) its therapeutic effects involved neuroplastic mechanisms involving BDNF secretion, alteration of cortical and corticospinal excitability, and potentiation of the descending pain modulating system. RESULTS: (1) The MANCOVA analysis, after adjustment for multiple comparisons by the Bonferroni Test, revealed that patients with disruption of the descending pain modulatory system had higher Intracortical Facilitation (ICF; mean ± SD) 1.43 (0.3) vs. 1.11 (0.12), greater corticospinal system excitability (MEP; μV) 1.93 (0.54) vs. 1.40 (0.27), and higher serum BDNF levels (pg / Ml) 32.56 (9.95) vs. 25.59 (10.24), ($P < 0.05$ for all), and (2) analysis of variance in a mixed-effect model showed a reduction in pain scores of -73.02% (95% CI = -95.28 to -52.30), and decreased dysfunction by -43,19% (95% CI, -57.23 to -29.39), decreased corticospinal excitability ($p = 0.02$), potentiation of the descending pain modulating system ($p = 0.01$) and increased levels BDNF serum levels ($p < 0.01$). In addition, the magnitude of the increase in BDNF leves was predictive of long-term effects on pain and dysfunction levels (Beta =

0.67; 95% CI = 0.07 to 1.26). These findings suggest that the weakening of the descending pain inhibitory system is associated with increased ICF, MEP, and BDNF serum levels, and the IMES-induced bottom-up effect improved pain and reduced dysfunction in this patient population. These effects may be mediated by improvement of corticospinal inhibitory mechanisms. Other findings suggest that the magnitude of the increase in IMES-generated BDNF predicted the long-term impact on clinical effects in this patient population..

Keywords: BDNF; Intramuscular electrical stimulation; Transcranial magnetic stimulation; Conditional modulation of pain; Myofascial pain syndrome.

## LISTA DE ILUSTRAÇÕES

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## Tabelas do artigo 1



## Tabelas do artigo 2

## LISTA DE ABREVIATURAS

| Sigla | Inglês | Português |
|---|---|---|
| **ACC** | *Anterior Cingulate Cortex* | Córtex do cíngulo anterior |
| **AMPc** | *Cyclic Adenosine Monophosphate* | Monofosfato cíclico de adenosina |
| **AMTP** | *Active Myofascial Trigger Point* | Ponto-gatilho miofascial ativo |
| **ASIC** | *Acid-Sensing Ion Chanel* | Canal iônico sensível a ácidos |
| **BDNF** | *Brain Derived Neurotriphic Factor* | Fator neurotrófico derivado do cérebro |
| **CGRP** | *Calcitonin Gene-Related Peptide* | Peptídio geneticamente relacionado à calcitonina |
| **CNTD** | *Chronic Non-Transmissible Diseases* | Doenças crônicas não transmissíveis |
| **CPM** | *Conditioned Pain Modulation* | Modulação condicionada da dor |
| **CPM-task** | *Conditioned Pain Modulation Task* | Teste da modulação condicionada da dor |
| **CS** | *Central Sensitization* | Sensibilização central |
| **CSP** | *Cortical Silent Period* | Período silente cortical |
| **DALY** | *Disability-Adjusted Life Years* | Anos de vida perdidos ajustados por incapacidade |
| **DLPFC** | *Dorso-Lateral Pre-Frontal Córtex* | Córtex pré-frontal dorsolateral |
| **DNIC** | *Diffuse Noxious Inhibitory Control* | Controle inibitório nóxico difuso |
| **EA** | *Electrical acupuncture* | Eletroacupuntura |
| **EPM** | *End-Plate Noise* | Ruído de placa motora |
| **EPS** | *End-Plate Spike* | Espícula de placa motora |
| **ES** | *Effect Size* | Tamanho de efeito |
| **HCPA** | | Hospital de Clínicas de Porto Alegre |
| **HPT** | *Heat Pain Treshold* | Limiar de dor ao calor |
| **IASP** | *International Association for the Study of Pain* | Associação Internacional para o Estudo da Dor |
| **IC** | *Insular Cortex* | Córtex insular |
| **ICF** | *Intra-Cortical Facilitation* | Facilitação intracortical |
| **IL** | *Interleukin* | Interleucina |
| **IMES** | *Intramuscular Electric Stimulation* | Estimulação elétrica intramuscular |
| **IMS** | *Intramuscular Stimulation* | Estimulação intramuscular |
| **LMTP** | *Latent Myofascial Trigger Point* | Ponto-gatilho miofascial latente |
| **LTD** | *Long Term Depression* | Depressão de longo termo |
| **LTP** | *Long Term Potenciation* | Potenciação de longo termo |

| | | |
|---|---|---|
| **M1** | *Primary Motor Cortex* | Córtex motor primário |
| **MEP** | *Motor Evoked Potential* | Potencial evocado motor |
| **mGlut** | *Metabotropic Glutamate Receptor* | Receptor metabotrópico do glutamato |
| **MPS** | *Myofascial Pain Syndrome* | Síndrome dolorosa miofascial |
| **MT** | *Motor Threshold* | Limiar motor |
| **Sigla** | **Inglês** | **Português** |
| **MTP** | *Myofascial Trigger Point* | Ponto-gatilho miofascial |
| **NK1** | *Neurokinin 1 (P Substance Receptor)* | Neurocinina 1 (receptor da substância P) |
| **NMDA** | *N-Methil-D-Aspartate Receptor* | Receptor N-metil-D-aspartato |
| **NPS** | *Numerical Pain Scale* | Escala numérica de dor |
| **P2X3** | *P2x Purinoceptor 3* | Receptor purinérgico p2x3 |
| **PAG** | *Peri-Aqueductal Grey* | Substância cinzenta periaquedutal |
| **PFC** | *Pre-Frontal Cortex* | Córtex pré-frontal |
| **PKA** | *Protein Kinase A* | Proteína cinase A |
| **PLA2** | *Phospholipase A2* | Fosfolipase A2 |
| **PNS** | *Peripheral Nerve Stimulation* | Estimulação de nervo periférico |
| **QST** | *Quantitative Sensory Test* | Teste sensorial quantitativo |
| **RNAm** | *Messenger Ribonucleic Acid* | Ácido ribonucleico mensageiro |
| **rTMS** | *Repetitive Transcranial Magnetic Stimulation* | Estimulação magnética transcraniana repetitiva |
| **S1** | *Primary Somatosensorial Córtex* | Córtex somatosensorial primário |
| **S100B** | *S100 Calcium-Binding Protein B* | Proteína ligante de cálcio s100B |
| **S2** | *Secondary Somatosensorial Córtex* | Córtex somatossensorial secundário |
| **SICI** | *Short Intra-Cortical Inhibition* | Inibição intracortical curta |
| **SMA** | *Supplementary Motor Area* | Área motora suplementar |
| **SMTP** | *Satellite Myofascial Trigger Point* | Ponto-gatilho miofascial satélite |
| **TMS** | *Transcranial Magnetic Stimulation* | Estimulação magnética transcraniana |
| **TNFα** | *Tumor Necrosis Factor A* | Fator de necrose tumoral α |
| **TRPV1** | *Transient Receptor Potential Cation Channel Subfamily V Member 1* | Receptor de potencial transitório vanilóide do tipo 1 |
| **VAS** | *Visual Analogue Scale* | Escala análogo-visual |
| **VAS-QS** | *Visual Analogue Scale - Sleep Quallity* | Escala análogo-visual - qualidade de sono |
| **WDR** | *Wide Dinamic Range* | Ampla faixa dinâmica |
| **WHOQOL** | *World Health Organization Quality of Life Questionaire* | Questionário de qualidade de vida da organização mundial da saúde |
| **YLD** | *Years Lived With Disability* | Anos vividos com incapacidade |

## TERMOS E DEFINIÇÕES

Abaixo estão descritos alguns termos usados com frequência nesta tese.

- ***Conditioned pain modulation*:** teste dinâmico que mede funcionalidade da modulação inibitória descendente da dor, avaliada através do paradigma "dor inibe dor" .

- **Estímulo supralimiar:** intensidade superior ao limiar capaz de despolarizar maior número de fibras nervosas do que o estímulo limiar e de elicitar respostas mais intensas.

- **Hiperalgesia:** aumento da percepção dolorosa provocada por um estímulo doloroso.

- **Hiperalgesia primária:** aumento da percepção dolorosa no local da lesão.

- **Hiperalgesia secundária:** aumento da percepção dolorosa em área adjacente ou remota do sítio da lesão.

- **Limiar de calor:** mínima energia necessária para atingir a sensação térmica.

- **Limiar de Dor:** mínima energia necessária para atingir a percepção da dor.

- **Método psicofísico:** mensuração da dor baseada na experiência subjetiva, medida em escalas ou limiares e dependente da cooperação dos sujeitos.

- **Neuromatriz:** múltiplas áreas cerebrais relacionadas com as respostas afetivas, cognitivas e avaliativas da dor.

- **Nociceptores:** subpopulação de neurônios localizados na pele, músculos, vísceras, articulações e vasos, sensíveis a estímulos agressores térmicos, químicos e/ou mecânicos. Em tecidos normais, os nociceptores são inativos até que sejam estimulados por energia suficiente para suplantar seu potencial de repouso.

- **Pensamento catastrófico:** pensamento catastrófico sobre dor é definido como uma persistente e negativa resposta cognitiva e emocional à "dor atual e à dor futura".

- **Plasticidade sináptica:** intrínseca propriedade que permite que o sistema nervoso central se ajuste as restrições do seu próprio genoma, para se adaptar a pressões ambientais, a mudanças fisiológicas e a diferentes experiências.

- **Potenciação sináptica de longa duração (*Long-term potentiation* - LTP):** aumento da eficácia sináptica que supera a duração do estímulo condicionado durante pelo menos 30 minutos (LTP precoce), algumas horas, dias ou meses (LTP tardia).

- **Teste de quantificação sensitiva (*Quantitative Sensory Testing* - QST):** método constituído por uma série de testes psicofísicos aplicados em diferentes tecidos para acessar a função sensitiva e as vias nociceptivas.

**SUMÁRIO**

# 1 INTRODUÇÃO

As doenças crônicas não transmissíveis (CNTD) compõem um grupo de patologias com longo período de latência, progressão lenta, etiologia não elucidada totalmente, lesões irreversíveis e complicações que acarretam graus variáveis de incapacidade ou óbito (Turrubiarte-Guillén et al. 2006; Orsatti et al. 2008). São responsáveis por mais de 58% de todas as mortes ocorridas no mundo e por mais de 45% da carga global de doença (World Health Organization 2003). As CNTDs passaram a ter mais destaque após o processo denominado transição demográfica, fenômeno mundial que indica a passagem de altas taxas de fecundidade e mortalidade para baixas taxas. Moura *et al.* (de Moura, de Carvalho, and da Silva n.d.) destacam que este fenômeno ocorreu no Brasil de forma sem precedentes, acarretando o envelhecimento populacional e o aumento da longevidade da população. Entre as décadas de 30 e 90, a proporção de mortes por CNTDs aumentou em mais de três vezes no Brasil, segundo dados do Ministério da Saúde. Schramm *et al.* (Schramm et al. 2004) apontam que as doenças crônicas foram responsáveis por 66% da carga de doenças no Brasil, no ano de 1998, usando o DALY (*disability-adjusted life years* – anos de vida perdidos ajustados por incapacidade).

Dentre as doenças que compõem as CNTDs a dor crônica tem alto impacto social, imputando um fardo ao indivíduo, ao sistema de saúde e à economia. Ela reduz a qualidade de vida, a capacidade de trabalho e a funcionalidade do paciente. Seus custos socioeconômicos diretos e indiretos superam o de doenças cardíacas, câncer e diabete melito somados, podendo superar 600 bilhões de dólares por ano (Breivik, Eisenberg, and O'Brien 2013). Estimativas de sua prevalência apontam para 37,3% da população em países desenvolvidos e para 41,1% em países em desenvolvimento (Tsang et al. 2008).

A dor crônica musculoesquelética é o tipo de dor crônica mais prevalente, estando entre as patologias com maior índice de anos vividos com incapacidade (YLD - *years lived with disability*) (Vos et al. 2012). Dentre as condições mais predominantes de dor musculoesquelética emerge a síndrome dolorosa miofascial (MPS) (Yap 2007), caracterizada por um conjunto de sinais e sintomas sensitivos, motores e autonômicos concorrentes com a presença de pontos-gatilho miofasciais (MTP). O MTP é um nódulo bem delimitado e altamente irritável localizado numa banda de fibras musculares tensas e sua compressão provoca dor local e/ou a distância. Embora a etiologia do MTP não esteja completamente entendida, sua perpetuação é acompanhada de alterações neuroplásticas mal-adaptativas que promovem um desbalanço entre os sistemas excitatórios e inibitórios promovendo uma forma de memorização implícita e de aprendizado da dor levando à intensificação da percepção dolorosa, ao aumento da área receptiva e à alodínia, quadro denominado de sensibilização central (CS). Este quadro tipicamente

apresenta aumento da excitabilidade cortical, caracterizado pelo aumento da atividade glutamatérgica e/ou diminuição da atividade gabaérgica (Vidor et al. 2014). O sistema modulador descendente também pode estar amplamente afetado, perdendo sua resposta inibitória e, por vezes, adquirindo atividade facilitatória (Caumo et al. 2016). O nível sérico do fator neurotrófico derivado do cérebro (BDNF - *Brain Derived Neurotrophic Factor*), um modulador neuronal regulador da potenciação de longa duração (LTP - *Long Term Potentiation*) encontra-se aumentado (Deitos et al. 2015). Esta neurotrofina tem se apresentado como um marcador de neuroplasticidade útil para ser usado para monitorizar a resposta terapêutica (Dall'Agnol et al. 2014).

A MPS crônica é multifacetada, com componentes periféricos e centrais, manifestos por dor segmentar, concorrente com sinais de distrofia segmentar, perda dos mecanismos de inibição avaliados por meio do sistema modulatório descentende da dor (CPM) e alteração de parâmetros de excitabilidade cortical, avaliados através da estimulação magnética transcraniana (TMS) (Dall'Agnol et al. 2014). Tem sido demonstrado que a perda da função dos sistemas de inibição está associada a elevados níveis de ansiedade traço, incapacidade e catastrofização (Vidor et al. 2014; Volz et al. 2013). Essas evidências sugerem que existe três sistemas neurais primariamente envolvidos na MPS: (i) o sistema cortico espinal; (ii) o sistema modulador descendente da dor: e (iii) o sistema regulador da neuroplasticidade. Portanto, no manejo dessa patologia, é necessário modular os múltiplos aspectos envolvidos no processo patológico, incluindo o estímulo nociceptivo e os processos de neuroplasticidade mal-adaptativa.

Nesse contexto, a estimulação eletrica intramuscular (IMES) e outras técnicas de neuromodulação periférica, como por exemplo o TENS e estimulação neural, interferem nesse sistema de forma aferente (*bottom-up*) promovendo alterações da plasticidade sináptica. Seu efeito é mediado por fenômenos de potencialização de longa duração (LTP) e depressão de longa duração (LTD - *Long Term Depression*) (C. et al. 2014).

Considerando os múltiplos mecanismos envolvidos no processo fisiopatológico e terapêutico, esta tese objetivou responder a duas questões principais:

- Compreender a relação entre a função do sistema modulador descendente de dor e a função do sistema corticoespinhal em pacientes com MPS com e sem o efeito de somação ao estímulo condicionado da dor.

- Comparar o impacto analgésico da IMES com estímulo *sham* na dor, disfunção, e nos parâmetros de excitabilidade cortical e do sistema modulador descendente da dor de pacientes com MPS.

Nossa hipótese era de que a disrupção do sistema modulador descendente da dor, aferida pelo CPM-task, estava correlacionada com disfunção na condução corticoespinal e desinibição no nível

cortical, acessados pela TMS, e nível sérico do BDNF. E que os efeitos neuromodulatórios da IMES se manifestariam como melhora clínica nos escores de dor, funcionalidade, além de que seu efeito terapêutico envolvesse mecanismos neuroplásticos implicando no aumento de BDNF sérico, alteração da excitabilidade cortical e corticoespinal, além de potencialização do sistema modulador descendente da dor.

Originou dois artigos: (1- página 57) ***A Framework for Understanding the Relationship between Descending Pain Modulation, Motor Corticospinal and Neuroplasticity Regulation Systems in Chronic Myofascial Pain*** publicado no jornal Frontier in Human Neuroscience, 2016 Jun 27;10:308 respondendo a primeira questão; e (2- página 95) ***Insights About the Neuroplasticity State on the Effect of Intramuscular Electrical Stimulation in Pain and Disability Associated With Chronic Myofascial Pain Syndrome (MPS): A Double-Blind, Randomized, Sham-Controlled Trial*** também publicado no jornal Frontier in Human Neuroscience, 2018 Oct 16;12:388 respondendo a segunda questão.

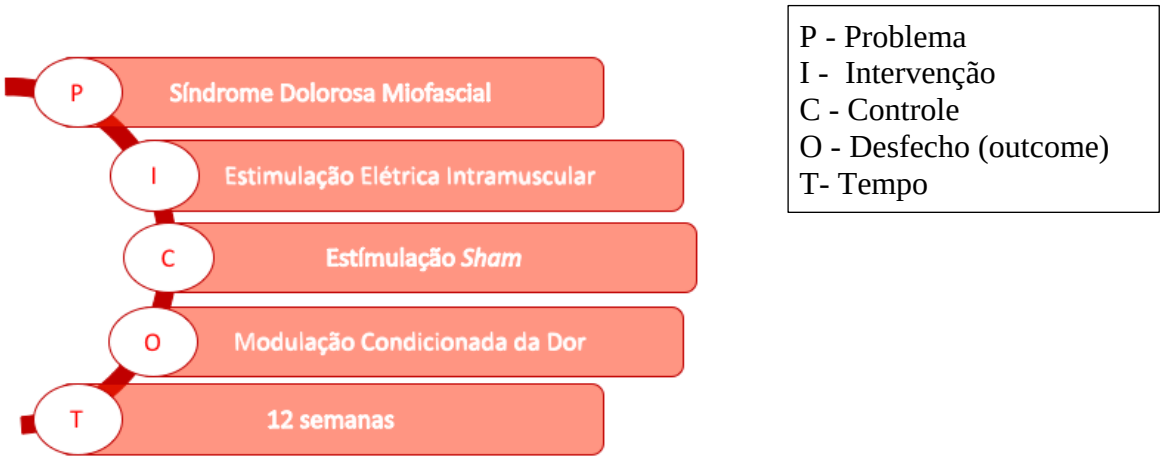
A estrutura de apresentação desta tese segue as normas do Programa de Pós-Graduação em Medicina: Ciências Médicas (PPGCM) da Universidade Federal do Rio Grande do Sul.

# 2 REVISÃO DA LITERATURA

## 2.1 ESTRATÉGIA PARA LOCALIZAR E SELECIONAR AS INFORMAÇÕES

Para formular a pergunta da pesquisa e desenvolver uma estratégia de busca efetiva foi utilizada a estratégia PICOT(Rios, Ye, and Thabane 2010), conforme a figura 1:

**Figura 1:** Estratégia PICOT




Inicialmente foram procurados os termos *MeSH* para síndrome dolorosa miofascial (*myofascial pain syndrome; Trigger point pain*), estimulação elétrica intramuscular (*intramuscular electric stimulation* □ IMES) e modulação condicionada da dor (*conditioned pain modulation* - CPM). A estratégia de busca envolveu as seguintes bases de dados: PubMed, EMBASE LILACS, e SCIELO. As referências bibliográficas dos artigos identificados foram revisadas para localizar outras não contempladas na busca. Também foram utilizados livros-texto.

Em relação ao termo MPS foram encontrados: 17388 no Pubmed dos quais 78 foram usados; 9.913 artigos no EMBASE dos quais 65 foram usados; 1005 no Lilacs dos quais 15 foram incluidos; e 49 artigos no Scielo, dos quais 7 foram incluidos.

Em relação ao termo IMES foram encontrados: 1208 artigos no PubMed dos quais 12 foram incluidos; 892 artigos no EMBASE dos quais 17 foram incluidos; 6 artigos no Lilacs, dos quais 2 foram incluidos; 3 artigos encontrados no Scielo dos quais apenas 1 foi incluído.

Em relação ao termo CPM, foram encontrados: 604 artigos no PudMed dos quais 5 foram incluidos; 592 artigos no EMBASE, dos quais 8 foram incluidos; 2 artigos no Lilacs, nenhum deles incluido; nenhum artigo no Scielo.

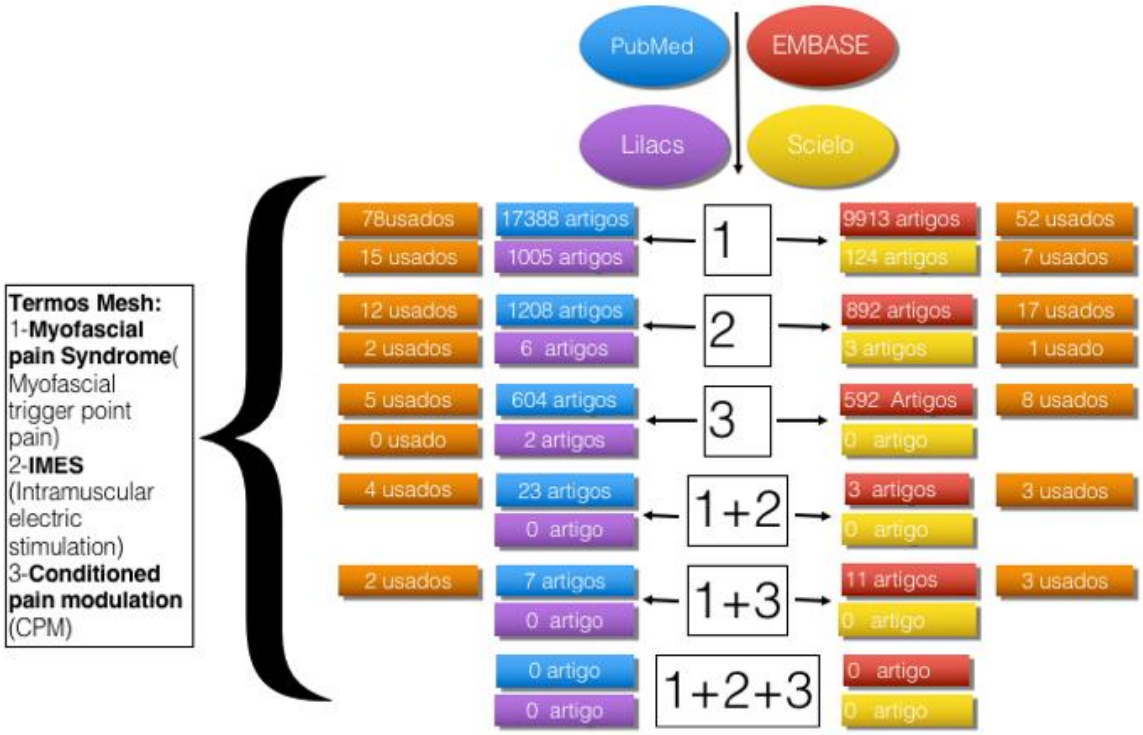
Quando os termos MPS e IMES foram somados, foram encontrados: 23 artigos no PubMed dos quais 4 foram incluidos; 3 no EMBASE, todos usados; nenhum artigo no Lilacs e no Scielo.

Quando os termos MPS e CPM foram somados, foram encontrados: 7 artigos no PubMed dos quais 2 foram incluidos; 11 artigos no EMBASE dos quais 3 foram incluidos; nenhum artigo no Lilacs e no Scielo.

Nenhum artigo foi encontrado quando somados os termos MPS, IMES e CPM.

A figura 2 apresenta a estratégia de busca de referências bibliográficas sobre as bases que fundamentam este estudo.

**Figura 2:** Estratégia de busca de referências bibliográficas



## 2.2 SÍNDROME MIOFASCIAL

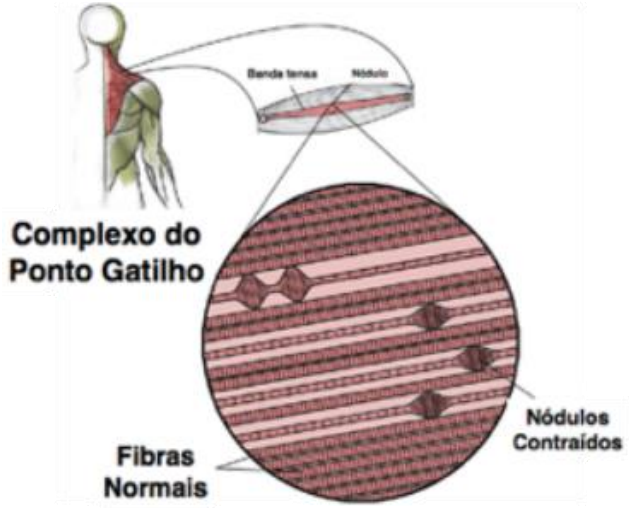
### 2.4.1 Definição e apresentação clínica

A MPS caracteriza-se por um conjunto de sinais e sintomas sensitivos, motores e autonômicos vinculados à existência de MTPs. O principal sintoma sensitivo é dor, normalmente de caráter regional, pouco localizada e profunda. Associado à dor, são observados espasmo, fraqueza, baixa tolerância ao efeito da carga laboral e perda de coordenação do segmento acometido. Adicionam-se a isso trofoedema, disestesia, parestesia, sinais distróficos e diminuição da amplitude de movimento.

MTP é classicamente definido como um nódulo bem delimitado, altamente irritável e sensível localizado numa banda de fibras musculares tensas, sentida como um cordão tenso no ventre muscular (Figura 3). Sua compressão provoca dor local e/ou a distância (zonas de dor referida), além de se observar uma contração involuntária da banda tensa na qual se localiza (*Twitch Response* - TR) (Gerwin, Dommerholt, and Shah 2004).

Clinicamente, os MTPs são divididos em ativos (AMTP) e latentes (LMTP) (Mense 2008a). Estes últimos apresentam todas as características clínicas dos primeiros, porém em menor intensidade. A única exceção é a reprodução da dor espontânea do paciente quando esses pontos são comprimidos (Gerwin, Dommerholt, and Shah 2004; Li et al. 2009).

**Figura 3:** Complexo do ponto-gatilho




Fonte: Adaptada de Shah(Shah et al. 2008a) .

A tabela 1 demonstra os critérios diagnósticos da MPS sugeridos pela Associação Internacional para o Estudo da Dor (IASP), em 2010, em virtude do Ano Global de Combate à Dor Musculoesquelética (International Association for the Study of Pain 2017). Outros transtornos associados à MPS são distúrbios do sono, humor, incapacidade física e redução da qualidade de vida (Gerber et al. 2013).

**Tabela 1.** Critérios diagnósticos da síndrome miofascial - IASP

| Critérios diagnósticos mínimos | Critérios de confirmação |
|---|---|
| Presença de banda tensa palpável em músculo esquelético | Evocação de reação contrátil visualmente ou à palpação da banda tensa |
| Presença de área de hipersensibilidade dentro da uma banda tensa muscular | Presença de "sinal do pulo", ou seja, reação de retirada à palpação dos nódulos |
| Reprodução da sensação de dor referida com estimulação do nódulo doloroso | Reconhecimento da dor sentida à palpação muscular |
| | Previsão de padrões de dor referida |
| | Fraqueza muscular e músculo tenso |
| | Dor com alongamento ou contração do músculo afetado |

O desenvolvimento e a ativação de um LMTP estão relacionados a alguma forma de sobrecarga do músculo, podendo ser um trauma agudo, uma contração sustentada ou movimentos repetitivos (David G. Simons et al. 1999; Lavelle, Lavelle, and Smith 2007). Ao promover dor a distância de sua origem, na área de referência, com frequência surgem novos MTPs, chamados de pontos-gatilho miofasciais satélites (SMTP) (David G. Simons et al. 1999; Mense 2008b).

### 2.4.2 **Epidemiologia**

Embora seja reconhecido que a MPS é uma causa prevalente de dor e incapacidade (David G. Simons et al. 1999), são escassos os estudos epidemiológicos, em parte devido ao incompleto conhecimento de sua fisiopatologia e à grande variabilidade de critérios diagnósticos ao longo do tempo. Estas razoes podem explicar por que sua real prevalência ainda é imprecisa.

Uma revisão sistemática recente demonstrou 19 diferentes critérios diagnósticos, porém sem nenhum padrão específico consistente de escolha individual ou em combinação para o diagnóstico da MPS (Tough et al. n.d.). Tentativas de padronização desses critérios têm sido feitas com o intuito de reduzir a variabilidade de critérios diagnósticos, fator que introduz potencial viés de seleção em estudos clínicos e na prática clínica (International Association for the Study of Pain 2017; Rivers et al. 2015). Fatores adicionais que contribuem para a imprecisão da prevalência são sua frequente associação com outras síndromes dolorosas musculoesqueléticas regionais, como lombalgia (Iglesias-González et al. 2013), cervicalgia (Muñoz-Muñoz et al. 2012), ombralgia (Bron et al. 2011), lesão em chicote (Freeman, Nystrom, and Centeno 2009), gonartrose (Alburquerque-García et al. 2015) ou com síndrome neuropáticas, como radiculopatia lombar (Adelmanesh et al. 2015) e cervical (Sari, Akarirmak, and Uludag 2012) neuralgia do trigêmeo (Ichida et al. 2015), cefaleia tensional (Couppé et al. 2007), migrânea (Giamberardino et al. 2007) e fibromialgia (Bennett and Goldenberg 2011; Alonso-Blanco et al. 2011; Ge 2010), dentre outras. Tal associação tem sido atribuída a um epifenômeno do desenvolvimento da CS, comum no processo de cronificação dessas patologias.

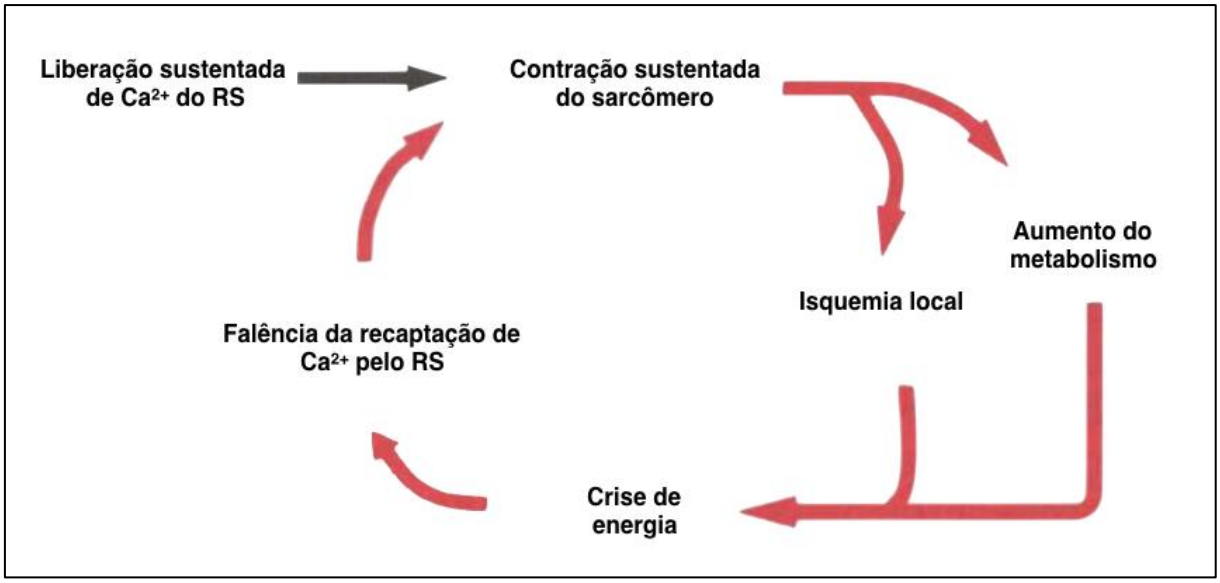
### 2.4.3 **Fisiopatologia da síndrome dolorosa miofascial**

A MPS ainda não tem sua fisiopatologia completamente elucidada. Dentre as teorias vigentes, a da crise energética e a integrativa de Simons são as mais aceitas. Adicionalmente, estudos recentes têm demonstrado que alterações neuroplásticas mal-adaptativas corroboram para o surgimento e manutenção da MPS.

### 2.4.4 **Teoria da crise energética**

Esta teoria postula que o gatilho da MPS seria consequência de uma crise de energia no MTP provocada pelo aumento da concentração do íon $Ca^{2+}$ citoplasmático, possivelmente em virtude da ruptura do retículo sarcoplasmático (RS) ou do sarcolema. Esse aumento do $Ca^{2+}$ intracelular incrementa a força contrátil entre actina e miosina, processo que concorre com aumento do efeito da acetilcolina na junção neuromuscular. Estes fatores levam ao aumento da demanda metabólica do músculo e à diminuição de aporte de oxigênio e nutrientes devido ao fechamento da rede capilar local. A diminuição de ATP promove redução da capacidade de recaptação do $Ca^{2+}$ citoplasmático para o interior do RS ou para o meio extracelular, um efeito mediado pela bomba de $Ca^{2+}$ ATP-dependente. A isquemia provoca o aumento local de substâncias inflamatórias que sensibilizam os nociceptores (Figura 4) (Gerwin, Dommerholt, and Shah 2004; David G. Simons et al. 1999).

**Figura 4:** Teoria da crise energética




Fonte: Adaptada de Simons (David G. Simons et al. 1999).

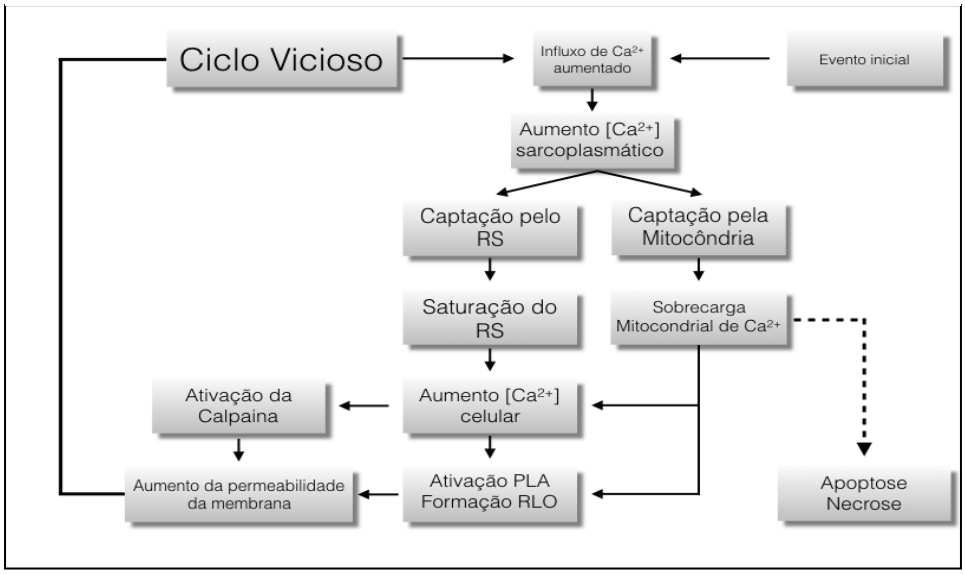
### 2.4.5 **Teoria integrativa**

Esta teoria postula que a disfunção primária se localiza na placa motora, processo que se caracterizaria pelo aumento da produção e liberação de acetilcolina na junção neuromuscular. A presença da aceticolina despolariza a membrana pós-juncional de modo sustentado, efeito que leva a liberação de $Ca^{2+}$ do RS resultando na contratura muscular e perpetuação da disfunção pelo aumento do metabolismo, pela diminuição do aporte energético, pela compressão da rede capilar e pela sensibilização de nociceptores. Embora sem especificar como, Simons especula que o sistema nervoso simpático também participa desse processo por modular a liberação de acetilcolina na placa motora (Gerwin, Dommerholt, and Shah 2004; David G. Simons et al. 1999).

### 2.4.6 **Lesão muscular desencadeada por $Ca^{2+}$**

É bem conhecido que o exercício físico pode produzir dando muscular com as contrações, musculares excêntrica e concêntrica máxima sustentada (Paschalis et al. 2007). O mecanismo envolvido nesse processo é dependente do $Ca^{2+}$ intracelular, de forma que o potencial de ação promove o influxo de $Ca^{2+}$ aumentando sua concentração citoplasmática. A magnitude desse processo depende da frequência da estimulação, da concentração de $Ca^{2+}$ extracelular, do tipo de fibra e da composição dos canais de sódio e $Ca^{2+}$ na membrana. O RS tende a armazenar o excesso de $Ca^{2+}$ normalizando sua concentração citoplasmática. No entanto, essa capacidade é limitada, principalmente nas fibras musculares do tipo 1. O esgotamento dessa capacidade segue aumentando o $Ca^{2+}$ intracelular, o qual ativa proteases e a fosfolipase A2 (PLA2). A principal protease ativada é a calpaína, que cliva proteínas do citoesqueleto, das miofibrilas e do sarcolema. Isso promove aumento da permeabilidade ao $Ca^{2+}$. A PLA2 degrada fosfolipídios do sarcolema e da mitocôndria. O acúmulo de $Ca^{2+}$ na mitocôndria leva ao aumento da produção de radicais livres, evento que induz a abertura de poros na membrana mitocondrial os quais desregulam o potencial desta membrana. Este processo reduz a produção de ATP, um efeito que culmina com apoptose ou necrose da célula (Figura 5) (Gissel and Clausen 2001; GISSEL 2005).

**Figura 5:** Ciclo da lesão muscular desencadeada por cálcio

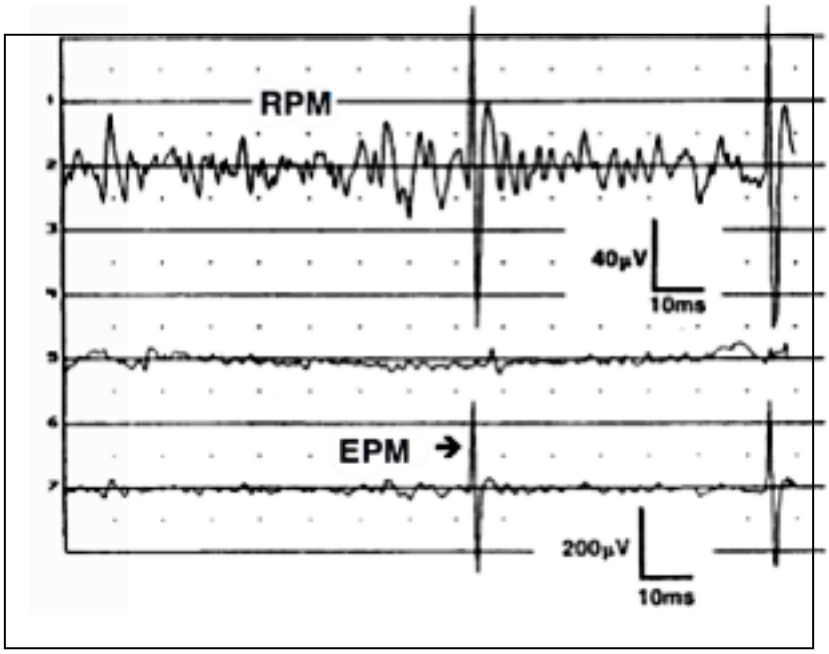



Fonte: Adaptada de Gissel (GISSEL 2005).

### 2.4.7 **Disfunção da placa motora**

Estudos eletrofisiológicos demonstraram ocorrer atividade elétrica espontânea em repouso no MTP. Tal atividade corresponde ao ruído de placa motora (EPN) e à espícula de placa motora (EPS). Inicialmente os eletrofisiologistas pensaram se tratar da atividade elétrica normal de placa motora. Posteriormente verificou-se que os EPNs com ou sem EPS são mais frequentes em MTP do que em regiões de placa motora (D G Simons 2001; David G Simons, Hong, and Simons 2002). Também, a prevalência de EPN correlaciona-se com maior intensidade da dor e menor limiar de dor do MTP (Figura 6) (Kuan et al. 2007).

**Figura 6:**Ruído e espícula de placa motora




Fonte: Adaptado de Simons (D G Simons 2001).

### 2.4.8 **Peptídeo geneticamente relacionado a calcitonina (CGRP)**

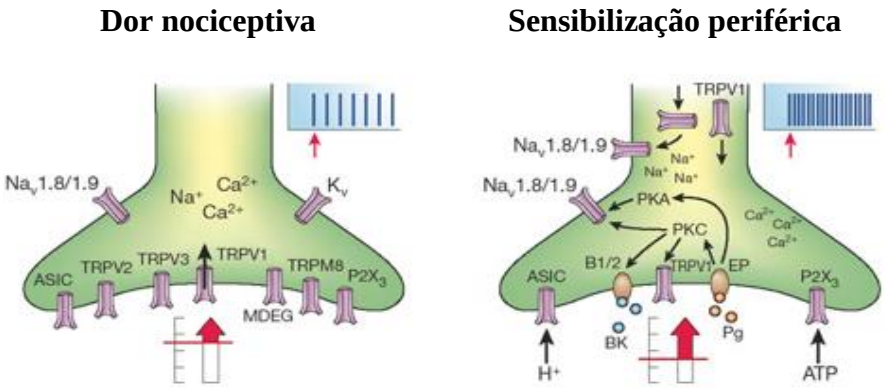
O CGRP, um neuropeptídio formado por 37 aminoácidos, encontra-se amplamente distribuído no sistema nervoso central e periférico e apresenta duas isoformas (αCGRP e βCGRP). O αCGRP é co-secretado em pequena quantidade com a acetilcolina na placa motora pelo terminal motor. Sua função parece sermodular o efeito da acetilcolina na placa motora, bem como sua força contrátil. Ao ser liberado, acopla-se no seu receptor R1-CGRP localizado na membrana pós-juncional promovendo a ativação da proteína cinase A (PKA) através do aumento de AMPc (Vega and Avila 2010; Rossi, Dickerson, and Rotundo 2003; Fernandez et al. 2003). O aumento do AMPc promove diminuição da expressão de acetilcolinesterase na placa motora (Rossi, Dickerson, and Rotundo 2003). A PKA promove aumento do tempo de abertura do canal do receptor de acetilcolina, bem como da concentração desse receptor no sarcolema. Também aumenta a concentração de $Ca^{2+}$ muscular por aumentar a concentração de canais de $Ca^{2+}$ voltagem dependentes no sarcolema bem como a captação de cálcio pelo RS. Estudos com microdiálise demonstraram que o meio bioquímico do MTP (Shah et al. 2005) apresenta diminuição do Ph, elevada concentração de bradicinina, substância P, noradrenalina, serotonina, TNF□, IL-6, IL-8, IL-1 e CGRP em relação a ao LMTP e ao músculo não afetado (Shah et al. 2008b; Shah and Gilliams 2008). Outro trabalho encontrou níveis elevados de lactato, piruvato e glutamato no repouso e durante atividade leve (Rosendal et al. 2004). Essas substâncias sabidamente sensibilizam os nociceptores promovendo o aumento do estímulo nociceptivo que desencadeia e sustenta

a CS. Outro fator que pode aumentar a excitabilidade é a ação da noradrenalina de modular o efeito do glutamato no motoneurônio (Shah et al. 2008b). Então, a elevada concentração de noradrenalina reduz o limiar da dor e aumenta a dor espontânea no nível dos nociceptores (Ge, Fernández-de-las-Peñas, and Arendt-Nielsen 2006).

### 2.4.9 **Sensibilização periférica**

Os nociceptores correspondem a 50% do volume de axônios no tecido muscular. Na MPS a produção de substâncias inflamatórias encontradas no AMTP sensibiliza as terminações nervosas livres (Shah et al. 2008b). Particularmente a ativação dos receptores ASIC leva a um aumento de seu RNA mensageiro no gânglio da raiz dorsal e de sua expressão na membrana do nociceptor, caracterizando uma forma de sensibilização periférica (Gautam, Benson, and Sluka 2010). Outros receptores também parecem participar desse processo, como o TRPV1 e o P2X3 (Figura 7) (Gregory and Sluka 2014).

**Figura 7:** Sensibilização periférica




Fonte: adaptado de Scholz (Scholz and Woolf 2002).

### 2.4.10 **Sensibilização central**

A ativação sustentada do nociceptor muscular desencadeia um efeito em cascata que induz a sensibilização periférica. Esta ativação leva a alterações neuroplásticas no processamento central da dor, as quais provocam reforço das sinapses excitatórias (LTP) e redução da inibição das vias modulatórias

(LTD). Clinicamente a CS se caracteriza por hiperalgesia, aumento da área receptiva e alodínia (McMahon 2013).

As fibras A delta e C originadas dos nociceptores periféricos fazem sinapse com neurônios de projeção localizados nas lâminas I, II, e V do corno posterior da medula. A liberação sustentada de glutamato associada à liberação de substância P pelo aferente primário reduz o limiar de despolarização dos neurônios de projeção. Esse mecanismo é dependente do aumento da concentração de $Ca^{2+}$ intracelular desencadeado pela abertura de canais NMDA, pela ativação de receptores NK1 e mGlut, pela fosforilação de canais de membrana, pela transcrição celular e pela produção de óxido nítrico e prostaglandinas. Os dois últimos difundem-se pelo meio extracelular contribuindo para ativação de sinapses vizinhas e da glia, aumentando a área receptiva (McMahon 2013).

A ativação da micróglia e do astrócito libera fatores inflamatórios que sensibilizam ainda mais o neurônio de projeção além de colaborar para a apoptose de interneurônios inibitórios; esta última compõe um dos mecanismos do desenvolvimento da alodínia (Watkins et al. 2007).

Associada a esses mecanismos intrínsecos no nível do corno dorsal da medula, observa-se também a perda de mecanismos inibitórios descendentes

O M1 parece ser um importante centro cortical de controle modulatório da dor. Vários resultados de estudos apontam para esse efeito: exercício físico promove efeito analgésico em indivíduos saudáveis (Henrik Bjarke Vaegter, Handberg, and Graven-Nielsen 2014) e esse efeito está diminuído em pacientes com dor crônica musculoesquelética (Henrik B Vaegter, Handberg, and Graven-Nielsen 2016); imaginação de movimentos e posturas corporais também em indivíduos saudáveis (Volz et al. 2015) e em pacientes com dor crónica (Bowering et al. 2013); estimulação elétrica invasiva do córtex motor promove resultados mais duradouros do que a estimulação de outras estruturas do cérebro (Ostergard, Munyon, and Miller 2014).

Nos pacientes com MPS, observa-se uma forma de reorganização do córtex motor caracterizada pelo aumento da atividade corticoespinhal acompanhada de desorganização de redes intracorticais (aumento de facilitação intracortical e diminuição da inibição intracortical) (Vidor et al. 2014; Dall'Agnol et al. 2014; Schabrun et al. 2016)

### 2.4.11 **Sistema modulador descendente da dor**

Esse sistema exerce função tanto anti-nociceptiva quanto pró-nociceptiva (Nir and Yarnitsky 2015). Seus centros efetores se localizam no tronco cerebral e hipotálamo (Chebbi et al. 2014;

Bouhassira, Bing, and Le Bars 1993), sofrendo influências de estruturas corticais e subcorticais, como a região orbital ventrolateral do córtex frontal, o córtex do cíngulo anterior (ACC), o córtex insular rostral, a amígdala (Ohara, Vit, and Jasmin 2005) e o córtex motor primário (M1) (Ye et al. 2014). Emitem seus axônios, direta ou indiretamente, para o corno posterior nos diversos segmentos da medula espinal, exercendo uma função moduladora sobre a transmissão nociceptiva (Benarroch 2008).

A estrutura pivô desse sistema parece ser a susbstância cinzenta periaquedutal (PAG)(Millan 2002a). Diversos estudos desde a década de sessenta tem demonstrado que esse é o principal sítio de atividade suprasegmentar da analgesia mediada pelos opioides (Loyd and Murphy 2009). Mais recentemente descobriu-se que, além do sistema opioidérgico, o sistema endocanabinoide promove analgesia por reduzir o tônus gabaérgico sobre a substância cinzenta periaquedutal levando a desinibição do sistema modulador descendente(Palazzo et al. 2010).

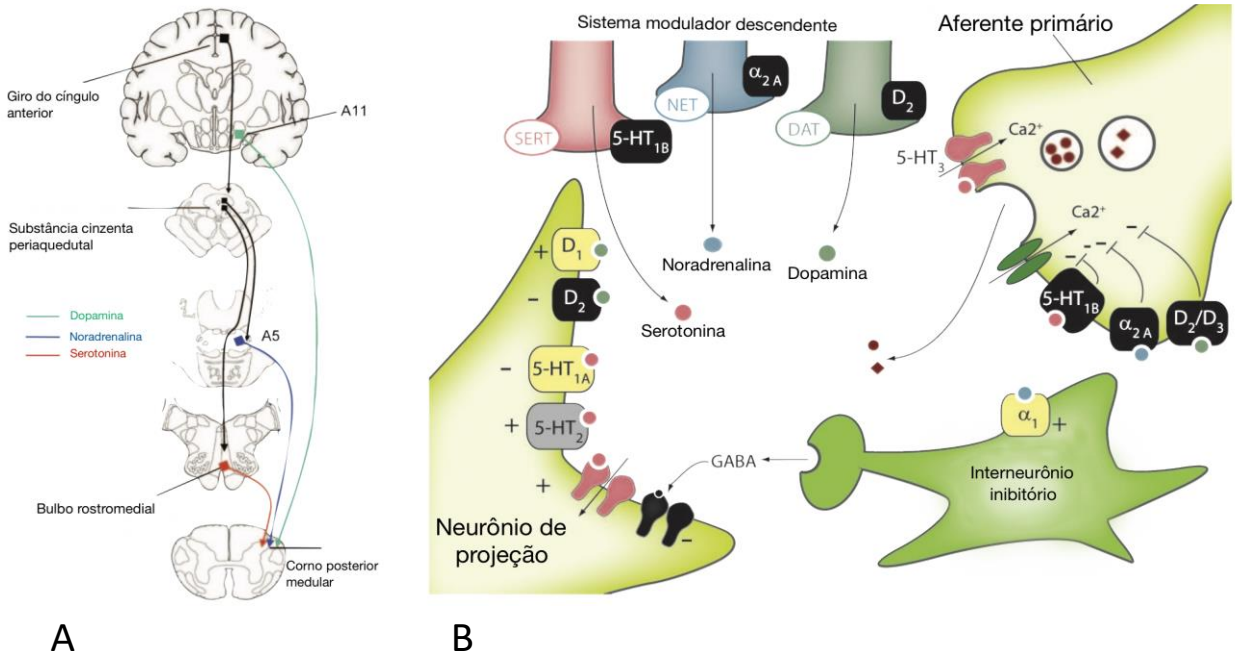
A PAG projeta axônios para a região do bulbo ventro-medial, principalmente ao núcleo magno da rafe e núcleo reticular magnocelular (Benarroch 2008). Esses neurônios serotoninérgicos emitem seu axônios via fascículo postero-lateral exercendo modulação sobre a primeira sinapse da via nociceptiva, no corno posterior da medula. Nesse nível a serotonina ativa receptores pós-sinápticos 5-$HT_{1A}$ inibindo neurônios de projeção e interneurônios glutamatérgicos facilitatórios além de ativar receptores pré-sinápticos 5-$HT_{1B/D}$ reduzindo a liberação de glutamato e substância P pelos aferentes primários, resultando num efeito anti-nociceptivo. Nesse nível, a serotonina também pode ter efeito pró-nociceptivo, ao estimular receptore 5-$HT_{2/3}$, diminuindo o potencial de membrana dos neurônios de projeção (Figura 8 A/B) (Kirkpatrick et al. 2015). Esses achados neurofisiológicos embasam os estudos clínicos que demonstram que os antidepressivos inibidores seletivos da recaptação da serotonina não possuem atividade antinociceptiva clinicamente relevante (Millan 2002b; Goffaux et al. 2007).

Outra área de projeção dos neurônios da PAG é o tegmento pontino, mais especificamente os núcleos A5, A6 e A7 (locus ceruleos) (figura 8A). Esses neurônios também trafegam em sentido descendente pelo fascículo póstero-lateral da medula e, através dos receptores $\alpha 2$ pré-sinapticos, reduzem a liberação de glutamato pelos aferentes primários. Especula-se que também atuem na membrana pós-sinaptica aumentando o potencial de membrana dos neurônios de projeção. No corno posterior da medula a noradrenalina também ativa interneurônios inibitórios através de rceptores $\alpha 1$( figura 8B). O sistema noradrenérgico exerce função estritamente antinociceptiva (Bannister and Dickenson 2017; Millan 2002a).

Outra parte do sistema modulador descendente tem origem na área A11 periventricular posterior do hipotálamo. Esses neurônios dopaminérgicos também modulam a primeira sinapse ao agirem em

receptores D2 diminuindo a liberação de glutamato no terminal axonal pré-sináptico. Da mesma forma que a serotonina a dopamina também pode ter uma ação prónociceptiva ao ativar receptores D1 aumentando a liberação de glutamato pelo aferente primário (figura 8 A/B) (Benarroch 2008; Millan 2002b).

**Figura 8:** Sistema modulador descendente da dor




Fonte: adaptado de Benarroch(Benarroch 2008)

### 2.4.12 **O Fator Neurotrófico Derivado do Cérebro (BDNF)**

O BDNF é uma neurotrofina expressa nos nociceptores amielínicos (fibras C) (Michael et al. 1997) e na micróglia (Brown and Vilalta 2015). Também é encontrado nos centros integrativos superiores, em várias camadas do córtex somatossensorial e nas vias descendentes relacionadas à modulação supraespinhal da dor (Akbarian et al. 2002; Conner et al. 1997). Regula a diferenciação, a sobrevida neuronal e é um potente modulador sináptico do sistema nervoso central e do sistema nervoso periférico, atuando na eficiência sináptica excitatória glutamatérgica e inibitória gabaérgica. Suas ações são basicamente mediadas pelo seu receptor de alta afinidade, o receptor tirosina quinase B (*Tropomyosine Receptor Kinase B* – trkB), amplamente distribuído no sistema nervoso central (Zanette et al. 2014; MERIGHI et al. 2008).

O BDNF tem sido considerado um importante modulador neuronal regulador da LTP no hipocampo e no neocórtex durante o aprendizado motor (Fritsch et al. 2010). Também desempenha papel importante na modulação da neurotransmissão nas vias nociceptivas, tanto no nível segmentar da coluna vertebral quanto supraespinal (MERIGHI et al. 2008). Demonstrou um efeito facilitador no limiar da dor em mulheres e inibidor em homens suportando a noção que é um efeito modificador do fator gênero no limiar da dor em indivíduos saudáveis (Stefani et al. 2012).

Pacientes com escores elevados no Inventário de Sencibilização Central apresentam níveis séricos de BDNF mais elevados (Caumo et al. 2017). Níveis mais altos de BDNF podem estar envolvidos nos processos que mediam a desinibição da excitabilidade do córtex motor, assim como na função do sistema modulador descendente da dor independentemente do mecanismo fisiopatológico em síndromes de dor musculesquelética (Caumo et al. 2016). Sujeitos portadores de condições de dor crônica, como fibromialgia e migrânea, evidenciam níveis aumentados de BDNF no sangue e no líquido cefalorraquidiano que podem ser regulados com intervenções terapêuticas. Tais relatos suportam a ideia de que os níveis de BDNF podem servir como marcador de neuroplasticidade bem como ser úteis no monitoramento de efeitos terapêuticos (Deitos et al. 2015; Dall'Agnol et al. 2014; Zanette et al. 2014; Fischer et al. 2012).

## 2.3 MODULAÇÃO CONDICIONADA DA DOR (CPM):

No contexto de pesquisa clínica a potência do sistema modulador descendente da dor é investigada através do paradigma psicofísico da CPM(Nir and Yarnitsky 2015). Seu mecanismo foi inicialmente investigado e descrito em ratos por Le Bars em 1979, demonstrando um circuito espino-bulbo-espinal no qual a aplicação de um estímulo condicionante heterotópico com intensidade para provocar a ativação dos neurônios *Wide Dinamic Range* (WDR) no corno posterior da medula espinal desencadeia um sinal ascendente para o subnúcleo reticular dorsal do bulbo levando então a uma inibição descendente difusa dos neurônios de projeção via fascículo postero-lateral da medula, reduzindo a percepção de dor ao estímulo teste(Le Bars et al. n.d.). À esse mecanismo de contrairritação, conhecido e descrito há mais de 100 anos (Mackenzie 1909), Le Bars deu o nome de *Diffuse Noxious Inhibitory Controls* (DNIC) (Le Bars, Dickenson, and Besson 1979a, 1979b).

Embora esse último termo tenha sido usado inicialmente para estudar esse mesmo fenômeno em seres humanos acordados, como o DNIC foi estudado em animais anestesiados ele não considera mecanismos corticais "influenciadores" do sistema modulador descendente (David Yarnitsky 2010). Em

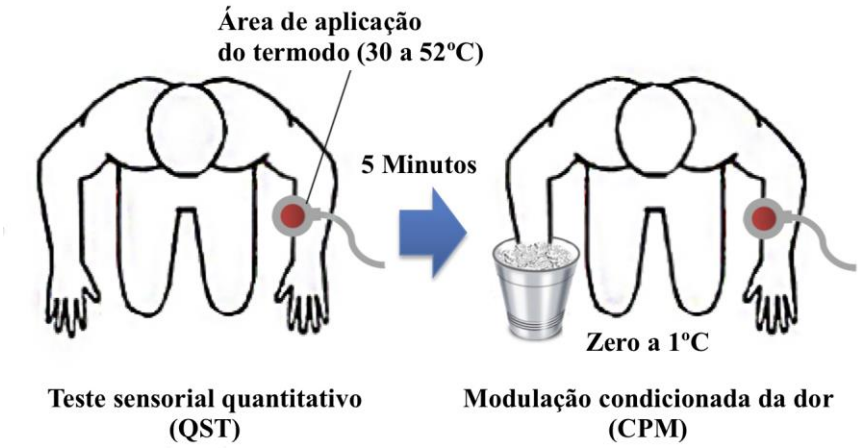
função dessa característica psicofísica, em 2009, um grupo formado por cientistas de pesquisa básica e clínica recomendou que o termo Modulação Condicionada da Dor seja utilizado em estudos com seres humanos(David Yarnitsky et al. 2010).

O teste caracteriza-se pela aplicação de um estímulo condicionante em uma área do corpo remota ao estímulo nociceptivo utilizado como teste. O estímulo condicionante pode ser de natureza variada, como: térmica (frio ou calor), elétrica, ou isquêmica (David Yarnitsky et al. 2010; L Arendt-Nielsen and Gotliebsen 1992; Motohashi and Umino 2001; Fujii, Motohashi, and Umino 2006). Assim como o estímulo teste: respostas eletrofisiológicas, limiar de dor à pressão, ou estímulo térmico aplicado por meio do teste quantitativo sensorial (*Quantitative sensory testing* - QST) (Pud, Granovsky, and Yarnitsky 2009; Valeriani et al. 2005; Lautenbacher, Kunz, and Burkhardt 2008; Schestatsky et al. 2011).

Para a avaliação, inicialmente mensura-se a intensidade do estímulo teste necessária para promover dor de intensidade 6 pela Escala Numérica da Dor (NPS) (tempo 1). Em um segundo momento aplica-se a mesma intensidade do estimulo teste concomitantemente com o estímulo condicionante em uma área remota do corpo, e questiona-se ao indivíduo a intensidade da dor do estímulo teste, pela NPS (tempo 2). A CPM é calculada subtraindo a NPS do tempo 2, pela definida no tempo1( Figura 9). Quando seu valor for negativo, indica que o sistema modulador descendente da dor está eficaz; quando esse valor for igual a zero, significa perda da função do sistema modulador descendente; e quando for positivo, indica efeito de somação (Popescu et al. 2010; D Yarnitsky et al. 2015).

Vários estudos têm demontrado a disfunção do sistema modulador descendente em diversas síndromes de dor crônica (Lars Arendt-Nielsen and Graven-Nielsen 2008; Paul-Savoie et al. 2012; King et al. 2009).

**Figura 9:** Modulação condicionada da dor




Fonte: adaptado do artigo 1.

## 2.4 ESTIMULAÇÃO MAGNÉTICA TTRANSCRANIANA (TMS) COMO INTRUMENTO DIAGNÓSTICO E TERAPÊUTICO

A estimulação magnética é uma técnica não invasiva de estimulação do tecido neural, incluindo o córtex cerebral, raízes medulares e nervos cranianos ou periféricos (M. Kobayashi and Pascual-Leone 2003; M. Kobayashi and Pascual-leone, n.d.). Quando usada para estimular o córtex cerebral, recebe o nome de TMS. Seu mecanismo remonta ao princípio da indução eletromagnética descoberta por Faraday em 1831.

A primeira tentativa conhecida de induzir estimulação magnética cerebral é datada de 1896, e foi feita pelo francês Arsène d'Arsonval. O físico aplicou uma corrente alternada de 110 volts, 30 amperes e frequência de 42 ciclos por segundo em volta da cabeça e induziu o aparecimento de fosfenos, síncope e vertigem (WALSH, BARLOW, and KOHN 1946; Miniussi, Paulus, and Rossini 2013).

A TMS, como conhecida atualmente, foi introduzida por Anthony Barker, em 1985, como uma técnica para estimular de forma indolor e não invasiva o córtex motor humano, usando um estimulador formado por um capacitor de descarga e uma bobina posicionada sobre o escalpo (Barker, Jalinous, and Freeston 1985).

A bobina é posicionada tangencialmente ao crânio do indivíduo de modo a fornecer um campo magnético perpendicular à mesma. De acordo com a lei de indução eletromagnética de Faraday, uma corrente elétrica de alta potência e curta duração que passa pela bobina induz a formação de um campo magnético ao redor da mesma, determinado pela taxa de mudança de corrente por unidade de tempo. O campo magnético atravessa o escalpo e o crânio e atinge o tecido cortical induzindo uma corrente elétrica cerebral (Pascual-Leone 2002). O fluxo de íons nesse campo elétrico nos dois lados da membrana despolariza ou hiperpolariza os neurônios. Usualmente, a corrente magnética necessária é da ordem de grandeza de 1,5 a 2 T (Wassermann and Lisanby 2001).

O campo elétrico induzido é mais intenso na proximidade do escalpo e decresce, rapidamente, à medida que se aprofunda no cérebro (Day et al. 1989). Pode-se dizer que o campo magnético alcança uma profundidade de 2 a 3 cm, atingindo, mais intensamente, os neurônios horizontais nas circunvoluções do córtex, uma vez que o campo é mais intenso e que os neurônios horizontais têm suas fibras dispostas paralelamente às linhas de campo (George and Belmaker 2007). A diferença entre os tempos de latência da resposta eletromiográfica da estimulação elétrica e da magnética pode ser explicada pelo tempo necessário para a estimulação ser transmitida dos neurônios horizontais nas circunvoluções corticais às células piramidais no fundo do sulco, ou seja, devido à ativação transináptica (Kernell and Chien-Ping 1967; PATTON and AMASSIAN 1954).

### 2.4.1 A estimulação magnética transcraniana como instrumento de medida da excitabilidade cortical (TMS)

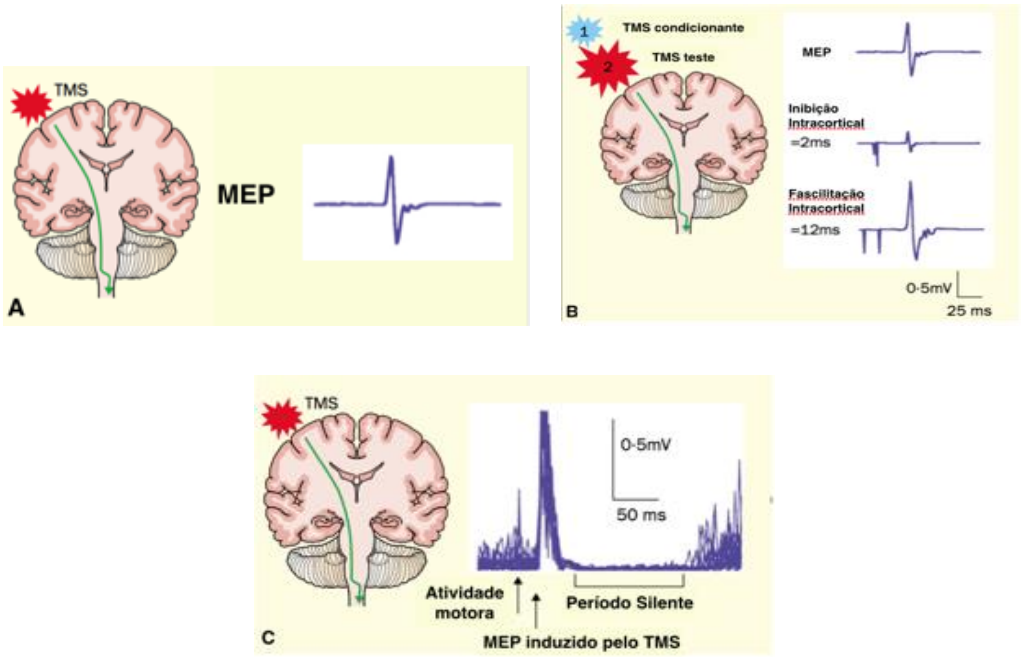
A aplicação da TMS sobre o sistema motor promove informações sobre a excitabilidade do córtex motor, a integridade funcional de estruturas neuronais intracorticais, a condução corticomedular, corticonuclear, transcalosa e da via motora periférica até o músculo. Os parâmetros de excitabilidade cortical medidos pela TMS incluem: MT, MEP, SICI, ICF e CSP.

O **limiar motor (MT**) é definido como a intensidade mínima para elicitar um MEP (*motor evoked potential*) de 50 μV pico a pico em pelo menos 50% dos ensaios (5 em 10). Este parametro está relacionado com a força das projeções corticoespinhais (Lefaucheur et al. 2008). A amplitude do **potencial evocado motor (MEP)** reflete a integridade e excitabilidade da via corticoespinhal motora até o orgão efetor, o músculo. A maior parte do **período silente cortical** (CSP) se deve a mecanismos inibitórios no córtex motor. Mecanismos de inibição na coluna vertebral, tais como a inibição Renshaw, contribuem apenas com os primeiros 50-60 ms desta supressão. O CSP é provavelmente mediado por

receptores de GABA B. A **facilitação intracortical (ICF)** e a **inibição intracortical curta (SICI)** refletem especificamente a ação de interneurônios excitatórios (glutamatérgicos) e inibitórios (gabaérgicos) no córtex motor (Figura 10) (Hwa and Avoli 1992; Ilić et al. 2002; Di Lazzaro et al. 1998; Kujirai et al. 1993; Ziemann, Rothwell, and Ridding 1996).

Várias síndromes neurológicas, inclusive síndromes dolorosas crônicas, demonstram alterações desses parâmetros, e sua normalização correlaciona-se com melhora clínica (Bunse et al. 2014; Ferreri et al. 2011; Schwenkreis et al. 2010; Vacherot et al. 2010).

**Figura 10:** Parâmetros de excitabilidade cortical




Fonte: Adaptado de Kobayashi (M. Kobayashi and Pascual-Leone 2003).

### 2.4.2 **A estimulação magnética transcraniana terapêutica: TMS repetitiva (rTMS)**

De acordo com a frequência dos estímulos, há dois tipos de rTMS: de baixa frequência (< 5 Hz) e de alta frequência (> 5 Hz). Tais padrões promovem, respectivamente, a diminuição ou o aumento da excitabilidade cortical basal (Fregni and Pascual-Leone 2007). A seleção do tipo de estimulação depende da função cortical basal e do objetivo terapêutico (Sandkühler 2009).

A estimulação é aplicada sobre o escalpo através da bobina do aparelho de TMS, que pode apresentar-se em diferentes formatos: em forma de 8, circular, em H ou em cone. A mais usualmente utilizada é a bobina em forma de 8, pois promove a estimulação de forma mais focal. A circular é preferencialmente utilizada para estimular áreas maiores (Ossipov, Morimura, and Porreca 2014; Niddam 2009). As bobinas em H ou em cone, entretanto, vêm sendo testadas para aumentar a profundidade da estimulação (Millan 2002a).

Sabe-se que os processos fisiológicos envolvidos com os efeitos da rTMS são relacionados a mecanismos primários e secundários. Os primários são referentes ao tipo de ativação neuronal (baixa ou alta frequência), à duração, à intensidade, à localização da excitação neuronal, à resolução espacial da excitação, à profundidade da estimulação e ao tempo de duração dos efeitos (Levandovski et al. 2011). Por outro lado, os mecanismos secundários dizem respeito à resolução funcional da estimulação, bem como à interação entre zonas excitadas ou não pela técnica (Fields, Rowbotham, and Baron 1998). Em razão da contiguidade do funcionamento neuronal, infere-se que esses dois diferentes mecanismos se relacionam entre si. Contudo, ressalta-se a importância em diferenciar que zonas ou que conjuntos de células responderão diretamente ao impulso da rTMS e quais as consequências dessa excitação para o aspecto perceptivo, cognitivo ou comportamental (S. Kobayashi 2012). Um importante tópico sobre os mecanismos primários da ação é a localização cerebral exata da ação desta técnica. Os mecanismos fisiológicos da TMS ainda não são completamente conhecidos. Essa inibição ou excitação refere-se ao desempenho de uma função cerebral que depende de uma vasta e complexa rede de neurônios. A aplicação da técnica pode induzir movimentos ou sensações (como os fosfenas) bem como elucidar efeitos disruptivos no desempenho de tarefas motoras ou perceptivas (Mobbs et al. 2009; Camarata and Yaksh 1985). Em nível celular individual, pode-se afirmar que o efeito inibitório tem por característica, dada uma frequência normal de potenciais de ação neuronal, interromper ou inibir essa cadência de impulsos. O excitatório, contudo, provoca uma repetição rápida de potenciais de ação em algumas células ou um só disparo num conjunto sincronizado de células (Pickering et al. 2016).

Alguns estudos sugerem que os efeitos induzidos pela rTMS podem interferir diretamente nas alterações da plasticidade sináptica bem como compartilhar mecanismos com o clássico fenômeno de LTP e LTD, caracterizado por evidenciar forte dependência com a frequência e com o período da estimulação (Yaksh 1979; Yaksh and Tyce 1979). Dados da literatura sugerem que indivíduos saudáveis que receberam rTMS triplicaram os níveis plasmáticos de BDNF em comparação com aqueles que receberam intervenção *sham*, da mesma forma que pacientes deprimidos (Woolf, American College of Physicians, and American Physiological Society 2004) ou com MPS (Adan et al. 2012; Levandovski, Sasso, and Hidalgo 2013) que receberam rTMS aumentaram os níveis séricos de BDNF após receber rTMS.

Outro mecanismo proposto para explicar os efeitos persistentes da TMS é a indução gênica (Levandovski et al. 2011). Os genes c-fos e c-jun são, provavelmente, os de expressão imediata e mais estruturados. Sua expressão é rotineiramente usada como marcador de atividade cerebral, pois são induzidos rápida e transitoriamente em resposta a estímulos aos neurônios. A TMS induz aumento na expressão de c-fos RNAm (ácido ribonucleico mensageiro) em áreas restritas, principalmente no núcleo talâmico paraventricular, cíngulo e região frontal (Mahdi et al. 2011).

A eficácia da rTMS no tratamento da depressão levou a *Food and Drug Administration*, nos Estados Unidos, e as agências reguladoras do Canadá e de Israel a aprovarem a rTMS como opção terapêutica de uso corrente para pacientes refratários ao primeiro curso de antidepressivos. Nas dores crônicas, a técnica tem apresentado um largo tamanho de efeito (ES) no tratamento de migrânea, de traumatismo raquimedular e de dor central (Vanegas and Schaible 2004). Essa magnitude de efeito tem sido observada com cinco sessões consecutivas (Lefebvre et al. 2000). Também se apresenta como opção às terapias convencionais, em quadros como fibromialgia, dores neuropáticas tipo neuralgia trigeminal, pós-herpética e dor visceral (Le Bars et al. n.d.; Le Bars, Dickenson, and Besson 1979a, 1979b; Schweinhardt 2011). Seu uso no tratamento da dor tem como suporte teórico evidências recentes, as quais sugerem que o método pode reduzir a dor e modificar correlatos neurofisiológicos da experiência dolorosa (Le Bars et al. n.d.). Nessa abordagem, a TMS é dirigida preferencialmente ao córtex motor (Figura 8) (Le Bars, Dickenson, and Besson 1979a; Pud, Granovsky, and Yarnitsky 2009; Botelho et al. 2016).

## 2.5 ESTIMULAÇÃO ELÉTRICA INTRAMUSCULAR (IMES)

Infelizmente, até a presente data, não existe um consenso sobre a nomenclatura das técnicas de estimulação elétrica periférica na literatura internacional (Chipchase, Schabrun, and Hodges 2011; Chakravarthy et al. 2016; Rossini et al. 2015). Na maioria das técnicas a corrente elétrica é administrada através de eletrodos de superfície posicionada sobre a pele do paciente, sendo denominadas de técnicas transcutâneas: estimulação elétrica transcutânea, estimulação nervosa periférica, eletroestimulação periférica (PES), eletroestimulação neuromuscular (NMES). As técnicas denominadas percutâneas utilizam agulhas de acupuntura, ou eletrodos implantáveis posicionados diretamente nas estruturas a serem estimuladas pela corrente elétrica: eletroacupuntura (EA), estimulação elétrica intramuscular (IMES), estimulação de nervo periférico (PNS); esta última podendo ter o gerador de corrente elétrica totalmente implantado ou externo.

Decidimos, nesta tese, usar o termo IMES para conotar o aspecto percutâneo da técnica utilizada, com posicionamento de agulhas de acupunturas nas estruturas escolhidas ligadas a um eletroestimulador externo a cada sessão de tratamento, evitando confusões com as técnicas mais invasivas da PNS que usam eletrodos implantáveis por punção ou cirurgicamente.

Estimulação intramuscular (IMS) é uma forma de estimulação neuro-muscular periférica, inicialmente descrita por Chan Gunn, em 1996, como tratamento da MPS baseado em sua teoria de origem radiculopática (Gunn 1996), segundo a qual a origem do MTP seria decorrente de uma forma de radiculopatia desencadeada por contratura da musculatura paravertebral e/ou doença degenerativa da coluna. Com base na "Lei de Canon da supersensibilidade após denervação", Gunn postulou que, quando uma parte de uma cadeia de unidades nervosas é lesada, a sensibilidade dos seus receptores se torna anormalmente aumentada. Também enfatizou que os locais mais comuns de supersensibilidade quando uma unidade nervosa é lesada são músculos esqueléticos, o que levaria ao "encurtamento muscular", desencadeado pela presença do MTP (Ga et al. 2007)

Sua técnica de escolha do sítio a ser agulhado, diferentemente da Medicina Tradicional Chinesa, baseia-se em conhecimentos de anatomia humana, neurofisiologia e fisiopatologia da MPS. A técnica preconiza estimular os MTPs achados no exame físico através de rotação ou movimentos de entrar e sair da agulha de acupuntura do nódulo muscular (IMS). Ou alternativamente, estimular elétricamente os pontos motores ou troncos nervosos dos músculos acometidos (IMES), além de estimular a musculatura paravertebral do segmento da medula que origina a raiz nervosa que inerva os músculos correspondentes ao segmento da disfunção (Gunn 1996; Chu et al. 2004).

Os mecanismos pelos quais a IMES promove analgesia ainda não estão completamente elucidados. No entanto, estudos tem demonstrado que ela promove o aumento da circulação sanguínea muscular (Janssen and Hopman 2003), recrutamento de unidades motoras (Leitch, Brown, and Macefield 2017), e a ativação da substância cinzenta periaquedutal, estimulando a via modulatória descendente (Niddam et al. 2007). Também eleva os níveis intramusculares de fatores neurotróficos promovendo reinervação após lesão de nervo periférico (Willand et al. 2015, 2016).

A frequência da corrente elétrica tem importante papel nos mecanismos centrais desse tipo de estimulação. Estimulação de baixa frequência (≤10 Hz) promove liberação no CNS de peptídeos opióides de alto peso molecular (principalmente β-endorfina) produzindo analgesia de longa duração, enquanto alta frequência (≥100 Hz) libera peptídeos opióides de baixo peso molecular (principalmente dinorfina) promovendo analgesia mais intensa, porém breve (Han 2004). Circuitos neuronais distintos parecem responder de maneira diversa a diferentes frequências da corrente, com ativação de diferentes

áreas talâmicas e corticais (Yang et al. 2005). Além dos peptídeos opióides endógenos, outros neurotransmissores como serotonina, norepinefrina, glutamato bem como outros neuropeptídeos estão implicados nos efeitos periféricos, e centrais da EA (Zhang et al. 2014).

Uma recente revisão sistemática sobre o uso da IMS em dores de origem musculosquelética investigou 416 publicações, restringindo a avaliação a apenas quatro delas, que ainda assim consideraram com moderado risco de vieses metodológicos, porém nenhuma conclusão metanalítica pôde ser estimada devido à heterogeneidade dos estudos (Kim et al. 2012). Os autores excluíram da investigação estudos que comparavam a IMS com técnicas de infiltração dos MTPs.

Este grupo de pesquisa, recentemente publicou um ensaio clínico randomizado em paralelo que demonstrou maior eficácia da IMS no tratamento da MPS quando comparada ao placebo e à infiltração com lidocaína. A IMS proporcionou maior redução dos escores de dor, maior aumento do limiar de dor à pressão (PPT), melhor qualidade de vida relacionada à saúde e menor uso de analgésicos de resgate durante o tratamento (Caumo. et al. 2014). Uma limitação desse estudo foi a falta de seguimento após o tratamento. Outro ensaio clínico randomizado, cruzado, demonstrou que a IMES promoveu aumento do BDNF sérico, correlacionado com a redução dos escores de dor em cefaleia tensional, demonstrando provável efeito em mecanismos de neuroplasticidade no sistema nervoso central (Chassot et al. 2015). Outro estudo deste grupo de pesquisa, com pacientes portadores de osteoartrite de joelho, demonstrou que pelo menos parte dos efeitos analgésicos da IMES é mediada no nível cortical pela redução da excitabilidade (redução do MEP e da ICF e aumento do CSP) e aumento da potência do sistema modulador descendente da dor (da Graca-Tarragó et al. 2015).

Sumen et. al demonstrou que a IMES de alta frequência e baixa intensidade associada a exercício de alongamento foi mais eficaz na redução da dor e aumento do limiar à pressão a longo prazo do que a associação de lasertarapia de baixa potência e alongamento em pacientes com MPS (Sumen et al. 2015).

A tabela 2 resume os principais ensaios clínicos randomizados sobre técnicas de neuromodulação periférica percutânea publicados até o momento

**Tabela 2.** Ensaios clínicos randomizados sobre técnicas de neuromodulação periférica percutânea

| Autores | Técnica (n) | Protocolo | Síndrome dolorosa | Efeitos na dor | Considerações |
|---|---|---|---|---|---|
| Ga 2007 | IMS/PSS (22) x Infiltração de PGM com lidocaina à 0,5% (21) | 1 sessão semanal por 3 semanas | MPS do trapézio superior | IMS reduziu mais a do rem todas as sessões que a infiltração | Após um mês do tratamento IMS foi superior em analgesia, mobilidade cervical e redução de sintomas depressivos |
| Couto 2014 | IMS/PSS x Infiltração de MTP com lidocaína | 2 sessões por semana por 4 semanas | MPS | Maior eficácia da IMS na redução da dor | Maior efeito da IMS também sobre a qualidade do sono, funcionalidade e melhora emocional |
| Chassot 2015 | EA x sham | Sessões de 30 minutos 2 x/semana por 5 semanas | Cefaleia tensional crônica | EA demonstrou maior efeito analgésico que estímulo sham | Aumento no nível sérico do BDNF correlacionou positivamente com maior efeito analgésico da EA |
| Sumen 2015 | LLLt/alongamento x IMES/alongamento x alongamento | 1 sessão de 20 minutos diária por 10 dias úteis consecutivos | MPS | Maior efeito nos grupos com os tratamentos combinados em comparação com tratamento único, porém sem diferença entre LLLt e IMES | Maior efeito no limiar de dor a pressão após um mês do tratamento no grupo IMES/alongamento |

**TABELA** 2**.** Ensaios clínicos randomizados sobre técnicas de neuromodulação periférica percutânea (continuação)

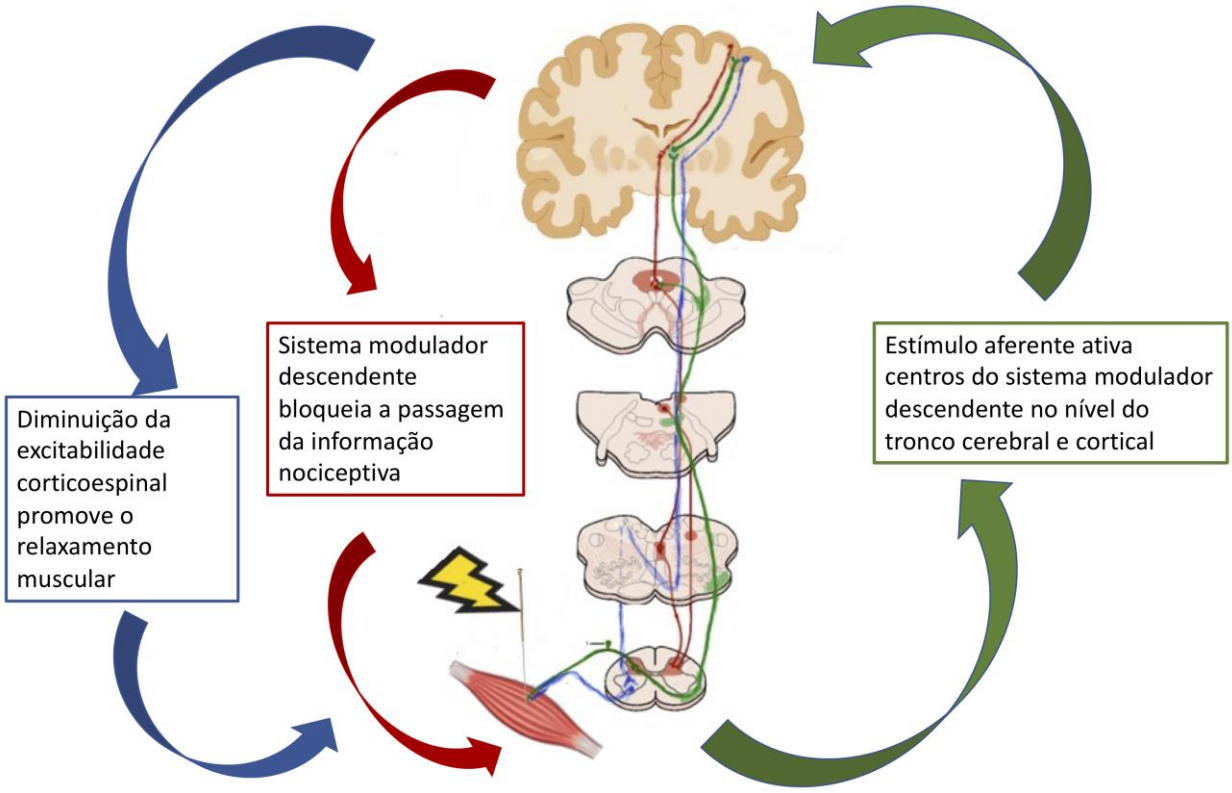
| Autores | Técnica | Protocolo | Síndrome dolorosa | Efeitos na dor | Considerações |
|---|---|---|---|---|---|
| Aranha 2015 | EA x ACP x sham | 2 sessões de 30 minutos por semana por 4 semanas | MPS do trapézio superior | Melhora da dor global com ambos tratamentos ativos em comparação com sham. Melhora da dor localizada apenas no grupo EA no trapézio esquerdo | Melhora na amplitude de movimento, embora fugaz, com os tratamentos ativos |
| Chung 2016 | EA/splint x sham/splint | 1 sessão semanal por 13 semanas | Síndrome do túnel do carpo | Pequena superioridade do tratamento combinado. MD -0,2 [IC -0,36 a -0,03] | A diferença encontrada é clinicamente irrelevante |
| Tarragó 2019 | IMES/TDCS x IMES/sham x sham/TDCS x sham/sham | 5 sessões diárias | Osteoartrite de joelho | IMES/TDCS foi superior que cada técnica isolada. Não houve diferença entre as duas técnicas isoladas quando comparadas ao estímulo sham | Em todos os grupos de tratamento ativo observou-se melhora da função do sistema modulador descendente da do, melhora da disfunção e diminuiu consumo analgésico |

**Legenda**- IMS- Estimulação intramuscular; PSS- Estimulação de nervo periférico; MPS- Síndrome dolorosa miofascial; MTP- Ponto-gatilho miofascial; EA- Eletroacupuntura; Splint-imobilização; LLLt- Terapia com laser de baixo nível; IMES- Estimulação elétrica intramuscular; TDCS- Estimulação elétrica com corrente contínua

# 3 MARCO TEÓRICO

A partir da base teórica, observa-se que o processo fisiopatológico da MPS é complexo e envolve mecanismos periféricos e centrais. O racional que embasa esta tese fundamenta-se na teoria de que o estímulo aferente (*bottom-up*) da IMES (em verde) ativa centros corticais e do tronco cerebral do sistema modulador descendente da dor (em vermelho) além de promover a diminuição da excitabilidade do sistema corticoespinal (em azul) promovendo o relaxamento da musculatura acometida e a melhora da dor (Figura 11).

**Figura 11:** Marco teórico



# 4 JUSTIFICATIVA

A MPS provavelmente seja a causa mais prevalente de dor crônica na população em geral. Ela gera altos índices de incapacidade, o que leva a elevados custos diretos e indiretos para o indivíduo e para a sociedade. Normalmente é pouco responsiva aos tratamentos analgésicos convencionais. Tal fato provavelmente ocorra em virtude de os tratamentos correntes não alterarem as principais vias do processo de doença, devido ao limitado conhecimento de sua fisiopatologia, aos escassos métodos diagnósticos e à falta de treinamento médico para seu reconhecimento.

Evidências sugerem que existe três sistemas neurais primariamente envolvidos na dor da MPS: (i) o sistema corticoespinal; (ii) o sistema modulador descendente da dor; e (iii) o sistema regulador da neuroplasticidade. Para se obter sucesso terapêutico, é necessário entender melhor essa inter-relação para poder intervir em todas as dimensões envolvidas no seu processamento, desde o processo nociceptivo periférico até os mecanismos modulatórios descendentes.

A IMES atua nos mecanismos periféricos da nocicepção (contratura/isquemia muscular, por exemplo), mas também ativa regiões do sistema nervoso central envolvidas no processamento da dor (neuromatriz da dor) potencializando a via modulatória de forma *bottom-up*.

Presumivelmente essa técnica neuromodulatória, por atuar em pontos diferentes na neuromatriz da dor, possa promover efeitos clinicamente relevantes. Assim, entende-se como propícia a realização de estudos que busquem as respostas para essas questões relacionada à eficácia clínica dessa técnica neuromodulatória no tratamento da MPS, bem como sua relação com outros fatores que possam estar vinculados com o processo nociceptivo.

# 5 OBJETIVOS

## 5.1 OBJETIVO GERAL

Avaliar possíveis mecanismos neuroplásticos envolvidos na dor crônica de origem miofascial do complexo craniocervicomandibular, bem como os efeitos de 10 sessões de IMES comparados a intervenção *sham* em 24 pacientes do sexo feminino com idade de 18 a 65 anos nesses mesmos mecanismos.

## 5.2 ESTUDO 1

### 5.4.1 Objetivo principal

Avaliar a relação entre o sistema modulador descendente da dor, o sistema corticoespinhal e o sistema modulador da neuroplasticidade em pacientes com MPS com e sem efeito de somação espacial ao estímulo condicionado da dor.

### 5.4.2 Objetivo específico

Comparar, em pacientes com e sem efeito de somação espacial ao estímulo condicionado, os seguintes desfechos:

– Primários: excitabilidade cortical - potencial evocado motor - MEP e facilitação intracortical - ICF

– Secundários: excitabilidade cortical - inibição intracortical curta - SICI e período silente cortical - CSP

– Incapacidade relacionada à dor

## 5.2 ESTUDO 2

### 5.4.1 Objetivo principal

Verificar a relação do efeito da IMES nos níveis de dor, nos limiares termoalgésicos, na capacidade funcional, nos níveis séricos de BDNF e na qualidade do sono em indivíduos com dor crônica miofascial do complexo craniocervicomandibular.

### 5.4.2 Objetivo Específico

Comparar o efeito da IMES com o da intervenção *sham* nos seguintes desfechos:

– Testes psicofísicos de dor: limiar da sensibilidade ao calor, limiar de dor ao calor e tolerância à dor ao calor (*Quantitative Sensory Testing* - QST);

– Capacidade funcional (Escala Funcional de Dor);

– Níveis de dor (Escala Análogo-Visual - VAS);

– Sistema modulatório descendente de dor (CPM);

– Níveis séricos do BDNF;

– Escala análogo-visual - qualidade de sono (VAS-QS).

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# 7 ARTIGO 1

## A Framework for Understanding the Relationship between Descending Pain Modulation, Motor Corticospinal and Neuroplasticity Regulation Systems in Chronic Myofascial Pain

**Short title:** Descending disinhibition correlates with higher BDNF

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## ABSTRACT

Myofascial pain syndrome (MPS) is a leading cause of chronic musculoskeletal pain. However, its neurobiological mechanisms are not entirely elucidated. Given the complex interaction between the networks involved in pain process, our approach, to providing insights into the neural mechanisms of pain, was to investigate the relationship between neurophysiological, neurochemical and clinical outcomes such as corticospinal excitability. Recent evidence has demonstrated that three neural systems are affected in chronic pain: (i) motor corticospinal system; (ii) internal descending pain modulation system; and (iii) the system regulating neuroplasticity. In this cross-sectional study, we aimed to examine the relationship between these three central systems in patients with chronic MPS of whom do/do not respond to the Conditioned Pain Modulation Task (CPM-task). The CPM-task was to immerse her non-dominant hand in cold water (0−1◦C) to produce a heterotopic nociceptive stimulus. Corticospinal excitability was the primary outcome; specifically, the motor evoked potential (MEP) and intracortical facilitation (ICF) as assessed by transcranial magnetic stimulation (TMS). Secondary outcomes were the cortical excitability parameters [current silent period (CSP) and short intracortical inhibition (SICI)], serum brain-derived neurotrophic factor (BDNF), heat pain threshold (HPT), and the disability related to pain (DRP). We included 33 women, (18–65 years old). The MANCOVA model using Bonferroni's Multiple Comparison Test revealed that non-responders ($n$ = 10) compared to responders ($n$ = 23) presented increased intracortical facilitation (ICF; mean ± $SD$) 1.43 (0.3) vs. 1.11 (0.12), greater motor-evoked potential amplitude (μV) 1.93 (0.54) vs. 1.40 (0.27), as well a higher serum BDNF (pg/Ml) 32.56 (9.95) vs. 25.59 (10.24), ($P < 0.05$ for all). Also, non-responders presented a higher level of DRP and decreased HPT ($P < 0.05$ for all). These findings suggest that the loss of net descending pain inhibition was associated with an increase in ICF, serum BDNF levels, and DRP. We propose a framework to explain the relationship and potential directionality of these factors. In this framework we hypothesize that increased central sensitization leads to a loss of descending pain inhibition that triggers compensatory mechanisms as shown by increased motor cortical excitability.


**Key words:** BNDF, cortical excitability, CPM, MEP, TMS, QST, chronic pain

# 1 INTRODUCTION

Myofascial pain syndrome (MPS) is a leading cause of chronic musculoskeletal pain (Simons et al., 1999). MPS has been associated with disability, and also with dysfunction of corticospinal conduction as assessed by motor evoked potential (MEP; Vidor et al., 2014). As with other chronic pain syndromes, the mechanisms of MPS are not entirely elucidated. A major barrier to the understanding of these mechanisms is that pain is an experience orchestrated by a network of cortical regions, elements of the limbic system and the spine-bulbospinal loop. The ascending portion of this circuit involves the spine reticular tract (Willer et al., 1999), which comprises modulatory systems such as the opioidergic (Le Bars et al., 1981; Willer et al., 1990), noradrenergic (Sanada et al., 2009; Makino et al., 2010), and serotonergic systems (Chitour et al., 1982). Given this complex interaction, our approach to provide insights into the neural mechanisms of pain was to investigate the relationship between neurophysiological, neurochemical, and clinical outcomes such as corticospinal excitability as indexed by transcranial magnetic stimulation (TMS) measurements, conditioned pain modulation (CPM) to measure the descendent endogenous inhibitory pain system and serum brain-derived neurotrophic factor (BDNF) as a critical marker of neuroplasticity. Corticospinal excitability as indexed by TMS has become a reliable marker in chronic pain syndromes, including MPS (Vidor et al., 2014). It has been shown that pain and disability are associated with an imbalance between excitatory and inhibitory systems as assessed by increased intracortical facilitation (ICF) and by a reduced current silent period (CSP; Vidor et al., 2014; a proxy of glutamatergic activity), a higher pain catastrophizing score (Volz et al., 2013a) and a higher trait anxiety score (Vidor et al., 2014).

The CPM (Yarnitsky, 2010) involves the diffuse noxious inhibitory control (DNIC) system. The DNIC system assesses the reduction in the pain sensation on the stimulus by a simultaneous pain input from distant sites of the body (Le Bars, 2002). While the CPM assesses how much, a conditioning stimulus can reduce the pain response evoked by the other strong, painful stimuli at a distant large body surface area (the test stimulus; Volz et al., 2013a). When the CPM-task increases pain, this indicates a disruption of endogenous pain-inhibitory processes and a summation effect (King, 2014), which amplifies the pain response and it is a process of the central sensitization (Boyer et al., 2014). It appears that these pain-related neural changes maintain the dysfunction of endogenous descending inhibitory mechanisms as

observed in many chronic pain syndromes including knee osteoarthritis (Arendt-Nielsen et al., 2010), chronic pancreatitis (Olesen et al., 2010), rheumatoid arthritis (Leffler et al., 2002a), long-term trapezius myalgia (Leffler et al., 2002b), irritable bowel syndrome (King et al., 2009), temporomandibular disorder (King et al., 2009), fibromyalgia (Staud et al., 2003), and MPS (Pielsticker et al., 2005).

BDNF, a critical molecule for the development and maintenance of cortical neurons and cortical synapses, interacts with the descendant modulatory system. Clinical studies have found higher levels of BDNF in the blood (Deitos et al., 2015) and cerebrospinal fluid in patients with chronic pain (Bø et al., 2009), and in fibromyalgia has been associated with a lower pain threshold (Zanette et al., 2014). This set of evidence demonstrates that there are three main neural systems involved in chronic pain: (i) the corticospinal motor system; (ii) the internal descending pain modulation system; and (iii) the system regulating neuroplasticity. Our hypothesis is that disruption of the infra cortical medulator system, as assessed by pain scores during the CPM-task, is correlated with dysfunction of corticospinal conduction and disinhibition at the cortical level, due to increases in the MEP amplitude, ICF, and serum BDNF level. We aimed to analyze the relationship between these three central systems in chronic MPS patients in responders and non-responders to Quantitative Sensory Testing (QST) during the immersion of her non-dominant hand in cold water (0−1◦C) to produce a heterotopic nociceptive stimulus (CPM-task). To determine the CPM we used the difference between the pain score on NPS (0–10) QST during cold water immersion (QST+CPM) at the temperature of the point at which subjects felt 6/10 pain on the NPS scale [during the initial time period (T0)]. Our primary outcomes were the MEP and ICF as assessed by TMS. The secondary outcomes were the cortical excitability parameters [current silent period (CSP) and short intracortical inhibition (SICI)], serum BDNF level, heat pain threshold (HPT), and the disability related to pain.

## 2 METHODS

This exploratory study was performed at the Hospital de Clinicas de Porto Alegre in Porto Alegre, Brazil. The study protocol was approved by the Institutional Review Board (IRB 0000921) at the Hospital de Clinicas de Porto Alegre and conducted according to the Declaration of Helsinki. All subjects provided written informed consent for their participation.

We administered clinical assessment scales validated in the Brazilian population. Additionally, we collected behavioral measurements (i.e., several pain assessments) and neurophysiological measurements (i.e., motor córtex excitability as indexed by TMS) to establish baseline data.

### 2.1 Design Overview, Settings, and Participant

We recruited the participants from the general population through public postings in different health care units and physicians' referrals from the Chronic Pain Service at the Hospital de Clínicas de Porto Alegre. The inclusion criteria included the following: (1) right-handed females (2) aged 19– 65 years old, (3) confirmed the diagnosis of MPS in the upper body segment for at least 3 months before enrollment, and (4) limitation in routine activities due to MPS. Furthermore, patients needed to present with a pain score of the visual analog scale (VAS) at least of 4cm (i.e., moderate or severe pain; Palos et al., 2006), associated with functional disability in most days of the 3 months before enrollment. Disability associated with MPS was evaluated using a questionnaire that included six specific questions (yes/no). These questions aimed at assessing interference with work, personal relationships, pleasure obtained during activities, personal goals, clear thinking (i.e., problem solving, concentrating, or remembering), and responsibilities at home during the past 3 months. For enrollment, an affirmative answer to one or more of these questions was necessary to ensure that chronic pain was decreasing the patient's quality of life. Moreover, the diagnosis of MPS was confirmed by a second experienced independent examiner with significant clinical experience related to chronic pain. MPS criteria were the presence of regional pain, normal neurological examination, stiffness in the target muscles; decreased the range of motion, the presence of palpable nodules, tender points, trigger points, taut bands, and pain characterized as hollow, dull, or deep that was exacerbated by stress. To standardize the severity of MPS and to distinguish neuropathic pain from ongoing nociception, were included only patients with the Neuropathic Pain Diagnostic Questionnaire (DN4) with a score equal to or higher than four (Bouhassira et al., 2005). The presence of previous surgery on the affected areas or other pain disorders such as rheumatoid arthritis, radiculopathy, and fibromyalgia; and frequent use of steroidal and non-steroidal anti-inflammatory medications were exclusion criteria.

Anticipating an effect size (f) of 0.4 for a multiple regression analysis allowing for two predictors and a type I and II errors of 0.05 and 0.20, respectively, and the minimum sample

size was 30 patients. Finally, considering the likely attrition rate and other unexpected factors, the required sample size was determined to be 33 patients (**Figure 1**).

INSERT FIGURE 1

### 1.2. Instruments and Assessments

The tools used to assess psychological state were validated in the Brazilian population (Staud et al., 2003; Kaipper et al., 2010; Sehn et al., 2012; Caumo et al., 2013). Two independent medical examiners that were blinded to the aim of the study were trained to conduct the psychological tests and to administer the pain scales. The patients' baseline depressive symptoms were assessed using the Beck Depression Inventory (BDI II; Warmenhoven et al., 2012), and the Pittsburgh Sleep Quality Index to assess the sleep quality (Buysse et al., 1989). To measure the anxiety, we used the refined version of the State- Trait Anxiety Inventory (STAI; Kaipper et al., 2010) obtained using the Rasch model, which derivates shorter state-trait STAI- Form X scales free of threshold disorders and for differential item functioning (DIF) problems. The scores in the state- and trait score ranges from 13 to 52, and 12 to 36, respectively. The catastrophic thinking related to pain was assessed using the Brazilian Portuguese Catastrophizing Scale (BP-PCS; Sehn et al., 2012). To measure the pain intensity during the most part of time in the last week was used the VAS, ranging from 0 cm (no pain) to 10 cm (worst possible pain). We used a standardized questionnaire to assess demographic data and medical comorbidities.

As subjects with chronic pain usually use rescue analgesics changes from week to week according to pain level, the analgesic use was defined as the self-reported average used per week during the last 3 months. For data analysis, we included the analgesic use as a dichotomous variable: the analgesic was coded one when they used more than 4 days per week while the analgesic uses less than 4 days per week it was coded as zero (reference value).

### 1.3. Outcomes

The primary outcomes were the MEP and ICF as assessed by TMS. The secondary outcomes were the cortical excitability parameters CSP and SICI, serum BDNF level, HPT, and the disability related to pain assessed by Brazilian Profile of Chronic Pain: Screen score

(B-PCP:S). The primary factor of interest, the score on NPS (0–10) during the conditioned pain modulated (CPM-task), are described in detail below.

(a) The Brazilian Profile of Chronic Pain: Screen (B-PCP:S; Caumo et al., 2013) was used for quick identification of an individual's multidimensional pain experience. The B-PCP:S includes a severity scale (four items; possible score range of 0–32), an interference scale (six items; possible score range of 0–36), and an emotional burden scale (five items; possible score range of 0–25). The disability related to pain (DRP) regarding severity, interference with daily activities, and the emotional burden was evaluated using the B-PCP:S (Caumo et al., 2013). It accepted as a criterion to define disability a presence of chronic or recurrent pain or discomfort causing restriction (Caumo et al., 2013); thus, we assumed that higher scores on the B-PCP:S indicated more severe disability or greater functional deficits at work, at home, and during social situations and a higher emotional burden (Vidor et al., 2014).

(b) To measure the cortical excitability parameters we used a surface electromyography. The recordings were gathered at the contralateral right first dorsal interosseous muscles using Ag/AgCl electrodes. First, the resting motor threshold (RMT) was determined by obtaining five motor evoked potentials (MEPs) with a peak-to-peak amplitude of 50 μV from 10 consecutive trials. To define the MEP we recorded 10 MEPs with an intensity of 130% of the individual RMT. Moreover, the cortical silent periods (CSPs) were assessed during muscle activity by a dynamometer to maintain them at ∼20% maximal force. Accordingly, the CSPs were 10 records using an intensity of 130% of the RMT. Short intracortical inhibition (SICI) using an inter-stimulus interval of 2 ms was also assessed. The conditioning stimulus was set at 80% of the RMT while the test stimulus was set at 100% of the individual MEP intensity. The intracortical facilitation (ICF) was assessed with an inter-stimulus interval of 12 ms. We conducted the paired-pulse in a randomized order for a total of 30 trials (ten each for ICF, SICI and control stimuli). To calculate the RMT we used the lowest stimulus intensity that was able to evoke an MEP of at least 50μV in 5 out of 10 consecutive trials. Off-line analyzes included the collection of the duration of the CSPs as well as the amplitudes of all of the MEPs, SICIs, and ICFs. The corresponding units for these parameters included MEP in μV, SICI, and ICF in their ratios to MEP and CSP in ms (Pascual-Leone et al., 1994).

c) The laboratory outcome measured was the serum level of BDNF. We collected the blood samples before starting the assessment. We centrifugate the blood samples for 10min at 4500 × g at 4◦C, and we stored the serum at −80◦ C for the hormone assay. We determined

the serum BDNF using an Enzyme-Linked Immunosorbent Assay (ELISA) using a ChemiKine BDNF Sandwich ELISA Kit, CYT306 (Chemicon/Millipore, Billerica, MA, USA). The lower detection limit of the kit for BDNF is 7.8 pg/mL.

d) We used the Quantitative Sensory Testing (QST) to assess HPT. This measure use the method of limits with a computer Peltier-based device thermode (30 × 30 mm; Schestatsky et al., 2011) attached to the skin on the ventral aspect of the mid-forearm. The set at 32◦C and was increased at a rate of 1◦ C/s to a maximum of 52◦ C. The heat pain threshold (HPT) of each patient was defined as the mean of three assessments performed with an inter-stimuli interval of 40 s (Schestatsky et al., 2011). The thermode position was slightly altered between trials, to avoid, either sensitization or response suppression of the cutaneous heat nociceptors.

e) To measure the CPM-task we evaluated the pain intensity in two tonics HPT test stimuli separated by a CPM-task. We used the HPT as conditioning pain stimulus to elicit a prolonged pain sensation to trigger CPM. The CPM-task consisted of immersion of non-dominant hand in cold water at a temperature of 0−1◦C for 1 min. To maintain the water temperature zero to 1◦C was used a thermostat to control the temperature variation. The QST procedure was introduced after 30 s of cold-water immersion. To determine the CPM we used the difference between the pain score on NPS (0– 10) QST during cold water immersion (QST+CPM) at the temperature of the point at which subjects felt 6/10 pain on the NPS scale [during the initial time period (T0)]. An accepted criterion to define responders to the CPM-task is the reduction of NPS pain scores under a heterotopic stimulus compared with NPS pain scores under a nociceptive stimulus without a heterotopic stimulus. If the patients did not report a reduction or report an increase in their pain score during the CPM-task, the descendent modulatory systems were considered to have failed to modulate the nociceptive response. For the data analysis, non-responders showed a difference in the score on NPS, HPT1–HPT0, of zero or higher, and for responders, these values were lower than zero.

## 2.4 STATISTICAL ANALYSIS

Descriptive statistics were used to summarize the main socio- demographic features of the sample. *T*-Tests for independent samples and Chi-squared and Fisher's exact tests were used

to compare continuous and categorical variables between groups respectively. To test for normality was used the Shapiro-Wilk test. To ensure that the data were normally distributed, we performed a log transformation for BDNF level.

After verifying the corresponding assumptions, the Pearson correlation coefficient (*r*) was used to assess the relationship between covariates (age, sleep quality, catastrophic thinking about pain; state-trait anxiety, and depressive symptoms) with the outcomes related to cortical excitability parameters, BDNF, and pain measures (see **Table 3**). To maintain the assumption of independence between covariates and to control for collinearity when the Pearson correlation coefficients (*r*) for two variables were higher than 0.5 (moderate), in the multivariate analysis model was included only one of the variables (see **Table 4**). Based on this criterion the catastrophizing thinking related to pain and trait-anxiety were included in the multivariate analysis model, taking into account that they have been shown to be correlated with cortical excitability in previous studies on MPS (Volz et al., 2013b; Vidor et al., 2014) (**Table4**). The covariates not included in the multivariate analysis model were age, depressive symptoms, sleep quality, and state- anxiety. A multivariate covariance analysis (MANCOVA) model was used to explore the relationship between the responders and non-responders to multiple outcomes [cortical excitability (MEP, ICI, ICF, CSP), BDNF, HPT, and disability related to pain on B-PCP:S. Bonferroni's Multiple Comparison Test was used to identify the source of significant differences. The data were analyzed using SPSS software version 22.0 (SPSS, Chicago, IL).

# 3 RESULTS

## 3.1 PATIENT CHARACTERISTICS

We screened 54 potential participants with a diagnosis of MPS, and we included 33 in the study. The reasons for exclusion were not fulfilling the diagnostic criteria for MPS, not present a neuropathic component according to the DN4 (Neuropathic Pain Diagnostic Questionnaire), lacking disability as defined in the protocol, and the presence of another diagnosis (fibromyalgia). All enrolled subjects participated in all aspects of the study and were included in all of analyses (**Table 1**)

INSERT TABLE 1

## 3.2 UNIVARIATE ANALYSIS

### 3.2.1 Relationships between the function of the corticospinal *modulatory system, motor córtex excitability, pain measures and BDNF level*

Relationships between the function of the corticospinal modulatory system, motor córtex excitability, pain measures, and BDNF level according to a spectrum of responders and no responder to CPM-task. The non-adjusted means and standard deviation (SD) of the cortical excitability parameters, BDNF, pain threshold and disability related to pain were presented in **Table 2**.

INSERT TABLE 2

### 3.2.2 Assessment of relationship between independent variables as to identify potential confounders

The Pearson correlation was used to identify potential confounding factors in the relationships between outcomes (cortical excitability, BDNF, HPT, and disability). The correlated parameters were the scores of the Brazilian Portuguese Catastrophizing Scale (B-PCS); Beck Depression Inventory (BDI); Pittsburgh Sleep Quality Index (PSQI); and Short State-Trait Anxiety Inventory (STAI-E-T), and age (**Table 3**). The covariates included in the multivariate analysis model (**Table4**) were the trait-anxiety and catastrophizing scores.

INSERT TABLE 3

INSERT TABLE 4

### 3.3 Multivariate Analysis of the Relationship between the Corticospinal Modulatory System, Cortical Excitability, BDNF, HPT, and Disability According to Spectrum of Responders and Non-Responders to CPM-task

The results of the MANCOVA model analysis with multiple outcomes as dependent variables, including cortical excitability parameters (MEP, ICF, SICI, CSP), BDNF, HPT, and disability related to pain according to spectrum of responders and non-responders to CPM-task, and the STAI-E-T score and catastrophizing score, as independent variables, are presented in **Table 4**. The MANCOVA model using Bonferroni's Multiple Comparison Test revealed a significant relationship between the responders and non-responders groups and the outcomes related to cortical excitability measurements (ICF and MEP), BDNF, disability related to pain and HPT [Hotelling's Trace = 1.84, F(34) = 6.05, $P < 0.001$]. This analysis presented a power of 0.99. The adjusted determination coefficient of this model was R2 = 0.57; thus, the variables included in the model explain 57% of the variance in the outcome variables. The results of this

adjusted multivariate model are presented in **Table 4**. Non-responders showed higher cortical excitability (ICF, MEP), greater disability related to pain, higher BDNF level, and lower HPT. However, no effect was observed in other cortical excitability parameters (CSP and ICI; see **Table 4**).

In **Figures 2A–C** are presented the relationships according to a spectrum of responders and non-responders to CPM-task and intracortical facilitation and MEP (primary outcomes) and BDNF (secondary outcome). The means were compared using MANCOVA with Bonferroni's Multiple Comparison test (the model was shown in **Figures 2A–C**; **Table 4**).

INSERT FIGURE 2

## 4 DISCUSSION

This study confirmed our hypothesis that the descending pain modulation system as assessed according to a spectrum of responders and non-responders to CPM-task is simultaneously correlated with a disinhibition at the cortical level, as measured by ICF and with global neuroplasticity levels as determined by serum BDNF. Also, the disengagement of descending pain modulatory system was correlated with a dysfunction of the corticospinal pathway as indexed by MEP, a lower HPT, and a greater disability.

The current study expanded on the data available in the literature showing that the magnitude of disinhibition in regulating sensory information was associated with changes in the cortical and subcortical levels. This disinhibition state occurs through multiple neurobiological systems, which can amplify sensory pain signals to the neural pain matrix. Additionally, the level disengagement of descending pain modulatory system was correlated with changes in serum BDNF level, which is involved in the modulation of the excitatory/inhibitory central nervous system balance. Thus, the variation in the spectrum of dysfunction of internal modulator system in chronic pain conditions could be understood as a signal from a balance in the neuroplasticity mediators involved in the modulation of the excitatory/inhibitory central nervous system (CNS; Deitos et al., 2015). While that the variation

of BDNF could be interpreted as a signal from a "diseased balance," once such balance differs between the spectrum of responders and responders to CPM- task. However, persists the concerns how good is this signal to identify the chronic pain imbalance in the CNS and how is its predictive properties for the evaluation of the MPS.

These results demonstrated that this integrative pattern to assess changes in the pain pathway highlights that a cross talk between the neural network of cortical regions and the spine-bulbospinal loop occurs along with changes in the BDNF secretion, which is the central marker of neuroplasticity process mechanisms. Thus, this set of changes reinforcing the hypothesis, that, if we improve the understanding of underlying neurophysiological mechanisms of chronic MPS, this could give support for the clinical decision based on practical approaches for its recognition (Nijs et al., 2010). Additionally, these findings provide some theoretical support for the mechanism involved in the effect of interventions that improved pain and enhanced the function of the descendent modulatory system in studies that used melatonin, amitriptyline (de Zanette et al., 2014), rTMS (Dall'Agnol et al., 2014), and the combination treatment of CPM and duloxetine (Yarnitsky et al., 2012). Although human studies permit us to determine only the effect in the network, our findings allow a new way to construct the rational to combine therapeutic approaches to improve functional of descending pain modulatory systems. Such techniques include pharmacological (i.e., antidepressant, anticonvulsant, etc.) and non-pharmacological approaches (i.e., Transcranial direct current stimulation (tDCS), TMS, electroacupuncture and other physical therapy).

We observed greater MEPs amplitude in non-responders to CPM-task (**Table 4**). Although a significant portion of the corticospinal input to the motoneuron pool is relay via lumbar group II interneurons (Marchand-Pauvert et al., 1999), the MEP amplitude is a reflection of the latency of depolarization of the spinal motor neuron pool. Its amplitude reflects the integrity and function of conduction along the efferent pathway, which form part of the lumbar propriospinal system and it express the excitability of the cortical and spinal motor neuron pool (Marchand-Pauvert et al., 1999; Pierrot-Deseilligny and Burke, 2005; Iglesias et al., 2008). Thereby, this result suggests that an enhanced activity of descending tracts, whose motor portion is assessed by the MEP, suggests that the inhibitory capacity of the corticospinal modulator system is reduced (Vidor et al., 2014), resulting in increased amplitude of MEP. One critical issue here is whether corticospinal excitability is a compensatory or a causal mechanism of pain. Given our data does not allow us to clarify the temporal relationship between these two variables; we can only hypothesize the correlation between these two variables (MEP and CPM response). We have proposed before that increased motor cortex excitability is a compensatory

mechanism aiming to reduce thalamic overactivity and thus pain (Castillo Saavedra et al., 2014); though this mechanism is not enough to control pain (an anology here would be increased insulin in a subject with hyperglycemia; increased insulin levels would be the compensatory mechanism). Therefore, increased pain increases corticospinal excitability and when pain is controlled this marker becomes normalized. The data from ICF supports this hypothesis. We have proposed before that increased motor cortex excitability is a compensatory mechanism aiming to reduce thalamic overactivity and thus pain (Castillo Saavedra et al., 2014).

Either increased ICF or decreased ICI suggest an involvement of cortical mechanisms in the dysfunction of the descendent modulatory system, which facilitate the activity of the corticospinal system. Although the ICF is a complex phenomenon, it reflects increase in the activity within glutamatergic circuits, it also may arise through a loss of GABA-A-mediated modulation (Di Lazzaro et al., 2000; Fedi et al., 2008). Additionally, the disinhibition involves the loss of inhibitory pyramidal cells. MEP amplitude is also an indicator of primary motor córtex excitability: larger amplitudes indicate higher excitability of the motor córtex, which may modulate intracortical excitability and the transmission efficiency of corticospinal neurons, resulting in less facilitation. Overall, as proposed above these changes in cortical plasticity could be explained as a compensatory mechanism to downregulate increased excitability in the pain neural networks such as thalamic structures.

The higher serum BDNF in non-responders suggests that this neurotrophin may be a marker of severity of CS. The CS involves a proliferation of synaptic activity due the trophic factors, to support maladaptive plasticity that perpetuates the sensation of pain. Our findings give neurophysiological support (MEP) to understand the link between serum BDNF and the severity of dysfunction of the descendent modulatory system. Even though this relationship is complex, they support the idea that the activity of the descending inhibitory system is related to central sensitization (Schwenkreis et al., 2003; Deitos et al., 2015) and a greater activation in the brainstem (Graven-Nielsen et al., 2012). This assumption, supported by an experimental study with rats exposed to chronic pain, demonstrates that the BDNF effect on pain pathways may change according to the region of central nervous system (i.e., spine, brainstem, hippocampus, and cortex, etc.; Spezia Adachi et al., 2015). The mentioned study demonstrated that the tDCS decreased the BDNF levels in the spinal cord and brainstem, whereas BDNF levels did not change in the hippocampus (Spezia Adachi et al., 2015). These differents effects according to site suggest that BDNF activates distinct pathways (i.e., descending systems) and that its effect is pleiotropic. Although previous findings show that the increase in excitatory

activity and the decrease in inhibitory synaptic activity in the córtex related to BNDF level (Ren and Dubner, 2007; Tao et al., 2014), the present results do not allow for a conclusion regarding a cause-effect relationship between BDNF level and descendent modulatory system dysfunction.

Overall, the findings of this study corroborate the idea that the BDNF modulates the synaptic plasticity in an activity- dependent manner to strengthen a nociceptive transmission, recruits non-nociceptive input to the pain pathways and it binds to high-affinity trkB receptors. This BDNF effect enhances the response that NMDA-mediated C-fibers evoke, which in turn causes activation of several signaling pathways in spinothalamic tract neurons. Thereby, this strength excitatory synapsis promotes the disinhibition of descending pathways (Zanette et al., 2014). This statement is also supported indirectly by clinical findings, where the serum BDNF was correlated inversely with the pressure pain threshold in fibromyalgia (Zanette et al., 2014). Equally, we showed that the BDNF increase would be favoring pain transmission because greater scores in the CPM indicates a lower function in the descending pain modulatory system and a higher propensity for pain. This finding is biologically plausible because the enhance in the BDNF activates signaling pathways in the spinothalamic tract, which reduces the GABAergic inhibitory effect (Spezia Adachi et al., 2015). These findings support the hypothesis that the chronic pain induces reorganization in circuits involved in pain processing at cortical and in descending pain modulatory system. Although the relationship between BDNF with the physiopathology of pain is complex, it has important functions in the processes of neurogenesis and neuroplasticity. Thereby, efforts are being made to understand its role in the pain modulatory system.

In the current study, a lower HPT and higher DRP in non-responders were observed (**Table4**). These results are congruent with evidence from previous studies that a lower pain threshold in patients with long-term chronic pain may be a signal of lack of function of the inhibitory system (Kwon et al., 2013; Defrin et al., 2015). Another explanation for this finding is a potential protective effect if one considers that the hippocampus amplifies signals to the neural representation (Ma et al., 2012).

A greater disability according to scores on the B-PCP:S was associated with the disinhibition of the descendent pain modulatory system. The B-PCP:S dominions indicate pain severity, restriction for daily activities (at work, at home, during social situations) and the emotional burden. According to a spectrum of responder and non-responders to CPM-task, the disability was correlated positively with the catastrophizing and trait-anxiety. In previous study we demonstrated the relationship between greater disability related to pain and a higher trait

anxiety in MPS (Vidor et al., 2014). While in another study with healthy subjects was observed that the perceived intensity of the conditioning stimulus was associated with the pain catastrophizing and trait anxiety (King, 2014). In fact, the current findings suggest that the relationship between the descending modulatory system and the disability related to pain is regulated by brain regions that are involved not only with pain but also with cognitive and emotional functioning in general (Pessoa, 2008). Similar dysfunction was observed when there were lesions in brain regions implicated in descending pain modulation (i.e., traumatic brain injury and multiple sclerosis), including the medial prefrontal córtex (PFC) and rostral anterior cingulate córtex (ACC; Bushnell et al., 2013). Additionally, it has been demonstrated that the alterations in the biological integrity and functioning of brain regions were involved in both pain control and cognitive and emotional functioning. Thereby, the changes in this network could explain the relationship between the severity disability and the dysfunction of the corticospinal pain modulatory system (**Table 4**).

This study had some limitations: Firstly, TMS is an indirect neurophysiological evaluation of neurotransmitter system activity. Secondly, only females were evaluated, as gender differences in pain perception and modulation are controversial. Thirdly, psychiatric disorders are a potential confounding factor in chronic pain syndromes, and they cannot have been adequately controlled. The psychiatric symptoms (anxiety, depression, catastrophizing, and psychiatric diagnosis) were equally distributed between the groups (responder vs. non-responder). In fact, 39.39% (14/33) of patients suffered from mental illnesses. However, this finding is expected, because the emotional disturbance is part of chronic pain syndromes, and they can worsen the sensitization and chronification. Moreover, the results of this study need to be carefully interpreted because it was an exploratory study. Further, research on chronic pain of different psychopathologies is required to confirm our initial findings and the impact of our findings on patients' responses to different therapeutic approaches.

These results suggest that a non-response to the CPM-task is likely associated increased plasticity in central structures associated with pain that control endogenous inhibitory control and that in this case compensatory mechanisms are activated as reflected by increased cortical excitability. This failure to respond to CPM-task is associated with higher serum BDNF, lower HPT, and a greater level of disability related to pain. Overall, these findings suggest that the CPM-task is a test that allows for inference regarding the loss of net descending pain inhibition. Thus, this short and simple test might be useful for predicting a patient's response to therapy, and it helps in the clinical decision-making process for individual patients. The results of this study may also assist in the development of individualized treatment.

## AUTHOR CONTRIBUTIONS

AB participated in the sequence alignment and drafted the manuscript. MT participated in the sequence alignment. ES participated in the design of the study and performed the statistical analysis. FG conceived the study, participated in its design and coordination and helped drafting the manuscript.

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**Declaration of conflict of interest:**

The authors declare that there are no financial or other relationships that might lead to conflicts of interest to any of the following arrangements: financial relationship to the work; employees of a company; consultants for a company; stockholders of the company; members of a speakers bureau or any other form of financial compensation.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Table 1 -** Demographic and clinical characteristics of the study sample. Values are given as the mean (SD) or frequency (n = 33*)*

| Variables | **Non responders (n = 23)** | **Responders (n = 10)** | **P** |
|---|---|---|---|
| Age (years) | 43.36 (14.78) | 48.30 (9.13) | 0.33 |
| Marital Status (married/unmarried) | 13/10 | 4/6 | 0.31 |
| Education (years) | 13.91 (4.25) | 12.57 (3.88) | 0.37 |
| Smoking (yes/no) | 1/22 | 0/10 | 0.69 |
| Alcohol consumption (yes/no) | 22/1 | 10/0 | 0.69 |
| Duration of pain (years) | 6.04 (1.64) | 6.4 (0.97) | 0.42 |
| Trait-anxiety (STAI-T) | 28.82 (6.14) | 25.17 (6.41) | 0.13 |
| State-anxiety (STA-T) | 30.0 (8.42) | 29.91 (5.75) | 0.98 |
| Beck Depression Inventory | 13.45 (7.04) | 15.83 (7.12) | 0.37 |
| Brazilian Portuguese Catastrophizing Scale (B-PCP:S) | 33.82 (6.98) | 31.52 (8.14) | 0.42 |
| Number of days analgesics were used per week in the last three months (< 4 times / ≥ 4 times)[a] | 8/15 | 2/8 | 0.33 |
| Presence of other chronic diseases before appearance of pain (yes/no)[b] | 4/19 | 2/8 | 0.6 |
| Diagnosis of psychiatric disorders (yes/no) | 8/15 | 5/5 | 0.32 |
| Active use of central nervous system medication (yes/no)[c] | 20/3 | 7/3 | 0.25 |

[a] The same patient may have used more than one medication.

[b] Chronic diseases other than pain: hypertension (n = 12); ischemic heart disease (n = 1); heart attack (n = 1); diabetes mellitus (n = 5); thyroid diseases (n = 2); other chronic diseases listed (n = 0).

[c] Central nervous medication: tricyclic antidepressant (n = 2); topiromate (n = 1) tylex (n = 1).

**Table 2 -** Measurements of motor córtex parameters by TMS, HPT, B-PCP:S and BDNF (n = 33)

| **Cortical Excitability Measures** | **Non-responders (n = 23)** | | **Responders (n = 10)** | | ***P&*** |
|---|---|---|---|---|---|
| | **Mean ± SD** | **Median (Q25, Q75)** | **Mean ± SD** | **Median (Q25, Q75)** | |
| Motor Threshold (MT) | 44.46 (8.04) | 44 (32: 65) | 41.1 (5.53) | 40.5 (32; 50) | 0.15 |
| Motor evoked potential (mV) | 1. 93 (0.54) | 2.06 (0.98; 3.14) | 1.40 (0.27) | 1.42 (1.03); 1.81) | 0.01 |
| Intracortical Facilitation (ratio: ICF/ test stimulus) | 1.42 (0,3) | 1.35 (0,71; 1.99) | 1.10 (0.12) | 1.09 (0.94; 1.24) | 0.00 |
| Short Interval Intracortical Inhibition (ratio: SICI/ test stimulus) | 0.25 (0.02) | 0.25 (0.23;0.27) | 0.27 (0.10) | 0.25 (0.08; 0.42) | 0.38 |
| Cortical Silent Period (CSP) | 69.36 (21.74) | 79.00 (38.0;120.0) | 61.91 (15.49) | 62.50 (33.25; 91.75) | 0.17 |
| Profile of Chronic Pain: Screen for Brazilian population (B-PCP:S) | 71.00 (10.02) | 73.00 (55.0;91. 0) | 59.22 (11.23) | 63.00 (51.0; 75.0) | 0.00 |
| Quantitative Sensory Testing ($^{o}$C) | 42.78 (4.27) | 44 (35;50) | 38.0 (3.03) | 38.00 (37: 41) | 0.00 |
| Brain-derived neurotrophic factor (BDNF) pg/ml (*log*) | 32.55 (9.95) | 33.0 (20.0;36. 0) | 25.59 (10.24) | 22.5 (5.5; 39.5) | 0.02 |

(Motor evoked potential: MEP); Interquartile interval (Q). Intra-cortical inhibition (ICI) expresses the relationship between the amplitude of wave and motor evoked potentials (relative amplitude, express in%), at inter-stimuli intervals (ISIs) of 2 ms with paired-pulse. The first is a sub-threshold stimulus [80% of the rest motor threshold (rMT)] followed by the second one which is a suprathreshold stimulus (130% rMT). **(B)** Cortical silent period (CSP) expressed in milliseconds (ms); **(C)** Motor-evoked potentials (MEP) expressed in mV, evoked by a stimulus of 130% the intensity of the rMT, and should have peak-to-peak MEP amplitude of at least 1 mV.

& Comparisons of mean using t test for independent samples.

**Table 3-** Pearson (r) correlation between potential confounding factors and outcomes (n = 33)

| | **Age** | **STAI-T** | **STAI-E** | **BPC-S** | **B-PCP:S** | **BDI** | **PSQI** | **MEP** | **ICF** | **SICI** | **CSP** | **BDNF** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age | *r = 0.05* | | | | | | | | | | | |
| STAI-T | *r* = 001 | *r = 0.15* | | | | | | | | | | |
| STAI-E | *r* = -0.04 | *r* = 0.65** | *r = -0.07* | | | | | | | | | |
| BPC-S | *r* = 0.06 | *r* = 0.32 | *r* = 0.29 | *r = -0.08* | | | | | | | | |
| B-PCP:S | *r* = -0.13 | *r* = 0.40* | *r* = 0.19 | *r* = 0.62** | *r = -0.11* | | | | | | | |
| BDI | *r* = 0.18 | *r* = 0.58** | *r* = 0.43** | *r* = 0.66** | *r* = 0.54** | *r = -0.25* | | | | | | |
| PSQI | *r* = -0.07 | *r* = 0.34* | *r* = 0.24 | *r* = 0.54** | *r* = 0.36* | *r* = 0.46** | *r = -0.11* | | | | | |
| MEP | *r* = -0.26 | *r* = 0.05 | *r* = -0.08 | 0.11 | *r* = 0.26 | *r* = -0.10 | *r* = -0.05 | *r = 0.33** | | | | |
| ICF | *r* = -0.01 | *r* = 0.15 | *r* = -0.06 | 0.14 | *r* = 0.45** | *r* = 0.09 | *r* = -0.07 | *r* = 042* | *r* = 0.25 | | | |
| SICI | *r* = -0.13 | *r* = -0.12 | *r* = -0.27 | -0.01 | *r* = -0.03 | *r* = -0.03 | *r* = -0.06 | *r* = -0.15 | *r* = 0.04 | *r = -0.27* | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CSP | *r* = -0.05 | *r* = -0.14 | *r* = 0.05 | -0.18 | *r* = -0.13 | *r* = -0.13 | *r* = -0.21 | *r* = 0.01 | *r* = 0.27 | *r* = 0.18 | -0.38* | |
| HPT | *r* = 0.32 | *r* = 0.03 | *r* = -0.08 | 0.28 | *r* = 0.07 | *r* = 0.20 | *r* = 0.11 | *r* = -0.35* | *r* = -0.05 | *r* = 0.34* | *r* = -0.11 | *r* = *0.20* |

** Correlation is significant at the 0.01 level (2-tailed).*. Correlation is significant at the 0.05 level (2-tailed). Brazilian Portuguese Catastrophizing Scale (B-PCS); Beck Depression Inventory (BDI); Pittsburgh Sleep Quality Index (PSQI); Short State-Trait Anxiety Inventory (STAI-E-T); Brazilian Profile of Chronic Pain: Screen (B-PCP:S)Intra-cortical inhibition (ICI); Cortical silent period (CSP): Motor-evoked potentials (MEP) expressed in mV, Brain-derived neurotrophic factor (BDNF) pg/ml (log).

**Table 4 -** Relationship between outcomes (cortical excitability parameters, pain measures and BDNF) and responders and no responders according change in NPS (0-10) during the CPM-task (n = 33)

| **Dependent Variable** | **Type III Sum of Squares** | **df** | **Mean Square** | **F** | **P** | ***Partial Eta Squared*** |
|---|---|---|---|---|---|---|
| Motor evoked potential (mV) | 1.15 | 3 | 0.38 | 2.79 | 0.03 | 0.22 |
| Intracortical facilitation (ratio: ICF/ test stimulus) | 2.52 | 3 | 0.84 | 5.81 | 0.00 | 0.38 |
| Short Intracortical inhibition (ratio: SICI/test stimulus) | 0.94 | 3 | 0.31 | 9.19 | 0.00 | 0.49 |
| Cortical silent period | 0.004 | 3 | 0.001 | 0.17 | 0.91 | 0.01 |
| Brazilian Profile of Chronic Pain: Screen (B-PCP:S) | 929.06 | 3 | 309.69 | 1.10 | 0.36 | 0.10 |
| Quantitative Sensory Testing ($^0$C) | 184.73 | 3 | 61.58 | 4.86 | 0.00 | 0.33 |
| Brain-derived neurotrophic factor (BDNF) pg/ml (*log*) | 2881.96 | 3 | 960.65 | 15.40 | 0.00 | 0.61 |
| **Parameter** | | **SEM** | **βa** | **t** | | **P** |
| **Motor evoked potential (mV)** | | | | | | |
| Conditioned pain modulation (CPM) during CPM/task | | | | | | |
| No responder [a] | | 0.61 | 0.15 | 4.09 | | 0.00* |
| Brazilian Portuguese Catastrophizing Scale (B-PCS) | | 0.007 | 0.009 | 0.74 | | 0.46 |
| State-anxiety (STAI-T) | | -0.01 | 0.01 | -1.50 | | 0.14 |

| | | | | |
|---|---|---|---|---|
| **Intracortical facilitation (ratio: ICF/ test stimulus)** | | | | |
| Conditioned pain modulation (CPM) during CPM/task | | | | |
| No responder [a] | 0.33 | 0.07 | 4.50 | 0.00* |
| Brazilian Portuguese Catastrophizing Scale (B-PCS) | 0.004 | 0.004 | 0.88 | 0.38 |
| State-anxiety (STAI-T) | 0.004 | 0.006 | 0.70 | 0.48 |

| **Dependent Variable** | **Type III Sum of Squares** | **df** | **Mean Square** | **F** | **P** | ***Partial Eta Squared*** |
|---|---|---|---|---|---|---|
| **Short Intracortical inhibition (ratio: SICI/test stimulus)** | | | | | | |
| Conditioned pain modulation (CPM) during CPM/task | | | | | | |
| No responder [a] | -0.02 | 0.03 | -0.62 | 0.54 | | |
| Brazilian Portuguese Catastrophizing Scale (B-PCS) | -0.01 | 0.02 | -0.34 | 0.80 | | |
| State-anxiety (STAI-T) | 1.85 | 0.003 | 0.007 | 0.99 | | |
| **Cortical silent period** | | | | | | |
| Conditioned pain modulation (CPM) during CPM/task | | | | | | |
| No responder [a] | 10.82 | 6.66 | 1.62 | 0.11 | | |
| Brazilian Portuguese Catastrophizing Scale (B-PCS) | -0.19 | 0.40 | -0.47 | 0.64 | | |
| State-anxiety (STAI-T) | -0.47 | 0.50 | -0.94 | 0.36 | | |
| **Brazilian Profile of Chronic Pain: Screen (B-PCP:S)** | | | | | | |

| | | | | |
|---|---|---|---|---|
| Conditioned pain modulation (CPM) during CPM/task | | | | |
| No responder [a] | 7.81 | 3.13 | 2.48 | 0.01* |
| Brazilian Portuguese Catastrophizing Scale (B-PCS) | 0.80 | 0.18 | 4.24 | 0.00* |
| State-anxiety (STAI-T) | 0.51 | 0.24 | 2.17 | 0.03* |
| **Quantitative Sensory Testing ($^0$C)** | | | | |
| Conditioned pain modulation (CPM) during CPM/task | | | | |
| No responder [a] | -3.82 | 1.41 | -2.70 | 0.01* |
| Brazilian Portuguese Catastrophizing Scale (B-PCS) | 0.08 | 0.08 | 0.99 | 0.33 |
| State-anxiety (STAI-T) | -0.19 | 0.10 | -1.82 | 0.07 |
| **Brain-derived neurotrophic factor (BDNF) pg/ml (*log*)** | | | | |
| Conditioned pain modulation (CPM) during CPM/task | | | | |
| No responder [a] | 0.39 | 0.14 | 2.66 | 0.01* |
| Brazilian Portuguese Catastrophizing Scale (B-PCS) | -0.05 | 0.09 | -0.61 | 0.54 |
| State-anxiety (STAI-T) | -0.01 | 0.01 | -1.47 | 0.15 |

[a] Reference category is responder, hence a positive value mean that the mean was higher in no responder.
CPM-task [no responder (NPS(0-10) HPT1-HPT0 ≥ 0) or responder (NPS(0-10) HPT1-HPT0 < 0]

**FIGURE 1**

**Fig 1-** The sequence of assessments

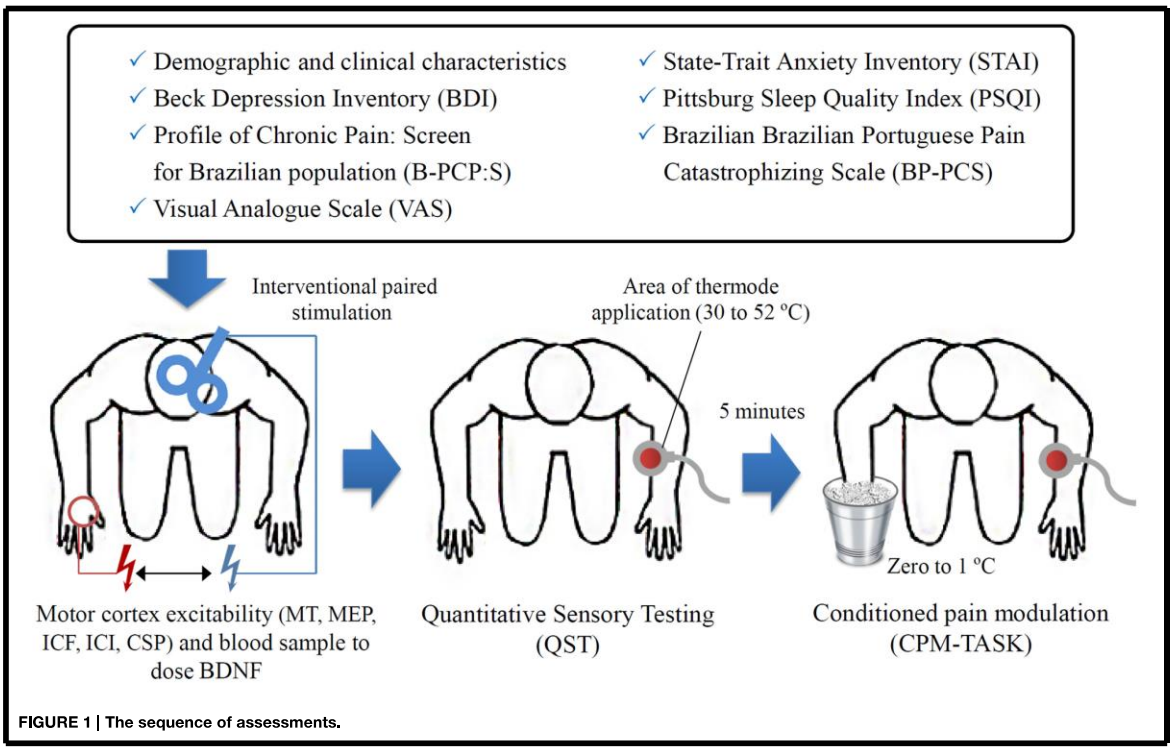
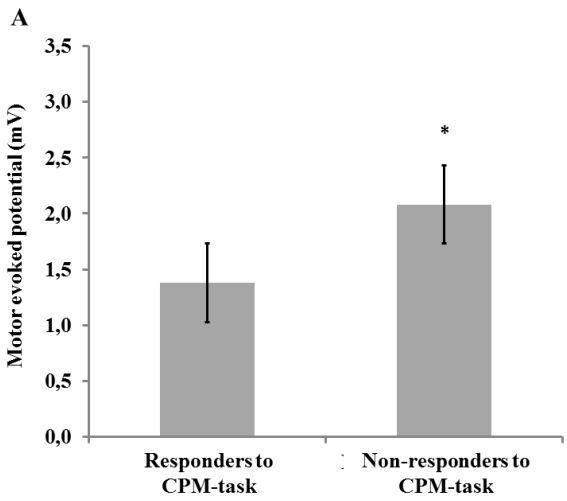
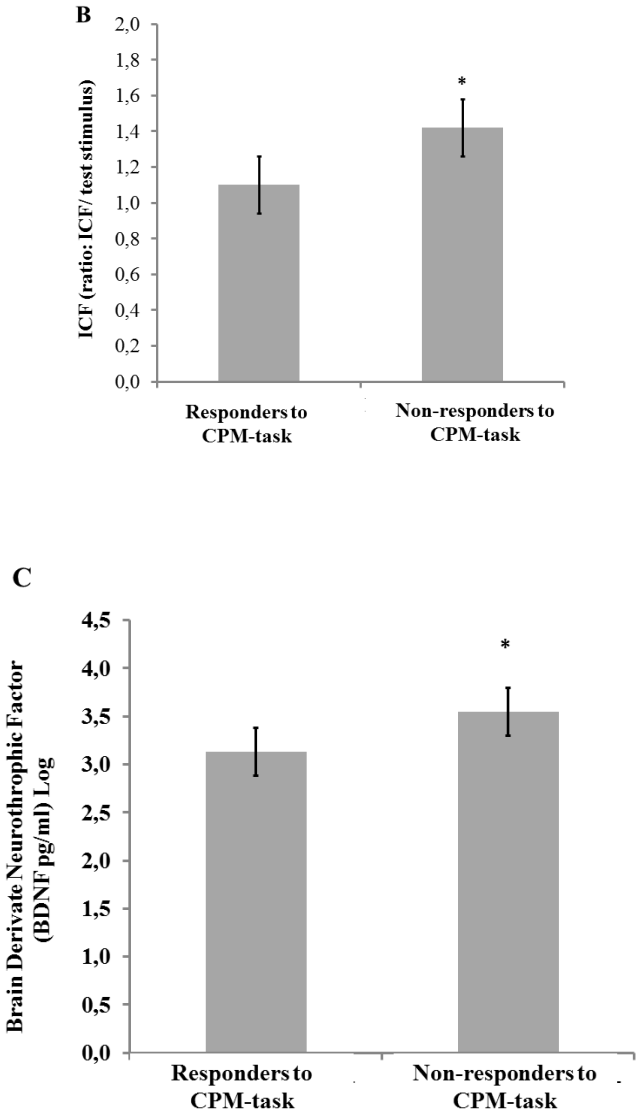



FIGURE 1 | The sequence of assessments.

**Fig 2-** Relationships between responders and non-responders to CPM-task and MEP(1a), ICF(1b) and BDNF(1c)






**Legend of Figure 2**

**Fig. 2 -** Comparisons between [non-responders ($NPS_{(0\text{-}10)}$ HTP1-HPT0 ≥ 0) (n = 10) and responders ($NPS_{(0\text{-}10)}$ HTP1-HPT0 < 0 (n = 23)]. (A) Motor evoked potential (mV); (B) Intra-cortical facilitation (amplitude/MEP amplitude ratio = ICF); and (C) Brain derived neurotrophic factor (BDNF) ng/ml (Log). Error bars indicate standard error of the mean (S.E.M.). * Asterisks positioned above the bars indicate differences between groups (responders and non-responders

to CPM-TASK) assessed by MANCOVA with post-hoc Bonferroni's Multiple Comparison test.

## 8 ARTIGO 2

# Insights about the neuroplasticity state on the effect of intramuscular electrical stimulation in pain and disability associated in chronic myofascial pain syndrome (MPS): a double-blind, randomized, sham-controlled trial

**Short running title:** Effect of the neuroplasticity state on the IMES clinical response

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**We declare that there are no conflicts of interest.**

**ABSTRACT**

**Background:** There is limited evidence concerning the effect of intramuscular electrical stimulation (IMES) on the neural mechanisms of pain and disability associated with chronic Myofascial Pain Syndrome (MPS).

**Objectives:** To provide new insights into the IMES long-term effect on pain and disability related to chronic MPS (primary outcomes). To assess if the neuroplasticity state at baseline could predict the long-term impact of IMES on disability due to MPS we examined the relationship between the serum brain-derived-neurotrophic-factor (BDNF) and by motor evoked potential (MEP). Also, we evaluated if the IMES could improve the descending pain modulatory system (DPMS) and the cortical excitability measured by transcranial magnetic stimulation (TMS) parameters.

**Methods:** We included 24 right-handed female with chronic MPS, 19–65 years old. They were randomically allocated to receive ten sessions of IMES, 2 Hz at the cervical paraspinal region or a sham intervention ($n$ = 12).

**Results:** A mixed model analysis of variance revealed that IMES decreased daily pain scores by −73.02% [95% confidence interval (CI) = −95.28 to −52.30] and disability due to pain −43.19 (95%CI, −57.23 to −29.39) at 3 months of follow up. The relative risk for using analgesics was 2.95 (95% CI, 1.36 to 6.30) in the sham group. In the IMES and sham, the change on the Numerical Pain Scale (NPS0-10) throughout CPM-task was −2.04 (0.79) vs. −0.94 (1.18), respectively, ($P$ = 0.01). IMES reduced the MEP −28.79 (−53.44 to −4.15), while improved DPMS and intracortical inhibition. The MEP amplitude before treatment [(Beta = −0.61, (−0.58 to −0.26)] and a more significant change from pre- to post-treatment on serum BDNF) (Beta = 0.67; CI95% = 0.07 to 1.26) were predictors to IMES effect on pain and disability due to pain.

**Conclusion:** These findings suggest that a bottom–up effect induced by the IMES reduced the analgesic use, improved pain, and disability due to chronic MPS. This effect might be mediated by an enhancing of corticospinal inhibition as seen by an increase in ICI and a decrease in MEP amplitude. Likewise, the MEP amplitude before treatment and the changes induced by the IMES in the serum BDNF predicted it's long-term clinical impact on pain and disability due MPS.

The trial is recorded in ClinicalTrials.gov: NCT02381171.

**Keywords:** MPS, IMES, TMS, clinical trial, BNDF, QST

## 1. INTRODUCTION

Myofascial pain syndrome (MPS) encompasses muscle and musculotendinous pain secondary to the development of myofascial trigger points (MTrPS). It is the principal source of pain in about 30% of individuals with musculoskeletal dysfunction, and its primary components are MTrPS, tender points, and taut bands. The trigger point induced central sensitization explains the referred pain and hyperalgesia phenomenon (Gerwin, 2014). Central sensitization (CS) represents an intensification in the activity of circuits and neurons in nociceptive pathways caused by an enhancement in membrane and synapses excitability. In patients with central sensitization, any sensory experience presents with higher amplitude, duration and spatial extent, which reflects a reduced excitatory-inhibitory balance (Schwenkreis et al., 2011; Botelho et al., 2016). Also, the reorganization of the cortex leads to na aberrant and extreme enhancement of pain (Nurmikko et al., 2016).

The transcranial magnetic stimulation (TMS) measures have proven to be useful to assess cortical physiological processes (e.g., inhibition, excitation). Previously, we found that hyperexcitability in the cortical-spinal pathway as measured by motor evoked potential (MEP) amplitude was positively correlated with the disengagement of the descending pain modulatory system (DPMS) (Botelho et al., 2016). Also, in other studies we showed that an imbalance between inhibitory and excitatory systems as indexed by a decreased cortical silent period (CSP) and an enhancement in the intracortical facilitation (ICF) were correlated with a higher levels of pain catastrophizing (Volz et al., 2013), higher trait anxiety and higher rate of disability due to pain (Vidor et al., 2014). Indeed, these set of results indicate that the primary motor cortex (M1) has turned into a target for assessing either neuroplasticity of the cortical-spinal pathway or, as well the cortical reorganization (Schwenkreis et al., 2011; Leite et al., 2017).

These changes in excitatory and inhibitory transmitter systems are confluent with a reduction in the descending inhibitory pathways (Botelho et al., 2016; Thibaut et al., 2017; Thapa et al., 2018). As aforementioned, the dysfunction in the inhibitory corticospinal system involves several different mechanisms, such as the strength of the glutamatergic synapses or the weakening of synapses of the GABA-ergic system. Actually, in this processes of sensitization, the brain-derived neurotrophic factor (BDNF) secreted by astrocytes and glial cells invert the influxes of the chlorite in GABA-ergic neurons and the GABA-ergic system paradoxically increase the excitability (Binder and Scharfman, 2004). Also, the BDNF sensitizes nociceptive neurons in the spinal cord, and it facilitates the activation of *N*-methyl-D- aspartate (NMDA) (Binder and Scharfman, 2004; Coull et al., 2005). Additionally, cumulative data indicate that BDNF effects are likely to be region-specific due to the fact that in the spinal cord it up-regulates pain pathways while in the hippocampus it down-regulates

synaptogenesis and neurogenesis (Duric and McCarson, 2007). Notably, according to the previous study, the electroacupuncture increased the serum BDNF around threefold compared to sham (Chassot et al., 2015). However, there is still a gap to advance in the comprehension of the BDNF role in the motor cortex excitability on chronic pain and how can it influence the results of therapeutic approaches, such as the electric intramuscular stimulation (IMES).

The IMES is a technique of electroacupuncture used to modulate pain processing in a bottom–up fashion. Although the neuroplasticity processes involved in its effect is not yet well comprehended (Couto et al., 2014; da Graca-Tarragó et al., 2015; Leitch et al., 2017), it has been used to treat some chronic pain conditions, as in MPS and chronic tensional headache) (Couto et al., 2014; Chassot et al., 2015). Among the possible mechanisms involved in its long-term effects points out by the change in the neuroplastic state as assessed by the serum BDNF, the reduced ICF and increased the CSP (Tarragó et al., 2016). However, to date have limited evidence to comprehend the IMES effect in an integrative assessment including neurophysiological measures that evaluate its impact on the ratio of the inhibitory/excitatory system at the cortical level, on the descending pain modulating system function and the BDNF. Thus, we need novel insights into the IMES effect into the neural pathways involved in the pathophysiology of chronic MPS.

We investigated the effect of 10 sessions of IMES to test the primary hypothesis: To provide new insights into the IMES long-term effect on pain and disability related to chronic MPS (primary outcomes). Also, we tested another secondary hypothesis: (i) if the neuroplasticity state at baseline could predict the long-term impact of IMES on disability due to MPS we examined the relationship between the serum brain-derived- neurotrophic-factor (BDNF) and by MEP. (ii) If the IMES could improve the DPMS assessed by the change in the score on Numerical Pain Scale (NPS0-10) at the Conditioned Pain Modulation test (CPM-task) induced by cold water (0–1◦C). (iii) And if it changes the cortical excitability parameters indexed by TMS parameters [MEP, ICF, CSP and short intracortical inhibition (SICI)]. We hypothesize that the IMES analgesia is related to changes at the pain pathways at cortical and infra- cortical levels and that TMS parameters and serum BDNF are reliable markers of neuroplasticity state.

## 2. METHODS

The methods and results are presented according to the CONSORT guidelines (Schulz et al., 2010). The flow chart of the study is displayed in **Figure 1**..

**2.1. Design Overview, settings, and participants**

The Research Ethics Committee approved the protocol of this study at the Hospital de Clínicas de Porto Alegre (HCPA) (Institutional Review Board IRB 100276) following the Declaration of Helsinki. Before participating in this randomized, double-blind, two-group parallel, clinical trial, all patients provided their oral and written informed consent.

Twenty-four right-handed female patients, aged 19–65 years with a diagnosis of MPS in the upper body part were recruited. They should have clinical criteria to MPS (i.e., restricted findings of regional pain, palpable nodules, taut bands, stiffness in the target muscles, as well as the existence of trigger points and tender points). They should report some restrictions for the routine activities due to pain for at least 3 months as assessed using a questionnaire with six specific questions (yes/no). These questions were intended to evaluate interference with personal relationships, work, personal goals, pleasure with activities, and clear thinking (i.e., concentrating, problem-solving, or remembering). To be enrolled, subjects had to give an affirmative answer to one or more of the six questions mentioned above. Moreover, a second independent investigator with more than 10 years of experience to care for patients with chronic MPS confirmed the diagnosis after a standard clinical examination. They should describe the pain as dull, hollow or deep and aggravated during stress. The Neuropathic Pain Diagnostic Questionnaire (DN4) was employed to distinguish neuropathic pain from continuing nociception. To standardize the diagnosis of MPS concerning the severity, only patients with a neuropathic pain component were included (i.e., score equal to or higher than four) on DN4 (Bouhassira et al., 2005).

The exclusion criteria comprised the presence any other pain diagnosis, such as radiculopathy, rheumatoid arthritis, fibromyalgia; previous surgery on the affected areas; constant usage of anti-inflammatory steroids (because they could interfere in TMS measures). Also, patients with oncologic or neurologic disease history, hepatic or kidney insufficiency, and ischemic heart disease or those with criteria to contra-indicated TMS use according to the international guideline were excluded. If they had a history of alcohol or substance abuse during the previous 6 months were also excluded.

----------Insert Fig. 1----------

**2.2. Sample size rationale**

The sample size was estimated based on previous clinical trial (Dao et al., 1991). A sample size of 22 patients divided into two groups of 11 would permit to detect a difference of 1.5 cm in the pain severity reported on the visual analog scale (VAS) of 10 cm, by a standard deviation ($SD$ = 1.2), for a error type I equal to 1% and an error type II equal to 20%, respectively. We included 24 patients (12 per groups) to account for possible dropouts.

### 2.3. Randomization

We used software to generate the sequence of randomization with a fixed block size of 6. Twenty-four patients were randomly allocated to receive treatment (IMES or sham). Before the recruitment phase, opaque brown envelopes containing the protocol were prepared. The envelope contained a numerical code corresponding to the allocated treatment. Each envelope was opened in sequence according to the number that existed externally after the participant agrees to engage in the study. Only the physician responsible for administering the interventions was not blinded to treatment.

### 2.4. Blinding

To guarantee the blinding, during the whole timeline protocol, two investigators who not involved in the patient's evaluations were responsible for handling the randomization code. Also, to reduce the potential to bias the IMES was administered by the same physician (with more than 10 years of practice in acupuncture). Furthermore, the participants were requested to debate treatment details only with the physician who administered the IMES sessions. Pain assessments, psychological tests and measures of cortical excitability by TMS was done by two trained examiners who were blinded to the interventions group.

### 2.5. Interventions

#### 2.5.1. Active electrical intramuscular stimulation (IMES)

The IMES was applied using acupuncture needles with guide tubes, 30 mm in length and 0.25 min diameter (Suzhou Huanqiu Acupuncture Medical Appliance Co. Ltd., 218, China) connected to an electroacupuncture device (Sikuro, São Paulo, Brazil). The current was delivered to IMES in the paraspinal region related to the nerve roots involved in the splenius capitis (C3 and C4) (**Figure 2**) and semispinalis capitis (C2 and C3). The needles were inserted at the paraspinal region at 1.5 cm from the spinous process line (Couto et al., 2014) at the anterior border of the sternocleidomastoid muscle (**Figure 2**). The accessory nerves were stimulated to record a motor response from the trapezius muscle (Fahrer et al., 1974). They received 10 treatment sessions during 20-min at a frequency of 2 Hz (da Graca-Tarragó et al., 2015).

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

#### 2.5.2. Sham of electrical intramuscular stimulation

The same electroacupuncture device (Sikuro, São Paulo, Brazil), described above was used for the sham control condition. However, the output jack plug was broken to avoid no electrical current could move to the patient. We fixed the electrodes at the same spots where active stimulation would be applied. That is, at the paraspinal region at 1.5 cm from the spinous process line, at the border of the sternocleidomastoid muscle and to stimulate the accessory nerves we fixed the electrodes at the anterior border of the trapezius muscle (**Figure 2**). While the stimulator was left in front of the participant for 20 min, to ensure that the flashing diode corresponding to the electrical stimulus was both audible and visible. All patients received ten sessions with 20-min of duration.

### 2.7. Instruments and assessments

### 2.8. Outcomes

The primary outcomes comprise disability due to pain in the Brazilian Profile of Chronic Pain: Screen (B-PCP:S) and the pain reported on the VAS registered in a diary. Secondary outcomes were the analgesics doses used weekly during the treatment period, the change on Numerical Pain Scale (NPS0-10) during the conditioned pain modulation (CPM-task) and heat pain threshold (HPT). Also, we assessed the cortical excitability measures (MEP, ICF, CSP, and SICI), and daily sleep quality. Below, we described the outcomes assessment in details.

***Primary outcomes***

**(**a) Pain intensity was evaluated using a 10-cm VAS. The scores in VAS ranged from zero (no pain) to 10 (worst possible pain). We request to patients to report the pain score in the most part of the time in the last 24 h? So, to increase the compliance, an examiner checked the patients' pain diary during the 12 weeks of follow-up.

(b) To assess the multidimensional pain experience we used the Brazilian Profile of Chronic Pain: Screen (B-PCP:S) (Caumo et al., 2013). The B-PCP:S comprehend three subscales: (i) the subscale to assess the pain severity comprise four items with a possible score extending from 0 to 32). (ii) Subscale to evaluate the pain interference in the routine activities by 6 issues and its score extending from 0 to 36). (iii) Subscale to assessed the emotional burden due to pain by five items and its rating extending from 0 to 25. It was employed at baseline, at the end of the intervention course, and at 2, 4, 6, 8, and 12 weeks after the end of the intervention. The disability related to pain is characterized to be associated with chronic or recurrent discomfort or pain causing restriction (Caumo et al., 2013). Thus, we considered that higher scores on the B-PCP:S indicated higher disability or functionality at home, at work, during social circumstances and when experiencing more significant amounts of the emotional burden**.**

***Secondary outcomes***

(c) Supplementary analgesia use: Patients could use additional analgesic drugs (acetaminophen, ibuprofen, Dorflex® , tramadol or codeine) for pain relief, if necessary. They were authorized used 750 mg of acetaminophen up to four times per day (QID) and 200 mg of ibuprofen up to QID as rescue analgesics. If these drugs were not sufficient, patients could use Dorflex (Sanofi Aventis, São Paulo, Brazil; 35 mg orphenadrine citrate combined with 300 mg dipyrone and 50 mg caffeine). If they persisted with pain, It was allowed to use 60 mg of codeine up to QID or tramadol three times per day (TID). Patients recorded the rescue of analgesic used in a pain diary, which was checked during every intervention session and each visit of the follow-up. For analysis, we considered the total analgesic dose used throughout treatment.

(d) The Quantitative Sensory Testing (QST) assessed the HPT. We used a thermode (30 × 30 mm) in a computer Peltier-based device using the method of limits. A temperature of 32◦C was set with an increased at a 1◦C/s rate, to a maximum of 52◦C. The thermode was fixed to the skin on the ventral surface of the mid- forearm. The HPT was defined as the mean of three following measures of painful temperature. The thermode remained on the left ventral forearm; its position was slightly altered between trials to prevent either response suppression of the cutaneous heat nociceptors or sensitization (Schestatsky et al., 2011).

(e) To assess descending pain modulatory by a heterotopic noxious stimulus (CPM), we set the temperature to the point that each patient rating 6/10 pain on the NPS(0-10). The CPM test was performed 30 s following 1-min immersion of the non-dominant hand in cold water (zero to one degree Celsius). The CPM- task was the score in the NPS 0-10 induced by the QST during the cold-water immersion (QST + CPM) at the temperature produced by the QST that they were rating 6/10. CPM-task test was performed after we measured the parameters of cortical excitability. To control for individual variability, the proportion of difference from baseline was used was used for the analysis (Botelho et al., 2016).

(f) Daily sleep quality was measured by a 10-cm visual analog sleep quality scale (VASQS) which scores ranged from zero (the worst sleep quality) to 10 (the best sleep quality). The following question was asked to patients to answer in their sleep diaries: “How well did you sleep last night compared with your habitual sleep?”

(g) The assessment of the cortical excitability parameters, recordings via surface electromyography placed at the contralateral right first dorsal interosseous muscles using Ag/AgCl electrodes were used. Firstly, the Resting Motor Threshold (RMT) was established by obtaining five MEPs with the peak-

to-peak amplitude of at least 50 μV out of ten successive trials. Afterward, we documented ten MEPs with an intensity of 130% of the individual RMT. Additionally, we determined the CSPs during muscle activity measured by a dynamometer at 20% of the maximal force. Ten CSPs were assessed using an intensity of 130% of the RMT. An inter-stimulus interval of 2 ms was used to evaluate the SICI. We set the conditioning (first) stimulus at 80% of the RMT, and we set the test stimulus at 100% of the individual MEP magnitude. We determined the ICF with an inter-stimulus interval of 12 ms. We measured the paired-pulse of TMS in a randomized arrangement for a total of 30 trials (ten for each control stimuli, ICF, and SICI). Off-line analyzes included the collection of the amplitudes of all of the MEPs, SICI and ICF as well as the extension of the CSPs. The equivalent units for these parameters included MEP in μV, ICF and SICI in their ratio to the MEP, and ms for the CSP (Pascual-Leone et al., 1994).

**Other Instruments and Assessment**

All psychological instruments used in this study had been validated for the Brazilian Portuguese (Caumo et al., 2013; Sehn et al., 2012). Two independent medical investigators blinded to the treatment group assignments were trained to apply the pain scales and to conduct the psychological tests. The patients' baseline depressive symptoms were estimated using the Beck Depression Inventory II (Wang and Gorenstein, 2013). The sleep quality was determined using the Pittsburgh Sleep Quality Index (Bertolazi et al., 2011). Anxiety was assessed using the refined version of the Rash analysis of the State-Trait Anxiety Inventory (STAI) (Kaipper et al., 2010). The pain-related catastrophic thinking was evaluated using the Brazilian Portuguese Catastrophizing Scale (BP-PCS) (Sehn et al., 2012). Demographic data and medical comorbidities were determined using a standardized questionnaire. At the baseline and the end treatment, blood samples were collected to measure to serum BDNF. These blood samples were centrifuged at 4500 × *g* in plastic tubes for 10 min at 4◦C and were stored at −80◦C for hormone assay. An Enzyme-Linked Immunosorbent Assay (ELISA) using a ChemiKine BDNF Sandwich ELISA Kit, CYT306 (Chemicon/Millipore, Billerica, MA, United States) was used to determine the serum BDNF. The lower detection limit of the kit is 7.8 pg/mL for BDNF.

**2.9. Statistical Analysis**

*T*-Tests for independent samples and Chi-squared or Fisher's exact tests were used to compare continuous and categorical variables between intervention groups, respectively. The analysis of the effect of the interventions on the outcomes (VAS for pain scores, B-PCP:S scores, daily sleep quality

and changes on NPS(0- 10) during CPM-task) was determined using a mixed ANOVA model. The independent variables were time, experimental group (IMES or sham), the interaction between time and experimental group, and the subject identification as a within-subject factor. (**Table 2** and **Figures 3**, **4**).

To evaluate the mean difference in the cortical excitability measures (MEP, SICI, ICF, CSP) we calculate the percentage change at baseline to end treatment, which were compared using the Wilcoxon–Mann Whitney. The cortical excitability measures at the end of treatment we lost in one patient of IMES group. Thus, to carry forward analysis using the intention-to- treat approach, we considered the effect observed in the worst case of the respective treatment group (**Table 3**). To assess if the neurophysiological state of the corticospinal pathway and the neuroplastic changes associated with the treatment, we analyze the effect of group in a mixed regression model (**Table 4**). The dependent variable was the cumulative mean B-PCP:S score from baseline to end follow up.

The covariates were the change in the average of serum BDNF expressed in percentage and the baseline MEP. All analysis were adjusted for multiple comparisons by the Bonferroni's test. The data were analyzed using SPSS version 22.0 (SPSS, Chicago, IL, United States).

## 3. RESULTS

### 3.1. Patients characteristics

Patients demographic and clinical features are shown in **Table 1**. Twelve patients were assigned to the sham group, and 12 were allocated to the IMES group. Twenty-three patients completed the study; one patient in the IMES group withdrew due to needle phobia. The baseline characteristics were similar across the IMES and sham groups (all *P*-values > 0.05) (**Table 1**). We did not observe any severe or moderate side effects from the interventions.

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

### 3.2. Primary outcomes: efficacy concerning pain scores and disability

#### 3.2.1. Pain Scores on VAS

After treatment, the IMES group had significantly lower pain scores in the VAS ($P < 0.001$) than the sham group (**Table 2**), and there was no interaction between time and intervention group ($P = 0.92$) (**Figure 2**). Compared to the sham group, the IMES group displayed a relative mean pain reduction of -73.02% (effect size of 0.55) at 12 weeks after the conclusion of the interventions (**Table 2**). Compared to baseline, the superior effect of IMES was also evident: in the second week of treatment the IMES group had a pain reduction of 55.31% compared to baseline.

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

----------Insert Fig. 3 ----------

**3.2.2. Disability due to pain assessed by the B-PCP: S score**

The IMES group had significantly higher improvement in the mean B-PCP:S score ($P$ < 0.000,1) (**Table 2**). There was effect of time and interaction between time and intervention group ($P$ = 0 < 001, for both). The changes on B-PCP:S in follow-up was presented in **Figure 4**. This effect persisted until the 8th week following the end of the intervention, with an effect size of 0.8.

----------Insert Fig. 4 ----------

**3.3. Secondary outcomes**

***Use of analgesics conditioned pain modulation, neurophysiological changes and sleep quality***

**3.3. 1. Use of analgesics**

Analgesic use was reported in 69.4% during the treatment period in the sham group and 30.6% in the IMES group. The relative risk for using analgesic during the 14 weeks (two treatment weeks and 12 weeks of follow-up) was 2.95 (95% CI, 1.36 to 6.30); that is, the sham group used almost threefold additional analgesics. There was a significant decrease in the analgesic doses for patients receiving IMES compared to those receiving sham ($P$ < 0.01).

**3.3. 2. Effects on HPT and conditional pain modulation by heterotopic stimulus**

The IMES increased the HPT (**Table 2**) and induced a 61.27% reduction in the pain scores on the NRS during the evoked pain by QST vs. QST + CPM (**Figure 5**). These findings suggest that the IMES-intervention also induced an effect on the bottom up inhibitory mechanisms.

The scores on numerical pain rating scale before treatment (QST vs. QST + CPM) in IMES and sham group was −1.49 (1.99) vs. −1.77 (3.09) ($P$ > 0.05), respectively. After ten sessions of treatment, the change on the NPS(0-10) during CPM-task in IMES and sham was −2.04 (0.79) vs. −0.94 (1.18), respectively. The difference mean was – 1.25 (−2.07 to −0.18), ($P$ = 0.01).

----------Insert Fig. 5----------

**3.3.3. Neurophysiological changes: assessment of IMES effect on cortical excitability - indexed in TMS parameters**

Compared to the group allocated to sham, the IMES decreased the MEP by 28.79% (**Table 3**) ($P$ = 0.02) and increased the SICI by 37.41% ($P$ = 0.005). However, the IMES did not induce significant changes in ICF and CSP (**Table 2**)

----------Insert Table 3 ----------

### 3.3.4. Assessment of sleep quality

There was no interaction between time and intervention group for the previous night sleep quality compared with the habitual sleep quality based on the VAS-QS scores ($P = 0.004$). However, the IMES improved the VAS-QS by 12.75% in the previous night sleep quality compared with habitual sleep.

### 3.3.5. Neuroplasticity markers that predict a long-term impact of IMES on disability due to pain

One crucial issue is to identify markers associated with the long- term effect on pain and disability assessed by B-PCP:S. To address this critical matter, we run a mixed regression model in which we controlled the change on the B-PCP:S score from the end of treatment for both parameters, MEP at baseline and the change on serum BDNF from baseline to end treatment (**Table 4**).

At the baseline, the serum BDNF in sham and IMES groups was 27.11 (10.97) vs. 26.21 (6.83), respectively. While at end treatment the serum BDNF in the sham and IMES groups was 25.05 (10.45) vs. 31.93 (10.74), respectively. Thus, to adjust to individual characteristics, we calculated the percentage of change from baseline to treatment end. The mean (SD) of the percentage of change in the sham and IMES groups was −6.06 (19.53) vs. 28.74 (25.57), respectively. The Mann–Whitney Test showed that sham group had a significantly lower serum BDNF ($P < 0.01$) compared to IMES when we compared the mean of percentage change from the baseline. Afterward, we ran a multivariate mixed regression model, which showed an interaction between treatment groups (IMES or sham) and BDNF ($P < 0.05$) (**Table 4**). This result suggests that the increase in the BDNF is intervention dependent. Also, it indicates that the change in BDNF induced by IMES may be a marker that predicts the long-term effect of treatment, as assessed by the B-PCP:S and the VAS 12 weeks after end treatment. Our findings showed that more substantial increase in serum BDNF induced by the IMES and that higher excitability in the corticospinal pathway as measured by the MEP at baseline, both were correlated negatively with pain and disability at the end of follow-up. Also, it indicates that IMES effect induced the more significant increase of serum BDNF.

---------- Insert Table 4----------

## 4. DISCUSSION

This study demonstrated that IMES induced a sustained improvement in pain and disability, as well an increase in the inhibition of the corticospinal system as indexed by the MEP amplitude. The effects of IMES improved the HPT, the sleep quality, and the DPMS function. Additionally, we observed that the MEP before treatment and the more significant change in the serum BDNF at the treatment end predicted the long-term effect of IMES in pain and disability at the follow-up end.

These findings extend the knowledge regarding the effects of IMES on pain and disability according to daily pain scores on VAS, the B-PCP: S score (**Table 2**) and reduced analgesic use at follow-up end. Assessed by different ways, these findings also highlight the long-term clinical impact of the bottom-up effects of IMES, which is associated with changes in serum BDNF, as well as neurophysiological and psychophysical measures. Overall, this indicates that neuroplastic changes in the pain pathways mediated the clinical result. The impact of IMES on pain scores is consistent with those of previous randomized clinical trials in which the IMES using a frequency of 2 Hz induced better effects than sham in a short time in MPS (Couto et al., 2014). Also similar to the results of another study, which showed that ten sessions of IMES using a frequency of 2 Hz in MPS improved the DPMS according to changes in scores on the NPS(0–10) during CPM-task (da Graca-Tarragó et al., 2015).

The effect of IMES reduced the MEP amplitude and increased the SICI (**Table 2**). Both indicate a reduction in the excitability of the motor cortex, as well as lower facilitation of the transmission at corticospinal neurons. These results showed that electrical stimulation of peripheral nerves could modulate cells of cortical networks (Miyata and Usuda, 2015). The MEP amplitude reflects the ratios of glutamine/glutamate and GABA/glutamate in the corresponding primary motor cortex. Thereby, larger MEP amplitude indicates hyperactivity in glutamatergic circuits or loss of GABA-ergic activity mediated by GABA-A receptor (Lin et al., 1996; Dall'Agnol et al., 2014). Thus, the current finding highlight that the M1 stimulation permits us to assess if the IMES effect might reduce the facilitation in the corticospinal pathway. Accordingly, IMES seems to be able to modify the inhibitory and excitatory interhemispheric interactions to enhance the neuroplasticity and possibly the balance between excitation and inhibition. Even though the mechanisms underlying of the IMES effect on cortical excitability are unclear, the neuroplasticity process induced by IMES was likely counter-regulated by the disinhibition state at the cortical level and within the interconnections involved in pain modulation. This finding is plausible because there is evidence that the cortex modulates the nociception by projections directly into the spinal dorsal horn neurons and trigeminal nucleus (Millan, 2002). These circuits can also be mediated by indirect projections from the cortex to the dorsal horn through the hypothalamus, amygdala, and PAG (Millan, 2002) or from the secondary somatosensory cortex through thalamus relay (Xie et al., 2009). In fact, these findings indicate that the modulatory effects produced by IMES were not limited to the spinal cord but also occur at distant interconnected sites including the motor cortex.

The present data also extend literature the larger MEP at baseline is inversely correlated with pain severity and disability at follow-up end (**Table 3**). This inverse correlation suggests that MEP at

baseline indicates higher excitability in a cortico- spinal way, that is part of central sensitization concurs to a higher impairment due to pain. This hypothesis is plausible because, in central sensitization (CS), the secretion of BDNF by astrocytes changes Gamma-aminobutyric acid (GABA-ergic) function, where it can induce excitability rather than inhibition. Indeed, the CS is a dysfunction which involves different mechanisms, such as a selective loss of GABAergic interneurons (Moore et al., 2002) and the collapse of the chloride gradient, which is correlated with enhanced excitability in postsynaptic neurons (Coull et al., 2003). Thus, this result suggests that the adjustment of the imbalance between excitability and inhibition is controlled by different neurobiological systems, and perhaps some processes are influenced by IMES. As the MEP is an index of the hyperexcitability in corticospinal pathways, it reflects that the motoneuronal excitability at baseline can predict at some level the clinical effect of IMES at the follow-up end.

In fact, our findings may hold critical clinical implications such as (i) to support an understanding of the bidirectional pathways between peripheral and central brain changes in MPS. (ii) To help to decide on a therapeutic approach based on the neurophysiological phase state of each patient, because a larger MEP amplitude at baseline predicted more considerable improvement in the disability due to MPS. (iii) To improve the understanding of underlying neurophysiological mechanisms of the limitation due to chronic MPS, which could give support to plan neuromodulatory approaches to induce a top–down (i.e., direct current stimulation-tDCS) and bottom-up modulation technique (i.e., dry-needling) or pharmacological interventions.

Also, we observed that the IMES effect increased the SICI by the reorganization of the somatosensory cortex. This hypothesis is acceptable because a similar effect has been demonstrated using electroacupuncture in neuropathic pain (Napadow et al., 2007). Also, electroacupuncture leads to a reduction in inhibitory postsynaptic currents mediated by GABA (Fu and Longhurst, 2009), through a process that is aided by BDNF released from microglia. Accordingly, the electroacupuncture can induce long- term depression (LTD) (Pyne and Shenker, 2008), which is an activity-dependent reduction in the efficacy of neuronal synapses that modify the expression of the postsynaptic receptor NMDA (*N*-methyl-D-aspartic acid) (Pérez-Otaño and Ehlers, 2005). Thereby, IMES may modulate the disinhibition process due to the loss of the postsynaptic potassium chloride cotransporter (KCC2).

This result is clinically relevant and suggests that the increase in serum BDNF levels underlie the therapeutic effect of IMES. This relationship between the increase in BDNF and pain is consistent with evidence provided by a study in patients with chronic tensional headache treated with electroacupuncture (Chassot et al., 2015). Another study patients with MPS found a similar effect in

patients undergone treatment using repetitive (r)TMS sessions (Dall'Agnol et al., 2014). Thereby, these findings give additional neurobiological support for the sustained impact of IMES on pain, and it suggests that the serum BDNF may be a useful marker to predict its therapeutic effects at long-term.

The IMES effect increased the HPT and improved the DPMS as evidenced by the changes in the NPS(0–10) during CPM- task. These findings suggest that the IMES-intervention induces an effect on the bottom–up inhibitory mechanisms. Its impact in the HPT is in agreement with an earlier study, in which IMES stimulation of myofascial trigger points (MTP) produced an increase in pain threshold and greater activation on the dorsal midbrain encompassing periaqueductal gray (PAG) (Niddam et al., 2007). In our findings, we demonstrated that IMES induced enhancement on descending pain control mechanisms (**Figure 5**). Accordingly, it is plausible that it activates the PAG, which is the primary control center for descending pain modulation (Pertovaaraa and Almeida, 2006). Further, our findings showed that IMES improved pain and disability, and these changes were concurrent with the improvement in the neurophysiological parameters (e.g., SICI, MEP and in DPMS [change in the NPS(0–10) during CPM-task]). In fact, these effects are congruent with the idea that the descending pain facilitation is a process mediated by structural plasticity. These changes occur in an activity-dependent manner in the pain pathways, an effect mediated by serotonin, glycine, or GABA neurotransmitters, which likely engage connectivity forces at cortical and infra-cortical levels (Basbaum and Fields, 1978; Cui et al., 1999).

The IMES effect also improved the restorative effect of sleep (**Table 2**) as in agreement with a previous study the IMESimproved sleep quality in a short-term (Couto et al., 2014). In fact, in the present study, the IMES effect on sleep quality presented a small effect size. However, it is possible that IMES changes the sleep quality by indirect effects such as modulation of the sleep/wake cycle secondary to an increase of melatonina secretion (Spence et al., 2004). This benefit may be a secondary effect of the needling procedure, as it could reduce the levels of circulating cytokines as shown in the experimental pain neuropathic model, where its effect decreased the inflammatory mediators (Cha et al., 2012). Thus, according to another evidence of a clinical study, the increase of cytokines could interrupt melatonin secretion by the pineal gland (Spence et al., 2004). Although, this a possible hypothesis to explain this association and further studies are needed before definitive conclusions are drawn.

Some issues concerning the design of our study must be addressed. (i) Our sample comprises only female because exist extensive literature that they are more prone to activation upon negative emotional responses (i.e., stress, fear, and anxiety) as well to physiological factors (i.e., the capability to endure pain) (Keefe et al., 2001; Wiesenfeld-Hallin, 2005). If for one side the homogeneity of this

study population is methodologically advantageous to reduce the effect of potential confounding factors, and thus, permit us to understand the impact of IMES in the corticospinal pain modulation system, it has the disadvantage to restricts the generalizability of results. Therefore, other researches with a higher number of patients are needed to more widely assess the potential benefits of IMES in different clinical settings. Hence, this could give support to therapeutic decision making in clinical settings. (ii) Even though we have included only patients without prior contact with acupuncture, the acupuncturist-physician knew the type of intervention that was applied. Also, IMES produces a muscle movement while sham stimulation not. Hence, the sensory perception could increase the chance of patients guess the type of intervention. However, we must realize that these limitations are intrinsic to technique. (iii) Given we did not formally measure the awareness of the allocation group (either active or sham), this is a limitation that one could consider. Despite these limitations, our outcomes were correlated with neurophysiological and psychophysical parameters, which are measurements less prone to bias. Furthermore, we measured the impact of IMES on pain and disability in the long term; this attenuates the likely impact that this bias could have in our conclusions. The strengths of the study include our assessment that IMES has a direct effect on the neuroplasticity process using markers of neuroplasticity such as BDNF. At the same time, we evaluated its impact on the clinical outcomes related to pain and disability with a follow-up according to the recommendations of the IMMPACT (Dworkin et al., 2005). Thereby, this study represents a significant contribution to evidence-based therapeutics for IMES in the treatment of MPS.

## CONCLUSION

In conclusion, these results revealed that ten sessions of IMES reduced pain score and improved disability due to chronic MPS, as well reduced the analgesic use. They also suggested that the IMES effects on chronic MPS are mediated by bottom– up regulation mechanisms, enhancing corticospinal inhibition and that this effect involves an increase in BDNF secretion. Additionally, they suggest that the MEP amplitude before treatment and the changes induced by the IMES in the serum BDNF predicted it’s the long-term impact on the chronic MPS symptoms.

## AUTHOR CONTRIBUTIONS

IT, FF, and LB were responsible for maintaining the study records. LB and IT participated in the sequence alignment and drafted the manuscript. LB administered the interventions. LA, AD, and AB realized the outcome measures. MZ participated in the sequence alignment. FF participated in the

design of the study and performed the statistical analysis. IT and WC conceived the study, participated in its design and coordination, and helped in drafting the manuscript.

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**Declaration of conflict of interest:**

The authors declare that there are no financial or other relationships that might lead to conflicts of interest to any of the following arrangements: *financial relationship to the work; employees of a company; consultants for a company; stockholders of the company; members of a speakers bureau or any other form of financial compensation.*

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**FG** conceived the study, participated in its design and coordination and helped drafting the manuscript.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Legends

**Figure 1 -** Flow chart showing participants recruitment and progress through the study.

**Figure 2** - Paraspinal intramuscular stimulation using acupuncture needles. Distance from the spinous process line is 1.5 cm at C3-C4 (splenius capitis muscle) ; C5-C6 (semispinalis capitis). Accessory nerves were stimulated in front of the anterior border of the sternocleidomastoid by recording the motor responses from the trapezius muscle (figure 2B).

**Figure 3 -** Weekly mean pain levels (as assessed by VAS) from baseline week (W) zero to W12 in the two experimental groups for the following question: "considering your chronic pain that motivated the treatment - how intense was your worst pain during the last 24 hours?". The error bars indicate the standard error of the mean (SEM). Asterisks (*) positioned above the bars indicate significant differences ($P<0.01$) at those time points between the sham and the IMES groups. All comparisons were performed by a mixed analysis of variance (ANOVA) model, followed by the Bonferroni correction for *post hoc* multiple comparisons.

**Figure 4 -** Weekly mean pain and disability related to pain (as assessed by B-PCP:S) from baseline week (W2, W4, W6, W8 and W12) in the two experimental groups. The error bars indicate the standard error of the mean (SEM). Asterisks (*) positioned above the bars indicate significant differences ($P<0.01$) at those time points between the sham and the IMES groups. All comparisons were performed by an analysis of Variance in the Mixed Model, followed by the Bonferroni correction for post hoc multiple comparisons.

**Figure 5 -** The change in NPS (0-10) during CPM-task, at baseline before intervention and in the end of treatment in the two experimental groups. The error bars indicate standard error of the mean. Asterisk (*) indicates differences between the sham and IMES groups. All comparisons were performed by a mixed analysis of variance model, followed by the Bonferroni test for post hoc multiple comparisons. Numerical Pain Scale (NPS0-10).

**Tables 1**.Characteristics of the study sample. Values are given as the mean (SD) or frequency (n=24).

| | **Placebo-sham (n=12)** | **IMES (n=12)** | ***P*-value** |
|---|---|---|---|
| Age (years) | 46.00 (13.55) | 48.36 (10.97) | 0.66 |
| Education (years) | 13.40 (3.48) | 12.18 (3.60) | 0.44 |
| Smoking (Yes) | 2 (16.67%) | 1 (8.33%) | 0.51 |
| Clinical Comorbidity (Yes) | 7 (58.33%) | 9 (75%) | 0.3 |
| Hypertension | 4 (33.33%) | 2 (16.67%) | |
| Hypothyroidism | 1 (8.33%) | ----- | |
| Other | 2 (16.67%) | 3 (25%) | |
| History of psychiatric disease (Yes) | 6 (50%) | 7 (58.33%) | 0.15 |
| Global pain on visual analogue scale | 5.89 (3.20) | 5.70 (3.49) | 0.9 |
| Pittsburgh Sleep Questionnaire | 17.6 (± 7.6) | 19.0 (± 5.9) | |
| Beck Depression Inventory | 14.40 (8.63) | 16.82 (10.90) | 0.31 |
| State-Anxiety on STAI | 23.80 (18.35) | 22.82 (7.47) | 0.74 |
| Trait-A anxiety on STAI | 26.80 (8.35) | 27.73 (8.93) | 0.92 |
| Brazilian Portuguese Catastrophizing Scale (B-PCS) | 29.00 (15.43) | 28.45 (11.86) | 0.93 |
| Profile of Chronic Pain: Screen for a Brazilian population (B-PCP:S) | 60.58 (14.96) | 60.78 (11.39) | 0.88 |
| Pain intensity reported on B-PCP:S | 24.75 (3.05) | 23.65 (3.80) | 0.47 |
| Interference with activities reported on B-PCP:S | 19.08 (7.26) | 21.70 (8.37) | 0.44 |
| Emotional burden due pain reported on B-PCP:S | 16.10 (7.61) | 16.08 (5.97) | 0.82 |

***Tables 2.*** *Treatment effect on pain, sleep quality, cortical excitability parameters and descendent modulator system between Groups: Mean ± SD, percentage on mean change before (B) to after (A) treatment, mean difference with the confidence interval (95% CI) and effect size(CI) (n=24).*

| Treatment | Mean (SD) before (B) tretament | Mean (SD) after (A) tretament | Percentage on mean change (B to A) $ | Mean difference of percentage change (B to A) placebo-sham vs. IMES | ES | P |
|---|---|---|---|---|---|---|
| **Primary outcomes** | | | | | | |
| Treatment effect on pain outcomes during 12 weeks of follow up period | | | | | | |
| **Pain reported on Visual Analogue Scale** *†* | | | | | | |
| Sham (n=12) | 5.46 (2.32) | 4.01 (2.58) | -26.00 (11.20) | -73.02 (-95.28 to – 52.30) | 0.55 | 0.0001 |
| IMES (n=12) | 5.53 (2.28) | 2.6 (2.40) | -47.02 (5.26) | | | |
| **B-PCP: S score and analgesic doses** *†* | | | | | | |
| **Brazilian Profile of Chronic Pain: Screen (B-PCP:S)** *†* | | | | | | |
| Sham (n=12) | 56.33 (16.23) | 49.89 (14.62) | -11.43 (10.08) | -43.19 (-57.23 to -29.39) | 0.80 | 0.0001 |
| IMES (n=12) | 55.85 (14.63) | 38.11 (19.86) | -31.76 (17.24) | | | |

$ Mean difference in the on mean change before (B ) to after (A) between treatment groups (rTMS vs. placebo-sham).

*† Mixed ANOVA model. Mean difference groups*

*¥ Compared using Wilcoxon-Mann Whitney.*

*Effect size (ES) (Mean difference IMES vs.* Placebo-sham*)/Standard deviation on placebo-sham] 12 weeks after conclude the treatment. The effect size was defined as small if lower than 0.20; moderate if between 0.50–0.60; and large if larger than 0.80.*

**Tables 3.** Treatment effect on sleep quality and cortical excitability parameters between Groups: Mean ± SD, percentage on mean change before (B) to after (A) treatment, mean difference with the confidence interval (95% CI) (n = 24).

| | | | | | |
|---|---|---|---|---|---|
| **Secondary outcomes** | | | | | |
| **Quantitative Sensory Testing ¥** | | | | | |
| Sham (n=12) | 42.31 (3.09) | 41.29 (4.53) | -3.29 (11.27) | 9.81 (0.92 to 18.69) | 0.02 |
| IMES(n=12) | 40.14 (2.67) | 43.03 (2.22) | 6.52 (8.05) | | |
| **Cortical excitability parameters** | | | | | |
| **Motor evoked-potential (MEP) ¥** | | | | | |
| Sham (n=12) | 1.75 (0.64) | 1.69 (0.45) | -4.54 (11.90) | -28.79 (-53.44 to -4.15) | 0.02 |
| IMES (n=12) | 2.26 (0.52) | 1.84 (0.74) | -33.34 (35.52) | | |
| **Intracortical facilitation (ICF)¥** | | | | | |
| Sham (n=12) | 1.10 (0.16) | 1.09 (0.28) | -8.78 (33.50) | 10.23 (-22.83 to 43.07) | 0.61 |
| IMES (n=12) | 1.14 (0.32) | 1.25 (0.38) | 1.45 (38.37) | | |
| **Short Intracortical inhinibition (SICI) ¥** | | | | | |
| Sham (n=12) | 0.35 (0.14) | 0.30 (0.18) | -28.03 (35.64) | 37.41 (9.24 to 65.69) | 0.005 |
| IMES (n=12) | 0.29 (0.15) | 0.29 (0.10) | 9.39 (22.98) | | |
| **Current silent period (CSP) ¥** | | | | | |
| Sham (n=12) | 71.64 (17.89) | 68.69 (13.64) | -5.16 (23.41) | 14.50 (-30.89 to 29.60) | 0.60 |
| IMES (n=12) | 69.64 (21.27) | 69.81 (19.50) | -5.30(42.38) | | |
| **Sleep quality *(Treatment effect during 12 weeks of follow up period). †*** | | | | | |
| **How well did you sleep last night – on visual analogue sleep quality scale (VASQS).** | | | | | |
| Sham (n=12) | 4.80 (2.56) | 6.03 (2.03) | 25.62 | 12.75 (4.65 to 21.02) | 0.004 |
| IMES(n=12) | 4.90 (1.4) | 6.78 (1.90) | 38.37 | | |

*Quantitative Sensory Testing (QST)*

*$ Mean difference in the on mean change before (B ) to after (A) between treatment groups (IMES vs. sham).*

*† Mixed ANOVA model. Mean difference groups*

*¥ Compared using Wilcoxon-Mann Whitney.*

**Tables 4.** Markers that predict the long term effect of treatment on pain and disability assessed in a multivariate mixed regression model (n=24).

| **Dependent variable: Brazilian Profile of Chronic Pain: Screen (B-PCP:S)** | | | | | |
|---|---|---|---|---|---|
| **Parameter** | ***β*** | ***SEM*** | ***t*** | ***P*** | ***95% CI*** |
| Intercept | 89.14 | 8.487 | 10.503 | .000 | 72.33 to 105.94 |
| ***Treatment group*** | | | | | |
| IMES | -9.89 | 2.865 | -3.454 | .001 | (-15.57 to -4.22) |
| Sham | 0[b] (reference) | | | | |
| Motor evoked potential (MEP) at baseline | -0.61 | 0.153 | -4.03 | .000 | (-0.58 to -0.26) |
| (BDNF)[&] | -0.08 | 0.132 | -0.70 | 0.48 | (-0.33 to 0.16) |
| ***Interaction between Change on serum brain derivate neural factor (BDNF)*** [&] ***vs. treatment group*** | | | | | |
| IMES* BDNF[&] | 0.67 | 0.29 | 2.24 | 0.02 | (0.07 to 1.26) |
| Sham * BDNF[&] | 0[b] (reference) | | | | |

*Confidence interval (CI);* Electrical intramuscular stimulation (IMES)

[&]*Change on serum brain-derived-neurotrophic factor (BDNF) [(BDNF pre-treatment – BDNF after treatment)/ BDNF pre-treatment]*100*

**Figure 1:** Flow chart showing participants recruitment and progress through the study

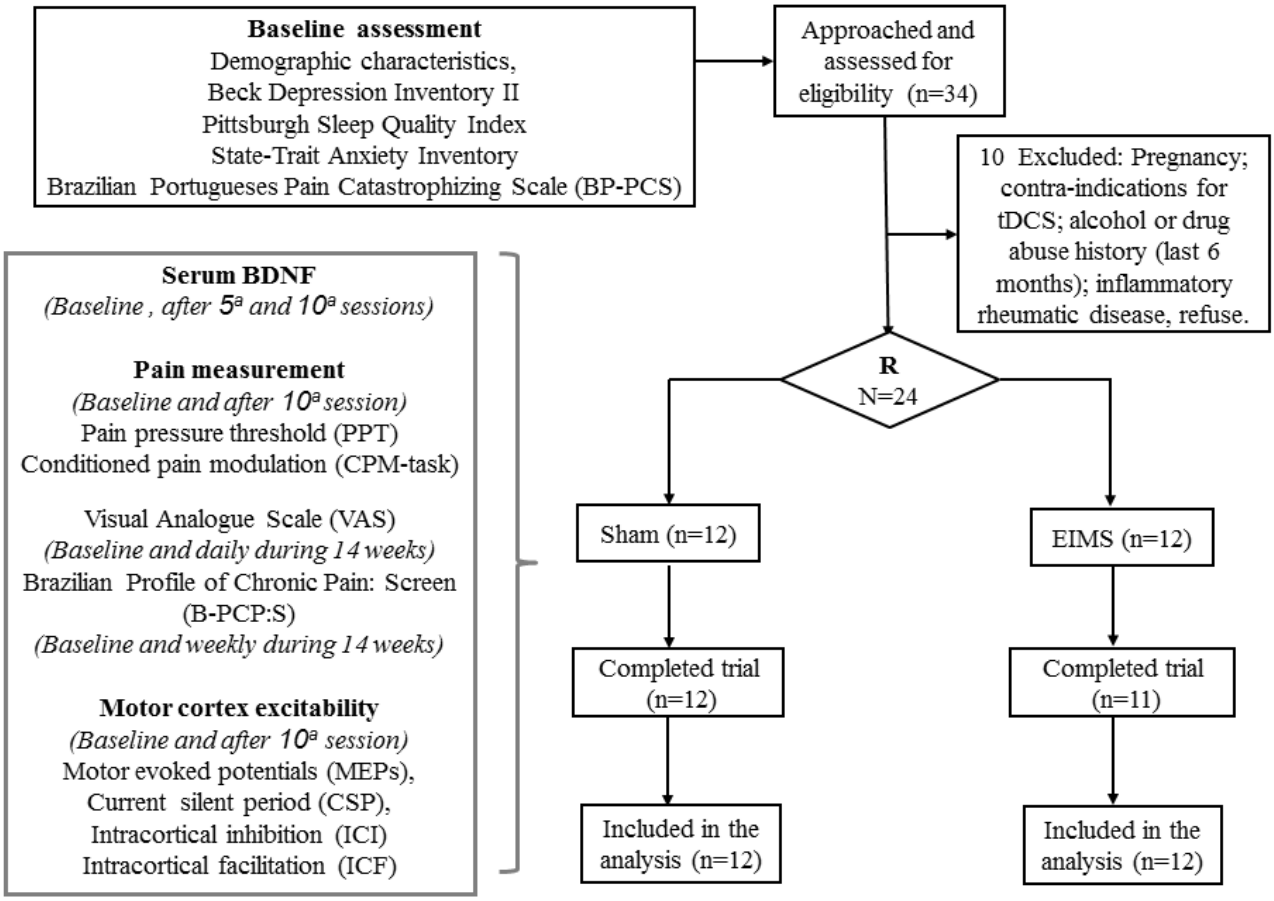


**Figure 2:** Paraspinal intramuscular stimulation using acupuncture needles. Distance from the spinous process line is 1.5 cm at C3-C4 (splenius capitis muscle and trapezius muscle); C5-C6 (splenius cervicis) and upper portion of the trapezius muscle at level of C7

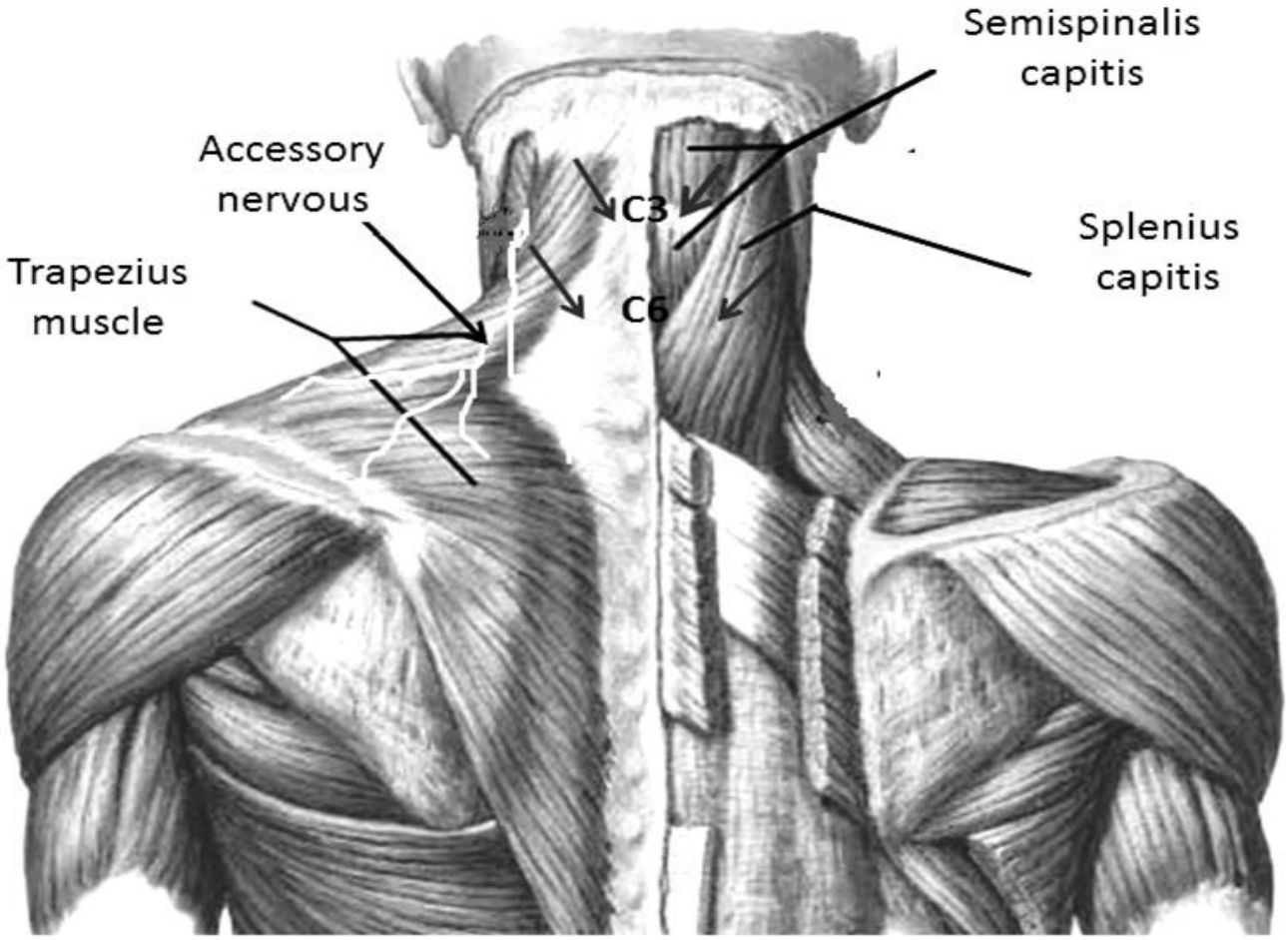


**Figure 3:** Weekly mean pain levels (assessed by VAS) from baseline week (W) zero to W12 in the two experimental groups for the following question: "considering your chronic pain that motivated the treatment - how intense was your worst pain during the last 24 hours

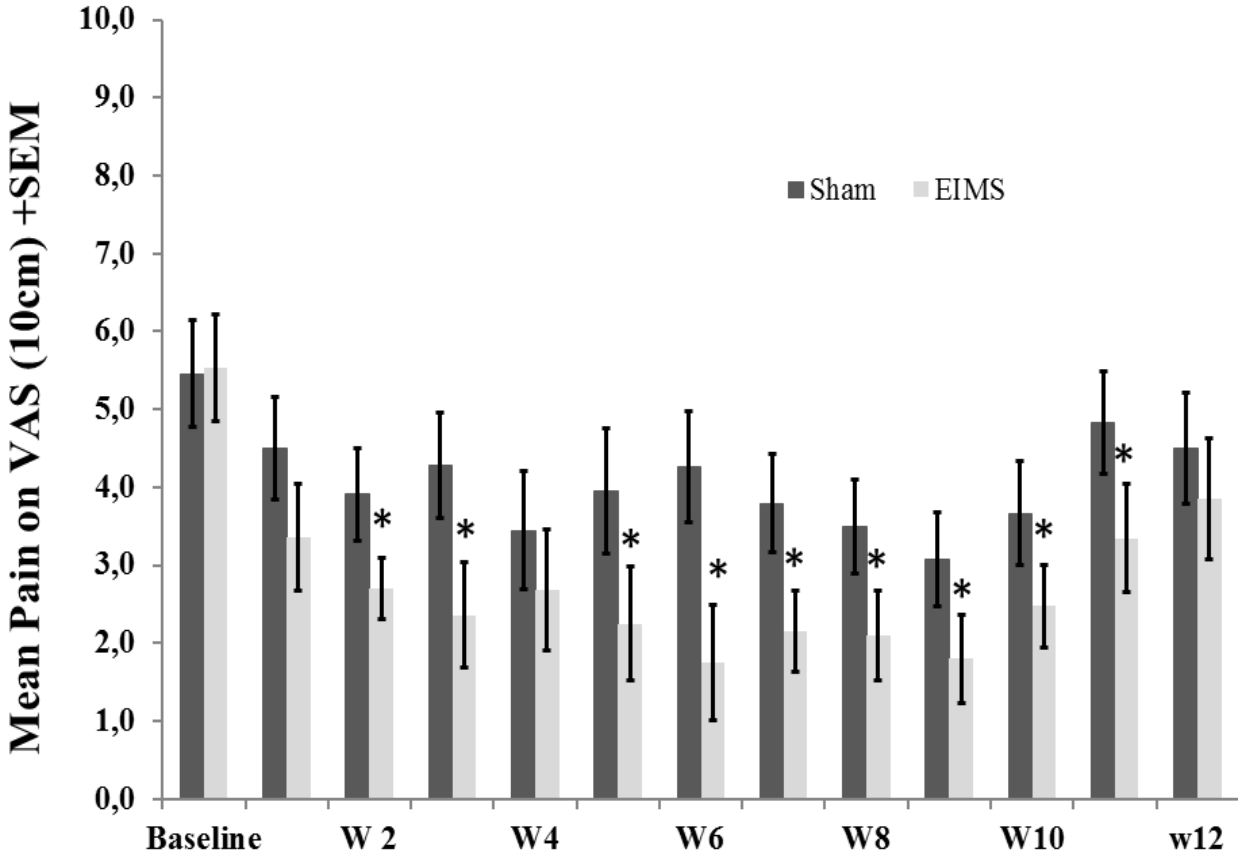


**Figure 4:** Weekly mean pain and disability related to pain (assessed by B-PCP:S) from baseline week (W2, W4, W6, W8 and W12) in the two experimental groups

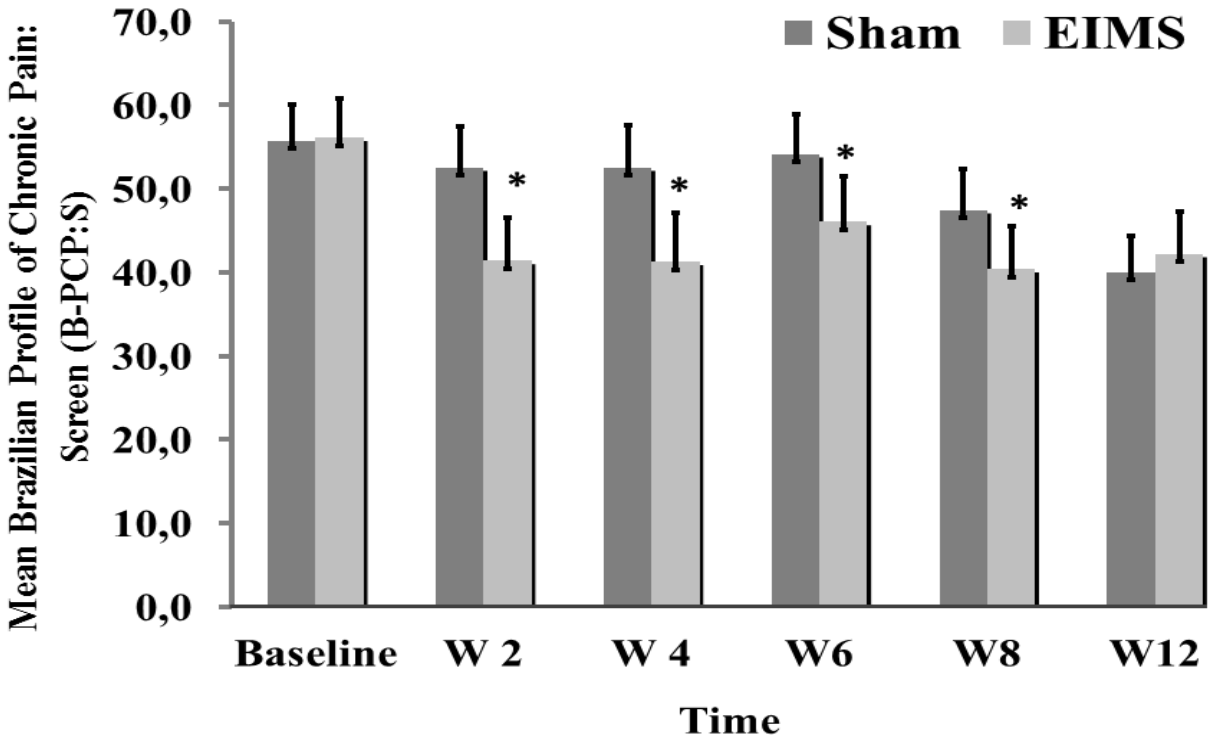
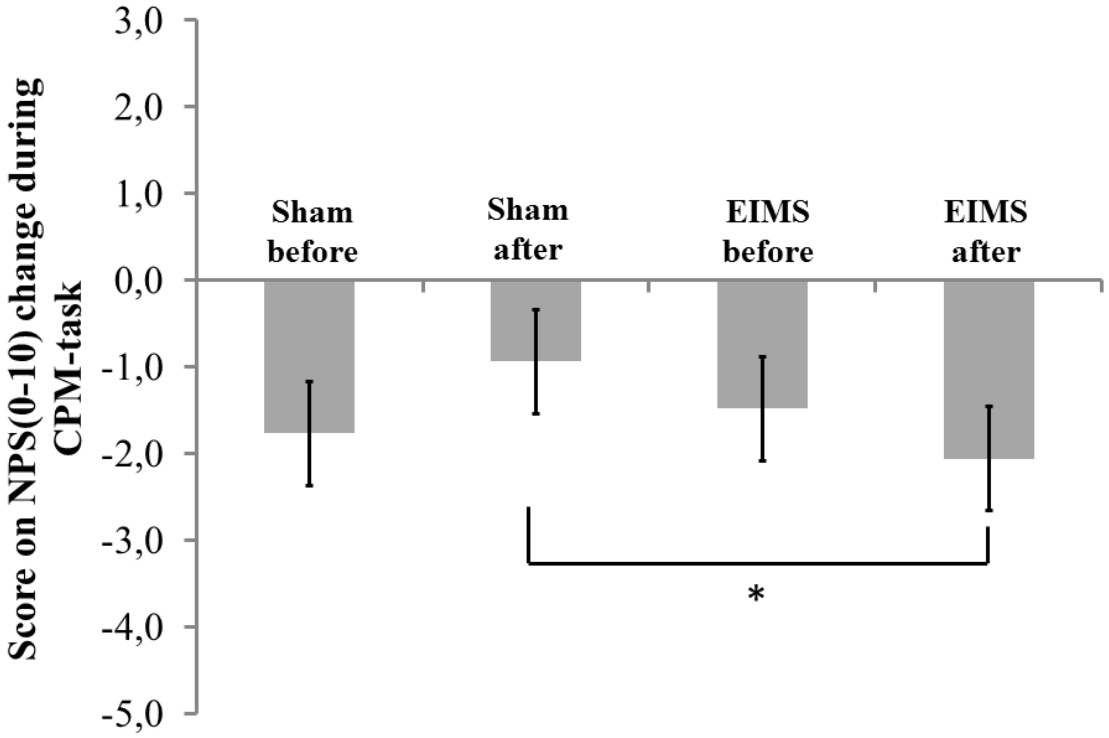


**Figure -** The change in NPS (0-10) during CPM-task, at baseline, before intervention and at the end of treatment, for the two experimental groups



## 9 CONSIDERAÇÕES FINAIS

Esses resultados sugerem que pacientes com MPS cursando com altos graus de disfunção relacionada a dor, provavelmente devido mecanismos neuroplásticos mal adaptativos, apresentam uma disrrupção nos sistemas inibitórios descendentes da dor e maior excitabilidade corticoespinal além níveis séricos do BDNF mais elevados. Também podemos concluir que 10 sessões de IMES reduziram a dor, melhoraram a funcionalidade nessa população de pacientes provavelmente por agir de forma aferente (*botton-up*) nos mecanismos inibitórios descendentes envolvendo, a produção do BDNF. Além disso o CPM-task demonstrou ser um teste relativamente rápido que pode ajudar a identificar essa disfunção e contribuir para a melhor escolha terapêutica desses pacientes.

## 10 PERSPECTIVAS FUTURAS

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Ainda que pudemos observar uma magnitude de efeito robusta da IMES no tratamento da MPS, os achados destes estudos correspondem apenas a parte dos mecanismos de ação desta técnica neuromodulatória. Estudos futuros associando instrumentos de medida que possam mapear a conectividade das redes corticais e infracorticais (ex. espectroscopia por infravermelho; ressonância magnética funcional) e atividade eletrofisiológica (ex. EEG quantitativo) podem contribuir para a melhor compreensão desses mecanismos nessa e em outras síndromes dolorosas. Também se a associação desta técnica a outras que atuem de maneira *top-down* (TDCS, rTMS) nestas e em outras vias do sistema nervoso possam apresentar resultados ainda mais intensos ou duradouros.

O Grupo de Pesquisa em Dor e Neuromodulação do Hospital de Clínicas de Porto Alegre, cujos estudos estão orientados à investigação dos mecanismos neuroplásticos, ao diagnóstico e às intervenções terapêuticas e sua intercessão com técnicas farmacológicas, não farmacológicas e neuromodulatórias, no contexto da dor crônica e aguda, se empenha em explorar essa área das neurociências

# 11 ANEXOS E/OU APÊNDICES

## 11.1 TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

**NOME DO ESTUDO:** TRATAMENTO DA SÍNDROME DOLOROSA MIOFASCIAL COM ESTIMULAÇÃO ELÉTRICA INTRA-MUSCULAR: ENSAIO CLÍNICO RANDOMIZADO DUPLO-CEGO *SHAM* CONTROLADO
Número do protocolo:_____
Instituição: Hospital de Clinicas de Porto Alegre- HCPA
Pesquisador Responsável: Dr. Wolnei Caumo – 9981-3977
Comitê de pesquisa e Ética em saúde do HCPA: (51) 3359-8304

O Sr(a) está sendo convidado (a) a participar do estudo: **"*Tratamento Da Síndrome Dolorosa Miofascial Com Estimulação Elétrica Intramuscular: Ensaio Clínico Randomizado Duplo-Cego Sham Controlado*".** Este estudo pretende avaliar o alívio de sua dor através da IMES.

**1. OBJETIVOS DO ESTUDO**

O objetivo primário do presente estudo é verificar a relação entre a estimulação elétrica intramuscular (IMES), limiares de dor, capacidade funcional e qualidade de vida em indivíduos com dor crônica miofascial do complexo craniocervicomaxilar

.

**2. EXPLICAÇÃO DOS PROCEDIMENTOS**

Para participar da pesquisa, será necessário que o senhor (a) responda várias perguntas antes do exame e durante o exame. Também existem perguntas que deverão ser respondidas em casa durante o período de tratamento.

Será necessário coletar duas amostra de sangue que avaliam a produção de hormônios e marcadores da função dos mecanismos de defesa do organismo para evitar que o Sr(a) sinta dor. O sangue será coletado sempre às 10hs. O volume de sangue será de 10 ml a cada coleta, o equivalente a duas colheres de sopa. As amostras de sangue serão coletadas antes de iniciar o tratamento e ao final do mesmo.

**3. TRATAMENTOS**

Neste estudo o senhor(a) poderá ser sorteado para um grupo para receber um dos tratamentos para aliviar sua dor. Conforme segue:

a) **Eletroestimulação periférica (IMES):** neste tratamento será aplicado um estímulo elétrico na área em que o Sr(a) sente dor ou próximas a ela. Este tratamento levará o tempo de 20 minutos.
b) **Grupo placebo**: este grupo receberá um tratamento chamado placebo em que o estímulo não provocará nenhum efeito.

O Sr(a) poderá ser sorteado para um dos 2 grupos:
Grupo 1: receberá eletroestimulação Periférica
Grupo 2: receberá placebo

Nem o Sr(a) nem a Profissional que lhe aplicará os questionários saberão qual tratamento o Sr(a) recebeu.

**3. POSSÍVEIS RISCOS E DESCONFORTOS**

A coleta de sangue poderá causar uma mancha escura no local da picada da agulha em seu braço ou um dolorimento local, desaparecendo após alguns dias.

Em alguns casos também podem aparecer algumas equimoses, manchas vermelho acastanhada na região do pescoço devido aos tratamentos e que desaparecem em poucos dias.

**4. POSSÍVEIS BENEFÍCIOS DESTES ESTUDOS**

O tratamento que será avaliado visa diminuir a dor e melhorar a qualidade de vida. Sendo essa hipótese verdadeira, a IMES poderá ser uma alternativa para o tratamento desse tipo de dor crônica miofascial. Com os resultados deste estudo poderemos obter informações importantes, sobre o quanto estes tratamentos poderão beneficiar outros pacientes com quadros de dor semelhantes ao seu.

**5. EXCLUSÃO DO ESTUDO**

O investigador responsável poderá excluí-la do estudo, sem o seu consentimento, quando julgar necessário, para o melhor encaminhamento do seu caso ou se o senhor (a) não cumprir o programa estabelecido.

**6. DIREITO DE DESISTÊNCIA**

O senhor (a) pode desistir de participar a qualquer momento da pesquisa. Sua decisão de não participar ou de deixar a pesquisa depois de iniciada não prejudicará o seu tratamento.

**7. PRIVACIDADE**

O Sr(a) não será identificado. Todas as informações obtidas no estudo serão mantidas em sigilo e o seu anonimato será preservado. Os resultados deste estudo poderão ser publicados com finalidade científica de forma anônima.

**8. CONTATO DOS PESQUISADORES**

Caso o senhor (a) tenha alguma dúvida poderá entrar em contato com os pesquisadores através dos telefones: Profº Dr Wolnei 9981-3977 (2º andar do HCPA Laboratório de Dor e Neuromodulação-sala 2201E – telefone 3359-8083) e Dr Leonardo Botelho (51) 9888-6550 ou ainda com o Comitê de Ética do Hospital de Clínicas este é um órgão composto por profissionais de diferentes áreas de conhecimento e por representantes da comunidade, são responsáveis pela avaliação ética e metodológica dos projetos de pesquisa que envolvam seres humanos - telefone 3359-8304.

**9. RESSARCIMENTO DE DESPESAS**

O senhor (a) não terá despesas com a sua participação na pesquisa.

**10. ASSISTÊNCIA INTEGRAL**- Será garantido ao Sr(a) acompanhamento, tratamento ou orientação, assistência integral e indenização durante sua participação na pesquisa. Em caso de dúvidas ou necessidade o Sr (a) poderá telefonar para os responsáveis pela pesquisa. Os números e nomes se encontram no item 8. Contato com os pesquisadores.

**11. ARMAZENAMENTO DE MATERIAIS BIOLÓGICOS**

O sangue coletado será avaliado no Laboratório de Pesquisa Experimental do Hospital de Clínicas de Porto Alegre. Este material não será armazenado nem utilizado em pesquisas futuras. Caso o senhor (a) tenha interesse em saber informação como, por exemplo, resultados de exames para acompanhamento clínico estes lhes serão fornecidos.

**12. CONSENTIMENTO**

Este termo de Consentimento Livre e Esclarecido será fornecido uma via para o Sr(a) e uma via será arquivada pelo pesquisador, sendo as duas vias assinadas e rubricadas todas as páginas por ambos.

Declaro ter lido – ou me foi lido – as informações acima antes de assinar este formulário. Foi-me dada ampla oportunidade de fazer perguntas, esclarecendo plenamente minhas dúvidas. Por este instrumento, torno-me parte, voluntariamente, do presente estudo.

Telefones para contato:_____

Nome do paciente: _____

Assinatura do paciente: _____

Nome do pesquisador responsável: _____

Assinatura do pesquisador responsável: _____

Porto Alegre,____de _____de 2015.

## 11.2 STROBE STATEMENT—CHECKLIST OF ITEMS THAT SHOULD BE INCLUDED IN REPORTS OF *CROSS-SECTIONAL STUDIES* (ARTIGO 1)

| | Item No | Recommendation | Nº Pag |
|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | 67 |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | 68 |
| **Introduction** | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 69-70 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 70 |
| **Methods** | | | |
| Study design | 4 | Present key elements of study design early in the paper | 71 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 70-72 |
| Participants | 6 | (*a*) Give the eligibility criteria, and the sources and methods of selection of participants | 71 |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 72-74 |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 72 |
| Bias | 9 | Describe any efforts to address potential sources of bias | 75-76 |
| Study size | 10 | Explain how the study size was arrived at | 71-72 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 74-75 |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | 74-75 |
| | | (*b*) Describe any methods used to examine subgroups and interactions | 74-75 |
| | | (*c*) Explain how missing data were addressed | n.c |
| | | (*d*) If applicable, describe analytical methods taking account of sampling strategy | n.a. |
| | | (*e*) Describe any sensitivity analyses | n.a. |
| **Results** | | | |
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | n.a. |

| | | | |
|---|---|---|---|
| | | (b) Give reasons for non-participation at each stage | n.a. |
| | | (c) Consider use of a flow diagram | n.c. |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 94 |
| | | (b) Indicate number of participants with missing data for each variable of interest | 76 |
| Outcome data | 15* | Report numbers of outcome events or summary measures | 76 |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 95-100 |
| | | (*b*) Report category boundaries when continuous variables were categorized | n.a. |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | n.a. |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | n.a. |
| **Discussion** | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 78 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 82 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 82 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 81-83 |
| **Other information** | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 83 |

*Legenda: n.a.- Não se aplica; n.c. – Não consta

## 11.3 CONSORT 2010 CHECKLIST OF INFORMATION TO INCLUDE WHEN REPORTING A RANDOMISED TRIAL. ( ARTIGO 2)

| Section/Topic | Item no | Standard CONSORT Checklist item | Page no. |
|---|---|---|---|
| **Title and abstract** | 1a | Identification as a randomised trial in the title | 104 |
| | 1b | Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts) | 106 |
| **Background and objectives** | 2a | Scientific background and explanation of rationale | 107-108 |
| | 2b | Specific objectives or hypotheses | 108 |
| **Trial design** | 3a | Description of trial design (such as parallel, factorial) including allocation ratio | 109 |
| | 3b | Important changes to methods after trial commencement (such as eligibility criteria), with reasons | n.a |
| **Participants** | 4a | Eligibility criteria for participants | 109 |
| | 4b | Settings and locations where the data were collected | 111 |
| **Interventions** | 5 | The interventions for each group with sufficient details to allow replication, including how and when they were actually administered | 110-111 |
| **Outcomes** | 6a | Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed | 111-113 |
| | 6b | Any changes to trial outcomes after the trial commenced, with reasons | n.a. |
| **Sample size** | 7a | How sample size was determined | 109 |
| | 7b | When applicable, explanation of any interim analyses and stopping guidelines | n.a. |

| | | | |
|---|---|---|---|
| **Sequence generation** | 8a | Method used to generate the random allocation sequence | 110 |
| | 8b | Type of randomisation; details of any restriction (such as blocking and block size) | 110 |
| **Allocation concealment mechanism** | 9 | Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned | 110 |
| **Implement-ation** | 10 | Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions | 110 |
| **Blinding (masking)** | 11a | If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how | 110 |
| | 11b | If relevant, description of the similarity of interventions | 110-111 |
| **Statistical methods** | 12a | Statistical methods used to compare groups for primary and secondary outcomes | 113-114 |
| | 12b | Methods for additional analyses, such as subgroup analyses and adjusted analyses | 114 |
| | | | |
| **4Participant flow (a diagram is strongly recommended)** | 13a | For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome | 114 |
| | 13b | For each group, losses and exclusions after randomisation, together with reasons | 114 |
| **Recruitment** | 14a | Dates defining the periods of recruitment and follow-up | n.c. |
| | 14b | Why the trial ended or was stopped | n.a. |
| **Baseline data** | 15 | A table showing baseline demographic and clinical characteristics for each group | 130 |
| **Numbers analysed** | 16 | For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups | 131-134 |

| | | | |
|---|---|---|---|
| **Outcomes and estimation** | 17a | For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval) | 131-134 |
| | 17b | For binary outcomes, presentation of both absolute and relative effect sizes is recommended | 131-134 |
| **Ancillary analyses** | 18 | Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory | 134 |
| **Harms** | 19 | All important harms or unintended effects in each group (for specific guidance see CONSORT for harms) | 114 |
| **Limitations** | 20 | Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses | 119-120 |
| **Generalisability** | 21 | Generalisability (external validity, applicability) of the trial findings | 119-120 |
| **Interpretation** | 22 | Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence | 116-120 |
| **Registration** | 23 | Registration number and name of trial registry | 109 |
| **Protocol** | 24 | Where the full trial protocol can be accessed, if available | n.c. |
| **Funding** | 25 | Sources of funding and other support (such as supply of drugs), role of funders | 121 |

Legenda: n.a. – Não se aplica; n.c. – Não consta