FACULDADE DE MEDICINA

PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA: CIÊNCIAS MÉDICAS

TESE DE DOUTORADO

EFEITO DA ESTIMULAÇÃO TRANSCRANIANA DE CORRENTE CONTÍNUA E DA ELETROESTIMULAÇÃO INTRAMUSCULAR NA DOR, NA CAPACIDADE FUNCIONAL E NA EXCITABILIDADE CORTICAL DE PACIENTES COM OSTEOARTRITE

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Porto Alegre

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UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

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“A chuva

Flocos líquidos transparentes descem

Pequenos, leves, inofensivos,

Um,depois mais um,depois tantos crescem

Que a terra toda alagam, e os rios

Se elevam, veias da natureza,

E levam a vida a todos os seres.

Ah, quem me dera ter essa firmeza:

De gota em gota construir saberes.”

Cícero Galeno Urroz Lopes

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RESUMO

Introdução: A osteoartrite de joelhos (KOA) apresenta alta prevalência, principalmente em mulheres. Com o envelhecimento da população esta prevalência irá aumentar. Os tratamentos conservadores apresentam limitada eficácia em expressivo número de pacientes no curso do tratamento . A cirurgia de protetização apresenta altos custos, possibilidade de complicações pós-operatórias graves e ainda que a correção anatômica seja perfeita, em torno de 20% dos pacientes persistem com dor crônica pós-operatória. Portanto, é preciso avançar no conhecimento dos mecanismos fisiopatológicos e estudar novas abordagens terapêuticas para agregar às existentes, visando melhor manejo da dor e para restabelecer a função de maneira mais efetiva. Estas questões motivaram três questões centrais que origiram os três estudos que compõem esta tese.

Estudo I:

No primeiro estudo avaliamos os mecanismos pelos quais há perpetuação da dor na KOA**.** Para responder a esta questão buscou respostas aos seguintes objetivos: I) Comparar se a função da via da dor inibitório descendente está associada com o estado de inibição no sistema corticospinal, indexado pelo potencial evocado motor (MEP) e o período de silêncio cortical (CSP) em pacientes com KOA e controles saudáveis. II) Determinar se há correlação entre as medidas de inibição intracortical (CSP, MEP) com alterações na escala de dor numérica (NPS 0-10) na KOA durante a tarefa de modulação condicionada de dor (CPM-*task*) considerando o efeito da capacidade funcional auto-relatada avaliada pelo Western Ontário and McMaster Universities Index (WOMAC) e uso de analgésicos. **Métodos:** Estudo transversal, foram incluídas 21 pacientes femininas com KOA e 10 controles saudáveis com idade entre 19 a 75 anos. Os parâmetros de excitabilidade do córtex motor (MEP e CSP) foram avaliados utilizando a estimulação magnética trasncraniana (EMT). Avaliação de dor e a incapacidade pelo WOMAC e a NPS (0-10) durante a CPM-*task*. **Resultados:** A média ajustada (DP) do CSP observada em pacientes com OA foi 23,43% menor do que em indivíduos saudáveis [54,54 (16,10) vs. 70,94 (22,87)], respectivamente (P = 0,01). A função do sistema modulador descendente de dor avaliado pela alteração do NPS (0-10) durante o CPM-*task* foi negativamente correlacionada com o parâmetro de excitabilidade cortical indexado pelo CSP (P = 0,001). O CSP foi negativamente correlacionado com a dor e incapacidade avaliada pelo índice WOMAC. **Conclusão:** Foi observado um sistema inibitório descendente de dor enfraquecido, corroborando com os achados em outras patologias de dor crônica.

Estudo II

O segundo estudo buscou determinar se na KOA, uma sessão de IMS (eletroestimulação intramuscular) ativa comparada com *sham* promove um efeito nos parâmetros de excitabilidade do córtex motor [MEP, inibição intracortical curta - SICI, facilitação intracortical (ICF) e CSP] e nas medidas de dor [limiar de dor a pressão (PPT); escala visual analógica de dor (VAS) e mudança na escala de dor numérica (NPS0-10) durante a CPM-*task*]. Esse estudo também se propôs a determinar se o fator neurotrófico derivado do cérebro (BDNF) sérico medeia o efeito desta estimulação no sistema cortico-espinhal, tal como avaliado pelo MEP e pelo PPT. **Métodos:** Foram incluídas 26 mulheres com KOA, com idade entre 50 a 75 anos. Elas foram divididas randomicamente para receber uma sessão de 30 minutos de IMS ativa (n = 13) ou IMS sham (n = 13) por meio de eletroestimulação com frequência de 2 Hz. As agulhas foram inseridas paravertebrais em nível da saída das raízes lombares de L1 a S2 e nos músculos cuja inervação corresponde a essas raízes e que sustentam a articulação do joelho (vasto medial, reto anterior, vasto lateral, tibial anterior e inserção da pata anserina). Os desfechos foram as medidas de dor (VAS, PPT, NPS durante CPM-*task*) e parâmetros de excitabilidade (MEP, CSP, SICI, ICF) realizados antes e imediatamente após a intervenção. **Resultados:** a IMS ativa comparado com *sham* diminuiu o MEP em 31,61% [intervalo de confiança (IC) 95%, 2,34-60,98]. Para os resultados secundários, IMS reduziu o ICF e aumentou o CSP. A IMS melhorou a dor relatada no VAS, o PPT e a pontuação do NPS (0-10) durante a CPM-*task*. O BDNF foi negativamente correlacionado com o PPT (r = 20,56). **Conclusão:** Obtivemos resultados demonstrando melhora da dor e reforço do sistema cortico-espinhal inibitório comparado ao tratamento *sham* com IMS.

Estudo III

O terceiro estudo buscou: 1) Avaliar se a utilização da ETCC (estimulação transcraniana de corrente contínua) combinada a IMS pode promover um resultado melhor de modulação da via cortico-espinhal de dor através da potenciação dos efeitos dos dois tratamentos; comparado a cada um deles isoladamente e ao tratamento *sham*. 2) Avaliar a capacidade da ETCC em reforçar o sistema inibitório descendente de dor e modular a excitabilidade neuronal através da VAS, PPT e NPS durante CPM-*task*. Além disso, avaliamos se o BDNF sérico poderia prever o efeito da terapia no final do tratamento. **Métodos:** 60 mulheres de 50 a 75 anos. Randomizadas em um de quatro grupos: ETCC+IMS, ETCC+IMS *sham*, ETCC *sham*+IMS, ETCC *sham*+IMS *sham*. Receberam 5 sessões de tratamento: ETCC anodal, lado contrário ao joelho acometido, 2mA, 30 min. IMS: estimulação

com freqüência de 2Hz, 30 min; agulhas colocadas a 2cm de L1 á S2, nos músculos vasto medial, vasto lateral, reto anterior, tibial anterior e na inserção da pata anserina. **Resultados:** O a-tDCS + a-IMS mostrou os melhores resultados com diferença significativa na dor (VAS) [média (DP) relacionadas ao tratamento (pós e pré): 0.46 (0.04) vs. 6.32 (1.97); 95%CI -5.42 (-8.24 to -4.36), p=.003] e funcionalidade. Esse resultado iniciou na primeira sessão e manteve-se ao longo do estudo. A-tDCS+a-IMS foi o único capaz de modificar o sistema inibitório descendente de dor. **Conclusão:** Obtivemos melhora da dor e capacidade funcional com IMS, ETCC e ETCC+IMS. Mas somente o grupo de tratamento ETCC+IMS demonstrou capacidade de modificação do sistema inibitório descendente de dor.

Palavras-chave: estimulação elétrica intramuscular, estimulação transcraniana de corrente contínua, estimulação magnética transcraniana, excitabilidade cortical, limiar de dor a pressão, modulação condicionada de dor.

ABSTRACT

Background: Knee osteoarthritis (KOA) has a high prevalence, especially in women. With the aging of the population this prevalence will increase. Conservative treatments have limited efficacy in expressive number of patients in the course of the treatment. The total knee replacement surgery presents high costs, possibility of serious postoperative complications and although the anatomical correction is perfect, around 20% persist with chronic postoperative pain. Therefore, it's necessary to advance in the knowledge of pathophysiological mechanisms and to study new therapeutic approaches to add to the existing ones, aiming to better manage pain and to restore function more effectively. These questions motivated three central questions that originated the three studies that compose this thesis.

Study I

In the first study we evaluated the mechanisms by which there is perpetuation of pain in knee osteoarthritis and to answer this question sought to answer the following objectives: I) To compare if the function of the descending inhibitory pain pathway is associated with the state of inhibition in the corticospinal system, indexed by the motor evoked potential (MEP) and the cortical silent period (CSP) in patients with KOA and healthy controls. II) To determine if there is a correlation between the intracortical inhibition measures (CSP, MEP) with changes in the numerical pain scale (NPS 0-10) in the KOA during the task of conditioned pain modulation (CPM-task) considering the effect of the self-reported function evaluated by the Western Ontario and McMaster Universities Index (WOMAC) and the use of analgesics. **Methods:** A cross-sectional study included 21 female patients with KOA and 10 healthy controls aged 19-75 years old. Motor cortex excitability parameters (MEP and CSP) were assessed using transcranial magnetic stimulation (TMS). Pain assessment and disability by WOMAC and NPS (0-10) during the CPM-task. **Results:** The adjusted mean (SD) of CSP observed in patients with OA was 23.43% lower than in healthy subjects [54,54 (16,10) vs 70.94 (22.87)], respectively (P = 0.01). The function of the descending pain modulatory system evaluated by the NPS (0-10) change during the CPM-task was negatively correlated with the cortical excitability parameter indexed by CSP (P = 0.001). CSP was negatively correlated with pain and disability assessed by the WOMAC index. **Conclusion:** It was observed a descending pain inhibitory system weakened, corroborating the findings of other chronic pain conditions.

Study II

The second study sought to determine if one active IMS session compared to sham promoted an effect on motor cortex excitability (MEP, short intracortical inhibition - SICI, intracortical facilitation (ICF) and CSP and in the pain measures [pressure pain threshold (PPT); Visual analogue pain scale (VAS) and numerical pain scale change (NPS0-10) during the CPM-task]. This study also aimed to determine whether serum brain-derived neurotrophic factor (BDNF) mediates the effect of this stimulation on the cortico-spinal system, as assessed by MEP and PPT. **Methods:** Twenty-six women with KOA, aged 50-75 years old, were included. They were randomly divided to receive a 30-minute session of active IMS (n = 13) or IMS sham (n = 13) by electrostimulation with a frequency of 2 Hz. The needles were inserted paravertebral at the level of the lumbar roots exit from L1 to S2 and in the muscles whose innervation corresponds to these roots and which support the knee joint (vastus medialis, rectus anterior, vastus lateral, tibialis anterior and insertion of the anserine paw). The outcomes were pain measures (VAS, PPT, NPS during CPM-task) and excitability parameters (MEP, CSP, SICI, ICF) performed before and immediately after the intervention. **Results:** the active IMS compared with sham decreased the MEP by 31.61% [confidence interval (CI) 95%, 2.34-60.98]. For the secondary outcomes, IMS reduced ICF and increased CSP. IMS improved pain reported in VAS, PPT, and NPS score (0-10) during the CPM-task. BDNF was negatively correlated with PPT (r = 20.56). **Conclusion:** We obtained results demonstrating improvement of pain and enhancement of the inhibitory corticospinal system compared to sham treatment with IMS.

Study III

The third study aimed to: 1) Evaluate if the use of the combined tDCS (transcranial direct current stimulation) to IMS can promote a better result of modulation of the corticospinal pain pathway through the potentiation of the effects of the two treatments; compared to each of them alone, and with the sham treatment. 2) To evaluate the ability of the tDCS to strengthen the descending inhibitory pain system and to modulate neuronal excitability through VAS, PPT and NPS during CPM-task. In addition, we evaluated whether serum BDNF could predict the effect of therapy at the end of treatment. **Methods:** 60 women aged 50 to 75 years old. Randomized in one of four groups: tDCS + IMS, tDCS + IMS sham, tDCS sham + IMS, tDCS sham + IMS sham. They received 5 sessions of treatment: anodal tDCS, opposite side to affected knee, 2mA, 30 min. IMS: stimulation with frequency of 2Hz, 30 min; needles placed at 2 cm from L1 to S2, in the vastus medialis, vastus lateralis, rectus anterior, tibialis anterior and insertion of the anserine paw. **Results:** a-tDCS + a-IMS

showed the best results with significant difference in pain (VAS) [mean (SD) related to treatment (post and pre): 0.46 (0.04) vs. 6.32 (1.97); 95% CI -5.42 (-8.24 to -4.36), p = .003] and functionality. This result started in the first session and was maintained throughout the study. A-tDCS + a-IMS was the only one able to modify the descending inhibitory pain system. **Conclusion:** We achieved improved pain and functional capacity with IMS, tDCS and tDCS + IMS. But only the tDCS + IMS treatment group demonstrated ability to modify the descending inhibitory pain system.

Key words: intramuscular electrical stimulation, transcranial direct current stimulation, transcranial magnetic stimulation, cortical excitability, pain pressure threshold, conditioned pain modulation.

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LISTA DE ABREVIATURAS

A	Amígdala.
ADAMTS	Família de proteases da matriz extracelular (agrecanases).
ADP	Difosfato de adenosina.
a-EIMS	Estimulação elétrica intramuscular ativa.
Ag-AgCl	Cloridrato de prata.
AIHs	Anti-inflamatórios esteróides.
a-IMS	Estimulação elétrica intramuscular ativa.
Alfa-HL	*Pore-forming toxin alpha haemolysin.*
AMP	Monofosfato de adenosina.
AMPA	*α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor.*
AMPcíclico	Adenosina 3',5'-monofosfato cíclico.
AMPc	Monofosfato cíclico de adenosina.
AMPK	*5' adenosine monophosphate-activated protein kinase*.
AINEs	Anti-inflamatórios não esteróides.
ASICs	Canais iônicos *acid-sensing.*
ATP	Adenosina trifosfato.
a-tDCS	Estimulação transcraniana de corrente contínua ativa.
AVC	Acidente vascular cerebral.
BDI	Inventário de Depressão de Beck.
BNDF	Fator neurotrófico derivado do cérebro.
BMI	Índice de massa corporal.
BP-PCS	Escala de catastrofismo de dor traduzida para o português brasileiro.
C	Córtex cingulado.
CaMK	Proteíno quinase Ca-calmodulina dependente.
CCLs	Ligantes das quimiocinas (CCL2, CCL7).
CBZ	Carbamazepina.
CE	Excitabilidade cortical.
CEE	Avaliação da excitabilidade cortical.
CGRP	Peptídeo relacionado ao gene da calcitonina.
CI	Intervalo de confiança.

Cl-	Cloro.
CNS	Sistema nervoso central.
COMP	Proteína oligomérica da matriz da cartilagem.
COMT	*Catechol-O- methyl transferase.*
CONSORT	Padrões consolidados de relatórios de ensaios clínicos.
COX-1	Ciclo-oxigenase 1.
COX-2	Ciclo-oxigenase 2.
CP	Dor crônica.
CPM	Modulação condicionada de dor.
CPM-task	*Cold Pressor Task.*
CRH	Hormônio liberador de corticotrofina.
CS	Sensibilização central.
CSP	Período de silêncio cortical.
CXCL1	Ligante 1 da quimiocina.
CXCL5	Ligante 5 da quimiocina.
DAMPs	Padrões moleculares associados ao perigo.
DLPFC	Córtex pré-frontal dorsolateral.
DNIC	Controle inibitório difuso nociceptivo.
DP	Desvio padrão.
DPMS	Sistema modulador descendente de dor.
D 1 á 5	Dias 1 á 5.
EACP	Eletroacupuntura.
EAC	Eletroacupuntura.
EAV	Escala visual-analógica.
EC	Estímulo condicionante.
EIMS	Estimulação elétrica intramuscular.
EMG	Eletromiografia.
EMT	Estimulação magnética transcraniana.
ET	Estímulo teste.
ETCC	Estimulação transcraniana de corrente contínua.
ER	Retículo endoplasmático.
F	Córtex frontal.
FIC	Facilitação intracortical.
FDI	Flexor do indicador.

fMRI	Ressonância magnética funcional.
FPS	Escala funcional de dor.
FPs	*Bacterial N-formylated peptides.*
FPR1	Receptor *formy peptide 1*.
GABA	Ácido gama-aminobutírico.
GABA-A	Receptor GABA-A.
GABA-B	Receptor GABA-B.
GEE	Equações de estimativas generalizadas.
GPCRs	Receptor da proteína G acoplada.
H	Hipotálamo.
H+	Hidrogênio.
HCPA	Hospital de Clínicas de Porto Alegre.
HCV	Vírus da hepatite C.
HD-ETCC	Estimulação transcraniana de corrente contínua de alta definição.
HMGB1	Proteína de alta mobilidade.
HETE	Ácido 5-hidroxieicosatrienóico.
HPA	Eixo hipotálamo-hipófise-adrenal.
HD-tDCS	Estimulação transcraniana de corrente contínua de alta definição.
HTLV	Vírus T-linfotrópico humano.
IC	Intervalo de confiança.
ICF	Facilitação intracortical.
ICI	Inibição intracortical.
IIC	Inibição intracortical curta.
IIE	Intervalo inter-estímulo.
ILs	Interleucinas (IL 1β, IL6, IL8, IL10).
IMS	Estimulação intramuscular.
JNK	Quinase do terminal Jun N.
K-L	Kellgren-Lawrence.
KOA	Osteoartrite de joelhos.
KOACP	Dor crônica da osteoartrite de joelhos.
LM	Limiar motor.
LMW-HA	Hialuronato de baixo peso molecular.
LTD	Depressão de longa duração.
LTP	Potenciação de longa duração.

MAC	Complexo de ataque á membrana.
MAPK	Proteíno-quinases ativadas por mitógenos.
MEP	Potencial evocado motor.
M1	Córtex motor primário.
MMPs	Metaloproteinases.
MT	Limiar motor.
NAc	Núcleo accumbens.
NA+/K+	Bomba de sódio e potássio.
Nav	Canais de sódio de tensão fechada (Nav 1.7, Nav 1.8, Nav 1.9).
NGF	Fator de crescimento nervoso.
NFkB	Fator nuclear kappa B.
NK1	*Neurokinin-1 receptor*.
NMDA	N-metil-D-aspartato.
NO	Óxido nítrico.
NPS	Escala numérica de dor.
NRS	Escala numérica de dor.
OA	Osteoartrite.
OR	Razão de chances.
PAG	Substância cinzenta periaquedutal.
PEM	Potencial evocado motor.
PICO	Estratégia PICO (paciente, intervenção, comparação, desfechos).
Piezo	Canal iônico ativado pelo alongamento.
PI3k	Fosfoionidase 3 quinase.
PKs	Proteinoquinases (PKA, PKC).
PPT	Limiar de dor à pressão.
PRISMA	Principais Itens para Relatar Revisões sistemáticas e Meta-análises.
PRRs	Receptor de reconhecimento padrão.
PSC	Período de silêncio cortical (período silente).
PSI	Índice de Qualidade de Sono de Pittsburgh.
PSQI	Índice de Qualidade de Sono de Pittsburgh.
P2X2	Receptor purinérgico.
P2X3	Receptor purinérgico.
RAGE	Receptor para glicagem avançada de produtos finais.
RNAm	RNA mensageiro.

ROS	Espécies que reagem ao oxigênio.
rTMS	*Repetitive transcranial magnetic stimulation.*
RTK	Receptor tirosinoquinase.
RVM	Medula rostroventral.
SD	Desvio padrão.
SDM	Desvio padrão da média.
SEM	Erro padrão da média.
SICI	Inibição intra-cortical curta.
s-IMS	Estimulação elétrica intramuscular placebo.
SHAM	Placebo.
SMD	Diferença de média padronizada.
SNC	Sistema Nervoso Central.
SNPS	Polimorfismo genético de base única.
SSPEs	Potenciais evocados somato-sensoriais.
s-tDCS	Estimulação transcraniana de corrente contínua placebo.
STROBE	Fortalecimento da Comunicação de Estudos Observacionais em Epidemiologia.
tDCS	*Transcranial direct current stimulation.*
TENS	Eletroestimulação nervosa transcutânea.
TGF-β	Fator β de transformação de crescimento.
TKR	Prótese total de joelho.
TLR	Receptor Toll-Like.
TL2	Receptor Toll-Like 2.
TL4	Receptor Toll-Like 4.
TMS	*Transcranial magnetic stimulation.*
TNFα	Fator de necrose tumoral α.
TPP	Limiar de dor á pressão.
TPP0	Limiar de dor á pressão 0.
TPP1	Limiar de dor á pressão 1.
TPs	Pontos gatilho.
Trk	Quinase de receptor de tropomiosina.
TRkB	Receptor de tropomiosina quinase B.
TRPA1	*Transient receptor potential A1.*
TRPV 1	*Transient receptor potential vaniloid 1.*
TRPV2	*Transient receptor potential vanilloid 2.*

UFRGS Universidade Federal do Rio Grande do Sul.

VAS Escala análogo-visual.

VEPs Potenciais evocados visuais.

WDR Wide dynamic range (amplo alcance dinâmico).

WHOQOL-BREF Questionário de qualidade de vida da Organização Mundial da Saúde.

WOMAC *Western Ontario and Mcmaster Universities Arthritis Index.*

5,6-EET Ácido 5,6-epoxieicosatrienóico.

5HT Serotonina.

4-HNE 4-hidroxinonenal.

TERMOS E DEFINIÇÕES

Abaixo a descrição de alguns termos utilizados com freqüência nessa tese.

Conditioned pain modulation (modulação condicionada de dor): teste que avalia a funcionalidade da modulação inibitória descendente de dor, através de mecanismo de contrairritação [1].

Nociceptores: Neurônios sensoriais com processos periféricos terminando na pele, músculos, articulações, vísceras e vasos; com corpos celulares localizados nos gânglios da raiz dorsal, trigeminal e gânglios nodosos. Sensíveis á estímulos agressores térmicos, mecânicos e químicos. Inativos até que sejam estimulados por energia suficiente que suplante seu potencial de repouso [2].

Hiperalgesia: Resposta aumentada à um estímulo doloroso [3].

Alodínia: Dor devida á um estímulo normalmente não doloroso [3].

Potenciação sináptica de longa duração (Long-term potentiation – LTP): Reforço sináptico, aumento da eficácia sináptica que vai além do estímulo condicionante por minutos (LTP precoce), horas, dias ou até meses (LTP tardia) [4].

Neuroplasticidade: Mudanças nas propriedades, estrutura e organização dos neurônios. É a forma que o sistema nervoso codifica novas experiências [5].

Limiar de dor: É caracterizado como o menor nível de intensidade de estímulo que é percebido como doloroso. Em psicofísica define-se como o nível de intensidade de estímulo percebido como doloroso durante 50% do tempo [3].

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APRESENTAÇÃO

Esta tese será estruturada em seis capítulos

Capitulo I – Introdução

Capitulo II – Revisão sistematizada da literatura

Capitulo III – Justificativa, mapa conceitual e objetivos

Referências da revisão da literatura (não constitui um capítulo)

Capitulo IV – Artigos científicos

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Capitulo V – Considerações finais e perspectivas

Capitulo VI - Apêndices

1 INTRODUÇÃO

Osteoartrite (OA) é a forma mais comum de artrite e a maior causa de dor e limitação funcional em idosos [6-8]. No Brasil afeta 6 a 12% dos adultos e um terço dos idosos acima de 65 anos [9]. É a principal causa de dor nos joelhos em pessoas a partir de 50 anos [10]. De uma forma geral a prevalência de osteoartrite de joelhos (KOA) em adultos é de 24% [11]. Heterogênea e multifatorial, dentre seus mecanismos o componente inflamatório contribui para os sintomas e a progressão da doença [9, 12, 13]. Evidências crescentes mostram que a sinovite induzida por produtos moleculares endógenos derivados do estresse celular e pela ruptura da matriz extra-celular podem influenciar a integridade e a função da cartilagem promovendo e acelerando a progressão da OA [14, 15]. Uma revisão sistemática associa a progressão da KOA á dor no joelho e altos níveis de ácido hialurônico e TNF α basais, nódulos de Heberden e o genu varo [16]. As recomendações atuais de tratamento, segundo os *guidelines* do *American College of Rheumatology* e a *European League of Associations of Rheumatology*, focam no alívio da dor, rigidez e melhora funcional. Não há terapias curativas. Muitas vezes os tratamentos são utilizados em combinação, na busca de melhores resultados [17]. Os guidelines de 2014 da *Osteoarthritis Research Society International* com relação ao tratamento conservador não farmacológico da KOA sugerem exercícios, reforço, auto-cuidado, intervenções biomecânicas e perda de peso [18].

A acupuntura é largamente utilizada para controle da dor em diversas patologias [19], como por exemplo, KOA [20]; prostatite crônica [21]; lombalgia e cervicalgia crônicas, cefaléia crônica [22]; e para alívio da dor após cirurgia de protetização de quadril e joelho [23]. Existe uma grande heterogeneidade de técnicas e critérios diagnósticos para indicá-la, fator que corrobora para resultados mistos [24, 25]. Este estudo utiliza inserção profunda de agulhas - terapia de estimulação intramuscular profunda (IMS) - dependente primariamente das propriedades neurofisiológicas das estruturas anatômicas a serem estimuladas. Acrescenta-se, também, eletroestimulação às agulhas, acelerando a liberação de encefalinas, beta-endorfinas e endomorfinas com a frequência de 2 Hz [26].

Recentemente desenvolveu-se, e é crescente o interesse pelos métodos de estimulação cerebral não invasiva, tais como, estimulação magnética transcraniana (EMT) e estimulação transcraniana de corrente contínua (ETCC) [27]. A estimulação cerebral não invasiva tem um grande espectro de uso (diagnóstico, prognóstico e terapêutico), podendo ser individualizada e focada na área-alvo, tem efeito imediato e a longo prazo, mínimas contra-indicações e efeitos adversos e é de baixo custo. Busca modificar e guiar a plasticidade neuronal, tornando o processo mais eficiente. A

EMT, através de campos magnéticos variáveis, induz correntes elétricas, sendo o princípio básico a modificação do potencial de membrana, promovendo novas descargas neuronais. As correntes atuam no interneurônio, concentrando-se mais em regiões corticais e subcorticais. A ETCC induz neuromodulação dos potenciais de ação já existentes nos neurônios. Promove estímulo sustentado sem morte neuronal, alteração da plasticidade e neuroproteção (efeito anti-apoptótico). Descobertas recentes no tratamento da dor crônica têm demonstrado que o córtex motor primário (M1) é uma área-alvo para as técnicas de estimulação cerebral [28]. Devido aos resultados promissores, a plasticidade do M1 tem sido explorada como um potencial marcador de dor crônica [29]. Estudos em diversas condições álgicas têm demonstrado a eficácia da ETCC na redução da dor, tais como na dor abdominal crônica devida á doença inflamatória intestinal [30]; dor miosfacial crônica [31]; lombalgia crônica [32]; dor do membro fantasma [33]; dor neuropática após lesão medular [34]; dor da fibromialgia [35]; dor da disfunção temporo-mandibular [36]; dor neuropática na esclerose múltipla [37]; dor da polineuropatia diabética [38] e na dor da radiculopatia [39]. Estudos utilizando EMT de pulso único e pareado com finalidade diagnóstica têm mostrado plasticidade alterada em M1 em indivíduos com dor neuropática [40], fibromialgia [41] e dor miofascial crônica [42]. Esses estudos mostraram atividade inibitória reduzida em estados de dor crônica levando a um estado desinibido, demonstrado pelas medidas da EMT: inibição intracortical (ICI) e período silente (CSP) [43].

Embora o efeito do agulhamento (ou acupuntura) no córtex motor tenha sido estudado em indivíduos saudáveis [44, 45], seu uso em indivíduos com dor crônica ainda necessita ser conhecido. Buscamos avaliar o efeito de uma abordagem ascendente – IMS − na plasticidade de M1 de sujeitos com dor crônica associada a processo inflamatório periférico (OA), bem como avaliar a plasticidade de M1 nesses mesmos sujeitos com uma abordagem descendente – ETCC – de tratamento, visto que se demonstrou que a OA promove sensibilização segmentar e central [46]. Além disso, os efeitos das síndromes de dor e inflamação periféricas na plasticidade de M1 são menos conhecidos. A inflamação crônica nas estruturas somáticas da OA leva, através de estímulos sensoriais aferentes sustentados, a modificações plásticas no sistema nervoso e à dor crônica. Nessa tese a proposta foi responder a três questões: 1- Avaliar a excitabilidade cortical em pacientes com KOA e o estado de inibição de seu sistema inibitório descendente de dor. 2- Avaliar se uma única sessão de IMS é capaz de modificar a dor (VAS) e o sistema descendente inibitório de dor enfraquecido das pacientes com KOA. 3- Avaliar se um curso de tratamento com ETCC combinada com IMS é superior na melhora da dor e reforço do sistema descendente inibitório de dor comparado aos tratamentos isolados (ETCC e IMS) e ao placebo-*sham*.

2 REVISÃO DE LITERATURA

2.1 Estratégias para localizar e selecionar as informações

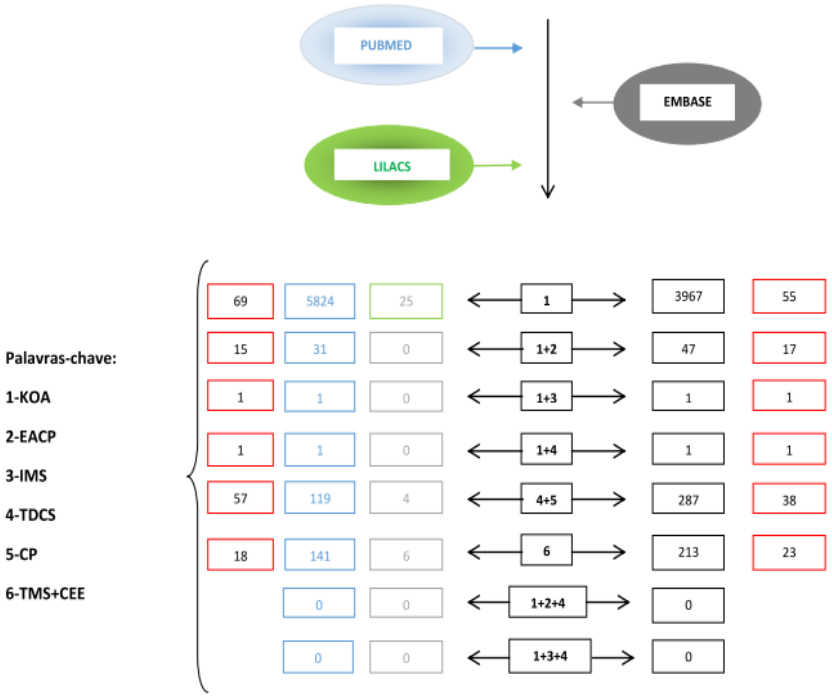
A busca foi realizada através da estratégia PICO, baseada no PRISMA, com utilização de filtros de busca. Os filtros de busca colocados foram: ensaios clínicos randomizados, revisões sistemáticas e meta-análises. As palavras-chave utilizadas constaram de: osteoartrite de joelhos (*knee osteoarthritis-KOA*), eletroacupuntura (*electroacupuncture-EACP*), estimulação intramuscular (*intramuscular stimulation-IMS*), estimulação transcraniana de corrente contínua (*transcranial direct current stimulation-TDCS*), estimulação magnética transcraniana (*transcranial magnetic stimulation-TMS*), dor crônica (*chronic pain-CP*), avaliação da excitabilidade cortical (*cortical excitability evaluation - CEE*).

No PUBMED foram encontrados 5824 publicações com a palavra-chave (*mesh term*) osteoartrite de joelhos, cruzando com eletroacupuntura apareceram 31 publicações, com estimulação intramuscular resultou 1 publicação, com estimulação transcraniana de corrente contínua também 1 publicação. Osteoartrite de joelhos combinada á estimulação transcraniana de corrente contínua e estimulação intramuscular nenhum achado; eletroacupuntura também sem achados. Estimulação transcraniana de corrente contínua combinada á dor crônica apareceram 119 publicações. O termo estimulação magnética transcraniana combinado á avaliação da excitabilidade cortical resultou em 141 publicações.

Na plataforma EMBASE o *emtree term* osteoartrite de joelhos localizou 3967 publicações, combinado á estimulação transcraniana de corrente contínua resultou em uma publicação, cruzado com eletroacupuntura obteve 47 publicações, com estimulação intramuscular resultou em uma publicação. Combinando os termos osteoartrite de joelhos com estimulação transcraniana de corrente contínua e eletroacupuntura; e estimulação intramuscular não houve resultado. Estimulação transcraniana de corrente contínua combinada á dor crônica resultou em 287 artigos. Estimulação magnética transcraniana combinada á avaliação da excitabilidade cortical obteve 213 publicações.

No LILACS osteoartrite de joelhos mostrou 25 publicações, combinado á estimulação transcraniana de corrente contínua zero publicação, combinado á eletroacupuntura também sem publicação, ocorrendo o mesmo na combinação com estimulação intramuscular. A combinação de osteoartrite de joelhos com estimulação transcraniana de corrente contínua com eletroacupuntura e estimulação intramuscular não obteve resultados.

A seguir descrição esquemática dos achados, sendo que os retângulos em vermelho mostram os artigos utilizados.



2.2. Aspectos epidemiológicos

OA é um grande problema de saúde pública e uma doença articular degenerativa bastante comum; estima-se que 85% da população acima de 65 anos apresente evidências radiológicas dela. As articulações mais afetadas são os joelhos, os quadris, as mãos, a coluna vertebral e os pés [47, 48]. Estima-se que 10% da população acima de 60 anos apresente sintomas de KOA [48]. Caracteriza-se por rigidez matinal menor que 30 minutos, limitação funcional, dor à palpação, dor à sobrecarga articular, ao final do dia e após período de repouso (dor protocinética). Observam-se alargamento ósseo, processo inflamatório leve, localizado, crepitação e limitação aos movimentos [7, 17, 48]. A dor crônica e o desequilíbrio biomecânico promovem síndrome miofascial secundária [49-51].

Na população idosa, KOA é a principal causa de morbidade, limitação das atividades, dificuldade de locomoção [52-54] e utilização dos serviços de saúde [7, 17, 52, 53, 55]. Associa-se a altos custos em saúde, principalmente indiretos, por faltas ao trabalho [17, 53, 54]. De acordo com o estudo de Nguyen ET AL, a prevalência de dor nos joelhos tem aumentado substancialmente nos últimos 20 anos, embora as evidências radiológicas de KOA não tenham sofrido incremento [10] . No Brasil afeta 6% a 12% dos adultos, mais de um terço da população com mais de 65 anos [9], mas estima-se que o número de pacientes com KOA dobre nos próximos 15 anos, o que causará substancial incremento de gastos ao sistema de saúde [53]. Acredita-se que a KOA seja a quarta maior causa de incapacidade entre as mulheres, dificultando a funcionalidade e reduzindo a qualidade de vida [47].

2.3. Aspectos fisiopatogênicos

A etiologia da OA envolve múltiplos fatores, com aspectos não completamente elucidados, mas sabe-se que há fatores genéticos, estresse biomecânico e o envelhecimento da composição da matriz cartilaginosa e sua estrutura [7, 56-58]. Ela afeta a cartilagem articular, a sinóvia, o osso periarticular e vários elementos de tecido conjuntivo adjacente com perda de cartilagem, redução do espaço articular, mudanças ósseas resultando em esclerose óssea subcondral, cistos e formação de osteófitos. Ocorrem mudanças na matriz da cartilagem, sinovite, lesões na medula óssea, mudanças degenerativas nos tecidos moles, incluindo ligamentos e meniscos [59, 60]. A progressão da doença está ligada a vários fatores como: instabilidade articular, mau alinhamento, obesidade, fraqueza muscular, deposição de cristais intra-articulares, neuropatia periférica e envelhecimento [58]. Ainda em relação à progressão da KOA, a meta-análise realizada por Bastick ET AL , 2015, sugere uma

forte relação com dor no joelho no baseline (OR, 2.38 [95% IC, 1.74-3.27]) e nódulos de Heberden (OR, 2.66 [95% CI, 1.46-8.84]).Também encontraram associação de progressão com alinhamento em varo, altos níveis de ácido hialurônico sérico e fator de necrose tumoral α [16] . Esse processo envolve degradação e reparo interativo entre cartilagem, osso e sinóvia, com mudanças bioquímicas na cartilagem articular, na membrana sinovial e no osso subcondral [7, 56-58].

O processo inflamatório leve e crônico da OA promove a sintomatologia e acelera a progressão da doença [15] e sua intensidade relaciona-se ao prognóstico [61]. Os condrócitos provavelmente são as células mais importantes, exibindo numerosas anormalidades metabólicas [62]. Residem na matriz da cartilagem, em condições de hipóxia relativa e sem suprimento vascular direto, apresentando baixa atividade metabólica. Respondem aos estímulos ambientais adversos promovendo degradação da matriz cartilaginosa e reduzindo os processos reparatórios [58]. A degradação se dá por mecanismos catabólicos (metaloproteinases-MMPs, agrecanases-ADAMTS) regulados pelas citocinas pró-inflamatórias [61], e anti-anabólicos (como por exemplo, maior geração de óxido nítrico) dos condrócitos. Certos mediadores como MMPs, TNF α, receptores Toll-like (TLR) e sinalizadores da proteína-quinase mitógeno-ativada p38 podem promover mecanismos neurais de dor articular crônica e modular o processo inflamatório articular e de degradação da cartilagem, mediados em parte pelo aumento do glutamato no fluido articular. Estudos transversais de imagem mostraram sinovite macroscópica em 75 % dos joelhos com OA, a disfunção do líquido sinovial sem inflamação grosseira também é importante no processo, com redução da produção de lubrificantes como ácido hialurônico e lubricina, suprimindo a proliferação sinovial. Ocorre estresse oxidativo através de citocinas convencionais (TNFα, IL1β, IL6 e várias quimiocinas) promovendo disfunção do condrócito. Alarminas como *high mobility group box protein 1* e calgradulinas (S100A8 e S100A9), produtos da degradação da matriz cartilaginosa (colágeno, fibronectina), proteoglicanos (como ácido hialurônico de baixo peso molecular), ácidos graxos livres e outros padrões moleculares associados ao perigo (DAMPs) estão aumentados, induzindo liberação de citocinas, mediados por vários receptores de reconhecimento padrão (PRRs) expressos na cartilagem e sinóvia. As respostas pró-catabólicas dos condrócitos através da reação inflamatória promovem a progressão da doença. Corroborando o processo há a ativação do complemento [múltiplas moléculas efetoras das vias clássica, alternativa e do complexo de ataque á membrana (MAC)], assim como a redução de seus inibidores sinoviais (por exemplo, a enzima inativadora C5a carboxipeptidase B) através de DAMPs, cristais de hidroxiapatita, cristais de pirofosfato de cálcio dihidratado e também por células apoptóticas e debris de células mortas. O extresse oxidativo inflamatório apresenta efeito citotóxico, comprometendo a viabilidade dos condrócitos e das células sobreviventes, ativando

sinalizadores transcricionais inflamatórios (NFκB, proteinocinases ativadas por mitógenos). Vários mediadores inflamatórios afetam a cartilagem por transdução em sinais ativadores que transcrevem a reprogramação dos condrócitos nas células catabolizadoras da matriz extracelular. Uma das manifestações é a maturação do condrócito para diferenciação hipertrófica, alguns mediadores são, o fator de crescimento endotelial e a sialoproteína óssea (podem promover angiogênese, inclusive na sinóvia). O extresse oxidativo também promove disfunção mitocondrial, as quais acrescem seus próprios processos inflamatórios relacionados. Há aumento da geração de óxido nítrico (NO) pelos condrócitos com supressão da fosforilação oxidativa mitocondrial promovendo calcificação. Há redução do ATP mitocondrial, perda da reserva de energia do condrócito, contribuindo para a função deficiente da matriz e viabilidade do condrócito. Ocorre redução do antioxidante mitocondrial SOD2 gerando disfunção mitocondrial e aumento da produção de espécies que reagem ao oxigênio (ROS). Ambos, extresse oxidativo e disfunção mitocondrial medeiam inflamação relacionada ao estresse do retículo endoplasmático (ER) e deficiente autofagia. Autofagia é um processo de homeostase celular, com remoção de organelas danificadas e geração de energia para a manutenção da sobrevivência celular; tem efeitos condroprotetivos, mas é deficiente no envelhecimento e na OA (figura 1) [15].

A proteinocinase AMP-ativada (AMPK) é um importante regulador da homeostase energética e se encontra reduzida na OA, assim como no envelhecimento. Os condrócitos que apresentam AMPK reduzida mostram resposta catabólica aumentada á ILβ, TNF e ao trauma biomecânico [15]. IL6 e IL10 encontram-se aumentadas na KOA. Lembrando que também estão relacionadas ao envelhecimento (produção de citocinas pró-inflamatórias resultando em um estado pró-inflamatório sistêmico leve) e á obesidade (tecido adiposo secreta citocinas pró-inflamatórias) e por sua vez, sua correlação com a KOA [9]. Dados recentes relacionam a síndrome metabólica a um risco aumentado de OA através da regulação deficiente de vias inflamatórias e metabólicas [61].

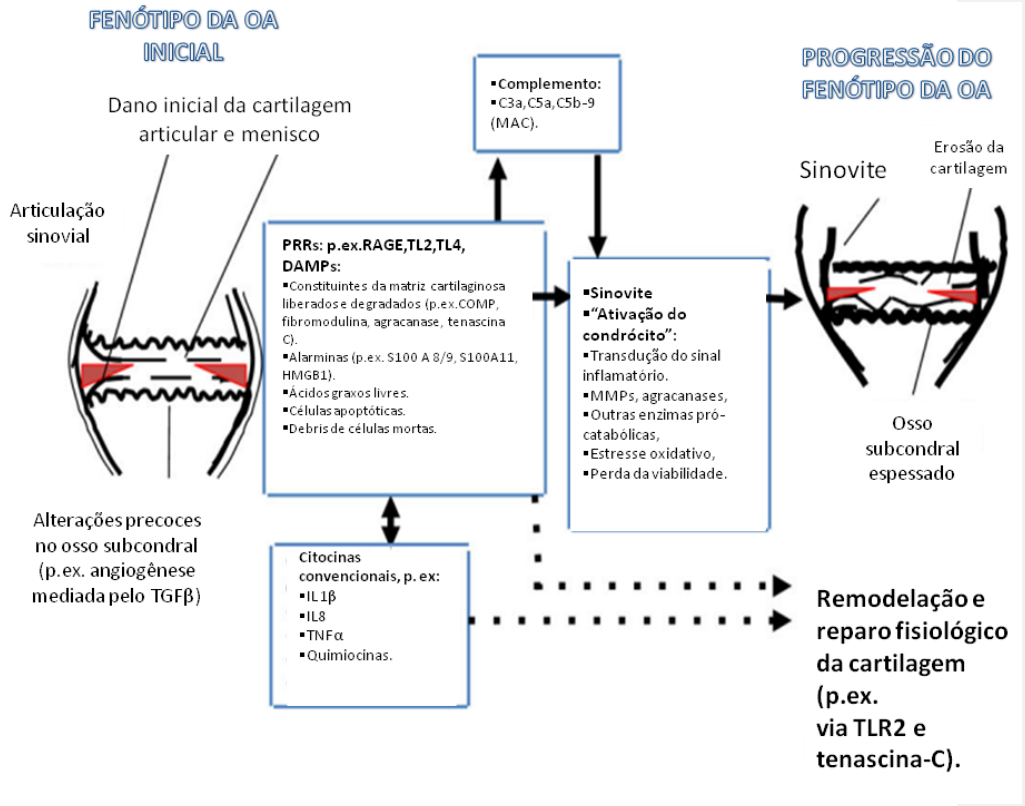


Figura 1: Adaptada de Liu-Bryan and Terkeltaub 2015. Relações entre os mediadores inflamatórios na OA. PRRs e seus ligantes DAMPS, citocinas inflamatórias, ativação do complemento C5a e C5b-9 aumentando o dano na cartilagem articular e meniscos, promovendo inflamação macroscópica, catabolismo/remodelamento da cartilagem. COMP: proteína oligomérica da matriz da cartilagem, DAMP: padrões moleculares associados a perigo, HMGB1: proteína de alta mobilidade, MAC: complexo de ataque a membrana, PRR: receptor de reconhecimento padrão, RAGE: receptor para glicagem avançada de produtos finais, TGF-β: fator β de transformação de crescimento, TLR: receptor Toll-Like.

O processo inflamatório crônico gera a progressão da doença [15] levando ao dano dos tecidos moles e do osso subcondral; e à dor crônica, promovendo alterações da aferência sensorial com modificações plásticas no sistema nervoso [63] propiciando a sensibilização segmentar [64] que promove a sensibilização central [65].

2.4. Mecanismos periféricos da dor

Os mecanismos periféricos da dor envolvem os neurônios aferentes primários, os nociceptores e as substâncias sensibilizadoras. Os aferentes primários são constituídos de projeções periféricas (axônios) dos neurônios somáticos, autonômicos e sensoriais. Os corpos neuronais dos aferentes primários (primeiros neurônios) localizam-se no gânglio da raiz dorsal e estão conectados ao sistema nervoso central pelas raízes ventral e dorsal [66]. Os nociceptores dos aferentes primários são geralmente as estruturas iniciais envolvidas no processo nociceptivo, transformando estímulos agressivos em potenciais de ação [4, 67]. Estão presentes em praticamente todos os órgãos, nos nervos periféricos encontram-se as fibras A δ e C e, nos órgãos inervados, as terminações nervosas livres [68]. Associadas com a transmissão da informação nociceptiva, as fibras A δ e C são polimodais. O dano tecidual induzido por doença, cirurgia, inflamação ou trauma causa destruição celular, com liberação de substâncias bioquímicas, os quais ativam nociceptores polimodais que respondem a estímulos térmicos, mecânicos e químicos [67]. As fibras A δ são mielinizadas, transmitem a primeira dor (rápida) e são classificadas em subtipos A δ 1 e A δ 2. As A δ 1 respondem a temperatura em torno de 52°C e a estímulos mecânicos e químicos, têm resposta mediada pelos receptores TRPV2 (*transient receptor potential vanilloid 2*) e são insensíveis a capsaicina. As A δ 2 são sensíveis a temperatura em torno de 43°C e a capsaicina. Através de receptores TRPV1 ativam canais catiônicos não seletivos permeáveis ao cálcio [4]. O TRPV1 é uma das moléculas-chave da nocicepção e é expressa somente em nociceptores. É um canal iônico de ligação fechado. Quando aberto, fluem cátions (em particular, cálcio) para o interior da célula e a despolarizam. Múltiplos estímulos o abrem, tais como temperaturas acima de 43°C, capsaicina e etanol. Suas características são modificadas pelos metabólitos da inflamação (metabólitos do ácido araquidônico produzidos pelas lipoxigenases) e por PH inferior a 5,9. Também é indiretamente sensibilizado, via segundos mensageiros, por mediadores inflamatórios como bradicinina, prostaglandina E2, ATP extracelular, glutamato, proteases, fator de crescimento nervoso (NGF − molécula-chave para a biologia do nociceptor). A elevação da expressão do TRPV1 na membrana, a fosforilação por proteíno-quinases e a desinibição do TRPV1 pelo fosfatidil-inositol-4,5-bifosfato promovem a sensibilização em nível celular. A partir dela, o limiar para abertura do receptor cai a ponto de a temperatura corporal normal ativar nociceptores e promover potenciais de ação. O significado funcional dos outros receptores é incerto e confuso. Infere-se seu envolvimento na

nocicepção devido à sua sensibilidade a irritantes, a mediadores inflamatórios ou pelo fato de serem hiperregulados em condições inflamatórias [68]. As fibras C são amielínicas, transmitem a dor secundária (lenta) e se classificam em C1 e C2. As fibras C1 respondem a capsaicina e a prótons, são peptidérgicas (liberam substância P e peptídio relacionado ao gene da calcitonina- CGRP) e expressam receptores tirocinase A para o NGF [4, 69]. As C2 expressam receptores purinérgicos P2X3 para adenosina e carboidratos de superfície, a α-D-galactose, com capacidade de ligação à lecitina IB-4 e sensibilidade a prótons [4]. Os receptores purinérgicos (P2X2 e P2X3) são abertos pelo ATP, que são canais de cálcio de ligação fechados. O ATP pode ser liberado por células lesadas ou por queratinócitos de pele inflamada. A ativação do P2X está implicada no processo de hiperalgesia inflamatória [68].

Os nociceptores traduzem os estímulos mecânicos, térmicos e químicos em um potencial de ação despolarizante. Quando o estímulo for suficiente, são abertos os canais de sódio voltagem dependentes (essenciais para geração e condutância de potenciais de ação), gerando potenciais de ação para o corno dorsal da medula e do tronco, causando uma resposta inflamatória neurogênica com liberação de substância P, neurocinina A e CGRP [68, 70]. A inflamação sensibiliza os receptores polimodais fazendo com que estímulos inócuos sejam vistos como nocivos, e os nóxicos tenham uma resposta exagerada. Também ativa os receptores chamados silentes (fibras C recrutadas somente no processo inflamatório) [68]. A liberação dessas substâncias produz uma mudança na excitabilidade das fibras sensoriais e autonômicas, tendo como resultado a vasodilatação local e o extravasamento de proteínas plasmáticas. Paralelamente, a substância P, a neurocinina A e o peptídio relacionado ao gene da calcitonina agem nas células inflamatórias para liberar os mediadores químicos teciduais da inflamação, tais como bradicinina, histamina, potássio, adenosina, prostaglandinas, leucotrienos, citocinas (podem promover efeitos excitatórios duradouros), substância P e NO [68, 71, 72]. Os canais de potássio e cálcio controlam a excitabilidade neuronal [2, 68]. O efeito dessas substâncias é mediado por receptores específicos, canais iônicos, sistemas de segundos mensageiros e neuropeptídios. O efeito desse conjunto de alterações produz descargas espontâneas dos terminais aferentes, redução do limiar de ativação dos nociceptores e descargas aumentadas a estímulos supralimiares. Esses fatores contribuem para as somações espacial e temporal da condução nociceptiva ao SNC, o que é denominado sensibilização periférica (figura 2) [67]. Durante o processo de sensibilização, as propriedades dos portões dos canais iônicos são modificadas pelos mediadores inflamatórios, que atuam nos receptores metabotrópicos de membrana ativando os sistemas de segundos mensageiros. Os sinais provenientes desse sistema especializado são conduzidos aos neurônios do corno dorsal da medula, onde a informação é modulada pelos sistemas pertencentes aos mecanismos centrais da dor –

sistemas descendentes facilitatórios ou inibitórios e interneurônios locais. A sensibilização periférica induz a hiperexcitabilidade dos neurônios nociceptivos do SNC, gerando a sensibilização central [68].

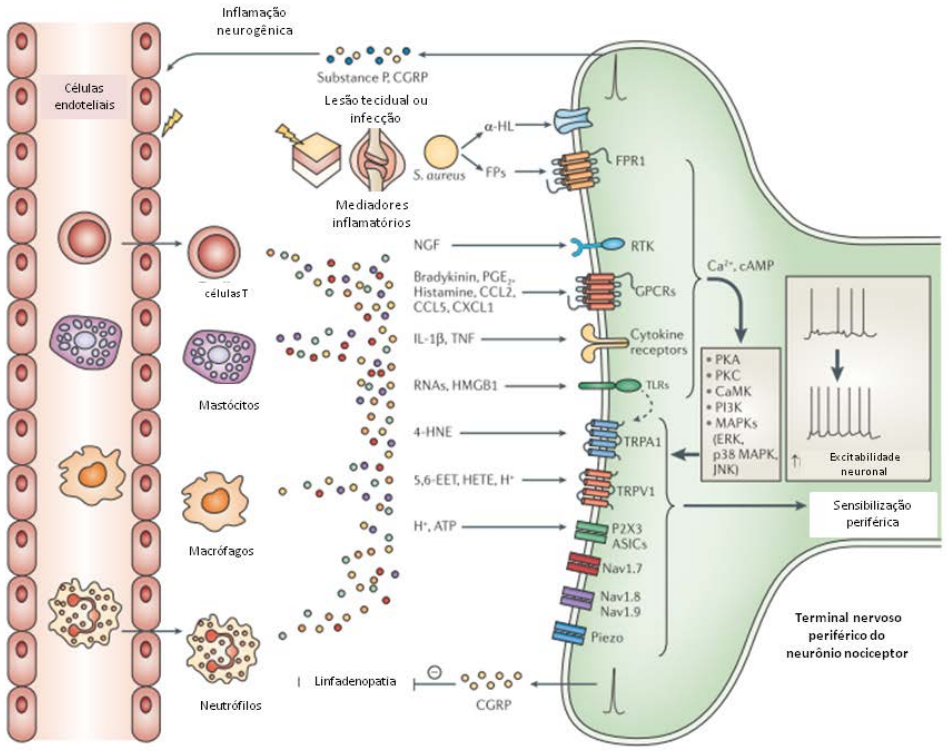


Figura 2: Modificada de Ji R-R, 2014. Processo inflamatório promovendo dor e sensibilização periférica [73] .

2.5. Mecanismos centrais da dor

Os mecanismos centrais da dor envolvem neurônios de segunda ordem, interneurônios e sistemas moduladores. O corno dorsal da medula é o sítio onde terminam os aferentes primários. Nesse nível, há uma complexa interação entre fibras aferentes, neurônios espinhais locais e fibras corticodescendentes [74]. Os neurônios aferentes primários terminam primariamente nas lâminas I, II (principalmente) e V [75, 76], onde fazem conexões com várias classes de neurônios de segunda ordem do corno dorsal da medula. Algumas fibras ascendem e outras descendem vários segmentos, no trato de Lissauer, antes de fazerem sinapses em neurônios que se projetam para os centros superiores [67, 77]. Nociceptores estimulados de forma persistente promovem dor espontânea, limiar

reduzido e hiperalgesia. A sensibilização central apresenta alterações dos impulsos periféricos, com adaptações positivas ou negativas. Ocorre aumento da resposta aos impulsos aferentes, descargas persistentes após estímulos repetidos, ampliação da receptividade de neurônios do corno dorsal e redução do limiar. A sensibilização do corno dorsal da medula pode ser de diferentes modalidades: *wind up*, sensibilização sináptica clássica, potencialização de longo termo, fase tardia da potencialização de longo termo e facilitação de longo termo. A sensibilização sináptica clássica ocorre a partir de uma estimulação nociceptiva assíncrona que gera uma sequência de estímulos periféricos nociceptivos, promovendo liberação de aminoácidos excitatórios (glutamato, aspartato), peptídeos e neurotrofinas no corno dorsal da medula, aumentando a resposta das fibras Aδ e C (potenciação homossináptica) e de eferentes de fibras Aβ não estimulados (potenciação heterossináptica). Após, ocorre a ativação de segundos mensageiros [AMPc (Adenosina 3',5'-monofosfato cíclico), PKA (proteína cinase A), fosfotidilinositol, fosfolipases C e A], promovendo abertura dos canais de cálcio com consequente produção de NO e prostaglandinas, migrando em direção à fenda sináptica liberando glutamato, aspartato, substância P e CGRP, ampliando o processo álgico [4]. Os estímulos provenientes desses terminais aferentes primários induzem a liberação de glutamato, substância P e neurocinina A do terminal pré-sináptico. Esses neurotransmissores excitatórios iniciam uma cascata de mudanças nos neurônios pós-sinápticos medulares [78, 79], incluindo a ativação de proteínas G, que medeiam a fosforilação de proteínas C. Essa por sua vez, leva à liberação de Ca^{++} dos compartimentos intracelulares, à produção de diacil-glicerol e à ativação de proteínas que modulam a atividade do canal iônico [80]. Essas mudanças aumentam a densidade de receptores N-metil-D-aspartato (NMDA) e a responsividade dos neurônios aos aminoácidos excitatórios [81]. Elas fazem com que a concentração do cálcio intracelular se eleve, o potencial transmembrana seja reduzido e os canais iônicos dos receptores NMDA fiquem ativos. A elevação da concentração de cálcio intracelular é o gatilho para a produção de NO, gás capaz de se difundir para fora da célula e intensificar a ativação dos neurônios aferentes primários [82]. O NO e as prostaglandinas por meio de sua difusão para o meio extracelular contribuem para a ativação das sinapses vizinhas e da glia; e aumento da área receptiva [83]. Micróglia e astrócitos ativados promovem uma sensibilização ainda maior no neurônio de projeção pela liberação de fatores inflamatórios, contribuindo também para a apoptose de interneurônios inibitórios [84]. Esse conjunto de mudanças é denominado sensibilização central (figura 3), e tanto sua eficácia sináptica quanto a excitabilidade dos neurônios denominados *wide dynamic range* (convergentes) estão aumentadas [85], contrapondo-se ao enfraquecimento dos sistemas moduladores descendentes [86]. Expressões clínicas desse fenômeno são a hiperalgesia e a alodinia. Estímulos repetitivos, provenientes das fibras

do tipo C, por tempo prolongado e baixa frequência, produzem um aumento sequencial no número de potenciais de ação dos neurônios do corno dorsal da medula, estimulando a liberação de neurotransmissores excitatórios, a remoção do bloqueio dos receptores NMDA, o aumento da condutividade ao cálcio e a resposta à dor, efeito denominado *wind-up* [4, 77, 87]; resultado de uma modulação positiva entre NMDA e receptores NK1 (neurocinina 1), promovendo uma amplificação das mensagens nociceptivas vindas dos nociceptores periféricos. A intensidade depende de mecanismos pré-sinápticos, receptores pré-sinápticos e propriedades pós-sinápticas das membranas. Os neurônios classe 2 tem papel importante, os localizados profundamente no corno dorsal geram um fenômeno mais intenso que os superficiais [88]. Nesse fenômeno, a atividade eletrofisiológica dos neurônios do corno dorsal da medula dura alguns segundos após o término do estímulo, enquanto na sensibilização central dura de minutos a horas [89]. Infere-se que o processo de potencialização de longo termo ocorra a partir de estímulos nociceptivos curtos de alta frequência ativando receptores AMPA (*α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor)* e NK1 e de canais de cálcio com resposta pós-sináptica prolongada e excitatória. A fosforilação dos receptores de membrana, a alteração do tempo de abertura dos canais iônicos, a formação de substâncias excitatórias no interior da célula e seu transporte para a fenda sináptica podem ser os mecanismos de melhora da eficácia da transmissão sináptica. Há ampliação da resposta nociceptiva através da ativação das proteinocinases por mitógenos (MAPK) modulando a fosforilação dos receptores NMDA e AMPA. Na facilitação de longo termo é acionada a mesma cascata da sensibilização sináptica clássica, mas ocorrem também a expressão de genes de formação imediata c-fos (B, C, D), c-jun, enzimas COX -2, genes de resposta lenta que codificam a pró-dinorfina e receptor NK1 e trkB no corno dorsal da medula espinhal. Há regulação ascendente das vias para síntese de citocinas, quimiocinas e moléculas de adesão com mudança no fenótipo do gânglio da raiz dorsal [4].

Sabe-se que a nocicepção prolongada promove liberação de peptídeos opióides endógenos e agonismo de seus receptores no SNC. Brown ET AL, verificaram que pacientes com dor crônica por osteoartrite apresentavam maior disponibilidade de receptores opióides nas regiões moduladoras de dor dos gânglios da base, insula e PAG, e mais intensamente na área subcalosa, núcleo acumbens e caudado. Devido á dor crônica ocorre uma super-regulação na disponibilidade dos receptores [90]. As alterações que ocorrem nos estados de dor crônica não são somente funcionais, mas também estruturais. Estudos com neuroimagem tem mostrado alterações no volume da substância cinzenta, integridade da substância branca e até na epigenética desses pacientes [76, 91].

A dor e os sinais nociceptivos podem exercer forte influência nas funções motora e autonômica e no estado emocional. É também claro que a percepção da dor pode ser fortemente modulada por

sistemas descendentes que se originam em várias zonas do cérebro que mantêm a inibição tônica do sistema nociceptivo [92]. Essa modulação pode levar tanto à *up regulation* quanto à *down regulation* da percepção da dor [74].

Estudos de imagem funcional têm mostrado evidências do envolvimento de múltiplos centros na ativação do sistema nociceptivo e sua associação com áreas que controlam as funções motora e autonômica e o estado emocional [93]. Essas regiões do cérebro abrangem o sistema límbico e áreas como a substância cinzenta periaquedutal, que fornecem substrato anatômico para as interações entre nocicepção, estado emocional e atividade autonômica [94]. Por exemplo, o gânglio basal e a região da substância cinzenta periaquedutal tanto recebem informações nociceptivas quanto coordenam importantes aspectos do movimento e do controle motor [95]. Há uma considerável sobreposição entre o sistema neuroanatômico, o sistema neurotransmissor modulador da percepção da dor e aqueles que controlam o estado emocional [94].

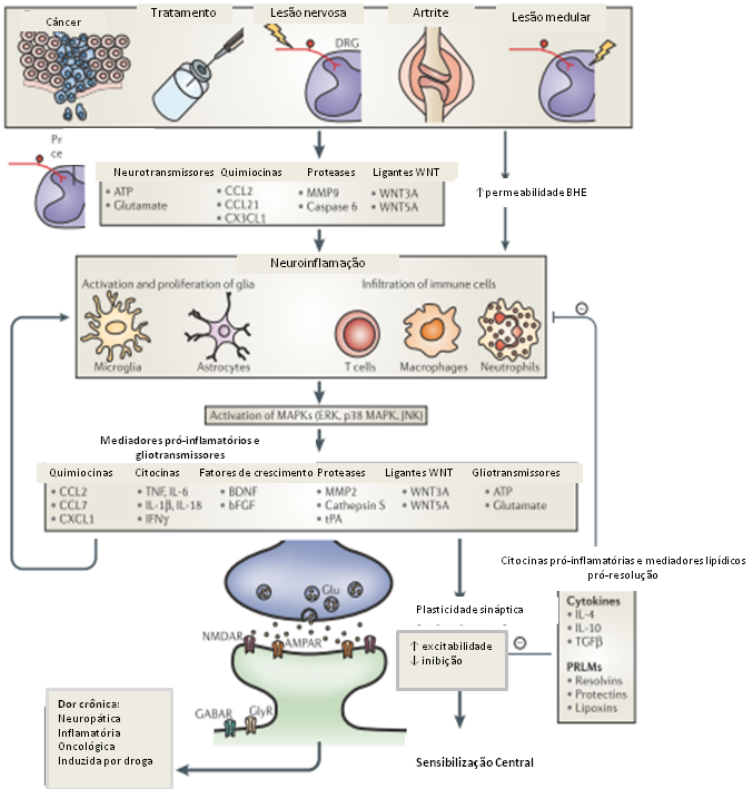


Figura 3: Adaptada de Ji R-R, 2014. Representação esquemática da sensibilização central.
BHE: Barreira hemato-encefálica [73] .

2.6. Interação dos Sistemas Nociceptivo e Neuromoduladores

A transmissão da informação nociceptiva dos terminais aferentes primários é modulada em vários níveis do neuroeixo, incluindo o corno dorsal da medula [96], os axônios desses neurônios sofrem decussação para o outro lado, transmitem os estímulos nociceptivos pelos tratos espinorreticular e espinotalâmico para o tronco cerebral e tálamo, após para o córtex somatossensorial primário, córtex somatossensorial secundário, ínsula e córtex cingulado anterior [97]. A inibição dos impulsos nociceptivos dos aferentes primários é modulada em níveis pré e pós-sinápticos, pela ação dos sistemas descendentes opióides, serotoninérgicos, noradrenérgicos e gabaérgicos [96]. A inibição também é mediada pelo efeito de interneurônios inibitórios locais. As estruturas supraespinhais que compõem o sistema modulador descendente incluem hipotálamo, substância cinzenta periaquedutal, *locus ceruleus*, núcleo *magnus* da rafe e os núcleos paragigantocelulares laterais. Esses sistemas atuam no nível do neuroeixo sobre sinais nociceptivos provenientes dos aferentes primários, que fazem parte dos mecanismos periféricos da dor (figura 4) [98].

Frente ao exposto, percebe-se que para compreender as respostas deste sistema complexo envolvendo a dor e seu processamento, necessitamos buscar biomarcadores com a finalidade de auxiliar no diagnóstico do nível de disfunção e com marcadores de plasticidade quantificar efeito terapêutico. Nessa tese a ênfase maior será na avaliação do sistema modulador descendente da dor, o BDNF e a excitabilidade do córtex motor.

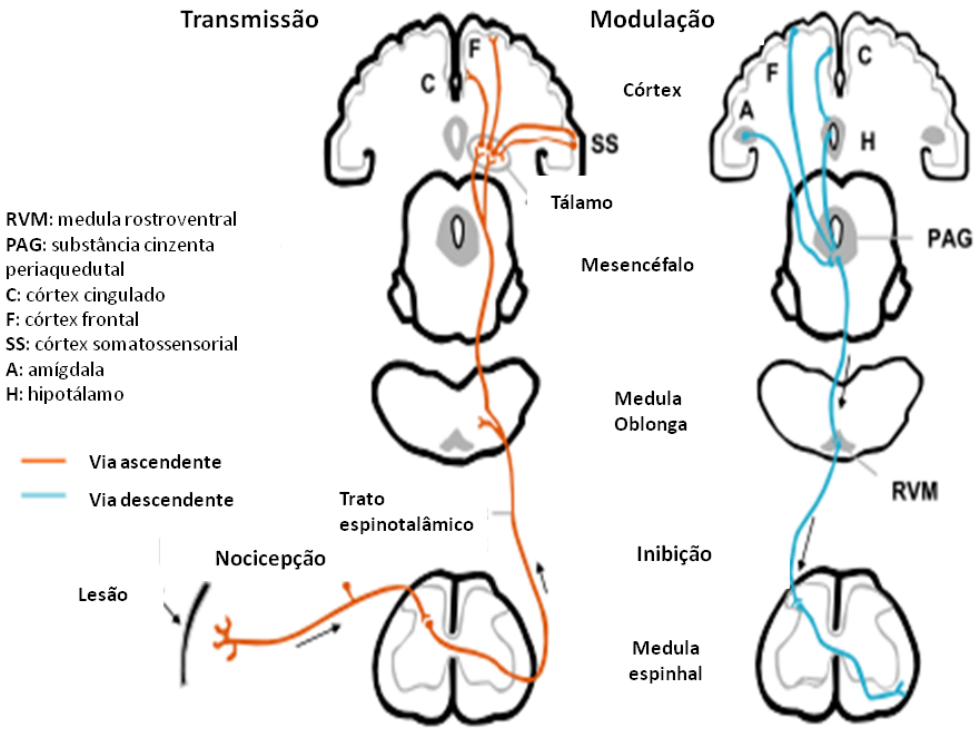


Figura 4: Adaptada de Hoffman GA, Harrington A, Fields HL [99].Exemplo esquemático das vias ascendente e descendente (inibitória) de dor.

2.7. Fator neurotrófico derivado do cérebro - *brain derived neurotrophic factor* (BDNF)

O BDNF é uma neurotrofina que regula a integridade e a diferenciação dos neurônios durante o desenvolvimento e está também envolvida em várias funções na vida adulta, incluindo o processo de plasticidade neuronal, sendo um regulador chave nesse processo. Possui forte ação protetora de sobrevivência neuronal, neurogênese, efeito anti-inflamatório e redução da morte celular. Apresenta efeitos na cognição, regula o desenvolvimento e função do circuito neuronal, medeia muitos processos (diferenciação neuronal, crescimento, formação sináptica, plasticidade). É liberado pelas fibras nociceptivas aferentes primárias, pela micróglia, astrócitos, neurônios sensoriais e motores; e células imunes, estando associado à modulação da propriocepção [100, 101]. Se localiza

principalmente nos terminais das lâminas I e II do corno dorsal da medula, armazenado junto a neuropeptídios como substância P e CGRP (peptídio relacionado ao gene da calcitonina), é encontrado também em centros integrativos superiores (córtex somatossensorial, vias descendentes relacionadas à modulação supraespinhal da dor). A expressão do BDNF no sistema nervoso central (SNC) é modificada por acometimentos diversos, tais como estresse, isquemia, hipoglicemia e depressão. Também pode funcionar em mecanismos adaptativos essenciais, exercendo papel crucial no processo de potenciação sináptica de longa duração, mecanismo de neuroplasticidade fundamental para desencadear e sustentar o processo de memória da dor, além de influenciar a eficiência sináptica de curta duração. Modula os sinais excitatórios (glutamatérgicos) e inibitórios (gabaérgicos/glicinérgicos), assim como a transmissão peptidérgica na medula espinhal [69]. O aumento de BDNF incrementa a potenciação sináptica de longa duração (*long term potentiation* - LTP), enquanto que a redução de seus níveis atenua este fenômeno. Com isso, observa-se que os quadros dolorosos crônicos dependem de um processo em cadeia de longo prazo [102-105]. Vários fatores parecem influenciar a ação modulatória do BDNF sobre a dor, entre eles o gênero. Altos níveis de BDNF foram relacionados com alto limiar de dor em mulheres, essa relação foi inversa nos homens. Já as mulheres no climatério apresentam a mesma relação dos homens [69]. Na fibromialgia encontra-se aumentado e correlaciona-se inversamente ao limiar de dor. Encontra-se também aumentado no plasma de pacientes com osteoartrite, mas normal na sinóvia [101]. Foram encontrados níveis elevados de expressão de RNAm de BDNF bem como a expressão dos seus receptores TRkB e p75 NTR (aos quais o BDNF se liga para agir) em células de fluido sinovial de pacientes com OA [106].

2.8. Estimulação magnética trancraniana como método de diagnóstico

A EMT é uma técnica de neuromodulação e neuroestimulação cerebral não-invasiva amplamente utilizada para examinar a fisiologia do córtex motor em seres humanos. Método baseado no princípio eletromagnético de indução, descoberto por Michael Faraday em 1838 [107, 108]. Funciona a partir de uma bobina magnética conectada a um aparelho de estimulação magnética promovendo mudanças constantes da orientação da corrente elétrica dentro da bobina, gerando um campo magnético, usualmente de 2 tesla, que atravessa alguns materiais relativamente isolantes, tais como pele e ossos. O campo magnético gera uma corrente elétrica dentro do crânio restrita a pequenas áreas dependendo da geometria e forma da bobina [109] . A corrente despolariza os neurônios orientados apropriadamente ao campo magnético [110]. A relação entre o campo elétrico

induzido e a orientação dos neurônios direciona a ativação dos axônios piramidais, podendo ser diretamente (mecanismo-D) na substância branca ou cinzenta, ou indiretamente (mecanismo-I) via interneurônios, na substância cinzenta [111, 112]. O limiar entre os mecanismos D e I é diferente possibilitando ajustar as contribuições relativas aos dois mecanismos. A diferença entre os mecanismos D e I provêm de estudos fisiológicos mostrando que os axônios são melhor ativados quando estão, pelo menos parcialmente, paralelos ao campo elétrico; e se estes axônios são sensíveis ao *hot spot* [113] e aos tipos de estudos [114-116]. *Hot spot* se define como a posição da bobina sobre M1 em que a intensidade mais baixa do limiar motor (LM) é necessária para induzir uma resposta em pelo menos 50% dos potenciais evocados no músculo primeiro interósseo dorsal em repouso [117, 118]. São utilizados pulsos únicos e pareados como parâmetros para obter as medidas de excitabilidade cortical. Os principais parâmetros de excitabilidade cortical são: limiar motor (LM), potencial evocado motor (PEM), período silente cortical (PSC), inibição intracortical curta (IIC) e facilitação intracortical curta (FIC). O LM é a menor intensidade de estímulo capaz de gerar um PEM com amplitude mínima de 50 μV no músculo em repouso ou 200 μV no músculo contraído, após pelo menos cinco dentre 10 pulsos magnéticos administrados [117, 118]. Informa sobre um núcleo central de neurônios da representação muscular no córtex motor. PEM representa a ativação das fibras musculares das unidades motoras estimuladas no hemisfério contralateral. Vários fatores influenciam o tamanho do PEM, dentre eles: intensidade do estímulo, número de neurônios motores recrutados no trato corticoespinhal, número de neurônios que disparam mais de uma vez o estímulo, sincronização do estímulo da EMT e ativação neuronal, localização do campo magnético e direção da indução do campo elétrico (figura 5).

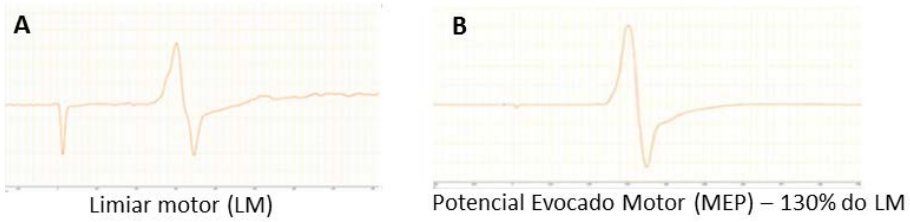


Figura 5: Medida do limiar motor e do potencial evocado motor (130% do LM), dado obtido no Laboratório de Dor e Neuromodulação, HCPA.

O PSC é reproduzido através da aplicação do estímulo transcraniano durante a contração voluntária do músculo efetor. Indica um silêncio transitório na atividade eletromiográfica isométrica voluntária do músculo efetor produzida pela EMT de pulso único no córtex motor contralateral. Ocorre logo após o PEM. A duração do PSC aumenta com a intensidade do estímulo. Seu

mecanismo se relaciona à atividade de circuitos inibitórios no córtex motor, resultando em uma falha transitória da saída cortical motora [119-121]. A duração da inibição cortical avaliada pelo PSC é consistente com as medições intracelulares de potenciais pós-sinápticos inibitórios de longa duração (na ordem de centenas de milissegundos) dependentes dos receptores GABA B [122].

A saída cortical motora provêm da interação entre vários sistemas que exercem influências excitatórias e inibitórias sobre os neurônios corticoespinhais. A EMT pode ser usada para investigar estes mecanismos, através de estímulos pareados, onde são aplicados um estímulo condicionante (EC) e um estímulo teste (ET). Os parâmetros de estimulação determinantes da interação entre o EC e ET são: intensidade do EC, intensidade do ET e tempo entre os dois estímulos (intervalo inter-estímulos - IIEs). O IIE entre 1-5ms é inibitório e entre 8-30ms é facilitatório [123] . O pulso pareado é normalmente expresso como a razão entre a amplitude de PEM (80% do LM) para produzir inibição intracortical curta ou facilitação intracortical curta, pela amplitude de PEM (130% do LM) produzida como ET. Índices abaixo de 1 representam inibição e superiores a 1 mostram facilitação [124, 125]. Em indivíduos saudáveis a administração aguda da maioria dos medicamentos GABAérgicos reforçam a IIC [126-128]. O efeito facilitador do lorazepam na IIC e a redução da FIC foi documentado por Di Lazzaro [129]. Estes resultados corroboram fortemente com as evidências de que a IIC reflete a inibição GABA-A medida no córtex motor [126-128]. Nos neurônios do córtex sensoriomotor do rato a latência do potencial excitatório pós-sináptico é na ordem de 10 ms, sendo mediada por receptores glutamatérgicos NMDA [130]. Essa latência é consistente com o tempo da FIC. Assim, a FIC pode refletir a transmissão glutamatérgica NMDA no córtex motor [131]. Esta hipótese é apoiada por dados experimentais, que mostram que os antagonistas NMDA reduzem a FIC [132, 133]. Tem sido sugerido que a FIC não é, exclusivamente, mediada por interneurônios excitatórios, mas por um equilíbrio entre os fenômenos inibitório e excitatório [134] (figura 6).

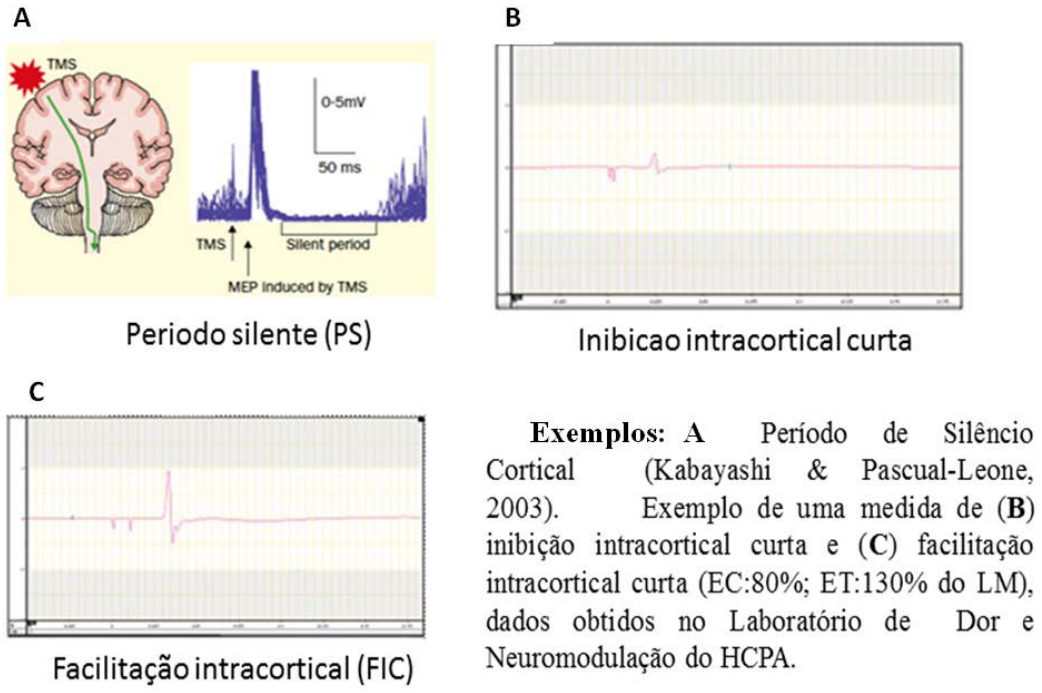


Figura 6: Exemplos de período de silêncio cortical, inibição intracortical curta e facilitação intracortical [135].

A bobina mais comumente utilizada é a em forma de oito, que permite estímulo focal. É posicionada paralelamente à cabeça do indivíduo na área correspondente ao resultado esperado conforme os objetivos do estudo. No caso de estudos de dor, o local mais comumente utilizado será o M1, marcado a partir do sistema 10-20 de eletroencefalografia. A indução eletromagnética promove um campo elétrico no cérebro, gerando neuroestimulação (promove novos disparos neuronais) e neuromodulação [107, 136]. Com profundidade de 2 a 3 cm, promove estimulação profunda, atigindo a substância cinzenta periaquedutal (PAG), substância cinzenta periventricular, tálamo sensorial e cápsula interna. EMT de baixa intensidade (igual ou abaixo de 1Hz) estimula neurônios inibitórios de baixo limiar. Na alta intensidade (igual ou acima de 5Hz) são excitados neurônios de projeção [137]. Podem ser utilizados pulsos únicos (avaliação da excitabilidade cortical) e repetitivos (tratamento). Para avaliação da excitabilidade cortical o pulso promove despolarização do motoneurônio e a captação da resposta é realizada por sinal eletromiográfico, o Potencial Evocado

Motor (MEP). Através de protocolos e estimulações diferentes pode se obter avaliação da excitabilidade cortical, processos inibitórios, facilitatórios e a organização somatotópica dos neurônios cortico-espinhais [5].

Variadas condições de dor crônica já foram avaliadas, como patologias crônicas músculo-esqueléticas, dentre elas a ruptura do manguito rotador, lombalgia crônica e insuficiência do ligamento cruzado anterior. Esses estudos mostraram modificações na excitabilidade cortical, no controle motor, reorganização do recrutamento da unidade motora, co-ativação de músculos e sobreposição da representação do músculo ou movimento no M1 [5]. Estudos de imagem sugerem que o desenvolvimento e a manutenção da dor crônica podem ser em parte, devido à atrofia cerebral, a química cerebral alterada e as funções cerebrais anormais. O efeito dessas modificações podem ser oriundas da alteração do delicado equilíbrio dos sistemas moduladores endógenos [138]. A cronificação da dor gera uma reorganização estrutural com alterações plásticas anatomofuncionais e metabólicas. São observadas alterações anatomofuncionais em regiões envolvidas no processamento da dor, em diferentes condições dolorosas, tais como: fibromialgia, dor lombar, dor facial, dor neuropática e dor fantasma. Elas apresentam redução da densidade da substância cinzenta no córtex cerebral, especificamente, no córtex cingulado, córtex insular, córtex pré-frontal, córtex pré-frontal dorsolateral, córtex somatossensorial, tálamo, córtex motor e tronco cerebral. O córtex cingulado é a região mais afetada [139-144]. O processo de aprendizado nos processos dolorosos ocasiona alterações plásticas, no entanto o motivo destas alterações ainda não foi esclarecido. Neurodegeneração ou redução tecidual podem estar relacionados com os quadros álgicos mais severos [40, 143]. São encontradas alterações de excitabilidade cortical, com aumento do PEM, redução do PSC e da IIC, sugerindo redução dos mecanismos inibitórios ou aumento da excitabilidade cortical [40, 139, 145].

2.9. Limiar de dor e sistemas moduladores

A dor é um processo complexo, nem sempre proporcional ao dano. A teoria do portão da dor de Melzack e Wall introduziu o conceito dos mecanismos de modulação, pelos quais o cérebro filtra, seleciona e modula aferências nociceptivas. A medula espinhal também é sede de modulação dinâmica. Em 1999, Melzack lançou a teoria da neuromatriz, segundo a qual o processo doloroso ativa múltiplas áreas cerebrais. Segundo estudos de imagem, o córtex somatossensorial primário e secundário, o córtex insular, o córtex pré-frontal, o córtex cingulado anterior e o tálamo são constantemente ativados no processo de nocicepção aguda. Essas regiões estão também envolvidas

na cognição, emoção e motivação, tendo papel na modulação da experiência nociceptiva [146]. Uma experiência dolorosa também pode ocorrer sem uma aferência nociceptiva primária, demonstrando a possibilidade de independência do processamento central em relação à injúria e dor [147]. A conceitualização da dor inclui uma complexa rede de interações neurais que incluem informações fisiológicas, psicológicas e também resultantes da experiência dolorosa individual [148]. "A dor depende de amplas redes neurais cerebrais e deve ser vista como um processo de percepção associado à consciência, influenciado por memórias, emoções, doenças, fatores genéticos e cognitivos" [69].

Sujeitos com a mesma condição clínica apresentam uma grande variação na quantificação de dor, assim como as respostas aos mesmos estímulos realizados em estudos experimentais variam de indivíduo para indivíduo [149]. Essa variabilidade decorre da interação de parâmetros biológicos e psicológicos que envolvem inúmeras proteínas implicadas nos diferentes estágios do processamento do sinal nociceptivo, desde a transdução até a percepção. A multiplicidade de respostas na sensibilidade à dor e na expressão da dor crônica pode ser parcialmente explicada pela interação de diferentes genes entre si com fatores ambientais. Os polimorfismos genéticos podem explicar parcialmente os mecanismos da relação percepção de dor e genética**.** Os genes apresentam inúmeras variações a partir dos polimorfismos genéticos de base única (SNPs) com alteração da expressão de proteínas e receptores implicados na sensibilidade à dor. A variabilidade na dor experimental, por exemplo, é explicada pelos polimorfismos no gene da COMT (*catechol-O-methyltransferase*) e do receptor opióide [69]. As mensurações de dor também sofrem influência do gênero devido á múltiplos mecanismos biopsicossociais, incluindo os hormônios sexuais, função do sistema opióide endógeno, fatores genéticos, capacidade de lidar com a dor, catastrofismo e até mesmo as diferenças nos papéis sexuais [69, 150] . Em diferentes estados dolorosos, os homens apresentam menos dor que as mulheres, tanto que a prevalência de dor pélvica crônica e síndrome do cólon irritável são maiores em mulheres. As mulheres apresentam maiores escores de dor em estudos experimentais, isto é especialmente marcante na dor à pressão. Também a sensibilidade térmica apresenta limiar menor nas mulheres. Parece que as variações hormonais seriam responsáveis por tais diferenças entre os sexos. Porém as flutuações hormonais que ocorrem ciclicamente nas mulheres são insuficientes para modificar os níveis de dor. No entanto, um estudo observou diferença significativa na modulação condicionada de dor (CPM) de mulheres em fase ovulatória comparadas a mulheres na fase menstrual ou lútea. Na fase ovulatória apresentaram inibição significativamente maior. Diferenças de distribuição, expressão ou sensibilidade dos receptores opióides também podem estar implicadas nas alterações de sensibilidade vistas entre os gêneros. Mulheres em repouso têm maior concentração de

receptores mu-opióides em regiões corticais e subcorticais variadas. Além disso, a "marca" afetiva associada à sinalização aferente da dor também pode estar implicada na diferença entre os gêneros (viu-se aumento de atividade cerebral em mulheres após estímulos álgicos padronizados em áreas associadas ao componente afetivo e emocional da dor). A utilização de testes padronizados para avaliar a dor busca ativar diferentes nociceptores e vias específicas de condução sensitiva e evocar dor em diferentes tecidos [69].

2.10. Modulação condicionada de dor (CPM: conditioned pain modulation)

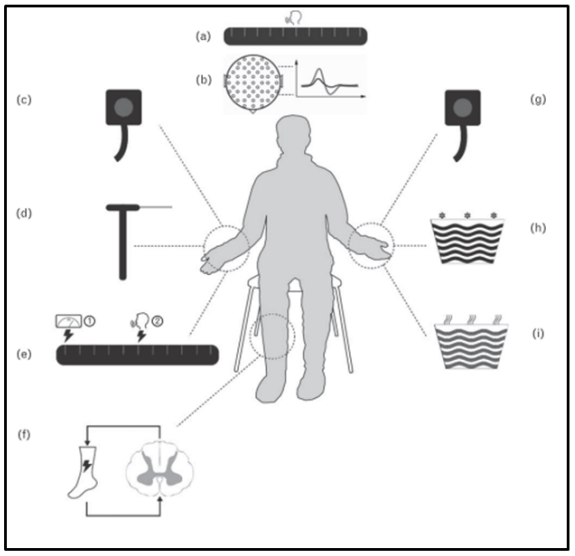


Figura 7: Adaptada de Nir R-R 2015 [151]. Exemplo esquemático do desenho para experimento de CPM. CPM: redução da dor pelo estímulo teste através da aplicação de um estímulo condicionante. (a) Avaliação subjetiva com escore numérico de dor. (b) Avaliação objetiva com potenciais evocados. (c) Estímulos testes: calor através de termodo, (d) pressão através de filamento de Von Frey, (e1) detecção elétrica de limiar de dor, (e2) avaliação de dor supralimiar, (f) respostas nociceptivas de reflexo de retirada. Estímulos condicionantes: (g) calor de contato com termodo, (h) teste de pressão com frio, (i) imersão em água quente.

O controle inibitório difuso nociceptivo, originalmente chamado de contrairritação, é o fenômeno pelo qual um estímulo nocivo inibe a percepção de dor promovida por outro estímulo nocivo [152] (figura 7). Atualmente esse fenômeno em humanos é chamado de modulação condicionada de dor (*conditioned pain modulation*, CPM) [153]. CPM é uma medida psicofísica experimental de avaliação dos mecanismos supra-espinhais inibitórios descendentes de modulação da dor [154-156]. Envolve um circuito espino-bulbo-espinhal, para o bulbo caudal com influências descendentes atingindo os neurônios do corno dorsal da medula [153]. A atividade dos neurônios sinalizadores de dor é atenuada em resposta a um estímulo nocivo aplicado em uma área remota do corpo [155]. Regiões corticais e subcorticais controlam estas vias descendentes [153]. Na prática aplica-se um estímulo nocivo constante (estímulo condicionante) em uma parte do corpo e um estímulo fásico (estímulo a ser testado) em uma parte distante do corpo antes, durante e após o estímulo condicionante. A testagem em paralelo avalia o estímulo teste antes e durante o estímulo condicionante. Na aplicação seqüencial a avaliação do estímulo teste é realizada antes e após a retirada do estímulo condicionante [154]. O CPM é avaliado através da redução que ocorre na percepção de dor do estímulo teste. Ele promove inibição generalizada através de neurônios de amplo alcance dinâmico (WDR) [152]. Não há um teste padrão para avaliá-lo; estudos já realizados utilizaram uma variedade de estímulos condicionantes, tais como, frio, calor e isquemia (figura 7) [152, 156].

Nem todas as pessoas saudáveis apresentam este fenômeno, na senescência ele provavelmente estará reduzido. Em condições de dor crônica o CPM estará reduzido ou ausente, podendo ser restaurado caso a condição de dor crônica seja tratada, como por exemplo, a cirurgia para protetização do quadril [152-154]. O efeito do CPM dependerá de qual estímulo (condicionante e teste) é utilizado, em média a resposta será de 29% em saudáveis. Mas o espectro de resposta pode variar desde boa inibição até algum grau de facilitação. É um importante biomarcador de dor crônica e um preditor de resposta ao tratamento. É uma medida replicável, mas o potencial de confiança dessa repetitividade depende dos parâmetros de estimulação, da população em estudo e da metodologia do estudo. Há evidências sugerindo o CPM como uma boa ferramenta prognóstica de resposta a tratamentos em condições de dor crônica e neuropática [154]. Nos items que seguem, após breve explanação das estratégias terapêuticas mais utilizadas no tratamento da OA do joelho, focaremos nas intervenções que são o alvo de interesse desta tese, a IMS e ETCC.

2.11. Estratégias terapêuticas

Os últimos guidelines de tratamento conservador da OA de joelhos são unânimes na utilização de exercícios aeróbicos de baixo impacto, reforço, alongamento e manutenção e/ou ganho da amplitude de movimentos como foco principal; além de redução do peso quando necessário e programas de auto-cuidado [11, 18, 157, 158]. Caso os sintomas persistam, a *Osteoarthritis Research Society International*, 2014, sugere: paracetamol, duloxetina, AINEs (vias oral e tópica), capsaicina, infiltrações intra-articulares com corticosteróides, uso de bengala, intervenções biomecânicas (órteses, palmilhas) e balneoterapia [18]. Os antiinflamatórios, incluídos nesse arsenal terapêutico, podem apresentar vários efeitos adversos, alguns graves [17, 159].

Bjordal JM et al., 2007, em uma metanálise com 36 ensaios clínicos randomizados, totalizando 2434 pacientes com dor crônica por OA de joelhos, encontraram resultados significativos de melhora em curto prazo (2-4 semanas) com TENS [escore na escala analógica-visual de dor (EAV) 18,8mm - 95% IC : 9,6 a 28,1; n=414]; eletro-acupuntura (EAV de 21,9mm - 95% IC: 17,3 a 26,5; n=73) e *laser* (EAV de 17,7mm - 95% IC: 8,1 a 27,3; n=343) [160]. O programa para controle da artrite através da educação e do exercício (*program for arthritis control through education and exercise – PACEex*) é um programa de autogestão que incorpora princípios e prática de autogerenciamento, definição de metas e exercícios em água aquecida. Mendelson AD *et al.*, avaliaram-no retrospectivamente em 347 sujeitos, com o objetivo de verificar a eficácia do autogerenciamento condicionado, da autogestão comportamental, dos níveis de objetivos alcançados, das deficiências auto relatadas, da dor e do estado de saúde. Os participantes mostraram melhora estatisticamente significativa no manejo de sua condição, na autogestão comportamental, no estado de saúde, nas deficiências e no nível de dor ($p<0,01$) apesar de terem apresentado aumento significativo no edema ($p<0,05$). Sessenta e oito por cento dos pacientes foram além de seus objetivos [161]. Brosseu *et al.*, implementaram um programa de caminhadas para portadores de OA de joelhos leve a moderada. Dividiram 222 sujeitos em três grupos: caminhada orientada, *PACEex* modificado (caminhada e educação) e caminhada autogerida através de panfleto educacional. Constataram que, em curto prazo, a adesão é maior nos grupos orientados, mas após 18 meses ela é maior no grupo autogerido. O grupo autogerido também adquiriu maior confiança em executar tarefas, comparado aos outros [162]. Cinesioterapia, ultrassom e TENS foram avaliados em um

ensaio clínico com 40 mulheres com OA de joelhos bilateral. Os autores encontraram melhora significativa nos três grupos quanto à dor (EAV), funcionalidade (WOMAC) e capacidade de se exercitar (teste de 6 minutos) [47]. Outra possibilidade de tratamento é a viscossuplementação, com infiltração intra-articular de ácido hialurônico, um glicosaminoglicano que promove lubrificação e absorção de choque e é base para os proteoglicanos da matriz extracelular [163]. Na pesquisa do uso de ortobiológicos o mais estudado até o momento é o plasma enriquecido de plaquetas. Chang ET AL em uma meta-análise verificou que os resultados do uso do plasma enriquecido de plaquetas são superiores e mais prolongados que os do ácido hialurônico em um seguimento de doze meses. Da mesma forma, Riboh ET AL observaram melhora significativa nos escores da WOMAC com plasma enriquecido de plaquetas comparado á ácido hialurônico [diferença média -21.14 (95% IC, -39.63 á -2.65)] e placebo [diferença média -17.84 (95% IC, -34.95 á -0.83)] [164]. A presente revisão terá como focos agulhamento seco (aqui definido como estimulação intramuscular – IMS) (figuras 8 e 9) e estimulação cerebral não invasiva (figuras 10 e 11).

2.12. Técnicas terapêuticas com agulhamento seco

As técnicas terapêuticas com o uso de agulhas são milenares. Existem múltiplas abordagens, denominações gerais que com frequência incluem um conjunto de técnicas distintas, o que dificulta o estudo e a compreensão dos mecanismos, técnica aplicada, contexto, etc. Nominalmente estas técnicas são conhecidas como acupuntura, que é largamente utilizada para controle da dor em diversas patologias [19], sendo este um dos seus efeitos mais proeminentes. Estudos têm sugerido que a analgesia pode ser iniciada pelo estímulo de fibras nervosas amielínicas finas e de alto limiar, que transmitem sinais através da medula espinhal e daí para centros neurológicos específicos, estimulando a liberação de neurotransmissores que bloqueiam a transmissão do estímulo doloroso [26, 165-168]. A liberação dessas substâncias modulatórias da dor parece estar relacionada ao aumento da expressão genética de neuropeptídios do SNC provocado pelo estímulo da agulha nos acupontos [169]. O estímulo das fibras táteis de grosso calibre (tipo A β), ativando neurônios da substância gelatinosa da medula espinal, inibe a transmissão das informações das fibras aferentes da dor, pelas fibras Aδ e C. O alívio da dor através de estímulos somáticos, dolorosos ou inócuos está relacionado a mecanismos conhecidos como de contrairritação, ou seja, analgesia por hiperestimulação ou por controle doloroso inibitório difuso. Também foram propostos outros mecanismos, como a modulação da função neuroimune. Ação antinociceptiva local por indução de

nucleotídeos adenina (adenosinas trifosfato–ATP, difosfato-ADP, monofosfato-AMP) e adenosina no músculo [170]. Park ET AL, sugere ativação da quinase extracelular regulada por sinal (ERK) dos fibroblastos e queratinócitos da pele agulhada como fator chave na analgesia da acupuntura utilizando modelo animal [171]. Várias estruturas do trato descendente inibitório da dor, tais como, hipotálamo, núcleo *acumbens*, mesencéfalo (incluindo a substância cinzenta periaquedutal e o núcleo da rafe) e sistema límbico estão envolvidos nos mecanismos analgésicos da acupuntura [172]. Estudos de neuroimagem mostraram ativação também do tálamo, córtex somatosensorial II, giro cingulado medial, lóbulo paracentral, giros frontais superior e inferior, precúneo, lóbulo parietal inferior, giros temporais superior e médio, giro fusiforme e cerebelo [173]. Evidências crescentes vêm demonstrando os mecanismos autonômicos da analgesia por acupuntura, com atividade parassimpática aumentada e simpática diminuída vinculada à atividade eletroencefalográfica aumentada [174-176]. Isto é especialmente interessante à luz de que algumas condições dolorosas crônicas têm marcado componente simpático [177]. Dentre as hipóteses sobre os possíveis mecanismos que explicam os efeitos terapêuticos da acupuntura, consideram-se eventos interdependentes locais, segmentares e centrais [178]. Carlsson aponta como eventos locais os reflexos axonais que desencadeiam aumento da circulação local e liberação de neuropetídeos e de endorfinas para receptores locais; como eventos segmentares, mecanismos de “portão”, possível *long-term depression* (LTD), inibição proprioespinal, reversão da *long-term potentiation* (LTP) para LTD (que reduz a sensibilização central) e inibição simpática com aumento da circulação segmentar; e como eventos centrais, inibição simpática, redução dos níveis de hormônios relacionados à resposta ao estresse, adrenalina e cortisona no plasma, além de uma possível ação da ocitocina na indução da elevação dos limiares de dor em longo prazo e efeitos antiestresse. LTP e LTD são mecanismos de plasticidade neuronal observados em conexões excitatórias [136] .

Maioli C et AL demonstraram que a estimulação com inserção de agulhas de acupuntura com ou sem manipulação promovem modificações plásticas na excitabilidade do sistema nervoso central, que perduram após a retirada das agulhas [179]. Em uma meta-análise realizada por Huang ET AL, avaliou-se a resposta encefálica ao estímulo da acupuntura através da ressonância magnética funcional verificando-se ativação de uma ampla rede de regiões de processamento somatossensorial, afetivo e cognitivo [180].

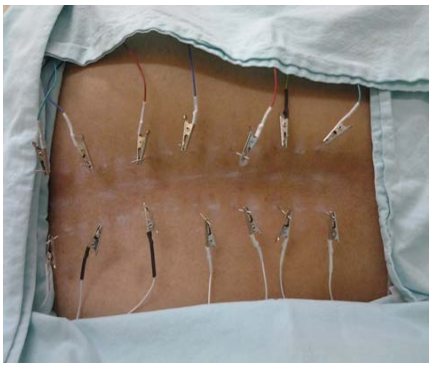
Com relação á eletroacupuntura na dor inflamatória, há liberação de opióides dos granulócitos, macrófagos, linfócitos e monócitos através da ativação simpática com aumento da expressão da molécula intracelular de adesão 1 nos vasos com migração de β endorfinas e meta-encefalinas. Incremento dos receptores canabinóides CB2 e anandamida endógena com redução da

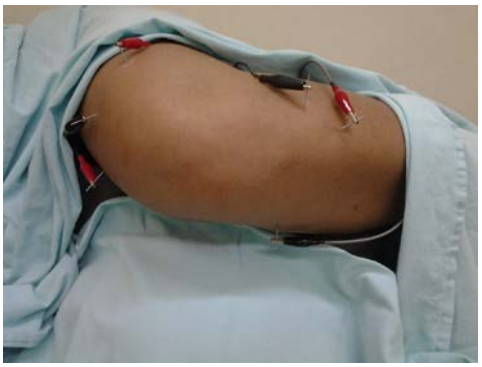
expressão de TNFα, interleucinas 1β e 6, COX 1 e 2 e prostaglandina E2.Na dor crônica, eletroacupuntura de alta e baixa intensidade promove o alívio da dor através dos receptores opióides μ e δ, via mecanismos pré e pós sinápticos.Induz liberação espinhal de receptores peptídeos nociceptina/orfanina FQ, atenuando a dor também por mecanismos pré e pós sinápticos. Eletroacupuntura também inibe a fosforilação do receptor n-metil-d-aspartato subunidade GluN1 promovendo a redução da liberação de glutamato pré sináptico pelo incremento da norepinefrina. Pela via pós sináptica reduz a dor através da ativação do receptor 5-HT1A. Reduz a liberação de substância P e síntese de citocinas da glia. Inibe receptores de neurocininas (NK1). Promove o trabalho interativo de vários neurotransmissores, incluindo dopamina e acetilcolina na redução da dor. Há liberação de endomorfinas ativando receptores μ opióides em neurônios gabaérgicos, inibindo a liberação de GABA proporcionando a ativação de neurônios serotonérgicos. Pode suprimir a atividade dos receptores NMDA através de opióides endógenos no córtex cingulado anterior intra-rostral [181].

O termo acupuntura pode ser confuso devido á sua imprecisão, assim como seu uso na literatura médica pode referir-se a um grande número de procedimentos, não necessariamente idênticos. Embora existam ensaios clínicos metodologicamente adequados a respeito dos efeitos da acupuntura na dor, existe uma grande heterogeneidade de técnicas e critérios diagnósticos [24, 182]. Para superar a confusão imposta pelo termo acupuntura e distingui-la de outras formas de agulhamento este estudo utiliza inserção de agulhas dependentes primariamente das propriedades neurofisiológicas das estruturas anatômicas a serem estimuladas, o que determina a dose e a técnica de agulhamento, esta técnica é denominada IMS.

2.13. Estimulação Intramuscular (IMS)

A “Estimulação intramuscular” (IMS), descrita por Gunn é uma técnica de agulhamento baseada inteiramente nos princípios da neurofisiologia e da neuroanatomia. Este método permite que o exame clínico, o diagnóstico, e o tratamento, bem como o progresso da terapia possam ser determinados de acordo com os sinais físicos da disfunção neural [183] (figuras 8 e 9). IMS é aplicada nos segmentos espinhais das raízes nervosas associadas ao dermátomo, ao miótomo, ou ao esclerótomo onde os pontos-gatilho forem encontrados [184, 185]. Alguns estudos anteriores revelaram que a IMS é superior ao agulhamento seco no ponto-gatilho para síndrome miofascial no alívio da dor [185, 186], e que a dor pode ser tratada mais eficazmente utilizando técnicas de IMS que pelos métodos clássicos [187-189].





Figuras 8 e 9: aplicação de IMS profunda a 2 cm dos processos espinhosos de L1-L5, S1-S2 (relacionados a sensibilização na KOA); e nos músculos dos dermátomos correspondentes acometidos.

Ga et al., 2007, compararam o efeito da injeção de lidocaína nos pontos-gatilho miofasciais com a IMS em 43 idosos. A IMS constou de agulhamento seco dos pontos-gatilho nos músculos trapézios e estimulação das raízes nervosas correspondentes segundo a técnica de Gunn (de acordo com o dermátomo, o miótomo e o esclerótomo acometido). Os resultados demonstraram melhora significativa nos parâmetros de dor, na amplitude de movimento cervical e nos sintomas depressivos com os dois tratamentos, mas a IMS apresentou resultados superiores [190].

Uma modalidade de IMS que permite quantificar a intensidade de estímulo é a eletroacupuntura (EAC), que, na intensidade de 2 Hz, acelera a liberação de encefalinas, beta-endorfinas e endomorfinas; na intensidade de 100Hz, induz a liberação seletiva de dinorfinas [26]. Embora o efeito da EAC, assim como o da acupuntura, tenha sido demonstrado em vários estudos, ele não é contundente na literatura. Considerando os múltiplos fatores implicados nestas divergências, propõe-se, a partir de uma perspectiva neurobiológica, o uso de um protocolo padronizado que inclui: o número de agulhas utilizadas, a técnica de agulhamento, a obtenção da sensação específica no agulhamento, o número de sessões de tratamento e a experiência do acupunturista.

2.14. Eletroestimulação cerebral não invasiva

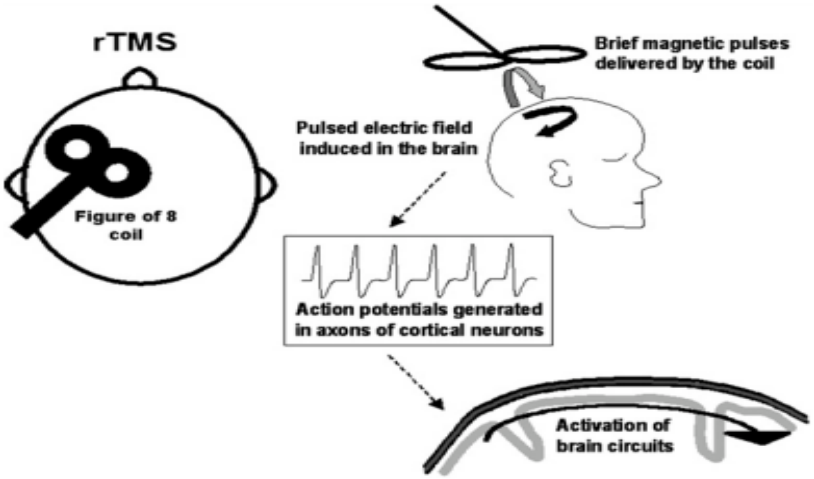


Figura 10: Exemplo esquemático de aplicação de EMT. **Fonte:** Veit Mylius et al, 2012 [191].

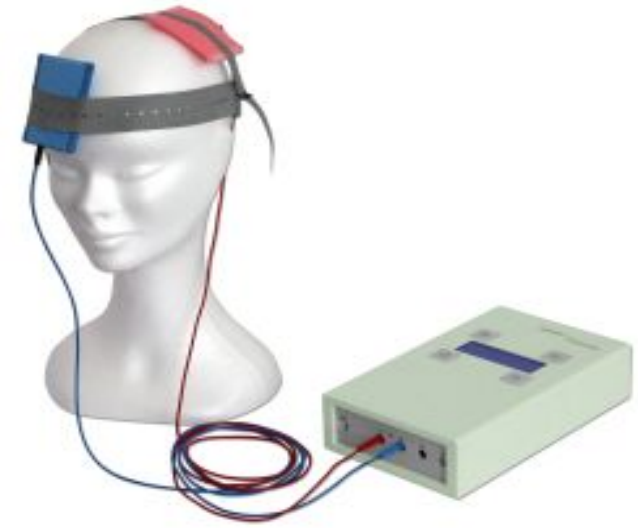


Figura 11: Exemplo esquemático de aplicação de ETCC. **Fonte:** drmueller-healthpsychology.com.

Aplicação de correntes elétricas para modificar a função cerebral é uma técnica bastante antiga [27], no tratamento da dor crônica é utilizada há mais de 200 anos [192]. Scribonius Largus,

médico do imperador romano Claudius, utilizava o peixe torpedo vivo no tratamento de cefaléias. No século XI, Ibn-Sidah, médico muçulmano, descreveu o uso do peixe-gato elétrico para tratar epilepsia. Os efeitos fisiológicos da eletricidade foram descritos no século XVIII, por Walsh (1773), Galvani (1791, 1797) e Volta (1792). Giovanni Aldini (sobrinho de Galvani) propôs o tratamento da melancolia com estimulação transcraniana. Zago et al, utilizaram correntes galvânicas nas desordens mentais. Eletroconvulsoterapia foi amplamente utilizada em psiquiatria, porém seu uso indiscriminado e sem marcadores neurofisiológicos confiáveis levou ao declínio da utilização. Não obstante, a corrente galvânica continuou a ser utilizada para patologias musculares e dor periférica [193].

Recentemente, reavivou-se o interesse pelos métodos de estimulação cerebral não invasiva para aplicações fisiológicas e terapêuticas [191]. Priori et al, Nitsche e Paulus descreveram a corrente elétrica transcraniana direta fraca para induzir mudanças corticais (bidirecional, polaridade dependente) [193]. Atualmente as principais são as estimulações magnética transcraniana (EMT) (figura 10) e transcraniana de corrente contínua (ETCC) (figura11), que têm demonstrado capacidade de modular vários processos neurológicos, inclusive dor [191]. Têm sido testadas em uma variedade de condições neuropsiquiátricas, como depressão maior, esquizofrenia, acidente vascular encefálico, dor crônica e doença de Parkinson [27] . Nos estudos de dor, o alvo principal é o córtex motor primário (M1), local validado para tratamento de dor refratária através de estimulação cortical [191]. A modulação de dor através da estimulação de M1 aparentemente se dá por efeitos corticais diretos nos núcleos ventro lateral e talâmico anterior, assim como tálamo medial, córtex cingulado anterior e porção superior do tronco [194]. Vários estudos em patologias de dor crônica têm demonstrado efeito clinicamente significativo na redução da dor com a aplicação de ETCC em M1 (por exemplo, fibromialgia, dor fantasma, neuralgia do trigêmio, migrânea crônica, dor lombar crônica e dor miosfascial crônica) [195] . Estudos de neuroimagem com EMTr em M1 têm demonstrado efeitos nos córtices pré-frontal, cingulado, orbitofrontal e insular, bem como tálamo e striatum [196]. Há mostras de que, conforme o mecanismo utilizado para promover a dor, diferentes fibras nervosas aferentes e circuitos neurais são recrutados, razão pela qual os resultados dos tratamentos obtidos através dos métodos de estimulação cortical não invasiva variam [191].

2.15. Mecanismos neurobiológicos da ETCC

A ETCC é baseada na aplicação transcraniana direta de corrente fraca (de 1 a 2mA), contínua, através de eletrodos escalpeanos de uma forma não invasiva, simples e indolor [197]. Outras

vantagens incluem o baixo custo, a possibilidade de um placebo confiável e a segurança (os estudos têm demonstrado efeitos adversos leves e transitórios) [198, 199]. Os efeitos são polaridade dependentes; de forma geral, a estimulação do anodo induz aumento da excitabilidade cortical e a estimulação do catodo a reduz [5, 193, 200, 201]. Os efeitos são explicados pela mudança no potencial de membrana de repouso (despolarização ou hiperpolarização). A estimulação anodal na primeira sinapse despolariza a membrana, hiperpolarizando nas sinapses subsequentes promovendo maior liberação de neurotransmissores através do aumento do cálcio intracelular. A liberação de neurotransmissores e o acoplamento de vesículas sinápticas aumenta mais pela ativação da quinase de receptor de tropomiosina (Trk), sugerindo um papel do BDNF. A liberação de neurotransmissores também facilita a abertura de canais AMPA e indiretamente NMDA com respostas de potenciação de longa duração (LTP). Uma vez ativado o receptor de Trk produz a LTP de fase tardia (L-LTP) favorecendo a abertura dos receptores NMDA. Ocorrendo o oposto com a ETCC catódica, com promoção de depressão de longa duração (LTD) (figura 12) [5]. Altera o limiar da membrana para gerar potenciais de ação na área estimulada e também para contra regular os processos disfuncionais causados pela doença em questão [5, 200, 202]. Porém, quando o tempo de estimulação no anodo é aumentado pode haver redução da excitabilidade e; quando a intensidade é aumentada no catodo pode haver aumento da excitabilidade. A técnica é, portanto, neuromodulatória, buscando homeostase [5]. Muda a excitabilidade de inputs sinápticos e a frequência de disparos espontâneos. Modifica o microambiente sináptico, alterando a força sináptica através dos receptores NMDA ou através da atividade gabaérgica [191, 193, 203]. Modula neurônios intracorticais e corticoespinhais e possui efeitos não sinápticos por mudanças transitórias na densidade dos canais de proteína [191, 193]. ETCC induz alterações da excitabilidade cortical promovendo efeitos duradouros [191] por aumentar a atividade espontânea levando a um aumento do *input* pré-sináptico e aumento da regulação do tônus sináptico mediado pelo NMDA. Esses efeitos duradouros também são dependentes de síntese de proteína e acompanhados por modificações no AMP cíclico intracelular e nos níveis de cálcio. Também se relaciona á promoção de neuroplasticidade dependente do uso, com mudanças de longo prazo semelhantes aos fenômenos de LTP e LTD das sinapses glutamatérgicas, como citado anteriormente [5, 27, 200]. Os efeitos podem ser explicados também pelo aumento da eficiência sináptica [200]. Promove mudanças neuroquímicas prolongadas, modulando oscilações neuronais espontâneas, altera MEPs, SSPEs e VEPs, modula a condução na medula espinhal e vias reflexas segmentares [204]. A eletrotaxia gerada pelos campos elétricos contribui para a neuromodulação assim como mudanças na expressão do BDNF. A orientação dos axônios no campo elétrico também é importante para ocorrência dos efeitos [5]. Outro mecanismo pelo qual ocorreria melhora da dor através da ETCC do anodo seria sua capacidade de restaurar o sistema inibitório

(gabaérgico) que se encontra deficitário nos pacientes portadores de dor crônica [191]. Há evidências que a ETCC atinja estruturas mais profundas como núcleo rubro e fascículo longitudinal medial, mas ainda não se sabe se é por efeito direto ou através do aumento da excitabilidade. Também se demonstrou sua capacidade modulatória em processos inflamatórios, liberação de fatores neurotróficos, crescimento e orientação dos dendritos e aumento de sua densidade (propriedade neuroprotetora e neurorestauradora). Vários neurotransmissores respondem pela neuroplasticidade promovida pela ETCC [5]. Influencia vários tecidos diferentes e em múltiplas estruturas celulares, como componentes não neuronais do SNC [193].

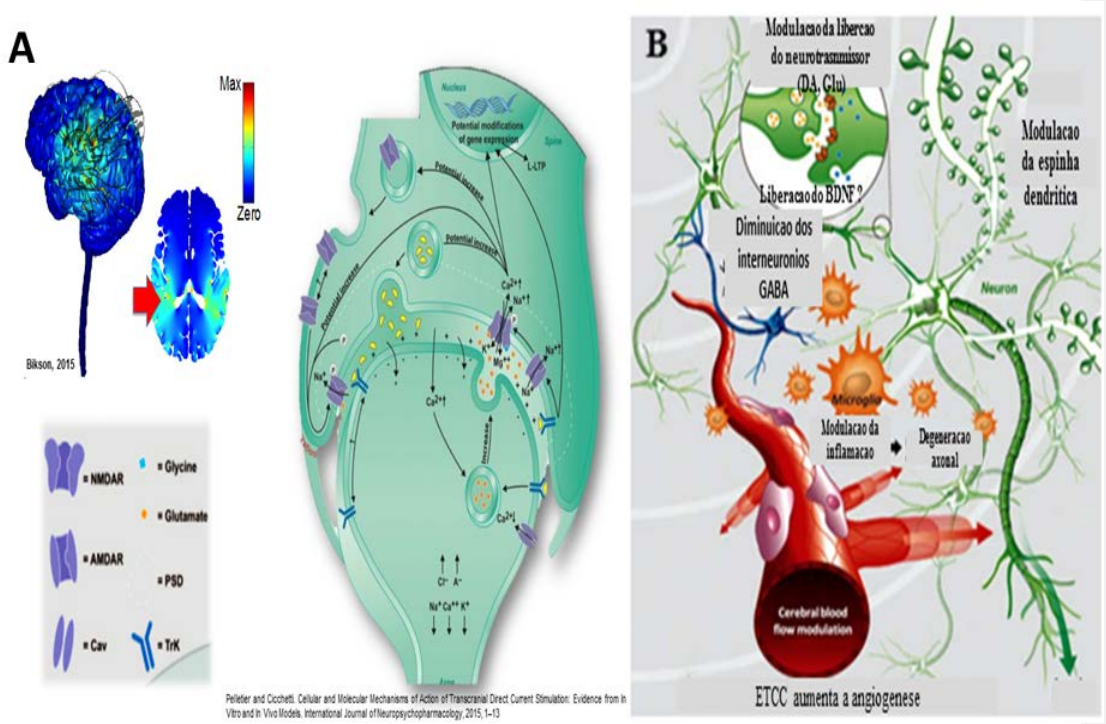


Figura 12: Adaptado de Pelletier 2015 [5] . (**A**) A ETCC anódica hiperpolariza a membrana dos interneurônios, com maior liberação do neurotransmissor, um efeito causada por um aumento no Ca2 + intracelular , enquanto que uma redução do Ca^{2+} leva a menor liberação do neurotransmissor. A ativação da Trk sugere um papel para o BDNF na ETCC anódica, seu efeito aumenta ainda mais a probabilidade de acoplamento de vesículas sinápticas e a liberação de neurotransmissor. Em geral, com respostas de LTP, existe uma regulação positiva da liberação de neurotransmissores que facilita a abertura de canais AMPA e indiretamente a de canais NMDA. O oposto ocorre na LTD. O influxo

de Ca^{2+} aumenta a fosforilação de AMPA e a sua incorporação à membrana. O aumento do Ca^{2+} aumenta ainda mais a liberação do fator neurotrófico para a fenda sináptica, enquanto na ausência do Ca^{2+} diminui. Uma vez ativado, o receptor de Trk pós-sináptico induz a LTP de fase tardia (L-LTP) e favorece a abertura de canais de NMDA, o que também promove L-LTP, enquanto ocorre o oposto na ETCC catódica, que promove a LTD. Tanto a L-LTP como a L-LTD dependem de modificações da expressão gênica. (**B**) A ETCC anódica está envolvida no remodelamento dendrítico, juntamente com aumento na densidade dendrítica, um mecanismo sugerido para suas propriedades neuroprotetoras e neuroreparadoras. Em um modelo de dor crônica induzida por estresse com ratos, o fator de necrose tumoral-α (TNF-α) também foi reduzido pela ETCC anódica. Foi observado também, aumento da angiogênese e dos níveis de fator de crescimento endotelial vascular (VEGF) em tecidos periféricos. A ETCC afeta uma série de processos fisiológicos tanto no sistema nervoso central quanto em nível periférico, os quais podem ser relevantes para os seus efeitos nos estados patológicos. AP, potencial de ação; BDNF, fator neurotrófico derivado do cérebro; DA, dopamina; Glu, glutamato.

2.16. ETCC – uso clínico e evidências de eficácia

O objetivo terapêutico orienta a técnica neuromodulatória, o sítio a ser estimulado e o tipo de estímulo a ser utilizado [29]. ETCC com o anodo localizado no M1 induz grande melhora da dor quando comparado com placebo e ETCC ativo do córtex pré-frontal dorsolateral (DLPFC). Estudos têm demonstrado que o efeito após um curso de tratamento pode perdurar até pelo menos três meses [205]. No estudo de Roizenblat o M1 e DLPFC foram os alvos. A estimulação de M1 promove analgesia significativa nas síndromes de dor central e DLPFC está associado a efeitos antidepressivos. ETCC também pode afetar alguns elementos do sono, específicos do sítio de estimulação [206]. Também é capaz de melhorar o aprendizado motor implícito e a coordenação vísuomotora [27]. ETCC induz uma modulação global da plasticidade cortical, abrangendo a modulação colinérgica global [207]. ETCC anodal de M1 sugeriu modulação inibitória do tálamo de forma direta ou indireta [208]. Essa modulação inibitória propõe-se a inibir a hiperatividade nas vias da dor, incluindo tálamo medial, córtex cingulado anterior e tronco superior [38]. Destacando-se o já citado papel chave do córtex cingulado anterior, que se mostra alterado em estados de dor crônica, com atrofia e conectividade reduzida [209].

A dor crônica é bastante prevalente e as opções terapêuticas baseadas em evidências são limitadas. ETCC é uma alternativa terapêutica atraente entre as terapias não farmacológicas, através

de seus possíveis efeitos na plasticidade mal adaptativa. Alguns de seus efeitos e parâmetros foram primeiramente demonstrados em saudáveis. Em busca de tratamentos eficazes a ETCC foi testada em várias condições de dor crônica como fibromialgia, dor neuropática, migrânea, dor miofascial e lombalgia [31, 32, 203, 210, 211]. A dor neuropática da lesão medular foi testada com resultados positivos [34, 210], ETCC em M1 também foi testada juntamente com ilusão visual [208]. Boggio *et al*., 2009 compararam em oito pacientes, em um estudo cruzado, o efeito do ETCC+TENS com somente ETCC e placebo. Obtiveram melhora significativamente maior no grupo ETCC+TENS da dor neuropática crônica localizada dos pacientes. Embora não se conheçam os mecanismos pelos quais ocorreu esta melhora os autores inferem que a aferência periférica do TENS poderia aumentar a resposta do ETCC[192]. Na esclerose múltipla a ETCC em M1 melhorou a dor [37]. Fibromialgia foi outra condição de dor crônica extensivamente avaliada com ETCC. O primeiro estudo foi em 2006, com 32 pacientes, mostrou melhora significativa da dor com ETCC anodal em M1, mas não no DLPFC [203]. Da mesma forma, a utilização da ETCC com exercício aeróbico em pacientes fibromiálgicas foi testada com sucesso em um estudo fase II que avaliou ETCC e exercício aeróbico separados e em conjunto. O grupo ETCC+exercício aeróbico mostrou melhora estatísticamente significativa em relação somente ao exercício ($F(13, 364) = 2.25$, $p=.007$); e somente ETCC ($F(13, 364) = 2.33$, $p=.0056$) [212]. Outros estudos confirmaram esse efeito analgésico [35, 201, 209, 213, 214]. Parece que o efeito de terapias combinadas é maior. Há evidências de melhora do sono e qualidade de vida [35, 201, 206]. A combinação de ETCC anodal no DLPFC e injeção dos pontos-gatilho mostrou melhora significativa na dor miofascial [31]. Outras condições de dor crônica apresentaram melhora significativa da dor com ETCC anodal em M1, tais como: lombalgia, dor induzida pelo interferon no tratamento da hepatite C, dor crônica pelo vírus T linfotrófico humano tipo I (HTLV I), dor da disfunção das articulações temporo-mandibulares, dor fantasma, dor da polineuropatia diabética, dor da doença inflamatória intestinal e dor neuropática dos membros superiores [30, 32, 36, 38, 192, 215-217]. Embora M1 esteja se consolidando como principal alvo para o tratamento da dor crônica [216] há pesquisas com boa resposta com o uso do DLPFC, como no estudo de Ayache ET AL, com pacientes com esclerose múltipla. Sugerindo analgesia através da modulação da rede emocional da dor (Tabela 1) [218].

A literatura mostra uma diversidade de técnicas de montagem: a com um eletrodo no encéfalo e outro fora é chamada unipolar. A com dois eletrodos, bipolar. O eletrodo “neutro” influencia o fluxo da corrente, conforme sua localização. Os efeitos adversos dependem da densidade de corrente, a redução da salinidade do meio condutor minimiza esses efeitos. Cabe salientar que a posição do eletrodo pode mudar de maneira dramática o fluxo de corrente. Os tamanhos de eletrodos mais

comumente utilizados são 7cm x 5cm e 5cm x 5cm, embebidos em solução salina para redução da resistência e melhor condutividade [203, 204, 218]. Esse padrão de eletrodos promove liberação de corrente em áreas difusas do encéfalo [204, 213]. A possibilidade de atingir uma área mais específica do encéfalo pode ser obtida através da utilização da ETCC de alta definição (HD-tDCS). Utiliza pequenos eletrodos em um formato 4 x 1 (adaptador multi-canais), com um eletrodo central e quatro distribuídos no entorno, sobre locais específicos e mantendo o mesmo raio; utiliza gel condutor para reduzir a resistência [213].

Tabela 1: Artigos de ETCC e dor crônica

Referência	Autores	Local da aplicação	Tipo de aplicação	Número de participantes/ patologia	Resultados
[30]	Volz MS ET AL 2016	M1 esquerdo Anodal Sham	2mA/20m/ 5s Seguimento 1 semana	20 Dor abdominal em doença inflamatória intestinal	↓VAS p=.014 -2.05±2.05 ↑PPT p=.000014 +.86kg
[31]	Choi YH ET AL 2014	M1 DLPFC Sham	2mA/20m/ 5s	24 3 perdas SDM cintura escapular	↓VAS DLPFC p=.008 -3.50
[32]	Schabrun ET AL 2014	M1 esquerdo Estimulação elétrica periférica Sham	1mA/30m 2hz/30m Cruzado 4 grupos Washout 1 semana	16 2 perdas Lombalgia crônica	↓VAS p=.03 2.3-2.1 Nos 3 grupos ativos
[34]	Yoon EJ ET AL 2014	M1 esquerdo anodal Sham	2mA/20m/ 2x ao dia/10dias	16 Dor neuropática Lesão medular	↓NPS -23% p=.016 pré 7.6±.5 pós 5.9±1.8
[35]	Fagerlund AJ ET AL 2015	M1 esquerdo Anodal Sham	2mA/20m/ 5s Seguimento 30 dias	50 2 perdas Fibromialgia	↓NPS tender points p=.012 13.6% (95%CI .36-.96)

[212]	Mendonça ME ET AL 2016	M1 esquerdo Anodal Exercício aeróbico ETCC sham	2mA/20min 5s Exercícios 4 semanas, 3x semana Seguimento 2 meses	45 12 perdas Fibromialgia	↓dor vs EA F=2.25 p=.007 vs ETCC F=2.33 p=.0056 cohen's effect size >.55
[217]	Brietzke AP ET AL 2016	M1 esquerdo Anodal Sham	2mA/20m/ 5s	28 3 perdas Dor crônica HCV	↓VAS 56% p<.01
[36]	Donell A ET AL 2015	HD-ETCC M1 esquerdo Anodal Sham	2mA/20m/ 5s Seguimento 4 semanas	24 Dor crônica disfunção ATMs	↓VAS >50% P=.04
[37]	Mori ET AL 2010	M1 esquerdo Anodal Sham	2mA/20m Seguimento 3 semanas	19 Dor neuropática Esclerose múltipla	↓VAS 45.5%±11 p<.05
[219]	Borckardt ET AL 2011	HD-ETCC DLPFC esquerdo Anodal Sham	Eletrodos 4x4 2mA/20m	21 2 perdas Dor pós-ERCP	↓VAS 22% menos hidromorfona F(2,13)=15.96 p=.0003
[213]	Villamar ET AL 2013	HD-ETCC M1 esquerdo Anodal Catodal Sham	Cruzado 2mA/20m Washout 1 semana	18 2 perdas Fibromialgia	Ativos: ↓VAS p=.004 Ef Sizes: Anodal .36 Catodal .30
[203]	Fregni F ET AL 2006	M1 DLPFC Anodal	2mA/20m/ 5s Seguimento 3 semanas	32 1 perda Fibromialgia	Anodal ↓VAS -58% .31 a cada sessão p=.001
[192]	Boggio P ET AL 2009	M1 esquerdo Anodal TENS Sham	2mA/30m 151µs/4hz/ 30m Washout 48h	8 2 perdas Dor neuropática nos membros superiores	ETCC+ TENS ↓VAS 36.5%± 10.7 p=.004

[201]	Valle ET AL 2009	M1 DLPFC Esquerdos Sham	2mA/20m/ 10s Seguimento 2 meses	41 Fibromialgia	Ativos ↓VAS M1 manteve efeito 2m -30% F(3.52)= 4.07 p=.011
[218]	Ayache ET AL 2016	DLPFC Esquerdo Anodal Sham	Cruzado 2mA/20m 3s ativas e 3s sham Washout 3 semanas	16 Dor esclerose múltipla	↓VAS Pré 51.2±19.2 Pós 43.1±26.2 p=.002
[216]	Bolognini ET AL 2013	M1 Anodal Catodo Córtex parietal posterior Sham	2mA/15m	8 Dor fantasma	M1 ↓VAS Pré 2.6cm Pós .8cm p<.02
[208]	Soler ET AL 2010	M1 Esquerdo Anodal Ilusão Visual Sham	2mA/20m/ 10s/2sem 4 grupos Marcha Seguimento 12 semanas	40 3 perdas Lesão medular Dor neuropática	Todo ativo: ↓NPS 29.7% p=.004
[215]	Souto ET AL 2014	M1 Esquerdo Anodal Sham	2mA/20m/ 5s	20 Dor HTLV	↓VAS 50% em 80% dos sujeitos p=.03
[38]	Kim ET AL 2013	M1 DLPFC Anodal Esquerdo Sham	2mA/20m 5s	60 Polineuropatia Diabética	M1 ↓VAS 33.91% (20-50%)
[209]	Foerster ET AL 2015	M1 Esquerdo Anodal Sham	Cruzado 2mA/20m 5s Washout 1 semana	12 Fibromialgia	↓VAS -35% P=.04
[210]	Fregni F ET AL 2006	M1 Esquerdo Anodal Sham	2mA/20m 5s Seguimento 16 dias	17 Dor neuropática Lesão medular	↓VAS -58% -2.6 (95%CI 1.44-3.74) Média 22.9%±3.6
[214]	Jales Junior et al. (2015)	M1 Esquerdo Anodal Sham	1mA/20m 10s 1x semana	20 Fibromialgia	↓VAS Pré=6.05 Pós=3.60 p<.032

3 JUSTIFICATIVA

A OA de joelhos é altamente prevalente, sendo um problema de saúde pública mundial. Já responde por altos custos em saúde e ausências ao trabalho. O envelhecimento da população tende a um crescimento exponencial da prevalência e dos custos, podendo tornar-se um ônus extremamente importante nos sistemas de saúde. Agrega-se a limitada eficácia das intervenções de tratamento conservador. Esse somatório de questões estimulou a busca de alternativas com o intuito de incrementar o sucesso terapêutico.

Sabendo que a KOA cursa com um processo inflamatório crônico com sensibilizações periférica e central, levantou-se a hipótese de alteração da excitabilidade cortical e enfraquecimento do sistema inibitório descendente de dor, mecanismo já visto em outras patologias de dor crônica. Essa hipótese motivou o primeiro estudo dessa tese: *"Descending control of nociceptive processing in knee osteoarthritis is associated with intracortical disinhibition"*.

A partir do resultado do primeiro estudo surgiram as hipóteses dos dois estudos seguintes, constituintes dessa tese, em busca do tratamento da KOA: ii) "*Electrical intramuscular stimulation in osteoarthritis enhances the inhibitory systems in pain processing at cortical and cortical spinal system"* (nº de registro no **NCT 01855958**). iii)"*Transcranial direct current stimulation potentiates the effects of electrical intramuscular stimulation at relieving pain and improving the descending pain modulatory system in patients with knee osteoarthritis: a randomised, double blinded, factorial designed and sham controlled study"* (nº de registro no **NCT 01747070**).

4 MAPA CONCEITUAL

A OA de joelhos é uma doença articular degenerativa caracterizada pela presença de dano articular e processo inflamatório. Os indivíduos afetados frequentemente sofrem de dor crônica, com causas não totalmente elucidadas. A correlação entre os achados radiológicos e a intensidade da dor é fraca. Porém, essa correlação é forte quando observamos a piora dos achados radiológicos em um mesmo indivíduo. A partir dessas constatações inferimos que a dor não é específica do joelho, existindo uma sensibilização devido a alterações no processamento central da dor, verificando-se perda da atividade inibitória analgésica descendente e aumento da atividade fascilitatória [220].

A OA de joelhos ainda carece de um tratamento conservador eficaz. As técnicas de estimulação transcraniana não invasiva, especialmente a ETCC e a EMT vislumbram um novo horizonte de diagnóstico e tratamento; e a eletroestimulação intramuscular pode tornar-se uma ótima opção terapêutica a partir de uma técnica de estimulação profunda com bases neurofisiológicas. O mapa conceitual apresentado integra as três questões centrais desta tese previamente mencionadas.

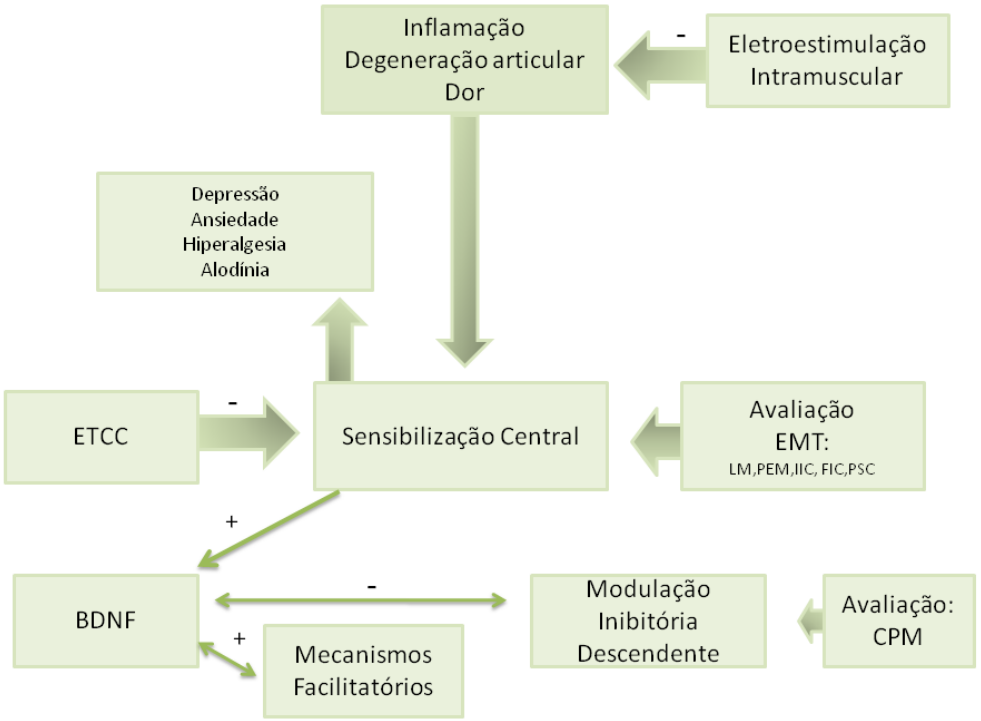


Figura 13: Representação esquemática da fisiopatologia da KOA com sensibilização central. Atuação da IMS (*bottom up*) e ETCC (*top down*) visando redução da dor,inflamação e melhora da funcionalidade.

5 OBJETIVOS

Essa tese teve como objetivo geral avaliar a relação entre a excitabilidade cortical, a função do sistema descendente inibitório de dor e a perpetuação de dor na KOA. Assim como avaliar o efeito de uma abordagem de tratamento *top down* (ETCC) e outra *bottom up* (IMS) na modificação da excitabilidade cortical, sistema modulador descendente da dor e os processos de neuroplasticidade relacionados, tendo como alvo as modificações associadas a doença e como os tratamentos se relacionam com estes biomarcadores de neuroplasticidade.

Objetivos do primeiro estudo:

Comparar se a função da via descendente inibitória de dor está associada com o estado de inibição no sistema corticoespinhal; e determinar se há correlação com alterações na NPS durante a *CPM-task.*

Objetivos do segundo estudo:

Determinar se uma sessão de IMS na KOA é capaz de promover mudança na excitabilidade cortical e na NPS durante a *CPM-task*, bem como na VAS e no PPT. Também se propôs a avaliar o papel do BDNF no efeito do tratamento.

Objetivos do terceiro estudo:

Avaliar a capacidade da ETCC (cinco sessões) e da IMS (cinco sessões) em reforçar o sistema descendente inibitório de dor, modular a excitabilidade cortical e modificar a dor através da VAS, NPS durante a *CPM-task* e PPT. Também se propôs a avaliar se o efeito dos dois tratamentos em conjunto tinha resultados superiores na melhora da dor e reforço do sistema inibitório descendente de dor através do “priming” da ETCC sobre a IMS.

6 REFERÊNCIAS BIBLIOGRÁFICAS

1. Gehling J, MainkaT., Vollert J, Pogatzki-Zahn EM,Maier C, Enax-Krumova EK., Short-term test-retest-reliability of conditioned pain modulation using the cold-heat-pain method in healthy subjects and its correlation to parameters of standardized quantitative sensory testing. BMC Neurol., 2016. 16(125).

2. Gold MF., Gebhard GF., Peripheral pain mechanisms and nociceptor seinsitization. Ch 3. In: Fishman SM, Ballantyne JC, Rathmell JP. Bonica's Management of pain,Fourth edition., 2010. Wolters Kluwer/Lippincott Williams e Wilkins.: p. 24-34.

3. Turk DC, Okifuji A., Pain terms and taxonomies of pain. In:Fishman SM, Ballaltyne JC, Rathmell JP. Bonica's Management of Pain., 2010. Wolters Kluwer Health / Lippincott Williams & Wilkins.: p. 13-23.

4. Rocha, A.P., et al., Pain: current aspects on peripheral and central sensitization. Rev Bras Anestesiol., 2007. 57(1): p. 94-105.

5. Pelletier SJ, Cichetti F. Cellular and molecular mechanisms of action of tDCS: evidence from in vitro and in vivo models. Int J Neuropsychopharmacol 2015; 1-13. doi: 10.1093/ijnp/pyu047.

6. Doherty, M.J., Pain in osteoarthritis., in Pain 2002: an updated review: refresher course syllabus. , M.A. Giamberardino, Editor 2002, IASP Press. p. 51-7.

7. Recommendations for the medical management of osteoarthritis of the hip and knee: 2000 update. American College of Rheumatology Subcommittee on Osteoarthritis Guidelines. Arthritis Rheum, 2000. 43(9): p. 1905-15.

8. Felson, D., The sources of pain in knee osteoarthritis. Curr Opin Rheumatol, 2005. 17(5): p. 624-8.

9. Imamura M, Esquerro F., Alfieri FM, Vilas Boas F, Tozetto-Mendoza TR, Chen J, Özçakar L, Arendt-Nielsen L, Battistella LR., Serum Levels of Proinflammatory Cytokines in Painful Knee Osteoarthritis and Sensitization. J Inflammation., 2015. doi.org/10.1155/2015/329792.

10. Nguyen U-S DT, Zhand Y., Zhu Y, Niu J, Zhang B, Aliabadi P, Felson DT., Increasing Prevalence of Knee Pain and Symptomatic Knee Osteoarthritis. Ann Intern Med. , 2011. 155(11): p. 725–732.

11. Fernandes L, Hagen K., Bijlsma JWJ, Andreassen O, Christensen P, Conaghan PG, Doherty M, Geenen R, Hammond A, Kjeken I, Lohmander LS, Lund H, Mallen CD, Nava T, Oliver S, Pavelka K, Pitsillidou I, da Silva JA, de la Torre J, Zanoli G, Vlieland TPMV., EULAR recommendations for the non-pharmacological core management of hip and knee osteoarthritis. Ann Rheum Dis 2013. 72: p. 1125–1135.

12. Conrozier, T., et al., Increased serum C-reactive protein levels by immunonephelometry in patients with rapidly destructive hip osteoarthritis. Rev Rhum Engl Ed, 1998. 65(12): p. 759-65.

13. Spector, T.D., Hart DJ,Nandra D,Doyle DV,Mackillop N,Gallimore JR,Pepys MB., Low-level increases in serum C-reactive protein are present in early osteoarthritis of the knee and predict progressive disease. Arthritis Rheum, 1997. 40(4): p. 723-7.

14. Liu-Bryan R., Inflammation and intracellular metabolism: new targets in OA. Osteoarthr Car, 2015. 23(11): p. 1835–1842. doi: 10.1016/j.joca.2014.12.016.

15. Liu-Bryan R, Terkeltaub R., Emerging regulators of the inflammatory process in osteoarthritis. Nat Rev Rheumatol., 2015. 11(1): p. 35–44.

16. Bastick AN, Belo J., Runhaar J, Bierma-Zeinstra SMA., What Are the Prognostic Factors for Radiographic Progression of Knee Osteoarthritis? A Meta-analysis. Clin Orthop Relat Res 2015. 473: p. 2969–2989.

17. Berman BM, Lao L., Langenberg P, Lee WL, Gilpin AMK, Hochberg MC., Effectiveness of acupuncture as adjunctive therapy in osteoarthritis of the knee. A randomized controlled trial. Ann Int Med, 2004. 141(12): p. 901-910.

18. McAlindon TE, Bannuru R., Sullivan MC, Arden NK, Berenbaum F, Bierma-Zeinstra SM, Hawker GA, Henrotin Y, Hunter DJ, Kawaguchi H, Kwoh K, Lohmander S, Rannou S, Roos EM, Underwood M., OARSI guidelines for the non-surgical management of knee osteoarthritis. Osteoarthr Car, 2014. 22: p. 363-388.

19. Biella G, Sotgiu M., Pellagata G, Paulesu E, Castiglioni I, Fazio F., Acupuncture produces central activations in pain regions. Neuroimage., 2001. 14: p. 60-66.

20. Mavrommatis CI, Argyra E., Vadalouka A, Vasilakos DG., Acupuncture as adjuntive therapy to pharmacological treatment in patients with chronic pain due to osteoarthritis of the knee: A 3-armed, randomized, placebo-controlled trial. Pain, 2012. 153: p. 1720-1726.

21. Qin Z, Wu J., Zhou J, Liu Z., Systematic Review of Acupuncture for Chronic Prostatitis/Chronic Pelvic Pain Syndrome. Medicine, 2016. 95(11).

22. Vickers AJ, Cronin A., Maschino AC, Lewith G, MacPherson H, Victor N, Foster NE, Sherman KJ, Witt CM, Linde K., Acupuncture for chronic pain: individual patient data meta-analysis. Arch Intern Med., 2012. 172(19): p. 1444–1453.

23. Crespin DJ, Griffin K., Johnson JR, Miller C, Finch MD, Rivard LR, Anseth S, Dusek JA., Acupuncture provides short-term pain relief for patients in a total joint replacement program. Pain Med., 2015. 16(6): p. 1195-1203.

24. Witt C, Brinkhaus B., Jena S, Linde K, Streng A, Wagenpfeil S, Hummelsberger J, Walther HU, Melchart D, Willich SN., Acupuncture in patients with osteoarthritis of the knee: a randomised trial. Lancet., 2005. 366(9480): p. 136-43. 25.

25. Irnich D, Beyer A., [Neurobiological mechanisms of acupuncture analgesia]. Schmerz, 2002. 16(2): p. 93-102.

26. Han, JS., Acupuncture and endorphins. Neurosci Lett, 2004. 361: p. 258-261.

27. Liebetanz D, Fregni F., Monte-Silva KK et al. , After-effects of transcranial direct current stimulation (tDCS) on cortical spreading depression. Neurosci Lett. , 2006. 398: p. 85-90.

28. Lima, M.C. and Fregni F., Motor cortex stimulation for chronic pain: systematic review and meta-analysis of the literature. Neurol, 2008. 70(24): p. 2329-37.

29. Lefaucher JP, Antal A., Ayache SS, Benninger DH, Brunelin J, Cogiamanian F, Cotelli M, De Ridder D, Ferrucci R, Langguth B, Marangolo P, Mylius V, Nitsche MA, Padberg F, Palm U, Poulet E, Priori A, Rossi S, Schecklmann M, Vanneste S, Ziemann U, Garcia-Larrea L, Paulus W., Evidence-based guidelines on the therapeutic use of transcranial direct current stimulation (tDCS). Clin Neurophysiol., 2017. 128: p. 56–92.

30. Volz M, Farmer A., Siegmund V., Reduction of chronic abdominal pain in patients with inflammatory bowel disease through transcranial direct current stimulation: a randomized controlled trial. Pain 2016. 157: p. 429–437.

31. Choi YH, Jung SJ., Lee CH, Lee SU., Additional Effects of Transcranial Direct-Current Stimulation and Trigger-Point Injection for Treatment of Myofascial Pain Syndrome:A Pilot Study with Randomized, Single-Blinded Trial. J Alt Compl Med., 2014. 20(9): p. 698–704.

32. Schabrun SM, Jones J., Cancino EE, Hodges PW., Targeting Chronic Recurrent Low Back Pain From the Top-down and the Bottom-up: A Combined Transcranial Direct Current Stimulation and Peripheral Electrical Stimulation Intervention. Brain Stim., 2014: p. 1-9. doi: 10.1016/j.brs.2014.01.058.

33. Bolognini N, Spandri V., Ferraro F, Salmaggi A, Molinari ACL, Fregni F, Maravita A., Immediate and sustained effects of 5-day transcranial direct current stimulation of the motor cortex in phantom limb pain. J Pain, 2015. 16(7): p. 657-65.

34. Yoon EJ, Kim YK., Kim HR, Kim SE, Lee Y , Shin HI. and M.P. Study, Transcranial Direct Current Stimulation to Lessen Neuropathic Pain After Spinal Cord Injury: A mechanistic PET study. Neurorehabil Neural Repair, 2014. 28(3): p. 250 –259.

35. Fagerlund AJ, Hansen OA., Aslaksen PM., Transcranial direct current stimulation as a treatment for patients with fibromyalgia:a randomized controlled trial. Pain, 2015. 156: p. 62–71.

36. Donnell A, Nascimento T., Lawrence M, Gupta V, Zieba T, Truong DQ, Bikson M, Datta A, Bellile E, DaSilva AF., High-Definition and Non-Invasive Brain Modulation of Pain and Motor Dysfunction in Chronic TMD. Brain Stimul., 2015. 8(6): p. 1085–1092. doi: 10.1016/j.brs.2015.06.008.

37. Mori F, Codecà C., Kusayanagi H, Monteleone F, Buttari F, Stefania Fiore S, Bernardi G, Giacomo Koch G, Centonze D., Effects of Anodal Transcranial Direct Current Stimulation

on Chronic *Neuropathic Pain in Patients With Multiple Sclerosis. J Pain., 2010 May;11(5):436-42. doi: 10.1016/j.jpain.2009.08.011.*

38. Kim YJ, Ku J., Kim HJ, Im DJ, Lee HS, et al., Randomized, Sham Controlled Trial of Transcranial Direct Current Stimulation for Painful Diabetic Polyneuropathy. Ann Rehabil Med., 2013. 37(6): p. 766-776. doi: 10.5535/arm.2013.37.6.766.

39. Attal N, Ayache SS., Ciampi de Andrade D, Mhalla A, Baudic S, Jatat F, et al., Repetitive transcranial magnetic stimulation and transcranial direct current stimulation in neuropathic pain due to radiculopathy : a randomized sham controlled comparative study . Pain, 2016. 157(6): p. 1224-31. doi: 10.1097/j.pain.0000000000000510.

40. Lefaucheur, JP., Drouot X., Menàrd-Lefaucher I, Keravel Y, Nguyen JP., Motor cortex rTMS restores defective intracortical inhibition in chronic neuropathic pain. Neurol, 2006. 67(9): p. 1568-74.

41. Mhalla A, Baudic S, Ciampi de Andrade D, Gautron M, Perrot S, Teixeira MJ, Attal N, Bouhassira D.,Long-term maintenance of the analgesic effects of transcranial magnetic stimulation in fibromyalgia. Pain, 2011. 152(7): p. 1478-85.

42. Volz MS, Medeiros LF, Tarragô Mda G, Vidor LP, Dall'Agnol L, Deitos A, Brietzke A, Rozisky JR, Rispolli B, Torres IL, Fregni F, Caumo W., The relationship between cortical excitability and pain catastrophizing in myofascial pain. J Pain., 2013. 14(10): p. 1140-7. doi: 10.1016/j.jpain.2013.04.013.

43. Zaghi S, Thiele B., Pimentel D,Pimentel T,Fregni F., Assessment and treatment of pain with non-invasive cortical stimulation. Restor Neurol Neurosci, 2011. 29(6): p. 439-51. doi: 10.3233/RNN-2011-0615.

44. Zunhammer, M., Eichhammer P,Franz J,Hajak G,Busch V., Effects of acupuncture needle penetration on motor system excitability. Neurophysiol Clin, 2012. 42(4): p. 225-30.

45. Lo Y.L, Cui SL., Acupuncture and the modulation of cortical excitability. Neuroreport, 2003. 14(9): p. 1229-31.

46. Imamura M, Imamura ST, Kaziyama HH, Targino RA, Hsing WT, de Souza LP, Cutait MM, Fregni F, Camanho GL., Impact of nervous system hyperalgesia on pain, disability, and quality of life in patients with knee osteoarthritis: a controlled analysis. Arthritis Rheum, 2008. 59(10): p. 1424-31.

47. Mascarin NC, Vancini R., Andrade MS, Magalhães EP, Lira CAB, Coimbra IB. , Effects of kinesiotherapy, ultrasound and electrotherapy in management of bilateral knee osteoarthritis: prospective clinical trial. BMC Musculoskel Dis, 2012. 13(182).

48. Chang WJ, Bennell KL, Hodges PW, Hinman RS, Liston MB, Schabrun SM , Combined exercise and transcranial direct current stimulation intervention for knee osteoarthritis: protocol for a pilot randomised controlled trial. BMJ Open 2015. 5(e008482.). doi: 10.1136/bmjopen-2015-008482.

49. Tukmachi E, Jubb R., Dempsey E, Jones P. , The effect of acupuncture on the symptoms of knee osteoarthritis - an open randomized controlled trial. Acupunct Med., 2004. 22(1): p. 14-22.

50. Brinkhaus B, Witt CM, Jena S, Linde K, Streng A, Hummelsberger J, Irnich D, Hammes M, Pach D, Melchart D, Willich SN.., Physician and treatment characteristics in a randomized multicentre trial of acupuncture in patients with osteoarthritis of the knee. Compl Ther Med, 2007. 15: p. 180-189.

51. Melzack R, Stillwell D., Fox EJ. , Trigger points and acupuncture points for pain: correlations and implications. Pain, 1977. 3(1): p. 3-23.

52. Emrani PS, Katz JN., Kessler CL, Reichmann WM, Wright EA, McAlindon TE, Losina E. , Joint space narrowing and Kellgren-Lawrence progression in knee osteoarthritis: An analytic literature synthesis. Osteoarthr Cartillage, 2008. 16(8): p. 873-882. doi: 10.1016/j.joca.2007.12.004.

53. Reinhold T, Witt CM., Jena S, Brinkhaus B, Willich SN., Quality of life and cost-effectiveness of acupuncture treatment in patients with osteoarthritis pain. Eur J Health Econ 2008. 9: p. 209-219.

54. Petersson IF, Boegard T., Saxne T, Silman AJ, Svensson B. , Radiographic osteoarthritis of the knee classified by the Ahlbäck and Kellgren & Lawrence systems for the tibiofemural joint in people aged 35-54 with chronic knee pain. Ann Rheum Dis 1997. 56: p. 493-496.

55. Witt CM, Jena S., Brinkhaus B, Liecker B, Wegscheider K, Willich SN. , Acupuncture in patients with knee osteoarthritis of the hip and knee. A randomized, controlled trial with an additional nonrandomized arm. Arthr & Rheum 2006. 54(11): p. 3485-3493. doi: 10.1002/art.22154.

56. Maly MR., Linking biomechanics to mobility and disability in people with knee osteoarthritis. Exerc. Sport Sci. Rev. , 2009. 37(1): p. 36-42. doi: 10.1097/JES.0b013e3181912071.

57. Hurley MV, Walsh NE., Effectiveness and clinical applicability of integrated rehabilitation programs for knee osteoarthritis. Curr Opin Rheumatol 2009. 21: p. 171-176. doi: 10.1097/BOR.0b013e3283244422.

58. Goldring MB, Goldring S., Osteoarthritis. J Cell Physiol. , 2007. 213(3): p. 626-34.

59. Loeser RF, Goldring SR., Scanzello CR, Goldring MB., Osteoarthritis: A Disease of the Joint as an Organ. Arthritis Rheum., 2012. 64(6): p. 1697–1707.

60. Sharma L, Chmiel JS., Almagor O, Dunlop D, Guermazi A, Bathon J, Eaton C, Hochberg M, Jackson R, Kwoh K, Mysiw WJ, Crema M, Roemer F, Nevitt M., Significance of Pre-Radiographic MRI Lesions in Persons at Higher Risk for Knee Osteoarthritis. Arthritis Rheumatol., 2014. 66(7): p. 1811–1819. doi: 10.1002/art.38611.

61. Berenbaum F, Griffin TM., Liu-Bryan R., Metabolic regulation of inflammation in osteoarthritis. Arthritis Rheumatol., 2017. 69(1): p. 9-21. doi: 10.1002/art.39842.

62. Kalunian KC, Tugwell P., Greene JM. , Pathogenesis of osteoarthritis. Up to Date., 2009. May.

63. Haavik-Taylor H, Murphy B., Cervical spine manipulation alters sensorimotor integration: a somatosensory evoked potential study. Clin Neurophysiol. , 2007. 118(2): p. 391-402.

64. Gwilym SE, Keltner JR., Warnaby CE, Carr AJ, Chizh, Chessell I, Tracey I., Psychophysical and functional imaging evidence supporting the presence of central sensitization in a cohort of osteoarthritis patients. Arth Rheum, 2009. 61(9): p. 1226-34. doi: 10.1002/art.24837.

65. Skou ST, Graven-Nielsen T., Rasmussen S, Simonsen OH, Laursen MB, Arendt-Nielsen L., Facilitation of pain sensitization in knee osteoarthritis and persistent post-operative pain: A cross-sectional study. Eur J Pain 2014. 18(7): p. 1024-31. doi: 10.1002/j.1532-2149.2013.00447.x.

66. Siddall PJ, Cousins MJ., Introduction to pain mechanisms implications for neural blockade. . In: Cousins MJ, Bridenbaugh PO, eds. Neural Blockade in Clinical Anesthesia and Management of Pain. 3a ed. , 1998. Philadelphia: Lippincott-Raven 1998: p. 675-714.

67. Byers MR, Bonica BJ., Peripheral pain mechanisms and nociceptor plasticity. In: Loeser JD, Butler SH, Chapman CH, Turk DC, editors. Bonica's managment of pain. 3a ed. , 2001. Philadelphia: Lippincott Williams & Wilkins.: p. 27-72.

68. Schaible H-G, Ebersberger A., Natura G. , Update on peripheral mechanisms of pain: beyond prostaglandins and cytokines. Arthr Res Ther., 2011. 13(210). doi: 10.1186/ar3305.

69. Stefani LPC., BDNF e efeito dose-resposta da melatonina no limiar de dor em indivíduos saudáveis. Tese (Doutorado), UFRGS, Faculdade de Medicina, Programa de Pós-Graduação em Medicina: Ciências Médicas, Porto Alegre, BR-RS, 2012. 2012(131f.).

70. Levine JD, Fields HL., Basbaum AI. , Peptides and the primary afferent nociceptor. J Neurosci 1993. 13: p. 2273-86.

71. Foster RW, Ramage AG., The action of some chemical irritants on somatosensory receptors of the cat. Neuropharmacol., 1981. 20: p. 191-8.

72. Dray A, Urban L., Dickenson A., Pharmacology of chronic pain. Trends Pharmacol Sci., 1994. 15: p. 190-7.

73. Ji R-R, Xu Z.-Z., Gao YJ., Emerging targets in neuroinflammation-driven chronic pain. Nature Reviews Drug Discovery . 2014: p. 1-16.

74. Cervero F, Laird JMA, From acute to chronic pain: mechanisms and hypotheses. . In: Carli G, Zimmerman M, eds. Progress in Brain Research 1996. 110: p. 3-15.

75. Light AR, Perl ER., Spinal termination of funcionally identified primary afferent neurons with slowly conducting myelinated fibers. J Comp Neurol 1979. 186: p. 133-50.

76. Kuner R., Central mechanisms of pathological pain. Nature Med., 2010. 16(11): p. 1258-66. doi: 10.1038/nm.2231.

77. Mednell LM, Physiological properties of unmyelinated fiber projection to the spinal cord. Exp Neul, 1996. 16: p. 316-32.

78. Duggan AW, Hope PJ, Jarro B, Schaible HG, Fletwood-Walker SM. , Release spread, and persistance of immunoreactive neurokinin A in the dorsal horn of the cat following noxious cutaneous stimulation. Study of antibody microbes. Neurosci, 1990. 35: p. 195-202.

79. Wilcox GL, Excitatory neurotransmitters and pain. In: Bond MR, Charlton JE, WoolfCJ, eds. Pain Research and Clinical Management. Proceedings of the VIth World Congress on Pain. , 1991. Amsterdan: Elsevier Science p. 97-111.

80. Mao J, Price DD, Mayer DJ., Mechanisms of hyperalgesia and morphine tolerance: a current view of their possible interactions. Pain, 1995. 62: p. 259-74.

81. Woolf CJ, Safieh-Garabedian B, Ma QP, Crilly P, Winter J. , Nerve growth factor contributes to the generation of inflammatory sensory hypersensitivity. Neurosci., 1994. 62: p. 327-31.

82. Meller ST, Gebhart GF, Nitric oxide (NO) and nociceptive processing in the spinal cord. . Pain, 1993. 52: p. 127-136.

83. Wall PD, Melzack R., Wall and Melzack‟s Textbook of Pain. 2006: p. 1.

84. Watkins LR, Hutchinson MR., Ledeboer A, Wieseler Frank J, Milligan ED, Maier SF. , Norman Cousins Lecture. Glia as the "bad guys": implications for improving clinical pain control and the clinical utility of opioids. Brain, behav immun., 2007: p. 131-46. DOI: 10.1016/j.bbi.2006.10.011.

85. Woolf CJ., Recent Advances in the Pathophysiology of Acute Pain. Br J Anaesth., 1989. 63: p. 139-46.

86. Woolf CJ, Mannion RJ., Neuropathic pain: etiology, symptoms, mechanisms, and management. Lancet., 1999. 353: p. 1959-64.

87. Woolf CJ., Evidence of a central component of post-injury pain hypersensivity. Nature, 1983. 306: p. 686-88.

88. Aronoff., G.M., What Do We Know About the Pathophysiology of Chronic Pain? Implications for Treatment Considerations. Med Clin N Am 2016. 100: p. 31-42. doi: 10.1016/j.mcna.2015.08.004.

89. Coderre TJ, Katz J., Peripheral and central excitability: differential signs and symptoms persistent pain. . Behav Brain Sci 1997. 20: p. 404-19.

90. Brown CA, Matthews J., Fairclough M, McMahon A, Barnett E, Al-Kaysi A, El-Deredy W, Jones AKP., Striatal opioid receptor availability is related to acute and chronic pain perception in arthritis: Does opioid adaptation increase resilience to chronic pain? . Pain, 2015. 156(11): p. 2267–2275.

91. Bushnell MC, Case LK., Ceko M, Cotton VA, Gracely JL, Low LA, Pitcher MH, Villemure C., Effect of environment on the long-term consequences of chronic pain. Pain, 2015. 156(1): p. S42-9. doi: 10.1097/01.j.pain.0000460347.77341.bd.

92. Stamford JA., Descending control of pain. Br J Anaesth., 1995. 75: p. 217-27.

93. Porro CA, Cavazzuti M., Functional imaging of the pain system in man and animals. In: Carli G. Zimmerman M, eds. Progress in Brain Research.Amsterdam: Elsevier Science., 1996. 110: p. 47-62.

94. Chapman CR., Limbic processes and the affective dimensions of pain. In: Carli G, Zimmerman M, editor. Progress in Brain Research., 1996. 1110: p. 63-81.

95. Chudler EH, Dong WK., The role of the basal ganglia in nociception and pain. Pain, 1995. 64: p. 3-38.

96. Terman GW, Bonica JJ, Spinal mechanisms and their modulation. In: Loeser JD, Butler SH, Chapman CH, Turk DC, eds. . Bonica's managment of pain. , 2001. 3th ed. Philadelphia: Lippincott Williams & Wilkins.: p. 73-152.

97. Russel IJ, Larsson AA., Neurophysiopathogenesis of fibromyalgia syndrome: a unified hypothesis. Rheum Dis Clin North Am., 2009. 35(2): p. 421-35.

98. Price DD. , General mechanisms of pain modulation. In: Price DD, editor.Psychological mechanisms of pain and analgesia., 1999. Seattle: IASP Press: p. 137-153.

99. Hoffman GA, Harrington A., Fields HL., Pain and the placebo: what we have learned? Perspect Biol Med., 2005. 48(2): p. 248-65.

100. Malenka RC, Bear MF., LTP and LTD: an embarassment of riches. Neuron, 2004. 44(1): p. 5-21.

101. Nijs J, Meeus M, Versijpt J, Moens M, Bos I, Knaepen K, Meeusen R. Brain-Derived neurotrophic factor as a driving force behind neuroplasticity in neurophatic and central sensitization pain: a new therapeutic target? Expert Opin Ther Targets 2015 Apr;19(4):565-76. doi: 10.1517/14728222.2014.994506.

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

103. Figurov A, Pozzo-Miller LD, Olafsson P, Wang T, Lu B. , Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. Nature 1996. 1996 Jun 20; 381 (6584): p. 706-9.

104. Korte M, Carroll P, Brem G, Thoenen H, Bonhoeffer T. , Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. Proc Natl Acad Sci USA. , 1995. 92(19): p. 8856-60.

105. Patterson SL, Abel T, Deuel TA, Martin KC, Rose JC, Kandel ER. , Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. Neuron, 1996. 16(6): p. 1137-45.

106. Klein K, Aeschlimann A, Jordan S, Gay R, Gay S, et al., ATP Induced Brain-Derived Neurotrophic Factor Expression and Release from Osteoarthritis Synovial Fibroblasts Is Mediated by Purinergic Receptor P2X4. PONE., 2012. 7(5): p. e36693. doi: 10.1371/journal.pone.0036693.

107. Rossi S, Hallett M., Rossini PM, Pascual-Leone A., The Safety of TMS Consensus Group. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin neurophysiol . 2009. 120: p. 2008–2039. doi: 10.1016/j.clinph.2009.08.016.

108. Chen R, Cros D., Curra A, Di Lazzaro V, Lefaucheur JP, Magistris MR, Mills K, Rösler KM, Triggs WJ, Ugawa Y, Ziemann U., The clinical diagnostic utility of transcranial magnetic stimulation: report of an IFCN committee. Clin Neurophysiol., 2008. 119(3): p. 504-32. DOI:10.1016/j.clinph.2007.10.014.

109. Hallet M., Transcranial magnetic stimulation and the human brain. Nature., 2000. 406(6792): p. 147-50.

110. Pascual-Leone A, Manoach DS., Birnbaum R, Goff DC., Motor cortical excitability in schizophrenia. Biol Psychiatry., 2002. 52(1): p. 24-31.

111. Terao Y, Ugawa Y., Basic mechanisms of TMS. J Clin Neurophysiol., 2002. 19(4): p. 322-43.

112. Di Lazzaro V, Oliviero A., Pilato F, Saturno E, Dileone M, Meglio M, Cioni B, Colosimo C, Tonali PA, Rothwell JC., Direct recording of the output of the motor cortex produced by transcranial magnetic stimulation in a patient with cerebral cortex atrophy. Clin Neurophysiol., 2004. 115(1): p. 112-5.

113. Kaneko K, Kawai S., Fuchigami Y, Shiraishi G, Ito T., Intracortical facilitation of the muscle response after transcranial magnetic double stimulation. Muscle & Nerve., 1996. 19: p. 1043-1045.

114. Rotem A., Moses E., Magnetic Stimulation of Curved Nerves. IEEE Trans Biomed Eng., 2006. 53(3): p. 414-20.

115. Tranchina D, Nicholson C., A model for the polarization of neurons by extrinsically applied electric fields. Biophys J., 1986. 50: p. 1139-1156.

116. Amassian VE., Eberle L, Maccabee PJ. and Cracco RQ., Modelling magnetic coil excitation of human cerebral cortex with a peripheral nerve immersed in a brain-shaped volume conductor: the significance of fiber bending in excitation. Electroencephal Clin Neurophysiol., 1992. 85: p. 291-301.

117. Rossini, P.M., Narici L, Martino G, Pasquarelli A, Peresson M, Pizzella V, Tecchio F,

Romani GL., Analysis of interhemispheric asymmetries of somatosensory evoked magnetic *fields to right and left median nerve stimulation. Electroencephalogr Clin Neurophysiol., 1994. 91(6): p. 476-82.*

118. Rothwell, J.C., Hallett M, Berardelli A, Eisen A, Rossini P, Paulus W., Magnetic stimulation: motor evoked potentials. The International Federation of Clinical Neurophysiology. Electroencephalogr Clin Neurophysiol Suppl., 1999. 52: p. 97-103.

119. Cantello R, Gianelli M., Civardi C, Mutani R., Magnetic brain stimulation: the silent period after the motor evoked potential. Neurology., 1992. 42(10): p. 1951-9.

120. Wassermann EM, Pascual-Leone A., Valls-Solé J, Toro C, Cohen LG, Hallett M., Topography of the inhibitory and excitatory responses to transcranial magnetic stimulation in a hand muscle. Electroencephalogr Clin Neurophysiol., 1993. 89(6): p. 424-33.

121. Chen R, Lozano AM., Ashby P., Mechanism of the silent period following transcranial magnetic stimulation. Evidence from epidural recordings. Exp Brain Res., 1999. 128(4): p. 539-42.

122. Roick H, vonGiesen HJ., Benecke R., On the origin of the postexcitatory inhibition seen after transcranial magnetic brain stimulation in awake human subjects. Exp Brain Res., 1993. 94(3): p. 489-98.

123. Di Lazzaro V, Restuccia D., Oliviero A, Profice P, Ferrara L, Insola A, Mazzone P, Tonali P, Rothwell JC., Magnetic transcranial stimulation at intensities below active motor threshold activates intracortical inhibitory circuits. Exp Brain Res., 1998. 119(2): p. 265-8.

124. Kujirai, T., et al., Corticocortical inhibition in human motor cortex. J Physiol., 1993. Nov(471): p. 501-19.

125. Sanger, T.D., Garg RR, and C. R., Interactions between two different inhibitory systems in the human motor cortex. J Physiol., 2001. 530(Pt 2): p. 307-17.

126. Ziemann U, Lonnecker S., Paulus W., Inhibition of human motor cortex by ethanol. A transcranial magnetic stimulation study. Brain., 1995. 118(6): p. 1437-46.

127. Ziemann U, L.onnecker S., Steinhoff BJ, Paulus W., The effect of lorazepam on the motor cortical excitability in man. Exp Brain Res., 1996a. 109(1): p. 127-35.

128. Ziemann, U., Lönnecker S, Steinhoff BJ, Paulus W., Effects of antiepileptic drugs on motor cortex excitability in humans: a transcranial magnetic stimulation study. Ann Neurol., 1996b. 40(3): p. 367-78.

129. Di Lazzaro V, Oliviero A., Meglio M, Cioni B, Tamburrini G, Tonali P, Rothwell JC., Direct demonstration of the effect of lorazepam on the excitability of the human motor cortex. Clin Neurophysiol., 2000. 111(5): p. 794-9.

130. Hwa GG, Avoli M., Excitatory postsynaptic potentials recorded from regular-spiking cells in layers II/III of rat sensorimotor cortex. J Neurophysiol. , 1992. 67(3): p. 728-37.

131. Ziemann U., TMS induced plasticity in human cortex. Rev Neurosci., 2004. 15(4): p. 253-66.

132. Ziemann U, Hallet M., Cohen LG., Mechanisms of deafferentation-induced plasticity in human motor cortex. J Neurosci., 1998. 18(17): p. 7000-7.

133. Schwenkreis P, Witscher K., Janssen F, Addo A, Dertwinkel R, Zenz M, Malin JP, Tegenthoff M., Influence of the N-methyl-D-aspartate antagonist memantine on human motor cortex excitability. Neurosci Lett., 1999. 270(3): p. 137-40.

134. Radhu, N., Ravindran LN, Levinson AJ, Daskalakis ZJ., Inhibition of the cortex using transcranial magnetic stimulation in psychiatric populations: current and future directions. J Psychiatry Neurosci., 2012. 37(6): p. 369-78.

135. Kobayashi M, Pascual-Leone A., Transcranial magnetic stimulation in neurology. Lancet Neurol., 2003. 2(3): p. 145-56.

136. Brasil-Neto JP., Learning, memory, and transcranial direct current stimulation. Front Psychiatry. , 2012. 3: p. 80. doi: 10.3389/fpsyt.2012.00080.

137. Klein MM, Treister R., Raij T, Pascual-Leone A, Park L, Nurmikko T, Lenz F, Lefaucheur JP, Lang M, Hallett M, Fox M, Cudkowicz M, Costello A, Carr DB, Ayache SS, Oaklander AL., Transcranial magnetic stimulation of the brain: guidelines for pain treatment research. Pain, 2015. 156(9): p. 1601-14. doi: 10.1097/j.pain.0000000000000210.

138. Niddam DM., Brain manifestation and modulation of pain from myofascial trigger points. Curr Pain Headache Rep., 2009. 13(5): p. 370-5.

139. Strutton PH, Theodorou S., Catley M, McGregor AH, Davey NJ., Corticospinal excitability in patients with chronic low back pain. J Spinal Disord Tech., 2005. 18(5): p. 420-4.

140. Apkarian AV, Sosa Y., Sonty S, Levy RM, Harden RN, Parrish TB, Gitelman DR., Chronic back pain is associated with decreased prefrontal and thalamic gray matter density. J Neurosci., 2004. 24(46): p. 10410-5.

141. Draganski B, Moser T., Lummel N, Gänssbauer S, Bogdahn U, Haas F, May A., Decrease of thalamic gray matter following limb amputation. Neuroimage., 2006. 31(3): p. 951-7.

142. Schmidt-Wilcke T, Leinisch E., Gänssbauer S, Draganski B, Bogdahn U, Altmeppen J, May A., Affective components and intensity of pain correlate with structural differences in gray matter in chronic back pain patients. Pain., 2006. 125(1-2): p. 89-97.

143. May A., Chronic pain may change the structure of the brain. Pain., 2008. 137(1): p. 7-15.

144. Younger JW, Shen YF., Goddard G, Mackey SC., Chronic myofascial temporomandibular pain is associated with neural abnormalities in the trigeminal and limbic systems. Pain., 2010. 149(2): p. 222-8.

145. Thayer JF, Friedmann BH., Stop that! Inhibition, sensitization, and their neurovisceral concomitants. Scand J Psychol., 2002. 43(2): p. 123-30.

146. Melzack R., From the gate to the neuromatrix. Pain, 1999. 6: p. S121-6.

147. Melzack R., Pain and the neuromatrix in the brain. J Dent Educ. , 2001. 65(12): p. 1378-82.

148. Voogt L, deVries J., Meeus M, Struyf F, Meuffels D, Nijs J., Analgesic effects of manual therapy in patients with musculoskeletal pain: A systematic review. Man Ther. 2015 Apr;20(2):250-6. doi: 10.1016/j.math.2014.09.001.

149. Mendell LM., Constructing and Deconstructing the Gate Theory of Pain. Pain, 2014. 155(2): p. 210–216. doi: 10.1016/j.pain.2013.12.010.

150. Bartley EJ, Fillingim RB., Sex differences in pain: a brief review of clinical and experimental findings. British Journal of Anaesthesia., 2013. 111(1): p. 52–8.

151. Nir R-R, Yarnitsky D., Conditioned pain modulation. Curr Opin Support Palliat Care 2015. Jun;9(2):131-7. doi: 10.1097/SPC.0000000000000126.

152. Edwards RR, Fillingim RB., Ness TJ. , Age-related differences in endogenous pain modulation: a comparison of diffuse noxious inhibitory controls in healthy older and younger adults. Pain Med, 2003. 101: p. 155–165.

153. Moont, R., Crispel Y, Lev R, Pud D, Yarnitsky D., Temporal changes in cortical activation during conditioned pain modulation (CPM), a LORETA study. Pain., 2011. 152(7): p. 1469-77. doi: 10.1016/j.pain.2011.01.036.

154. Kennedy DL, Kemp HI., Ridout D, Yarnitsky D, Rice ASC., Reliability of conditioned pain modulation:a systematic review. Pain, 2016. 157: p. 2410–2419. DOI: 10.1097/j.pain.0000000000000689.

155. Moont R, Pud D., Sprecher E, Sharvit G, Yarnitsky D. , „Pain inhibits pain" mechanisms: Is pain modulation simply due to distraction? . Pain, 2010. 150: p. 113–120. doi: 10.1016/j.pain.2010.04.009.

156. Popescu A, LeResche L., Truelove EL, Drangsholt MT. , Gender differences in pain modulation by diffuse noxious inhibitory controls: A systematic review. Pain, 2010. 150(309–318.). doi: 10.1016/j.pain.2010.05.013.

157. Hochberg, M.C., Altman RD, April KT, Benkhalti M, Guyatt G, McGowan J, Towheed T, Welch V, Wells G, Tugwell P., American College of Rheumatology 2012 recommendations for the use of nonpharmacologic and pharmacologic therapies in osteoarthritis of the hand, hip, and knee. Arthritis Care Res (Hoboken). 2012. 64(4): p. 465-74.

158. Jevsevar DS., AAOS Clinical Practice Guideline Summary. Treatment of Osteoarthritis of the Knee: Evidence-Based Guideline,2nd Edition. J Am Acad Orthop Surg., 2013. 21(9): p. 571-576.

159. Itoh K, Hirota S., Katsumi Y, Ochi H, Kitakoji H. , A pilot study on using acupuncture and transcutaneous electrical nerve stimulation (TENS) to treat knee osteoarthritis (OA). . Chin Med., 2008. 29: p. 3-2.

160. Bjordal JM, Johnson MI., Lopes-Martins RAB, Bogen B, Chow R, Ljunggren., Short-term

efficacy of physical interventions in osteoarthritic knee pain. A systematic review and meta-analysis of randomized placebo-controlled trials. Musculoskel Dis., 2007: p. 8-51. doi: 10.1186/1471-2474-8-51.

161. Mendelson AD, McCullough C., Chan A. , Integrating self-management and exercise for people living with arthritis. Health Ed Res., 2011. 26(1): p. 167-177.

162. Brosseau L, Wells GA., Kenny GP, Reid R, Maetzel A, Tugwell P, Huijbregts M, McCullough C, De Angelis G, Chen L. , The implementation of a comunity-based aerobic walking program for mild to moderate knee osteoarthritis (OA): a knowledge translation (KT) randomized controlled trial (RCT): Part I: The uptake of the Ottawa Panel clinical practice guidelines (CPGs). . BMC Public Health., 2012: p. 12-871. doi: 10.1186/1471-2458-12-871.

163. Filardo G, Kon E., Di Martino A, Di Matteo B, Merli ML, Cenacchi A, Fornasari PM, Marcacci M. , Platelet-rich plasma vs hyaluronic acid to treat knee degenerative pathology: study design and preliminary results of a randomized controlled trial. . BMC Musculoskel Dis., 2012: p. 13-229. doi: 10.1186/1471-2474-13-229.

164. Crane DM, Oliver KS., Bayes MC., Orthobiologics and Knee Osteoarthritis:A Recent Literature Review, Treatment Algorithm, and Pathophysiology Discussion. Phys Med Rehabil Clin N Am., 2016. 27: p. 985–1002. doi: 10.1016/j.pmr.2016.07.004.

165. Han, J.S., Electroacupuncture: an alternative to antidepressants for treating affective diseases? . Int J Neurosci. , 1986. 29(79–92.).

166. Han JS, Xie GX., Dynorphin: important mediator for electroacupuncture analgesia in the spinal cord of the rabbit. . Pain, 1984. 18: p. 367-376.

167. Mayer DJ, Price DD., Rafii A., Antagonism of acupuncture analgesia in man by the narcotic antagonist naloxone. . Brain Res., 1977. 121: p. 368-372.

168. Pomeranz B, Stux SG., Acupuncture treatment: Rheumatoid arthritis. In:Basics of acupuncture, 1° edição Berlin:Springer. , 1987: p. 276.

169. de Medeiros MA, Canteras NS., Suchecki D, Mello LE., Analgesia and c-Fos expression in the periaqueductal gray induced by electroacupuncture at the Zusanli point in rats. Brain Res, 2003. 973(2): p. 196-204.

170. Goldman N, Chen M., Fujita T, Xu Q, Peng W, Liu W, Jensen TK, Pei Y, Wang F, Han X, Chen JF, Schnermann J, Takano T, Bekar L, Tieu K, Nedergaard M. , Adenosine A1 receptors mediate local anti-nociceptive effects of acupuncture. Nature Neurosci., 2010 Jul;13(7):883-8. doi: 10.1038/nn.2562.

171. Park JY, Park JJ., Jeon S, Doo AR, Kim SN, Lee H, Chae Y, Maixner W, Lee H, Park HJ., From peripheral to central: the role of ERK signaling pathway in acupuncture analgesia. J Pain, 2014 May. 15(5): p. 535-549. doi: 10.1016/j.jpain.2014.01.498.

172. Hsieh JC, Tu CH., Chen FP, Chen MC, Yeh TC, Cheng HC, Wu YT, Liu RS, Ho LT., Activation of the hypothalamus characterizes the acupuncture stimulation at the analgesic point in human: a positron emission tomography study. Neurosci Lett., 2001.

307: p. 105-108.

173. Li C, Yang J., Park K, Wu H, Hu S, et al. , Prolonged Repeated Acupuncture Stimulation Induces Habituation Effects in Pain-Related Brain Areas: An fMRI Study. PONE 2014. 9(5): p. e97502. doi: 10.1371/journal.pone.0097502.

174. Haker E, Egekvist H., Bjerring P. , Effect of sensory stimulation (acupuncture) on sympathetic and parasympathetic activities in healthy subjects. J Auton Nerv Syst 2000. 79: p. 52–9.

175. Hsu CC, Weng CS., Sun MF, Shyu LY, Hu WC, Chang YH. , Evaluation of scalp and auricular acupuncture on EEG, HRV, and PRV. Am J Chin Med 2007. 35: p. 219–30. doi: 10.1142/S0192415X0700476X.

176. Sakai S, Hori E., Umeno K, Kitabayashi N, Ono T, Nishijo H., Specific acupuncture sensation correlates with EEGs and autonomic changes in human subjects. Auton Neurosci 2007. 133: p. 158–69.

177. Ge HY, Fernández-de-las-Peñas C., Arendt-Nielsen L. , Sympathetic facilitation of hyperalgesia evoked from myofascial tender and trigger points in patients with unilateral shoulder pain. Clin Neurophysiol, 2006. 117: p. 1545-1550.

178. Carlsson C., Acupuncture mechanisms for clinically relevant long-term effects reconsideration and a hypothesis. . Acupunct Med 2002. 20(2-3): p. 82-99.

179. Maioli C, Falciati L., Marangon M, Perini S, Losio A., Short- and long-term modulation of upper limb motor-evoked potentials induced by acupuncture. Eur J Neurosci., 2006. 23(7): p. 1931-8.

180. Huang W, Pach D., Napadow V, Park K, Long X et al., Characterinzing acupuncture stimuli using brain imaging withfMRI- A systematic review and meta-Analysis of the literature. POne, 2012. 7(4): p. e32960.

181. Zhang R, Lao L., Ren K, Berman BM., Mechanisms of Acupuncture-Electroacupuncture on Persistent Pain. . Anesthesiol., 2014. 120(2): p. 482-503. doi: 10.1097/ALN.0000000000000101.

182. Irnich D, Behrens N., Molzen H, Konig A, Gleditsch J, Krauss M, et al., Randomised trial of acupuncture compared with conventional massage and „„sham" laser acupuncture for treatment of chronic neck pain. BMJ, 2001. 322: p. 1574–8.

183. Gunn, C.C., The Gunn approach to the treatment of chronic pain.1996. 3-49.

184. Cheng KJ., Neuroanatomical basis of acupuncture treatment for some common illnesses. . Acupunct Med 2009. 27: p. 61-4. doi: 10.1136/aim.2009.000455.

185. Srbely, J.Z., Dickey JP,Lee D,Lowerison M., Dry needle stimulation of myofascial trigger points evokes segmental anti-nociceptive effects. J Rehabil Med, 2010. 42(5): p. 463-8. doi: 10.2340/16501977-0535.

186. Byeon HT, Park SH., Ko MH, Seo JH. , Effects of intramuscular stimulation in myofascial pain syndrome of upper trapezius muscle. . J Korean Acad Rehab Med., 2003. 27: p. 753-756.

187. Karakurum B, Karaalin O., Coskun O, Dora B, Ucler S, et al. , The 'dry needle technique': intramuscular stimulation in tension-type headache. . Cephalalgia 2001. 21: p. 813-817.

188. Ahn K., What is IMS? What is pain? . J Korean Acad Fam Med, 1999. 20: p. 1496-1509.

189. Itoh K, Katsumi Y., Hirota S, Kitakoji H. , Randomised trial of trigger point acupuncture compared with other acupuncture for treatment of chronic neck pain. Complement Ther Med 2007. 15: p. 172-179.

190. Ga, H., Koh HJ, Choi JH, Kim CH., Intramuscular and nerve root stimulation vs lidocaine injection to trigger points in myofascial pain syndrome. J Rehabil Med, 2007. 39(5): p. 374-8.

191. Mylius V, Borckardt JJ., Lefaucheur J-P. , Noninvasive cortical modulation of experimental pain. Pain, 2012. 153(7): p. 1350-63. doi: 10.1016/j.pain.2012.04.009.

192. Boggio, P.S., Amancio EJ, Correa CF, Cecilio S, Valasek C, Bajwa Z, Freedman SD, Pascual-Leone A, Edwards DJ, Fregni F., Transcranial DC stimulation coupled with TENS for the treatment of chronic pain: a preliminary study. Clin J Pain. , 2009. 25(8): p. 691-5. doi: 10.1097/AJP.0b013e3181af1414.

193. Brunoni AR, Nitsche MA., Bolognini N, Bikson M, Wagner T, Merabet L, et al. , Clinical research with transcranial direct current stimulation (tDCS): challenges and future directions. Brain Stim. , 2012. 5(3): p. 175-95. doi: 10.1016/j.brs.2011.03.002.

194. Reidler JS, Mendonça ME., Santana MB, Wang X, Lenkinski R, Motta AF, Marchand S, Latif L, Fregni F., Effects of Motor Cortex Modulation and Descending Inhibitory Systems on Pain Thresholds in Healthy Subjects. J Pain., 2012. 13(5): p. 450-58. doi: 10.1016/j.jpain.2012.01.005.

195. Jardim da Silva NR, Laste G., Deitos A, Stefani LC, Cambraia-Canto G, Torres ILS, Brunoni AR, Fregni F and Caumo W., Combined neuromodulatory interventions in acute experimental pain: assessment of melatonin and non-invasive brain stimulation. Front Behav Neurosci., 2015. 9(77). doi: 10.3389/fnbeh.2015.00077.

196. Dall'Agnol, L., Medeiros LF, Torres IL, Deitos A, Brietzke A, Laste G, de Souza A, Vieira JL, Fregni F, Caumo W, Repetitive transcranial magnetic stimulation increases the corticospinal inhibition and the brain-derived neurotrophic factor in chronic myofascial pain syndrome: an explanatory double-blinded, randomized, sham-controlled trial. J Pain. , 2014. 15(8): p. 845-55. doi: 10.1016/j.jpain.2014.05.001.

197. Vaseghi B, Zoghi M., Jaberzadeh S., How Does Anodal Transcranial Direct Current Stimulation of the Pain Neuromatrix Affect Brain Excitability and Pain Perception? A Randomised, Double-Blind, Sham-Control Study. . PLoS One. 2015 Mar 4;10(3):e0118340. doi: 10.1371/journal.pone.0118340.

198. O'Connell NE, Wand BM., Marston L, Spencer S, Desouza LH. , Non-invasive brain stimulation techniques for chronic pain. A report of a Cochrane systematic review and meta-analysis. . Eur J Phys Rehabil Med. , 2011. 47(2): p. 309-26.

199. O'Connell NE, Cossar J., Marston L, Wand BM, Bunce D, Moseley GL, De Souza LH. , Rethinking clinical trials of transcranial direct current stimulation: participant and assessor blinding is inadequate at intensities of 2mA. PLoS One. 2012;7(10):e47514. doi: 10.1371/journal.pone.0047514.

200. Nitsche MA, Cohen LG., Wassermann EM, Priori A, Lang N, Antal A, Paulus W, Hummel F, Boggio PS, Fregni F, Pascual-Leone A. , Transcranial direct current stimulation: State of the art 2008. Brain Stimul. , 2008. 1(3): p. 206-23. doi: 10.1016/j.brs.2008.06.004.

201. Valle A, Roizenblatt S., Botte S, Zaghi S, Riberto M, Tufik S, Boggio PS, Fregni F., Efficacy of anodal transcranial direct current stimulation (tDCS) for the treatment of fibromyalgia: results of a randomized, sham-controlled longitudinal clinical trial. J Pain Manag., 2009. 2(3): p. 353–361.

202. Brunoni AR, Ferrucci R., Bortolomasi M, Vergari M, Tadini L, Boggio PS, Giacopuzzi M, Barbieri S, Priori A. , Transcranial direct current stimulation (tDCS) in unipolar vs. bipolar depressive disorder. . Prog Neuropsychopharmacol Biol Psychiatry. , 2011. 35(1): p. 96-101. doi: 10.1016/j.pnpbp.2010.09.010.

203. Fregni F, Gimenes R., Valle AC, Ferreira MJ, Rocha RR, Natalle L, Bravo R, Rigonatti SP, Freedman SD, Nitsche MA, Pascual-Leone A, Boggio PS., A randomized, sham-controlled, proof of principle study of transcranial direct current stimulation for the treatment of pain in fibromyalgia. Arthritis Rheum., 2006. 54(12): p. 3988-98.

204. Brunoni AR, Vanderhasselt MA., Boggio PS,Fregni, F.Dantas E M, Mill JG, Lotufo PA, Bensenor IM., Polarity- and valence-dependent effects of prefrontal transcranial direct current stimulation on heart rate variability and salivary cortisol. Psychoneuroendocrinology, 2012. doi: 10.1016/j.psyneuen.2012.04.020.

205. Mendonça M, Fregni F., Dor crônica. In: Fregni F, Boggio PS, Brunoni AR. Neuromodulação Terapêutica. Princípios e avanços da estimulação cerebral não invasiva em neurologia, reabilitação, psiquiatria e neuropsicologia., 2012. São Paulo: Sarvier, 2012.: p. 199-217.

206. Roizenblatt S, Fregni F., Gimenez R, Wetzel T, Rigonatti SP, Tufik S, Boggio P, Valle AC. , Site-specific Effects of Transcranial Direct Current Stimulation on Sleep and Pain in Fibromyalgia: A Randomized, Sham-controlled Study. . Pain Practice 2007. 7(4): p. 297–306.

207. Kuo MF, Grosch J., Fregni F, Paulus W, Nitsche MA. , Focusing Effect of Acetylcholine on Neuroplasticity in the Human Motor Cortex. . J Neurosci., 2007. 27(52): p. 14442–14447.

208. Soler, M.D., Kumru H,Pelayo R,Vidal J,Tormos JM,Fregni F,Navarro X,Pascual-Leone A., Effectiveness of transcranial direct current stimulation and visual illusion on neuropathic pain in spinal cord injury. Brain, 2010. 133(9): p. 2565-77. doi: 10.1093/brain/awq184.

209. Foerster BR, N.T., DeBoer M, Bender MCA, Rice IC, Truong DQ, Bikson M, Clauw DJ, Zubieta JK, Harris RE, DaSilva AF., Excitatory and Inhibitory Brain Metabolites as Targets and Predictors of Effective Motor Cortex tDCS Therapy in Fibromyalgia. Arthritis Rheumatol., 2015. 67(2): p. 576–581.

210. Fregni F, B.P., Lima MC, Ferreira MJL, Wagner T, Rigonatti SP, Castro AW, Souza DR, Riberto M, Freedman SD, Nitsche MA, Pascual-Leone A., A sham-controlled, phase II trial of transcranial direct current stimulation for the treatment of central pain in traumatic spinal cord injury. Pain, 2006. 122: p. 197–209.

211. Antal A, Kriener N., Lang N, Boros K and Paulus W., Cathodal transcranial direct current stimulation of the visual cortex in the prophylactic treatment of migraine. Cephalalgia., 2011. 31(7): p. 820–828. doi: 10.1177/0333102411399349.

212. Mendonca ME, Simis M., Grecco LC, Battistella LR, Baptista AF and Fregni F., Transcranial Direct Current Stimulation Combined with Aerobic Exercise to Optimize Analgesic Responses in Fibromyalgia: A Randomized Placebo-Controlled Clinical Trial. . Front. Hum. Neurosci. , 2016. 10: p. 68. doi: 10.3389/fnhum.2016.00068.

213. Villamar MF, Wivatvongvana P., Patumanond J, Bikson M, Truong DQ, Datta A, Fregni F. , Focal Modulation of the Primary Motor Cortex in Fibromyalgia Using 41-Ring High-Definition Transcranial Direct Current Stimulation (HD-tDCS): Immediate and Delayed Analgesic Effects of Cathodal and Anodal Stimulation. . J Pain, 2013. 14(4): p. 371-383. doi: 10.1016/j.jpain.2012.12.007.

214. Jales Junior LH, Costa MDL., Jales Neto LH, Ribeiro JPM, Freitas WJSN, Teixeira MJ., Estimulação elétrica transcraniana por corrente contínua em fibromialgia: efeitos sobre a dor e a qualidade de vida, avaliados clinicamente e por cintilografia de perfusão cerebral. Rev Dor., 2015. 16(1): p. 37-42.

215. Souto G, Borges IC., Goes BT, Mendonça ME, Gonçalves RG, Garcia LB, Sa´ KN, Coutinho MR, Galvão-Castro BG, Fregni F, Baptista AF., Effects of tDCS-induced Motor Cortex Modulation on Pain in HTLV-1: A Blind Randomized Clinical Trial. Clin J Pain 2014. 30(9): p. 809–815. doi: 10.1097/AJP.0000000000000037.

216. Bolognini N, Olgiati E., Maravita A, Ferraro F, Fregni F., Motor and parietal cortex stimulation for phantom limb pain and sensations. Pain 2013. 154(8): p. 1274-80. doi: 10.1016/j.pain.2013.03.040.

217. Brietzke AP, Rozinsky JR., Dussan-Sarria JA, Deitos A, Laste G, Hoppe PFT, Muller S, Torres ILS, Alvares-da-Silva MR, de Amorim RFB, Fregni F,Caumo W., Neuroplastic Effects of Transcranial Direct Current Stimulation on Painful Symptoms Reduction in Chronic Hepatitis C: A Phase II Randomized, Double Blind, Sham Controlled Trial. Front. Neurosci. , 2016. 9(498). doi: 10.3389/fnins.2015.00498.

218. Ayache SS, Palm U., Chalah MA, Al-AniT, Brignol A, Abdellaoui M, Dimitri D, Sorel M, Créange A, Lefaucheur JP., Prefrontal tDCS Decreases Pain in Patients with Multiple Sclerosis. Front. Neurosci. , 2016. 10(147). doi: 10.3389/fnins.2016.00147.

219. Borckardt JJ, Romagnuolo J., Reeves ST, Madan A, Frohman H, Beam W, George MS., Feasibility, safety, and effectiveness of transcranial direct current stimulation for decreasing post-ERCP pain: a randomized, sham-controlled, pilot study. Gastrointest Endosc 2011. 73(6): p. 1158-64. doi: 10.1016/j.gie.2011.01.050.

220. Lee YC, Nassikas NJ., Clauw DJ., The role of the central nervous system in the generation and maintenance of chronic pain in rheumatoid arthritis, osteoarthritis and fibromyalgia. . Arthritis Res Ther., 2011 Apr 28;13(2):211. doi: 10.1186/ar3306.

CAPITULO IV – ARTIGOS CIENTÍFICOS

7 Artigo 1

Fator de Impacto: 2.13

Descending Control of Nociceptive Processing in Knee Osteoarthritis is Associated with Intracortical Disinhibition: An Exploratory Study

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ABREVIATIONS LIST

BMI: body mass index.

BP-PCS: Brasilian portuguese pain catastrophising scale.

CNS: central nervous system.

CPM-task: conditioned pain modulation task.

CS: central sensitization.

CSP: cortical silent period.

EMG: electroneuromyography.

FDI: first dorsal interosseous.

fMRI: Functional magnetic resonance imaging

GABA: gamma aminobutyric acid.

HCPA: Hospital de Clinicas de Porto Alegre.

ICF: intracortical facilitation.

K-L: Kellgren-Lawrence.

KOACP: knee osteoarthritis chronic pain.

MEP: motor evoked potential.

MT: motor threshold.

M1: primary motor cortex.

NAc: nucleus accumbens.

NPS: numerical pain scale.

OA: osteoarthritis.

PPT: pressure pain threshold.

tDCS: transcranial direct current stimulation.

TKR: Total knee replacement.

TMS: Transcranial Magnetic Stimulation.

SICI: short interval intracortical inhibition.

STROBE: Strengthening the Reporting of Observational Studies in Epidemiology

WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index.

ABSTRACT

Background: Based on the hypothesis that an imbalance in excitatory and inhibitory input is a central mechanism of knee osteoarthritis chronic pain (KOACP), this exploratory study had the following aims: i) to compare whether the function of the descending inhibitory pain pathway is associated with the state of inhibition in the corticospinal system indexed by the motor evoked potential (MEP) and the cortical silent period (CSP) in patients with severe osteoarthritis (OA) and healthy controls. ii) To determine if there is correlation between the measures of intra-cortical inhibition (CSP, MEP) with changes on the numerical pain scale (NPS 0-10) in KOACP during a conditioned pain modulated (CPM)-task considering the

effect of self-reported function assessed by the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and analgesic use.

Methods: In a cross-sectional study, we included females (n=21), with disability by pain or stiffness due to KOACP and healthy controls (n = 10), aged 19 to 75 years old. The motor cortex excitability parameters (MEP and CSP) were assessed using the Transcranial Magnetic Stimulation (TMS). We assessed the pain and disability by the WOMAC, and change on Numerical Pain Scale (NPS0-10) during CPM-task.

Results: A MANCOVA revealed that the adjusted mean (SD) on the MEP amplitude was 13.53% higher in the OA than in healthy subjects [1.33(0.49) vs. 1.15(0.13)], respectively (P=0.16). The adjusted mean (SD) on the CSP observed in OA patients was 23.43% lower than in healthy subjects [54.54(16.10) vs. 70.94(22.87)], respectively (P=0.01). The function of the descending pain modulatory system assessed by change on NPS (0-10) during a CPM-task was negatively correlated with the cortical excitability parameter indexed by the CSP (P=0.001). Also, the CSP was negatively correlated with the pain and disability assessed by the WOMAC index.

Conclusion: These findings support the hypothesis that the change in cortical plasticity in KOACP is associated with less intra-cortical inhibition, as measured by the CSP. These results show that the neural change in the motor cortex in KOACP is associated with pain and disability levels as well as with decreased activation of the endogenous pain modulating system by a CPM-task.

Key Words: CPM, osteoarthritis, transcranial magnetic stimulation.

Perspective: The motor cortex plasticity changes in knee osteoarthritis chronic pain are associated with less ICI, higher pain scores, pain and disability, as well as with decreased activation of the endogenous pain modulating system by the CPM-task.

INTRODUCTION

Osteoarthritis (OA) is the most important cause of pain and limitation in elderly (1). It is associated with chronic inflammation in somatic structures, which alters the afferent sensory inputs and leads to plastic changes in the nervous system (2). OA might lead to segmental sensitization (3) and promote central sensitization (CS) (4), a phenomenon that comprises expansion of the receptive field, a lower pain threshold, hyperalgesia inside and outside of sensitized areas (5,6) and the presence of widespread pain (6). Total knee replacement (TKR) is indicated in end-stage osteoarthritis (OA) to reduce pain and disability (7).

Although TKR surgery may produce a complete resolution of pain in a large percentage of patients (up to 73%) within the first 2-7 years following surgery (7), persistent pain associated with physical disability has been reported in approximately 15-20% of patients (8). While particular variables have been consistently associated with poor pain outcomes, such as pain catastrophizing and preoperative pain (9), the dysfunction of endogenous pain modulatory systems also provide insight to identifying patients prone to developing increased postsurgical pain (10) and postoperative chronic pain. Convincing evidence exists to support that the descending modulatory systems in chronic pain are disrupted; shifting from a state of inhibition to a mal-adaptive state of facilitation (11). Also, functional magnetic resonance imaging (FMRi) studies have demonstrated that compared to healthy subjects, patients with OA demonstrate an increased vigilance and a decreased ability to disengage from pain (12). These changes were associated with abnormal activity in the cingulate cortex, the amygdala, the insula, the nucleus accumbens (NAc) and pre-frontal areas (11).

In fact, long-term pain induces cortical reorganization involving the primary motor cortex (M1), which has been a target to assess the cortical excitability and to treat chronic pain conditions. Several studies have shown that M1 stimulation improved pain management

outcomes in patients with chronic pain, such as patients with fibromyalgia (13-14), trigeminal neuralgia (15), phantom pain (16), chronic migraine (17), low back pain (18) and Myofascial Pain Syndrome (19). Also, the M1 is a target that allows us to characterize pathophysiological consequences associated with chronic pain at the motor cortex by neurophysiological measurements made by Transcranial Magnetic Stimulation (TMS) (20). Among these parameters, the increase in the motor evoked potential (MEP) is considered a basic index of corticospinal excitability (21) and its amplitude has been observed after painful experiences (22) and under experimental pain (23). Also, it has been reported that neuropathic pain leads to a disinhibited state indicated by a shortened cortical silent period (CSP) (24). TMS protocols using paired-pulse suggests that the inhibition in the motor cortex assessed by CSP tap into different mechanisms. GABA-B agonists, such as baclofen was shown to enhance the CSP (25). Indeed, the early part of the CSP relies on spinal inhibition (26).

Considering that the chronic pain associated with OA reduces quality of life and given the expected exponential increase in the number of primary TKA, a better comprehension of the relationship between the intra-cortical inhibition and the potency of the descending inhibition system could improve future therapeutic approaches in OA. Thus, based on the hypothesis that an imbalance in excitatory and inhibitory inputs plays a role in the central mechanism of knee osteoarthritis chronic pain (KOACP), this exploratory study had the following aims: i) to compare whether, in patients with severe OA and healthy controls, the function of the descending inhibitory pain pathway is associated with the state of inhibition in the corticospinal system assessed by both the MEP amplitude and the CSP. ii) To determine if there is correlation between the measures of intra-cortical inhibition (CSP, MEP) with changes on the Numerical Pain Scale (NPS 0-10) in KOACP during a conditioned

pain modulated (CPM)-task considering the effect of self-reported function assessed by the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and analgesic use.

PATIENTS AND METHODS

Methods

The Methods and Results sections are reported according to STROBE guidelines (27). The Ethics Committee at the Hospital de Clínicas de Porto Alegre (HCPA) approved the (Protocol No. 11-0013). According to the Declaration of Helsinki, all patients provided written informed consent to participate.

Design overview, setting, and participants

A cross-sectional study was performed at Hospital de Clinicas de Porto Alegre (HCPA), Brazil, between March 2014 and December 2014. Patients were recruited from the general population through public postings in different health care units and referrals from physicians in the Physiatry and Chronic Pain Service at HCPA. Eligibility criteria were designed to study a group of patients who were potentially appropriate candidates for unilateral knee arthroplasty. The sample was comprised of 21 right-handed women meeting inclusion criteria of being aged 50 years or older (4) and experiencing moderate or intense pain or stiffness in the knee. Also, they needed to present functional impairments for at least six months that were not controlled with medical therapy (28). The baseline interview included the WOMAC, a validated instrument to assess pain, stiffness and functional limitations related to OA (29). To be eligible, they could report “moderate,” “severe,” or “extreme” pain or stiffness in response to at least one of the five pain questions (pain with walking, climbing stairs, reclining, sitting or standing). The needed to report a positive answer for two stiffness questions (morning stiffness, stiffness later in day), as well

as reporting whether they experience “moderate,” “severe,” or “extreme” difficulty with at least 1 of the 17 activities. Additionally, the radiographs of all knees were evaluated for the degree of osteoarthritis by one physiatrist with over ten years of experience in OA rehabilitation. This was conducted using the Kellgren-Lawrence (K-L) grading scale of 3–4 because it proved to be highly reproducible to grading severity of knee osteoarthritis (30). The exclusion criteria were as follows: accompanied orthopedic, rheumatic or neurological pathologies; surgery on the affected areas in the prior six months; habitual use of corticosteroids or other uncompensated chronic pathologies. Additionally, patients were excluded if they had a body mass index (BMI) of > 35 kg/m2 or if they had contraindications to TMS (31).

Healthy right handed controls were recruited from the general population using public postings. They were asked to complete screening questionnaires, and were excluded if they were experiencing any painful condition (either acute or chronic); used analgesics or corticosteroids; had any rheumatologic, psychiatric, or neurological disorder; had abused alcohol or psychotropic substances during the six months previous to the screening; or if they were using medications with known effects on the central nervous system (CNS). In addition, they were excluded if presented contraindications to TMS (31). The sequence of assessments is presented in figure 1.

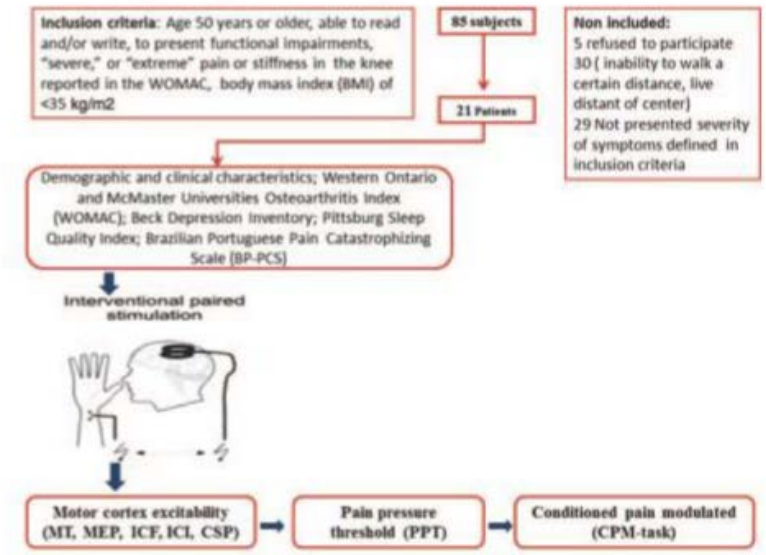

Figure 1. The sequence of assessments. Healthy controls were underwent the same sequence of assessments, excepting the questionnaires regarding, sleep, pain and depression.

Dependent variables

The dependent variables of interest were measurements of the intra-cortical inhibition indexed by CSP and MEP.

The left primary motor cortex (M1) parameters were assessed using the Transcranial Magnetic Stimulation (TMS) MagProX100 stimulator (MagVenture Company, Lucernemarken, Denmark) through a figure-eight coil (MagVenture Company). It was assessed prior to the pain pressure threshold assessment. Ag-AgCl electrodes were placed over the first dorsal interosseous (FDI) belly muscle and in its corresponding tendon on the distal phalanx of the index finger. The responses to stimuli were recorded from the FDI muscle of the right hand by surface electromyography (EMG).

Each patient was seated in a comfortable chair and informed about the TMS procedure,

including possible sensations that might be experienced. The amplitudes of the single and paired pulse TMS and the latency and the measures of the cortical silent period during the experiment were recorded on an Excel spreadsheet. The data were analyzed offline on a personal computer. To identify the motor "hot spot"; the coil was placed over the left M1 tangentially to the scalp at a 45° angle to the sagittal line. The motor threshold (MT) was defined using the lowest stimulus to induce 50% of the evoked potentials of the resting FDI (32). To ensure constant placement of the coil throughout the TMS assessments, the site was marked with a soft-tipped pen. Firstly, the MT was determined using the lowest stimulus to elicit evoked potentials in the resting FDI, with minimum amplitude of 50 μV peak-to-peak, in at least 5 of 10 (at least 50% of successive trials) (32).

Single-pulse measures including the MEP and the CSP were recorded at an intensity of 130% of the MT. The MEP value was the elicited evoked potential with a 1mV peak-to-peak amplitude. The mean of 10 consecutive trials were recorded. For the CSP, patients were instructed to perform isometric voluntary contractions with approximately 10% of maximal contraction of the FDI. The transient silence during the isometric voluntary EMG activity was elicited in the tonically contracting FDI muscle at approximately 10% of the maximal voluntary contraction, and the CSP was preceded by the MEP (33). Ten consecutive trials were recorded. The paired-pulse measurements included the SICI with interstimulus intervals of 2 ms and the ICF with interstimulus intervals of 12 ms (34).To define the individual MT, the first sub-threshold stimulus was set at 80% while the supra-threshold stimulus was set at 130% of the MT. The intensity of the supra-threshold test stimuli was adjusted to elicit the test stimuli with peak-to-peak amplitude of approximately 1 mV. At the level of the primary motor cortex, the reduction of the test MEP elicited by TMS is considered to reflect inhibition (34) and the increase of the test MEP elicited by TMS is considered to

reflect facilitation at the level of the primary motor cortex (21). Thirty recordings (10 for each ICI, ICF, and the test stimulus) were produced in a random order with an interval of approximately 8 seconds between each pulse. The paired-pulse measurements were analyzed by calculating their individual index (mean ICI/mean of the test stimulus; mean ICF/mean of the test stimulus) (35). These parameters were assessed before and after 2 min of rest (36).

Independent variables

All psychological tests used in this study were validated for the Brazilian population (37-38). The patients‟ depressive symptoms were assessed using the Beck Depression Inventory (39), and sleep quality was assessed using the Pittsburgh Sleep Quality Index (40). Pain catastrophizing was assessed using the Brazilian Portuguese Pain Catastrophizing Scale (BP-PCS) (41). A standardized questionnaire was used to assess comorbidities and demographic data. The WOMAC was used to evaluate self-report of knee-specific impairment based on symptoms during the preceding 48 h. The WOMAC assesses the pain, joint stiffness and perceived disability associated with OA to determine the overall impact on a patient‟s perceived function. It comprises 24 questions with responses given to each using a Likert scale. The pain subscale consists of five possible responses: no (0), mild (1), moderate (2), severe (3) or extreme (4) problems. This 20-point scale (range of 0–20) assesses pain in everyday situations (e.g., walking on flat surfaces). The stiffness subscale is a two-item (range 0–8), 10-point scale that assesses perceived knee stiffness after walking and at the end of the day. The disability subscale is a 17-item assessment of perceived physical function in a variety of everyday situations (e.g., getting into and out of a car). The WOMAC is a valid, reliable and responsive instrument that is commonly used to assess pain and disability in studies of knee OA (29). A total WOMAC score, (ranging 0-96),

is calculated by summing the items for all three subscales (42).

To measure the pressure-pain threshold (PPT), we asked patients to differentiate the perception of pressure versus the perception of "onset of pain". The patient was instructed to report the perception of pain onset verbally. A trained investigator assessed the pain threshold. An experienced rehabilitation physician (MGT) systematically evaluated superficial and deep hyperalgesia by assessing the PPT using an electronic algometer (J Tech Medical Industries,USA). Three successive readings taken at intervals of 3-5 min were used to define the PPT in kgf/cm2 (lb/cm2). The PPT was recorded at the site of greatest sensitivity where the devicehad a 1-cm2 hard-rubber probe applied to the myotome and sclerotome structures at the L1- L5 and S1-S2 dermatomes at the knee with greater pain (knee hyperalgesia).

To test the CPM, we used the Tousignant-Laflamme et al. protocol (43) and the experimental pain stimulus used was in accordance to the guidelines for the cold-pressor task (CPM-task) (44). The CPM-task is a strong nociceptive stimulus applied over a large body surface area (44) that takes place over a lengthy time span (45). The CPM-task allows us to modify the descendent pain modulatory system.

To assess the CPM-task, the patient immersed the non-dominant hand in cold water (zero to 1°C) for 1 min. During the last 30 sec of the cold-water immersion, the PPT procedure was administered to the dominant forearm. During the entire experiment, the cold-water temperature was maintained constant. The PPT that elicited pain ratings of 6/10 on the NPS (0-10) (PPT60) was used for the first PPT before the CPM-task (PPT0). After a short break, the PPT0 was applied at the S1-S2 dermatome at the knee of the leg with higher hyperalgesia. Following PPT0, the CPM-task was used to trigger the CPM. One minute after the CPM-task, we applied the second PPT (PPT1). To quantify the CPM, the mean pain rating of PPT1 was subtracted from the first PPT0 before the CPM-task (PPT1); negative values indicate

inhibitory conditioned pain modulation.

Analgesic use was defined by an average of analgesics used per week during the previous month. For data analysis, analgesic use was included as a dichotomous variable (the use of analgesics less than four days per week or the use on more than four days per week). This approach was chosen because patients with chronic pain rescue analgesic use changes each week, depending on their level of pain.

Efforts to address potential sources of bias

To reduce assessment bias, only one researcher (MGT) was involved in all of the assessments. The evaluator (MGT) is a practicing physiatrist of the outpatient clinic at HCPA with vast clinical expertise, who is well trained to make the TMS measures. Also, the evaluator was trained to apply clinical scales and PPT assessment, as well as in the care of chronic pain patients. In our study, all patients were submitted to a clinical evaluation by the same physician (MGT), who had many years of experience in treating patients with OA to revise the severity of OA and inclusion criteria. The algometer used to make measurements was manufactured by (J Tech Medical Industries, USA).

Sample size

The number of patients was estimated based on a type I and type II error of 0.05 and 0.20, respectively, and in anticipation of an effect size (f2=determination coefficient) of 0.4 for the multiple hierarchical regression analysis allowing for two predictors (the Post-hoc Statistical Power Calculator for Hierarchical Multiple Regression: http://www.danielsoper.com/statcalc3/calc.aspx?i (46). A sample of 18 patients was chosen to account for unexpected factors that would decrease the study power such as increased variability of the sample or missing data. A sample of 21 patients would detect an effect size for correlations of 0.4, with a power of 88% at a 0.05 alpha level.

Statistical analysis

Descriptive statistics were used to summarize the main characteristics of the sample. To evaluate if continuous variables presented criteria to normal distribution, we used Skewness/ Kurtosis tests. To compare continuous variables, we used the t test for independent samples and the Qui-Square or Ficher's exact test for categorical variables A MANCOVA was used to assess the relationship between the dependent variables, the cortical excitability parameters (MEP, CSP) with the main interest independent variable, and the change on the NPS (0-10) during thr CPM-task in patients with OA and healthy subjects. The covariate included in the model was age. We used Bonferroni‟s Multiple Comparison Test to adjust the differences for multiple comparisons.

A regression multiple analysis was used to explore the relationship between the change on NPS (0-10) during the CPM-task and cortical excitability parameters (CPS and MEP) in OA patients. This procedure was done to adjust this analysis for potential confounding factors in OA patients, such as analgesic use and disability assessed by the WOMAC index. Also, a regression analysis was used to generate the scatter plot of correlation between CSP and change on NPS (0-10) during the CPM-task. The data were analyzed using SPSS version 22.0 (SPSS, Chicago, IL).

3. RESULTS

3.1. Baseline characteristics

Twenty-one women with OA participated in this study along with ten healthy women. The baseline demographics, psychological characteristics, and cortical excitability parameters are shown in Table 1. A statistical significant difference was observed between OA patients and healthy subjects in age, years of formal education, and reduction on NPS (0-10) during the CPM-task and resting motor threshold.

TABLE 1. Clinical and Demographic Characteristics, Psychological State, and Measures of Intracortical Inhibition of the Sample (n = 31)

	Patients With OA (n = 21)	Healthy Subjects (n = 10)	
Characteristics	**Mean (SD)**		***P****
Age, years	64.50 (7.72)	34.10 (11.64)	0.001†
Formal education, years	11.29 (4.0)	16.70 (1.76)	0.01†
Body index	27.53 (5.11)	—	—
Smoking, yes/no (%)	0/21 (yes: 0%)	—	—
Alcohol, yes/no (%)	3/18 (yes: 14.28%)	—	—
Number of chronic disease	0.8 (0.67)	—	—
Hypertension (yes/no)	10 / 11 (yes: 47.6%)	—	—
Diabetes mellitus (yes/no)	2 / 19 (yes: 9.5%)	—	—
Asthma (yes/no)	2/19 (yes: 9.5%)	—	—
Other chronic disease than listed (yes/no)	3/18 (yes: 14.3%)	—	—
Psychotropic medication (yes/no)	11/10 (yes: 52.38%)	—	—
Analgesic drugs used more than 3 times per week during the past 3 months (yes/no) (%)	14/6 (yes: 66.66%)	—	—
Glucosamine/chondroitin, %	6/15 (yes: 28.57 %)	—	—
Working, yes/no (%)	4/17 (yes: 19.04%)	—	—
Education, years	10.38 (5.69)	—	—
Time of disability related to pain, years	6.73 (2.53)	—	—
WOMAC (global score)	57.92 (13.25)	—	—
WOMAC dominions			
Pain	14.54 (3.59)	—	—
Stiffness	4.19 (1.88)	—	—
Physical activity	39.19 (12.13)	—	—
Beck Depression Inventory	10.27 (7.42)	—	—
Catastrophizing thinking related to pain	23.19 (9.6)	—	—
Pittsburgh Sleep Quality Index	42.88 (15.83)	—	—
PPT in the knee area, kgf/cm^2	5.93 (2.34)	—	—
Reduction on NPS during CMP/task	−0.48 (3.22)	−3.16 (2.86)	0.03†
Resting motor threshold	44.29 (8.05)	39.2 (3.88)	0.02†
Motor-evoked potential amplitude, mV	1.11 (0.8)	1.26 (0.27)	0.46
Cortical silent period, ms	54.54 (16.10)	70.94 (22.87)	0.06
Short intracortical inhibition (ratio: MEP/SICI)	0.56 (0.36)	0.48 (0.14)	0.30
Intracortical facilitation (ratio: MEP/ICF)	0.89 (0.33)	1.06 (0.34)	0.21

CPM = conditioned pain modulation, ICF = intracortical facilitation, MEP = motor-evoked potential, NPS = numerical pain scale, OA = osteoarthritis, PPT = pressure pain threshold in the knee area (L1-L5 and the S1-S2 dermatome), SICI = short-interval intracortical inhibition, WOMAC = Western Ontario and McMaster Universities Osteoarthritis Index.

*Comparisons using *t* test for independent samples.

†$P < 0.05$.

3.2. Relationship between cortical excitability parameters and descending pain modulatory system in patients with OA and healthy subjects

A MANCOVA was used to adjust, by age, the relationship between the groups (OA patients or healthy subjects) and their outcomes related to cortical excitability measurements (CSP and MEP amplitude) with the descending pain modulatory system as assessed by the reduction on NPS (0-10) during CPM-task (Wilks‟ $\lambda = 0.43$ F (4) =5.08, P<0.01). The power of this analysis was 0.92%. The adjusted determination coefficient of this model is R2= 0.47 (i.e., the variables included in the model explain 47% of the variance in the outcomes variables). This analysis revealed that the function of the descending pain modulatory system, assessed by change on NPS (0-10) during CPM-task, is negatively correlated with the cortical excitability parameter indexed by the CSP (P<0.05) (Table 2). This analysis

revealed that the MEP amplitude was not statistically different between OA patients and healthy subjects (P> 0.05). The adjusted mean (SD) on the MEP amplitude observed in OA was 13.53% higher than in healthy subjects [1.33 (0.49) vs. 1.15 (0.13)], respectively (Figure 2).

TABLE 2. Multivariate Analysis of the Relationship Between Measures of Intracortical Excitability and Change on NPS (0–10) During CPM-Task in Patients with OA and Healthy Subjects (n = 31)

Dependent Variable	Type III Sum of Squares	df	Mean Square Error	*F*	*P*	Partial Eta Squared
Motor-evoked-potential amplitude, mV	2.34	4	0.58	5.34	0.01*	0.45
Cortical silent period, ms	6295.63	4	1573.98	7.55	0.001*	0.54
	B	**SEM**	***t***	***P***		**Partial Eta Squared**
Motor-evoked-potential amplitude, mV						
OA patients vs healthy subjects	−1.32	0.77	−1.70	0.10		0.10
Reduction on NPS (0–10) during CMP-task	−0.06	0.02	−2.61	0.01*		0.20
Age, years	0.01	0.01	0.96	0.34		0.04
Interaction (group × age)						
OA patients vs healthy subjects	0.02	0.01	1.42	0.16		0.07
Cortical silent period, ms						
OA patients vs healthy subjects	−60.63	33.50	−2.43	0.02*		0.18
Reduction on NPS (0–10) during CMP-task	−2.44	0.92	−2.64	0.01*		0.21
Age, years	−1.00	0.44	−2.25	0.03*		0.16
Interaction (group × age)						
OA patients vs healthy subjects	1.58	0.63	2.50	0.01*		0.19

Adjusted $R^2 = 0.47$.

B = beta coefficient, *F* for the MANCOVA, CPM = conditioned pain modulation, df = degrees of freedom, ICF = intracortical facilitation, MEP = motor-evoked potential, NPS = numerical pain scale, OA = osteoarthritis, PPT = pressure pain threshold in the knee area (L1-L5 and the S1-S2 dermatome), SEM = standard error of mean.

*$P < 0.05$.

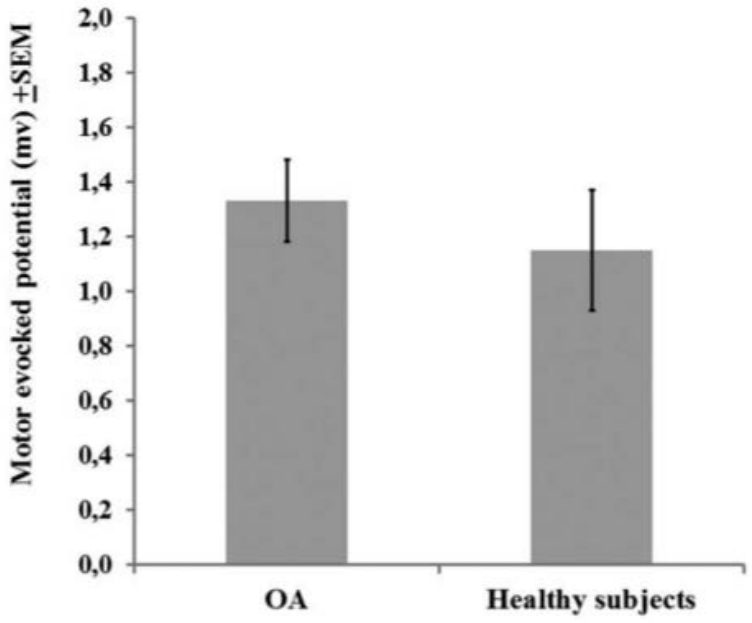

Figure 2. Comparisons between groups osteoarthritis (OA) or healthy subjects, on motor evoked potential (MEP) (n=31). The error bars indicate standard error of the mean (S.E.M.). The bars indicate means of each groups (OA or healthy subjects) compared by MANCOVA.

Also, the MANCOVA analysis (Table 2) shows that age was negatively correlated to the CSP. Accordingly, healthy subjects presented a longer CSP. The adjusted mean (SD) on the CSP observed in OA patients was 23.43% lower than in healthy subjects [54.54 (16.10) vs. 70.94 (22.87)], respectively (Figure 3). We observed that in healthy subjects the age was negatively correlated to the CSP, while in OA patients the direction of this relationship was inverse. Thus, even having older OA patients in comparison to healthy subjects, the older age was not enough to prolong the CSP as would do in healthy subjects of the same age, and in turn, OA patients presented shorter CSP (Table 2). Thus, this suggests that the inhibition within OA patients due to the neuroplastic changes induced by chronic pain.

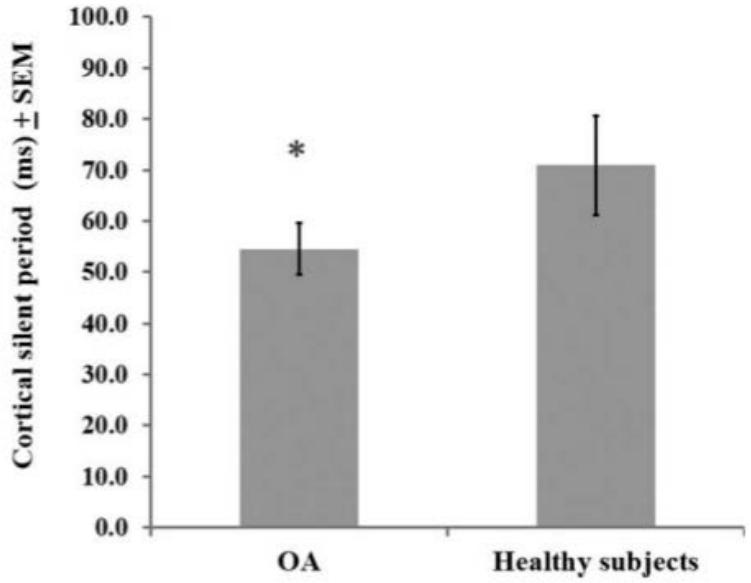

Figure 3. Comparisons between groups osteoarthritis (OA) or healthy subjects, on cortical silent period (CSP) (n=31). The error bars indicate standard error of the mean (S.E.M.). * Asterisks positioned above the bars indicate differences between groups (OA or healthy subjects) assessed by MANCOVA with post-hoc Bonferroni‟s Multiple Comparison test.

The MANCOVA analysis (Table 2) showed that the MEP amplitude and the CSP were negatively correlated with change on the score of the NPS (0-10) during the CPM-task. That is, the increase in the CSP was correlated with a higher change on the NPS (0-10) during the CPM-task, or vice-versa (Table 2). It is important to remember that a higher change on the NPS (0-10) during the CPM-task indicates that the heterotopic stimulus was more effective, thus, the difference on NPS (0-10) (PPT1 minus PPT0) produced a higher negative value. Thus, this explains the coherence this negative correlation.

Also, the increase of the MEP amplitude was negatively correlated with the change on the

NPS (0-10) during the CPM-task (Table 2). Considering that higher MEP amplitude indicates higher excitability on the cortical-spinal pathway, it should be plausible to expect a positive correlation, because it would be less prone to modulating the nociceptive stimulus. However, the MEP amplitude was not statistically different between groups (OA and healthy subjects) (Table 2).

An important question is to identify if this result could be explained by other confounding factors. Thus, we run a multiple regression analysis only with the OA patients (Table 3). In this model, the relationship between the change on the NPS (0-10) during the CPM-task and cortical excitability parameters (CPS and MEP) was adjusted by analgesic use and the self-reporting of pain and disability assessed by the WOMAC index. The multiple regression analysis confirmed an inverse correlation between the CSP with the change on the NPS (0-10) during the CPM-task, but not with the MEP amplitude (Table 3). The scatter plot of the raw CSP and change on the NPS (0-10) during the CPM-task is presented for illustrative purposes in Figure 3. The Pearson correlation coefficient (r) was -0.72 [confidence interval (CI) 95% -0.87 to -0.38] and the coefficient of determination (R2), that is the proportion of the variance explained by the association between the change on NPS (0-10) during CPM-task and the CSP was 52% (R2 = 0.52).

TABLE 3. Linear Regression of the Relationship Between Measures of Intracortical Excitability and the Function of Descending Pain Modulation Adjusting by Potential Confounding Factors (n = 21)

Parameters	*B*	SEM	*t*	*P*
Motor-evoked potential amplitude, mV				
Western Ontario and McMaster Universities Osteoarthritis Index	−0.009	0.01	−0.63	0.53
Change on NPS (0–10) during the CPM-task	−0.06	0.06	−1.13	0.27
Analgesic drugs used more than 3 times per week during the last 3 months (yes/no)	−0.08	0.39	−0.20	0.84
Cortical silent period, ms				
Western Ontario and McMaster Universities Osteoarthritis Index	−0.32	0.14	−2.22	0.04*
Change on NPS (0–10) during the CPM-task	−2.88	0.60	−4.79	0.001*
Analgesic drugs used more than 3 times per week during the last 3 months (yes/no)	5.28	3.90	1.35	0.19

Adjusted $R^2 = 0.62$.
B = beta coefficient, t statistic, CPM = conditioned pain modulation, NPS = numerical pain scale, SEM = standard error of mean.
*$P < 0.05$.

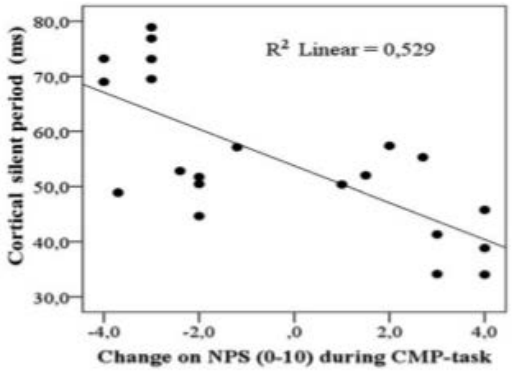

Figure 4. Scatter plot of the correlation between changes on NPS (0-10) during CPM-task and the current silent period (CSP) in patients with osteoarthritis (OA) (n=21).

15 DISCUSSION

This study extends the literature about the correlation between measures of the intracortical disinhibition as indexed by CSP and the dysfunction in the descending control of nociceptive processing as indexed by lower activation of the CPM-task in KOACP. Whereas, a lower CSP indicates that the trigger to induce a membrane potential is lower, thereby, there is less inhibition. A higher value of the change on the NPS (0-10) during the CPM-task indicates that the heterotopic stimulus to induce CPM was less effective. This assignment on descending pain modulatory system may explain the inverse correlation between the CSP with the disability related to pain as assessed by the total WOMAC. Our finding suggests that sustained peripheral inflammation increases the excitability of ascending nociceptive pathways, which induces dysfunction in descending modulatory systems, as assessed by the change on the NPS (0-10) during the CPM-task. At the cortical level, a shorter CSP reflects the decreased excitability of the inhibitory interneurons. This disinhibited state observed in OA, but not in healthy subjects, support the hypothesis that the chronic pain in OA induces

a disinhibitory effect at the intra-cortical level as well as in the descending pain modulatory system. Whereas, the intra-cortical disinhibition is in agreement with what has previously been described in neuropathic and fibromyalgia pain (47). The intra-cortical disinhibition state observed in this study is in disagreement with findings of a previous report, which did not find any significant changes in the motor cortical excitability in OA patients compared to healthy subjects (48). It is possible that the severity of osteoarthritis and the long-term pain level could explain this divergence. Our sample patients were shown to have moderate to severe knee osteoarthritis, intense chronic pain and disability during more than five years, while the mentioned study above noted OA pain in the hands, which carries a mild severity (49). In the present study, higher disability was inversely correlated with the CSP. Given that the CSP index assesses the function of GABAergic transmission (50), it is conceivable that sustained pain – in this case, triggered by peripheral inflammation – could lead to a cascade of events resulting in dysfunctional inhibitory function. Part of this defective cortical inhibition could be explained by recent evidence showing decreased gray matter volume associated with chronic pain syndromes, including OA (51). Central reorganization in chronic pain appears to lead to an inhibitory state that might facilitate activation in non-pain networks, such as the primary motor cortex. Thus, disturbances in the GABAergic and glutamatergic intra-cortical networks might explain the disinhibition found in KOACP.

The shorter CSP suggests a decrease of GABAergic neurons because they are responsible to exert rapid synaptic inhibition via GABA-B receptor (52). This activation of the GABAergic system governs the state of inhibitory interneurons within M1 (52). Also; it is known that the balance between inhibitory and excitatory systems is influenced by age. Elderly subjects present a slower motor response and a decline in the modulation of the

corticospinal activity system (53). Whereas this association is consistent in healthy subjects (54), our results suggest that chronic pain leads to changes in favor of excitability, as demonstrated by a shorter CSP. Also, the adjusted analysis shows that age is intrinsically associated with slower intra-cortical excitability, because the direction of correlation between the CSP and age changed when we analyzed the interaction of age with the group (OA or healthy subjects) (Table 3). However, the independent association between the CPM-task and the CSP persisted even after adjustment by age (Table 2). Thereby, it is unlikely that controls‟ age would modify the present findings because the relationship between the CPM-task and the CSP persisted even when we included only patients with OA (Table 3). However, we cannot assume that the effect of age as a confounding factor was entirely controlled. While an ideal strategy to validate our results is to compare them with healthy subjects, in a real life scenario it is complex to find controls to match the profile of our sample of OA.

Thus, changes in cortical plasticity in OA could be explained by a steady pain induced by a peripheral neural lesion, which increases the synaptic efficacy of neural structures involved in pain processing likewise occurring on a neuropathic lesion. The CSP in this study suggests that ongoing nociception from knee-related structures is essential to the chronic nature of this process and the development of sensitization (55). The association between the severity of pain and the disinhibition at cortical and infra-cortical levels highlights that knee hyperalgesia is an important generator of pain and sensitization as previously demonstrated in knee arthroplasty (4). However, cumulative evidence suggests that inflammation leads to increased hyperalgesia, which concurs with a lack of descending inhibitory pain mechanism (9). This finding was demonstrated in the present study and it is also supported by previous reports (9). It has been demonstrated that this diminished activity of the descending inhibitory interneuron

activity is a consequence of a decreased synthesis of neurotransmitters (GABA and glycine), a diminished activity of serotonin and norepinephrine (56). In this study, the lower activation of the CPM induced by the CPM-task was correlated negatively with the CSP (Figure 3), which suggests that the lower activation of the CPM by the CPM-task is related to higher intra-cortical disinhibition. Based on a cross-sectional analysis, this finding indicates that worsening of the descending pain inhibitory system function is associated with a loss of cortical pain inhibition. This is likely a consequence of higher pain intensities and longer durations, which induces more facilitated temporal summation compared to lower pain intensities and shorter durations of pain (57). The downward negative spiral of pain and central disinhibition has severe clinical consequences, as follows: it increases pain and local knee hyperalgesia and it is associated with a loss of cortical inhibitory mechanisms. Although the design of this study prevents determining the deterioration in the central pain modulatory system, it does permit us to better understand the dysfunctional process of disinhibition at cortical and intra-cortical regions in severe KOACP. Thus, the pieces of evidence these findings possess, may hold important clinical implications such as (i) to support an understanding of the bidirectional pathways between peripheral inflammation and central brain changes in OA; (ii) to select the best therapeutic approach based on the neurophysiological phase state of each patient, because chronic pain has been associated with unfavorable pain outcomes after knee arthroplasty (58); (iii) to determine strategies to manage patients with a higher risk of more severe chronic pain after knee arthroplasty, which includes anesthetic and analgesic approaches (4). Also, it improves the understanding of underlying neurophysiological mechanisms of chronic pain in OA, which could give support to plan new 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.

The small sample size is a limitation of this study. The study design is a limitation because it is not possible to determine a causative effect. The use of TMS assesses the neurotransmitter system activity in an indirect manner, and it has been shown to have relatively low specificity. However, TMS is a useful tool for neurophysiological assessment because it induces activity and evaluates the response of the subject. In this study, only females were evaluated, taking into account that gender differences in pain perception and modulation are a controversial topic (59). This limitation restricts the possibility of a direct comparison with other studies but has the advantage of avoiding possible contamination of the data. Because OA is more prevalent in females (60), these results might have greater clinical implications. Although we recruited healthy volunteers to assess the relationship between the cortical inhibitory function and the descendent pain inhibitory system, it is worth noting that our control sample was younger on average.

These findings support the hypothesis that a change in cortical plasticity in KOACP is associated with less intra-cortical inhibition, as measured by the CSP. These results show that this neural change in the motor cortex in KOACP is associated with pain and disability levels as well as with decreased activation of the endogenous pain modulating system by the CPM-task.

REFERENCES

1. Recommendations for the medical management of osteoarthritis of the hip and knee: 2000 update. American College of Rheumatology Subcommittee on Osteoarthritis Guidelines. Arthritis Rheumatol. 2000; 43(9):1905-1915.

2. Haavik-Taylor H, Murphy B. Cervical spine manipulation alters sensorimotor integration:

a somatosensory evoked potential study. Clin Neurophysiol. 2007; 118 (2):391-402.

3. Gwilym SE, Keltner JR, Warnaby CE, et al. Psychophysical and functional imaging evidence supporting the presence of central sensitization in a cohort of osteoarthritis patients. Arthritis Rheumatol. 2009; 61 (9):1226-1234.

4. Skou ST, Graven-Nielsen T, Rasmussen S, Simonsen OH, Laursen MB, Arendt-Nielsen L. Facilitation of pain sensitization in knee osteoarthritis and persistent post-operative pain: a cross-sectional study. Eur J Pain. 2014;18 (7):1024-1031.

5. Bajaj P, Graven-Nielsen T, Arendt-Nielsen L. Osteoarthritis and its association with muscle hyperalgesia: an experimental controlled study. Pain. 2001; 93 (2):107-114.

6. Graven-Nielsen T, Wodehouse T, Langford RM, Arendt-Nielsen L, Kidd BL. Normalization of widespread hyperesthesia and facilitated spatial summation of deep-tissue pain in knee osteoarthritis patients after knee replacement. Arthritis Rheumatol. 2012; 64 (9):2907-2916.

7. Hawker G, Wright J, Coyte P, et al. Health-related quality of life after knee replacement. J Bone Joint Surg Am. 1998; 80 (2):163-173.

8. Becker R, Doring C, Denecke A, Brosz M. Expectation, satisfaction and clinical outcome of patients after total knee arthroplasty. Knee Surg Sports Traumatol Arthrosc. 2011; 19 (9):1433-1441.

9. Lewis GN, Rice DA, McNair PJ. Conditioned pain modulation in populations with chronic pain: a systematic review and meta-analysis. J Pain. 2012;13 (10):936-944.

10. Landau R, Kraft JC, Flint LY, et al. An experimental paradigm for the prediction of Post-Operative Pain (PPOP). J Vis Exp. 2010 (35).

11. Pelletier R, Higgins J, Bourbonnais D. Is neuroplasticity in the central nervous system the missing link to our understanding of chronic musculoskeletal disorders? BMC

Musculoskelet Disord. 2015;16:25.

12. Davis KD, Moayedi M. Central mechanisms of pain revealed through functional and structural MRI. J Neuroimmune Pharmacol. 2013; 8 (3):518-534.

13. Knijnik LM, Dussan-Sarria JA, Rozisky JR, et al. Repetitive Transcranial Magnetic Stimulation for Fibromyalgia: Systematic Review and Meta-Analysis. Pain Pract. 2015.

14. Fregni F, Gimenes R, Valle AC, et al. A randomized, sham-controlled, proof of principle study of transcranial direct current stimulation for the treatment of pain in fibromyalgia. Arthritis Rheumatol. 2006; 54 (12):3988-3998.

15. Hagenacker T, Bude V, Naegel S, et al. Patient-conducted anodal transcranial direct current stimulation of the motor cortex alleviates pain in trigeminal neuralgia. J Headache Pain. 2014;15:78.

16. Bolognini N, Olgiati E, Maravita A, Ferraro F, Fregni F. Motor and parietal cortex stimulation for phantom limb pain and sensations. Pain. 2013;154 (8):1274-1280.

17. Dasilva AF, Mendonca ME, Zaghi S, et al. tDCS-induced analgesia and electrical fields in pain-related neural networks in chronic migraine. Headache. 2012;52 (8):1283-1295.

18. O'Connell NE, Cossar J, Marston L, et al. Transcranial direct current stimulation of the motor cortex in the treatment of chronic nonspecific low back pain: a randomized, double-blind exploratory study. Clin J Pain. 2013;29 (1):26-34.

19. Dall'Agnol L, Medeiros LF, Torres IL, et al. Repetitive transcranial magnetic stimulation increases the corticospinal inhibition and the brain-derived neurotrophic factor in chronic myofascial pain syndrome: an explanatory double-blinded, randomized, sham-controlled trial. J Pain. 2014; 15 (8):845-855.

20. Leo RJ, Latif T. Repetitive transcranial magnetic stimulation (rTMS) in experimentally induced and chronic neuropathic pain: a review. J Pain. 2007; 8 (6):453-459.

21. Di Lazzaro V, Pilato F, Dileone M, et al. The physiological basis of the effects of intermittent theta burst stimulation of the human motor cortex. J Physiol. 2008;586(16):3871-3879.

22. Conte A, Barbanti P, Frasca V, et al. Differences in short-term primary motor cortex synaptic potentiation as assessed by repetitive transcranial magnetic stimulation in migraine patients with and without aura. Pain. 2010; 148 (1):43-48.

23. Suppa A, Biasiotta A, Belvisi D, et al. Heat-evoked experimental pain induces long-term potentiation-like plasticity in human primary motor cortex. Cereb Cortex. 2013; 23 (8):1942-1951.

24. Zaghi S, Thiele B, Pimentel D, Pimentel T, Fregni F. Assessment and treatment of pain with non-invasive cortical stimulation. Restor Neurol Neurosci. 2011; 29 (6):439-451.

25. Werhahn KJ, Kunesch E, Noachtar S, Benecke R, Classen J. Differential effects on motorcortical inhibition induced by blockade of GABA uptake in humans. J Physio. 1999; 517 (Pt 2):591-597.

26. Inghilleri M, Berardelli A, Cruccu G, Manfredi M. Silent period evoked by transcranial stimulation of the human cortex and cervicomedullary junction. J Physiol. 1993;466:521-534.

27. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. J Clin Epidemiol. 2008; 61 (4):344-349.

28. Hochberg MC, Altman RD, Brandt KD, et al. Guidelines for the medical management of osteoarthritis. Part II. Osteoarthritis of the knee. American College of Rheumatology. Arthritis Rheum. 1995; 38 (11):1541-1546.

29. Bellamy N, Buchanan WW, Goldsmith CH, Campbell J, Stitt LW. Validation study

of WOMAC: a health status instrument for measuring clinically important patient relevant outcomes to antirheumatic drug therapy in patients with osteoarthritis of the hip or knee. J Rheumatol. 1988; 15 (12):1833-1840.

30. Ravaud P, Auleley GR, Amor B, Dougados M. Radiographic assessment of progression in knee osteoarthritis. Rheumatol Eur. 1995; 24: S129-131.

31. Rossi S, Hallett M, Rossini PM, Pascual-Leone A. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol. 2009; 120 (12):2008-2039.

32. Rothwell JC, Hallett M, Berardelli A, Eisen A, Rossini P, Paulus W. Magnetic stimulation: motor evoked potentials. The International Federation of Clinical Neurophysiology. Electroencephalogr Clin Neurophysiol Suppl. 1999; 52:97-103.

33. Fuhr P, Agostino R, Hallett M. Spinal motor neuron excitability during the silent period after cortical stimulation. Electroencephalogr Clin Neurophysiol. 1991; 81 (4):257-262.

34. Kujirai T, Caramia MD, Rothwell JC, et al. Corticocortical inhibition in human motor cortex. J Physiol. 1993; 471:501-519.

35. Sanger TD, Garg RR, Chen R. Interactions between two different inhibitory systems in the human motor cortex. J Physiol. 2001;530(Pt 2):307-317.

36. Maioli C, Falciati L, Marangon M, Perini S, Losio A. Short- and long-term modulation of upper limb motor-evoked potentials induced by acupuncture. Eur J Neurosci. 2006; 23(7):1931-1938.

37. Collins SL, Moore RA, McQuay HJ. The visual analogue pain intensity scale: what is moderate pain in millimetres? Pain. 1997; 72 (1-2):95-97.

38. Fleck MP, Louzada S, Xavier M, et al. [Application of the Portuguese version of the abbreviated instrument of quality life WHOQOL-bref]. Rev Saude Publica. 2000; 34 (2):178-

183.

39. Warmenhoven F, van Rijswijk E, Engels Y, et al. The Beck Depression Inventory (BDI-II) and a single screening question as screening tools for depressive disorder in Dutch advanced cancer patients. Support Care Cancer. 2012; 20 (2):319-324.

40. Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 1989; 28 (2):193-213.

41. Sehn F, Chachamovich E, Vidor LP, et al. Cross-cultural adaptation and validation of the Brazilian Portuguese version of the pain catastrophizing scale. Pain Med. 2012;13(11):1425-1435.

42. Berrada K, Wariaghli G, Idrissi Z, et al. [FRI0133] Normative values for the western ontario and mcmaster in the general population compared with knee osteoarthritis patients. Ann Rheum Dis. 2011; 70 (3):388.

43. Tousignant-Laflamme Y, Page S, Goffaux P, Marchand S. An experimental model to measure excitatory and inhibitory pain mechanisms in humans. Brain Research. 2008; 1230:73-79.

44. von Baeyer CL, Piira T, Chambers CT, Trapanotto M, Zeltzer LK. Guidelines for the cold pressor task as an experimental pain stimulus for use with children. J Pain. 2005; 6 (4):218-227. 22

45. Willer JC, De Broucker T, Le Bars D. Encoding of nociceptive thermal stimuli by diffuse noxious inhibitory controls in humans. J Neurophysiol. 1989; 62 (5):1028-1038.

46. Preacher KJ, Kelley K. Effect size measures for mediation models: quantitative strategies for communicating indirect effects. Psychol Methods. 2011; 16 (2):93-115.

47. Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. Pain.

2011;152 (3 Suppl):S2-15.

48. Schwenkreis P, Scherens A, Ronnau AK, Hoffken O, Tegenthoff M, Maier C. Cortical disinhibition occurs in chronic neuropathic, but not in chronic nociceptive pain. BMC Neurosci. 2010; 11:73.

49. Visser AW, Boyesen P, Haugen IK, et al. Radiographic scoring methods in hand Osteoarthritis - A systematic literature search and descriptive review. Osteoarthritis Cartilage. 2014; 22 (10):1710-1723.

50. Jacobs KM, Donoghue JP. Reshaping the cortical motor map by unmasking latent intracortical connections. Science. 1991; 251(4996):944-947.

51. Smallwood RF, Laird AR, Ramage AE, et al. Structural brain anomalies and chronic pain: a quantitative meta-analysis of gray matter volume. J Pain. 2013; 14 (7):663-675.

52. McDonnell MN, Orekhov Y, Ziemann U. The role of GABA (B) receptors in intracortical inhibition in the human motor cortex. Exp Brain Res. 2006;173 (1):86-93.

53. Fujiyama H, Hinder MR, Schmidt MW, Tandonnet C, Garry MI, Summers JJ. Age-related differences in corticomotor excitability and inhibitory processes during a visuomotor RT task. J Cogn Neurosci. 2012; 24 (5):1253-1263.

54. Klein-Flugge MC, Nobbs D, Pitcher JB, Bestmann S. Variability of human corticospinal excitability tracks the state of action preparation. J Neurosci. 2013; 33 (13):5564-5572.

55. Apkarian AV, Bushnell MC, Treede RD, Zubieta JK. Human brain mechanisms of pain perception and regulation in health and disease. Eur J Pain. 2005; 9 (4):463-484.

56. Ossipov MH, Dussor GO, Porreca F. Central modulation of pain. J Clin Invest. 2010; 120(11):3779-3787.

57. Arendt-Nielsen L, Graven-Nielsen T. Translational musculoskeletal pain research. Best Pract Res Clin Rheumatol 2011; 25 (2):209-226.

58. Kennedy LG, Newman JH, Ackroyd CE, Dieppe PA. When should we do knee replacements? Knee. 2003; 10 (2):161-166.

59. Racine M, Tousignant-Laflamme Y, Kloda LA, Dion D, Dupuis G, Choiniere M. A systematic literature review of 10 years of research on sex/gender and pain perception - part 2: do biopsychosocial factors alter pain sensitivity differently in women and men? Pain. 2012; 153 (3):619-635.

60. Kacar C, Gilgil E, Urhan S, et al. The prevalence of symptomatic knee and distal interphalangeal joint osteoarthritis in the urban population of Antalya, Turkey. Rheumatol Int. 2005;25(3):201-204.

8 Artigo 2

Fator de Impacto: 2.339

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Electrical Intramuscular Stimulation in Osteoarthritis Enhances the Inhibitory Systems in Pain Processing at Cortical and Cortical Spinal System

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Conflict of interest: The authors declare that there are no relationships, financial or otherwise,

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.

Trial registration: Clinical trials.gov: NCT01855958.

These findings highlight that for knee osteoarthritis (KOA) with severe pain and disability, the a-EIMS enhanced the corticospinal inhibitory systems in cortical and infra-cortical pain processing sites. Also, these results showed that serum BDNF had an inverse relationship with PPT independent of the treatment group.

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Abstract

Objective. To determine if in knee osteoarthritis (KOA), one session of active electrical intramuscular stimulation (a-EIMS) compared with sham causes an effect on the motor cortex excitability parameters [motor evoked potential (MEP; the primary outcome), short intracortical inhibition (SICI), intracortical facilitation (ICF) and cortical silent period (CSP)] and pain measurements [pain pressure threshold (PPT); visual analog scale (VAS) and change

in numerical pain scale (NPS0-10) during the conditioned pain modulation (CPM)-task]. This study also set out to determine if serum brain-derived neurotrophic factor (BDNF) mediates the effect of treatment on the cortical spinal system as assessed by MEP and PPT.

Design. Randomized clinical trial.

Subjects and methods. Women with KOA, 50–75 years old received a 30-min session of either sham (n=13) or a-EIMS (n=13) with 2 Hz. The pain measures and excitability parameters were measured before and immediately after a-EIMS or sham.

Results. The a-EIMS group compared with sham decreased the MEP by 31.61% [confidence interval 95 (CI) 95%, 2.34–60.98]. For the secondary outcomes, the a-EIMS reduced the ICF and increased the CSP but not changed the SICI. The a-EIMS improved the pain reported on VAS, the PPT, and the score of the NPS (0–10) during the CPM-task The BDNF was negatively correlated with the PPT (r =20.56).

Conclusions. The serum BDNF revealed an inverse relationship with PPT independent of the treatment group. These results suggest that a-EIMS enhanced the corticospinal inhibitory systems in cortical and infracortical pain processing sites most likely by bottom-up regulation mechanisms.

Key Words. Osteoarthritis; Intramuscular Stimulation; Transcranial Magnetic Stimulation; Pain Pressure Threshold; Conditioned Pain Modulation; Brain-Derived Neurotrophic Factor.

Introduction

The leading cause of disability and pain in the elderly patients is osteoarthritis (OA) of the knee joints [1]. The sustained input originating in the nociceptor sensory fibers, C and A-d, induced by chronic inflammation leads to peripheral sensitization [2], which interrupts descending inhibition. Kosek [3] demonstrated that the reduced descending analgesic activity was partially normalized following arthroplasty. A study using functional neuroimaging [4]

demonstrates augmentation of the processing of pain in the central nervous system (CNS) in OA patients. Also, in a separate study [5], they discovered atrophy of the thalamus, which was partially reversed with arthroplasty. These changes concur with central sensitization, which is characterized by allodynia, hyperalgesia, and an expansion of the receptive field. Thus, the pain that extends beyond the area of peripheral nerve supply persists long after the stimulus has been removed [6]. It has been demonstrated that chronic pain is a dynamic phenomenon resulting from the activity of both endogenous pain excitatory and inhibitory systems, as well as cortical reorganization, which leads to an abnormal and intense enhancement of pain [7]. The primary motor area (M1) has become a target for assessing neuroplasticity coupled with cortical reorganization [8]. The descending corticospinal pathways can be assessed applying magnetic pulses over the M1 using the transcranial magnetic stimulation (TMS), a noninvasive technique used to stimulate neurons in the brain cortex based on electromagnetic pulses. These pulses depolarize nerve cells that induce a motor response in contralateral muscle(s) of interest called "motor evoked potential" (MEP). This parameter is a reliable measure of change in corticospinal excitability in a range of research protocols [9–11]. In literature on chronic pain, it is suggested that the inhibitory capacity of the corticospinal modulator system is reduced [12]. The dysfunction of the corticospinal pain modulatory system is a phenomenon seen in chronic pain, which involves many different factors. One of these factors is the brain-derived neurotrophic factor (BDNF), a neurothrophin capable of strengthening excitatory (glutamatergic) synapses whereas weakening inhibitory (GABAergic) synapses [13]. It increases the central serotonergic activity and modulates neurons of dorsal raphe nucleus through a sprouting of mature serotoninergic neurons [14]. Also, BDNF sensitizes nociceptive neurons in the dorsal horn of the spinal cord facilitating the activation of N-methyl-D-aspartate (NMDA) [15]. Thus, a better comprehension of the

intersection of BDNF‟s contribution to motor cortex excitability and its ability to alter the descending pain modulatory system, can support challenges hindering the development of further optimized therapeutic options [16,17]. Additionally, it is important to understand how this mediator is related to the effect of neuromodulatory techniques used to treat pain in OA, such as needling techniques (i.e., acupuncture and electroacupuncture) [18]. Despite the use of needling techniques over millenniums to treat pain, studies have demonstrated that acupuncture at acupoints and nonacupoints were equally effective in improving pain. Thus, we selected electric intramuscular stimulation, which is efficacious and easy to reproduce [19] because the needling is applied in the spinal segment of the nerve roots associated with the dermatome corresponding to the pathology [20]. Nevertheless, its effect on motor system excitability and the descending inhibitory system, considering the influence of factors involved in the excitatory/inhibitory balance in the CNS, such as BDNF, has yet to be explored. We hypothesize that the active electrical intramuscular stimulation (a-EIMS) alters the excitatory/inhibitory balance in the CNS. Thereby, this effect can improve the descendent inhibitory influx, which also means the excitatory/inhibitory balance mechanism is modulated by BDNF. The aim of this study was determine if in knee osteoarthritis (KOA), one session of active electrical intramuscular stimulation (a-EIMS) compared with sham causes an effect on the motor cortex excitability parameters [MEP (the primary outcome), short intracortical inhibition (SICI), intracortical facilitation (ICF), and cortical silent period (CSP)] and pain measurements [pain pressure threshold (PPT); visual analog scale (VAS) and change in NPS0–10 during the conditioned pain modulation (CPM)-task]. This study also set out to determine if serum BDNF mediates the effect of treatment on the cortical spinal system as assessed by MEP and PPT.

Material and Methods, Design Overview, Setting, and Participants

The study protocol was approved by the Ethics Committee at the institution in which the work

was carried out. All of the patients gave their written informed consent to participate. The study was approved by the Research Ethics Committee at the Hospital de Clínicas de Porto Alegre (HCPA); it is in accordance with the Declaration of Helsinki. Our Hospital serves a population dependent on the Brazilian public health system. Patients were recruited from the general population through public postings in different health care units and referrals from physicians in the Physiatrist and Chronic Pain Service at the HCPA. The eligibility criteria were designed to study a group of patients who were potentially appropriate candidates for unilateral knee arthroplasty. The sample consisted of 26 right-handed women, who were included if they were the age of 50–75-years old and had moderate or severe pain or stiffness of the knee and functional impairment for at least 6 months that was not controlled with medical therapy. The baseline interview included the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), a validated instrument used to assess pain, stiffness, and functional limitations related to OA [21]. Patients were eligible for the study if they reported “moderate,” “severe,” or “extreme” pain or stiffness in response to at least 1 of the 5 pain questions (pain with walking, climbing stairs, reclining, sitting, or standing) and the 2 stiffness questions (morning stiffness, stiffness later in day) and if they reported “moderate,” “severe,” or “extreme” difficulty in response to at least 1 of the 17 activities. Additionally, the radiographs of all knees were evaluated with regard to the degree of osteoarthritis by one physiatrist physician who has worked in OA rehabilitation for more than 10 years. This evaluation was conducted using the Kellgren–Lawrence (K–L) grading scale of 3–4 [22], which has previously been validated and has been proven to be highly reproducible when used in the grading of KOA [23,24]. The exclusion criteria were as follows: other orthopedic, rheumatic, or neurological pathologies; surgery on the affected areas in the prior 6 months; the habitual use of corticosteroids; or other uncompensated chronic pathologies or a prior experience with acupuncture. Additionally, patients were excluded if they had a body mass

index (BMI) of >35 kg/m2. We did not include patients who had received physical therapy for the knee within the previous 30 days or those who were unable to read and/or write (Figure 1). The sample size was defined by the primary outcome: MEP. The sample calculation for MEP was based on one of our studies assessing M1 excitability and plasticity associated with chronic pain [25]. The sample size calculation was done using a mean difference between groups of 0.6 [pool of standard deviation (0.25)]. We assumed a type I error of 5%, a type II error of 20%, and a power of 80%. Assuming a normal distribution, we determined that a sample size of 24 patients would be necessary. Finally, considering the likely attrition rate and other unexpected factors, the required sample size was determined to be 26 patients.

Randomization

The patients were randomly assigned to one of the two treatment groups: [1] the a-EIMS group or [2] the sham group. We used sealed envelopes for allocation concealment. Before the recruitment phase, the envelopes containing the protocol materials were prepared. Each envelope was sealed, numbered sequentially, and contained an allocated treatment. After a participant agreed to participate in the trial, the next envelope in the sequence was opened, and the results were communicated by the investigator to the clinician administering the intervention.

Blinding

The following steps were taken in this study to control for possible measurement bias: the treatment sessions were administered by the same trained senior acupuncturist physician (with 10 years of experience) to ensure homogeneous treatment. In addition, all participants were asked to avoid interacting or discussing their group assignment with the investigator collecting the outcome data and with other participants. Furthermore, the patients were instructed to only discuss treatment details with the treating physician during the treatment

session. Two independent evaluators blind to the group assignments were trained to administer the pain scales, to assess the cortical excitability parameters and to conduct the psychological tests.

Interventions

Active Electrical Intramuscular Stimulation

This study used acupuncture needles with guide tubes (Suzhou Huanqiu Acupuncture Medical Appliance Co. Ltd., China) that were 40 mm in length and 0.25 mm in diameter. The needling in EIMS was applied using an electroacupuncture device (Sikuro, Sao Paulo, 298 Brazil) in the dermatomes corresponding to the nerve roots involved in the knee (L1, L2, L3, L4, L5, S1, and S2). A paraspinal EIMS using an acupuncture needle was administered maintaining a distance from the spinous process line of 2 cm at L1–L5 and S1–S2 [20]. The anatomic sites of peripheral EIMS were the vastus medialis, rectus femoris, vastus lateralis, and tibialis anterior muscles, as well as the pes anserinus bursae (Figure 2). All patients received one 30-min session with a frequency of 2 Hz.

Sham of Electrical Intramuscular Stimulation

For the sham control condition, we used an electroacupuncture device (Sikuro, Sao Paulo, Brazil), which was adjusted beforehand to prevent the current from passing through the electrodes. The electrical connection between the stimulator and the patient was broken at the output jack plug of the stimulator so that no current could pass to the patient. The electrodes were placed over the same points where active stimulation was applied, while the nerve stimulation unit was left in front of the patient for 30 min. This positioning ensured that the flashing diode that simulated the electrical stimulus was both visible and audible.

Instruments and Assessments

All psychological tests are validated for the Brazilian population. Baseline depressive symptoms were assessed using the Beck Depression Inventory [26], sleep quality was assessed using the Pittsburgh Sleep Quality Index [27], and the catastrophizing of pain was assessed using the Brazilian Portuguese Catastrophizing Scale (BP-PCS) [28].

Outcomes

The primary outcome was the MEP. Secondary outcomes were the SICI, CSP, ICF, and pain measures (PPT, CPM, and VAS). The function of the descendent modulator system was determined by the score on the (NPS) 0/10 during CPM-task.

Parameters of Cortical Excitability

TMS of the left motor cortex (M1) was performed using a MagPro x100 stimulator (MagVenture Company, Lucernemarken, Denmark) through a figure-eight coil (MagVenture Company). It was assessed before the tonic pressure pain (TPP) assessment. Ag–AgCl electrodes were placed over the first dorsal interosseous (FDI) muscle belly and its corresponding tendon on the distal phalanx of the index finger. The responses to stimuli were recorded from the FDI muscle of the right hand by surface electromyography (EMG).

The patients were seated in a comfortable chair and were informed about the TMS procedure, including all of the sensations that might be felt. The amplitudes of the single and paired pulse TMS and the latency of the CSP were measured during the experiment and recorded on an Excel spreadsheet. The data were analyzed offline on a personal computer. The coil was placed tangentially to the scalp over the left M1, at a angle to the sagittal line to identify the motor “hot spot”; the “hot spot” was defined as the coil position over the left M1 in which the lowest motor threshold (MT) intensity was required to elicit an acceptable response in at least

50% of the evoked potentials of the resting FDI. This site was marked with a soft tipped pen to ensure constant placement of the coil throughout the TMS assessments. First, the MT was determined, which was defined as the lowest stimulus intensity sufficient to elicit a response of at least 5 of 10 evoked potentials (at least 50% of successive trials), with a minimum amplitude of 50 mV peak-to-peak in the resting FDI [29,30]. Single-pulse measures including the MEP and CSP were recorded at an intensity of 130% of the MT. The MEP value was the value that evoked potential with an 1 mV peak-to-peak amplitude. The mean of 10 consecutive trials was recorded. For the CSP, the patients were instructed to perform isometric voluntary contractions with approximately 10% of maximal contraction of the FDI. The transient silence in the isometric voluntary EMG activity was elicited in the tonically contracting FDI muscle of approximately 10% of the maximal voluntary contraction, and the CSP was preceded by the MEP [31]. Ten consecutive trials were recorded. The paired-pulse measures included the SICI with interstimulus intervals of 2 ms and the ICF with interstimulus intervals of 12 ms. The first subthreshold stimulus was set at 80% of the individual MT, and the second suprathreshold stimulus was set at 130% of the MT. The intensity of the suprathreshold test stimuli was adjusted to elicit the test stimuli with peak-to-peak amplitude of approximately 1 mV. The reduction of the test MEP elicited by TMS is considered to reflect inhibition at the level of the primary motor cortex [32], and the increase in the test MEP elicited by TMS is considered to reflect facilitation at the level of the primary motor cortex [33]. Thirty recordings (10 for each SICI, ICF, and the test stimulus) were produced in a random order with an interval of approximately 8s between each pulse. The paired-pulse measures were analyzed by calculating their individual index (mean SICI/mean of the test stimulus; mean ICF/mean of the test stimulus) [32,34]. These parameters were assessed before and immediately after treatment.

PPT and Pain during the CPM

PPT: Prior to the test trial, the patient learned to differentiate the perception of pressure vs the perception of the onset of pain. The patient was instructed to verbally report the perception of pain onset. The investigator who assessed the pain threshold levels was trained. An experienced rehabilitation physician systematically evaluated the superficial and deep hyperalgesia by assessing the PPT using an electronic algometer (J Tech 407 Medical Industries, USA). The average values of the PPT in kgf/cm2 (lb/cm2) for three successive readings taken at intervals of 3–5 min were used as the parameter. The PPT in the site of the most sensitive area was taken where the device had a 1-cm2 hard-rubber probe that was applied over the myotomal and sclerotomal structures at L1–L5 and the S1–S2 dermatome at the knee of the leg with the higher pain (knee hyperalgesia). To test the CPM [we use the term CPM rather than diffuse noxious inhibitory control/DNIC because of the recent recommendations by Yarnitsky et al. [35]], we used the protocol of Tousignant-Laflamme et al. [36] and the guidelines for the cold-pressor task (CPM-task) as an experimental pain stimulus [37]. The CPM-task activates the diffuse noxious inhibitory control-like effect because it is a strong nociceptive stimulus that takes place over a lengthy time span [38] and is applied to a large body surface area [39]. The CPM-task allows us to modify the endogenous pain-modulating system. To quantify the CPM, we evaluated the pain intensity of three TPP test stimuli separated by a CPM-task. Although the TPP might lead to habituation and sensitization according to the dual process theory, cold water to zero is a reliable stimulus for inducing CPM [36].

CPM-Task: The cold-pressor task was used as a conditioning stimulus to elicit a strong and prolonged pain sensation to trigger the CPM. The CPM-task consisted of immersing the nondominant hand in cold water (zero to 18C) for 1 min. During the last 30s of the cold-

water immersion, the TPP procedure was administered over the right forearm (dominant forearm). The temperature was held constant during the experiment for each subject. The TPP that elicited pain ratings of 6/10 on the Numerical Rating Pain Scale [(NRS) 0/10] (TPP 60) was used for the first TPP before the CPM-task (TPP 0). After a short break, the TPP 0 was applied at the S1–S2 dermatome at the knee of the leg with greater hyperalgesia. Following the TPP 0, the CPM-task was used to trigger the CPM. One minute after the CPM-task, we applied the second TPP (TPP 1). We quantified the efficiency of the CPM by subtracting the mean pain rating of the TPP 1 from the TPP 0 before the CPM-task (TPP 1), where negative values indicate inhibitory CPM.

Functional Capacity and Pain Intensity

The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) was used to evaluate self-reported knee-specific impairment in patients with knee OA. The WOMAC responses are based on symptoms in the preceding 48 h. The WOMAC assesses the pain, joint stiffness, and perceived disability associated with OA to determine the overall impact on a patients‟ perceived function. It is composed of 24 questions with responses to each given on a Likert scale. The pain subscale has a 5-item Likert scale: none (0), mild (1), moderate (2), severe (3) or extreme (4) problems. This 20-point scale (ranging from 0 to 20) assesses pain in everyday situations (e.g., walking on flat surfaces). The stiffness subscale is a 2-item (ranging from 0 to 8) 10-point scale that assesses perceived knee stiffness after walking and at the end of the day. The disability sub-scale uses 17 items to assess perceived physical function in a variety of everyday situations (e.g., getting into and out of a car). The WOMAC is a valid, reliable, and responsive instrument that is commonly used to assess pain and disability in studies of knee OA. A total WOMAC score is calculated by summing the items for all three subscales, for a total score of between 0 and 96 [40]. Pain intensity was uniformly assessed

using the 100mm VAS, which ranged from no pain (0 mm) to the worst pain possible (100 mm). The pain was assessed before the intervention with the question, "what was the pain level in the knee during the majority of the last 24 h?" and at the end of session by the question, "what is the pain level in the knee at this moment?"

Analgesic use was defined as the self-reported average of analgesics used per week during the last 3 months. For data analysis, analgesic use was included as a dichotomous variable in which the use of analgesics for less than 4 days per week was coded as zero (the reference value) and use of more than 4 days per week was coded as one. This strategy was chosen because subjects with chronic pain typically use rescue analgesics irregularly and their frequency of use changes each week according to their pain level.

Serum BDNF

All of the trials used standard procedures for biological samples, which were reproduced for the healthy volunteers‟ sample and included samples gathered in fasting patients (for at least 8 h) early in the morning. All biological materials were gathered during the trials before applying any intervention. Plastic tubes were centrifuged for 10 min at 5,000 x g at 4C. Serum was frozen at -80C until the assays were performed. Serum neuroplasticity mediator concentrations were determined using BDNF (Chemicon/Millipore, catalog no. CYT306, lower detection limit of the kit=7.8 pg/mL). The personnel involved in sample handling and analysis were unaware of the other trial‟s results.

Statistical Analysis

The mean differences between the groups (a-EIMS and sham) at baseline were assessed using unpaired t-tests, and categorical variables were examined using $\chi 2$ or Fisher‟s exact tests, given that our main factor (intervention) was also categorical. Continuous variables were tested for normality using the Shapiro–Wilk test. To ensure normally distributed data,

we performed a log transformation for BDNF. A multivariate covariance analysis (MANOVA) model was used to explore effects between the interventions groups (a-EIMS and sham) and multiple outcomes [cortical excitability (SICI, ICF, CSP), VAS, the reduction on the NPS (0–10) induced by the CPM-task and the TPP]. We used Bonferroni‟s Multiple Comparison Test to adjust the differences for multiple comparisons. The results were evaluated using the absolute mean variation of pain measurements and cortical excitability parameters were evaluating using delta values. The results were evaluated using the absolute mean variation for MEPs of the percentage of variation [(post-treatment minus pre-treatment)/post-treatment] x100. We also calculated adjusted mean differences, which were defined as the relative changes in the a-EIMS 530 group compared with those of the sham group. This measurement was used to describe the a-EIMS treatment effect, and was calculated as the mean difference divided by the mean sham group outcome, which was further expressed as a percentage (%). The confidence intervals (95% CI) and associated P-values were also calculated. Within groups, the standardized mean difference (SMD) was computed in terms of the ratio between the mean change and the pool of baseline standard deviation (SD). The SMD was interpreted as follows: small, 0.20–0.4; moderate, 0.50–0.70; and large, 0.80 or higher, with respective CI [41]. All of the analyses were performed assuming intention-to-treat with the last observation carried forward and thus included all of the randomized patients for whom there were observations in the study outcomes. We conducted a group analysis by running a mixed ANOVA model in which the dependent variables were the MEP and the score on NPS 0-10 during the CPM task and independent variables were time (before and after), experimental group (a-EIMS and sham), the inter-action between time and experimental group and the subject identification. If appropriate, we then performed Bonferroni‟s test for post hoc multiple comparisons to identify the differences

between the groups at each time point and used a paired t-test to assess the effects of the variables on each experimental group. A Stepwise multiple linear regression analysis was conducted, with the PPT and the MEP as the dependent variables. The independent variables included in this model were the intervention group and the serum BDNF as independent variables. The data were analyzed using SPSS version 22.0 (SPSS, Chicago, IL).

Results

Baseline Characteristics

Twenty-six women participated in this study. The baseline characteristics are shown in Table 1. The clinical and demographic characteristics of the patients according to the randomization groups of receiving one session of a-EIMS or sham are shown in Table 1. Thirteen patients were allocated to the sham group, and 13 were allocated to the a-EIMS group. Twenty-five patients completed the protocol; one patient in the a-EIMS group withdrew due to needle phobia. The characteristics were similar across the a-EIMS and sham groups (all P values > 0.1; Table 1). We did not observe any serious or moderate side effects from the intervention.

Analysis of the Main Outcome: MEP

Patients receiving a-EIMS compared with the sham group demonstrated a mean decrease of the MEP of 31.61%. The effect size assessed by the SDM within the a-EIMS group was 0.56, whereas in the sham group, it was 0.28 (Table 2). A MANOVA analysis revealed a significant relationship between the intervention group (a-EIMS or sham) and the outcomes related to cortical excitability measurements and psychophysical measures (Hotelling‟s Trace=0.97, F [21] =10.17, P < 0.001). The power of this analysis was 96%. The adjusted determination coefficient of this model is R^2=0.43 (i.e., the variables included in the model explain 43% of the variance in the outcomes variables). The results of this adjusted multivariate model are

presented in Table 2. To analyze the changes of the MEP before and after the intervention, we ran a mixed model analysis (Figure 3).

The a-EIMS group significantly reduced the MEP amplitude (P=0.03) compared with the sham group. The effect of time and of the interaction between time and treatment group was significant ($P < 0.05$ for both). The a-EIMS group compared with the sham group demonstrated a mean pain reduction of 31.67%.

Secondary Outcomes: Pain Measures [PPT, Score on VAS and NPS (0-10) During CPM-Task] and Cortical Excitability Parameters (ICF, SICI, and CSP).

The between-group changes in pain, changes on the NPS during the CPM-task, and changes in the ICF and CSP are shown in Table 2. The post hoc analysis indicated significant differences between the a-EIMS group and the sham group in terms of the pain measurements [VAS, PPT, and score on NPS (0-10) during CPM-task]. Also, the a-EIMS group produced a significant effect compared with sham on the ICF and CSP. No significant difference was observed between groups in the SICI. We observed a large effect size within groups considering the change pre- to post-treatment in pain measures, and cortical excitability parameters (Table 2). To analyze the changes on the NPS 0-10 during the CPM-task before and after the intervention, we ran a mixed model analysis (Figure 4). The means (SD) on NPS (0–10) before intervention during the test-stimulus and conditioned-stimulus in a-EIMS was 7.11(1.53) vs 5.14(1.56), and in the sham group was 7.00 (1.73) vs 5.38 (1.23), respectively. Means (SD) are presented in Table 2. The change in NPS (0–10) during CPM-task is presented in Figure 4. After intervention, the a-EIMS group had significantly lower pain scores on the NPS (0–10) (P=0.01) during conditioned-stimulus than the sham group. The effect of time and of the interaction between time and treatment group was significant ($P < 0.05$ for both). The a-EIMS group compared with the sham group demonstrated a mean pain

reduction of 57.18%.

Multivariate Analysis: The Relationship Between the Serum BDNF and the Effect of A-EIMS On Cortical–Spinal System Indexed by MEP and PPT

One important issue is whether the effect of the intervention (a-EIMS or sham) on the outcomes [pain measures (PPT) and cortical spinal excitability system assessed by MEP] is mediated by the BDNF. The regression model demonstrated that the serum BDNF was not correlated with the MEP. It revealed a negative and marginal significant correlation with the PPT (P=0.05). In the analysis of interaction, we observed a statistically significant effect of BDNF on PPT in the sham. Thus, to understand this relationship, we ran a simple regression analysis. The scatter plot of this correlation is presented in Figure 5.The Pearson correlation coefficient was r = -0.56, CI 95% (-0.80 to -0.22); P = 0.003. The determination coefficient of this model was R^2= 0.31 (i.e., the BDNF explained 31% of the variance in the PPT).

Discussion

This study suggests that a single session of a-EIMS temporarily reduced the excitability of the cortical spinal system as assessed by MEP on the primary motor cortex area. Additionally, it demonstrated that the intervention increased the PPT and the function of the descendent pain modulatory system. The a-EIMS reduced the ICF and increased the CSP while the increase in serum BDNF was negatively correlated with the PPT independently of treatment group.

This study extends data demonstrating that the a-EIMS induced changes in the cortical spinal plasticity. Also, it shows a weakened pain inhibitory system as indexed by the cortical excitability parameters (MEP amplitude, ICF, and CSP). These findings provide us with a better comprehension of the possible mechanisms involved in the imbalance between excitatory and inhibitory systems, which leads to sustained chronic pain in OA. The lack of

intracortical inhibition is a result of the dysfunction of the descendent pain modulatory system. This finding is supported by compelling evidence that spinal input to the periaqueductal gray elicits pain inhibition through a spinal–supraspinal–spinal loop, whose functionality can be assessed by the pain threshold and the CPM [42]. Thus, this dysfunction on pain processing gives us some insights to understand why pain and disability persists in 15–20% of patients after total knee replacement surgery [43, 44].

The dissection of these mechanisms involved in chronic pain associated with OA might allow the development of an individualized and more effective therapy based on the diagnosis of dysfunction in the pain network system. In fact, this electrophysiological evidence contributes to advances in therapeutic approaches, which, in general, limit to provide general pain relief despite changing the maladaptive neuroplasticity.

Furthermore, it was also observed that the a-EIMS reduced the cortical excitability as demonstrated by a reduction in the ICF and an increase in the CSP. Several neurobiological processes may explain these findings, including the fact that the ICF originates from excitatory postsynaptic potentials mainly mediated by glutamatergic NMDA receptors [12, 45]. Their latency is approximately 10 ms, consistent with the time course of ICF. Pharmacological studies support such an observation, as NMDA receptor antagonists (i.e., dextromethorphan) decrease ICF. Conversely, GABA-B agonists (e.g., Baclofen) increase ICF [46]. Also, the ICF is not exclusively mediated by excitatory interneurons, but rather by a balance between inhibition and excitation [12]. There is also evidence that the mechanism underlying ICF depends on both N-methyl-D-aspartic acid and non-N-methyl-D-aspartic acid receptors and that these receptors can serve as global markers of glutamatergic receptor function [47].

The increase of CSP suggests an increased excitability of the inhibitory interneurons, while the reduction of MEPs and shortening of the ICF probably reflect decreased activity of

NMDA and GABA-A receptors [48]. In spite of this, the underlying mechanisms are unclear. The a-EIMS may modulate intracortical excitability as well as the transmission efficiency of cortico-spinal neurons, resulting in reduced both amplitude of MEP and ICF facilitation. These effects on MEP amplitude are consistent with previous studies in humans [49,50]. Finally, the CSP is the duration of the cortical inhibition, which is associated with the intracellular inhibitory postsynaptic potential from the direct stimulation of the GABA-A recepto [51]. According to a previous study, a diazepam injection induced a transient reduction on facilitatory thalamocortical inhibitory interneurons of the motor cortex [52], thereby, this effect is mediated by GABA-A inhibition. Hence, the changes in the balance between cortical inhibitory and facilitatory processes observed in this study can be explained by impairments in neurotransmission mediated by GABA-B and NMDA receptors [53].

The second potential hypothesis is that cortical reorganization in the motor cortex in chronic pain is mediated by circuits involved in pain processing. If this assumption is true, then cortical excitability responses induced by a-EIMS would represent changes in pain-related neural circuits. In fact, the motor cortex excitability modulation with repeated TMS leads to pain reduction, which is associated with the restoration of intracortical 733 inhibition [25,51]. Alternatively, a-EIMS augments the preceding processing centers of peripheral modulation, such as thalamic structures, as well as the excitability of cortical spinal pathways as shown by a reduction in the amplitude of MEP [25]. This potential insight supports the idea to use the a-EIMS combined with central modulatory approaches, such as tDCS with visual illusion [52], tDCS or TMS.

The a-EIMS increases the PPT; an effect mediated through myelinated A–d fibers and unmyelinated C-fibers. According to Baldry, the A-d nerve fibers are stimulated for as long as 72 h after needle insertion and could improve the clinical effects by stimulating afferent A-d

fibers, with the subsequent activation of enkephalinergic, serotonergic, and noradrenergic inhibitory systems [53]. In spite of this, in this study, the effect on the neuroplasticity process was assessed for a short period. Thereby, it is not possible to affirm whether this effect persists long term as occurs with electrical stimulation of the Na1/K1 pumps induced by electroacupuncture [54]. Conversely, the a-EIMS might be accelerating the synthesis and release of serotonin (5-HT) and norepinephrine in the CNS [55]. This may explain the effectiveness of a-EIMS at improving the function of the deficient pain modulatory pathway. In fact, it is hard to restrict the a-EIMS to a selective channel as its bottom-up effect on sensory processing occurs in some circuits located far from the electrical field created by the treatment.

Higher levels of serum BDNF were marginally correlated with lower PPT (Table 3). Despite the interaction between BDNF and sham group had been significant; this effect is possibly independent of the intervention group. We hypothesize that a type II error explains these marginal differences. Even though the present finding is congruent with a study in females with fibromyalgia [13], we must be careful in the interpretation of this relationship because the BDNF is widely distributed in the CNS. The BDNF enhances a C-fiber-evoked response, which activates signaling pathways in the spinothalamic tract and subsequently strengthens excitatory synapses. This process induces the accumulation of the Cl- in the neurons of the dorsal horn, which reduces the GABAergic inhibitory effect and it promotes the disinhibition [56]. Accordingly, the attenuation of pain behaviors has been associated with a decrease in BDNF in the spinal cord microglia and in neurons [56–58]. The inhibitory effects caused by the CPM-task involve specific supraspinal and spinal [59] components with a major top-down effect that lasts for a few minutes [35, 60]. The a-EIMS inhibitory effects persisted for over 1h after treatment. Although our follow-up period was short, this finding enforces the

hypothesis that a weakening of inhibitory processes within the descending motor system can occur in OA. This result suggests that the increase in analgesia is more related to a-EIMS than to the CPM-task. Additionally, the effect on ICF, MEP, PPT, and the CPM-task suggests that the a-EIMS analgesia might be mediated by cortical (decrease MEP amplitude and less ICF) and infracortical mechanisms. This effect induces local hyperpolarization of the axon and the electrical stimulation of Na+/K+ pumps, which activate C-fibers and A-beta fibers and alters pain transmission at the spinal cord, midbrain, and hypothalamic levels [54, 55]. Also, the tissue trauma caused by dry needling will liberate adenosine triphosphate (ATP) and adenosine, each inducing inhibitory and excitatory effects on unmyelinated human C-fibers by activating purinergic receptors [54,61].

Some issues concerning the design of our study must be addressed. Although the effect of one neuromodulatory therapy session was evaluated, it is important to notice that our objectives were to understand the acute effect of a single session of a-EIMS in the neuroplasticity process in pain pathways. Additionally, we aimed to better understand the relationship between the BDNF and the excitatory/inhibitory balance system. Thus, these findings might support the planning of new approaches to treating chronic OA pain, such as the use of combined techniques to induce top-down and bottom-up modulatory effects. A possible advantage of assessing only one session of a-EIMS is the maintenance of blinding. Even though we included only patients without prior experience with acupuncture, as the a-EIMS produces a sensory perception, a full blinding of subjects is less likely. Given we did not formally measure the awareness of the allocation group (either active or sham); this is a limitation that needs to be considered. Despite this limitation, our outcomes were neurophysiological and psychophysical parameters. These measurements are less prone to bias and thus will less likely impact our conclusions. While the present findings are important

in understanding the possible neurobiological mechanisms of peripheral neuromodulatory techniques on cortical spinal pain modulation, they do not support therapeutic decision making in clinical settings.

These findings demonstrated that a-EIMS reduced the excitability of the cortical spinal system as assessed by MEP and ICF in OA, and it increased the potency of the descendent pain modulatory system and the PPT. These results suggest that a-EIMS enhanced the bottom-up regulation mechanisms in the cortical and infracortical inhibitory systems during pain processing. The BDNF was negatively correlated with pain threshold, but its effect is independent of the treatment group. Overall, this research may lead to novel therapeutic avenues for pain management in OA.

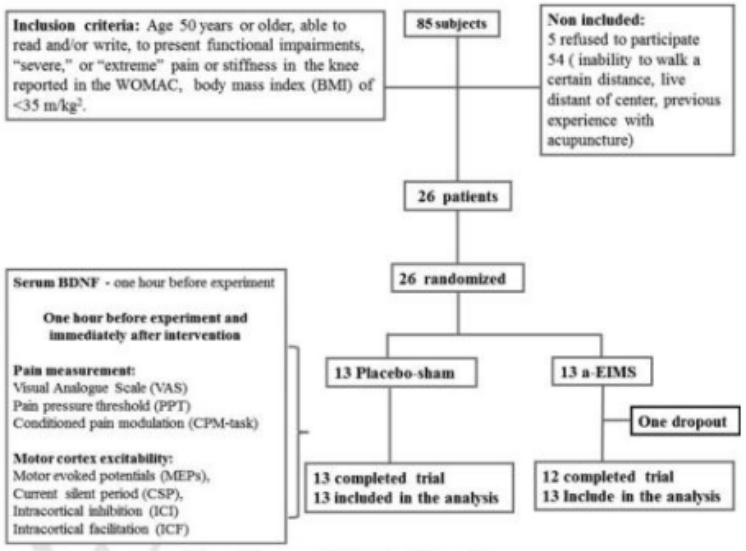

Figure 1 Flow chart of the study eligibility and the number of patients at each point. The two experimental groups were as follows: deep intramuscular stimulation (a-EIMS); sham.

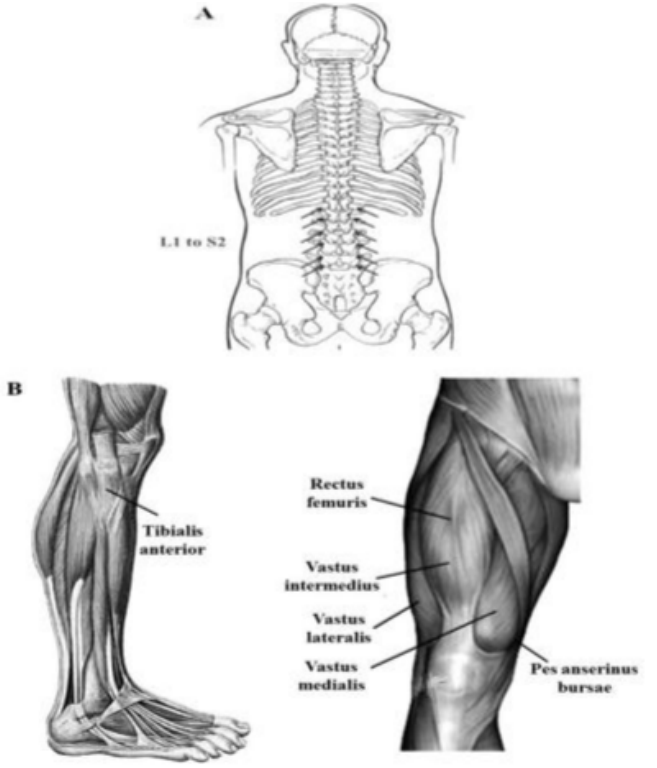

Figure 2 (**A**) Paraspinal intramuscular stimulation using acupuncture needles. Distance from the spinous process line is 2 cm at L2–L5 and S2. **(B)** The anatomic sites for peripheral EIMS were the vastus medialis muscle; the rectus femoris muscle; the vastus lateralis muscle; the tibialis anterior muscle; and the pes anserinus bursae muscle. For the sham group, the electrodes were placed in the same anatomic sites.

Table 1 Demographic and clinical characteristics of the study sample

Variable	a-EIMS (n=13) Mean (SD)		Sham (n=13) Mean (SD)		*P*
Age (years)	62.15	(7.44)	66.85	(7.53)	0.29
Body mass index (kg/m^2)	29.16	(6.65)	27.47	(4.20)	0.45
Employed (yes/no)	5 (38.5)/8 (61.5)		1 (7.7)/12 (92.8)		0.08
Time that report pain in the most part of days (years)	6.67 (1.59)		6.49 (1.48)		0.70
Time since diagnosis					
Between 1 and 2 years ago	1		0		
Between 2 and 5 years ago	1		4		
More than 5 years ago	11		9		
Time of pain that need to take pain medication weekly (years)†	6.69 (0.48)		6.90 (0.30)		0.20
Psychotropic medication yes/no (%)‡	4 (33.3)/8 (66.7)		5 (38.5)/8 (61.5)		0.56
Smoking (yes/no)	0 (0)/13 (100)		1(7.7)/12 (92.3)		0.5
Alcohol (yes/no)	2 (15.4)/11 (84.6)		5 (38.5)/8 (61.5)		0.2
Chronic disease (yes/no)	3 (23.1)/10 (73.9)		4 (30.8)/9 (69.2)		0.5
Diabetic (yes/no)	3/10		1/12		
Hypothyroidism (yes/no)	3/10		1/12		
Hypertension (yes/no)	7/6		7/6		
WOMAC	54.92	18.05	52.46	(11.56)	0.40
VAS score of the Pain in the last 24 h	6.85	(0.38)	6.77	(0.43)	0.63
Beck depression inventory	13.67	(8.12)	12.60	(8.37)	0.75
Brazilian Portuguese pain catastrophizing	24.76	11.85	19.46	(10.01)	0.23
Pittsburgh sleep quality index score	41.69	(15.46)	44.07	(16.72)	0.70
BDNF	19.4	(6.79)	18.11	(4.96)	0.54

Values are given as the mean (SD) or frequency (n = 26).

Table 2 Percentual change mean [(post-treatment values minus pretreatment values)/post-treatment values) × by 100] [standard deviation (SD)] on cortical excitability parameters excitability parameters (MEP, ICF, SICI, CSP), PPT and change in NPS (0–10) during the CPM-task (n = 26)

Dependent Variables	Type III Sum of Squares	df	*F*	*P**	*Partial eta squared*
Motor evocate Potential (MEP) mV	6,495.12	1	4.96	0.03	0.17
Pain pressure threshold (kgf/cm[2])	5,120.21	1	7.71	0.01	0.24
Pain score on VAS†	30,132.00	1	15.24	0.001	0.39
Change in NPS (0–10) during the CPM-task	21,251.70	1	6.31	0.01	0.20
Intracortical facilitation	9,053.41	1	5.68	0.02	0.19
Intracortical inhibition	541.85	1	0.18	0.66	0.008
Cortical silent period (CSP) (ms)	3,392.39	1	15.59	0.001	0.34

Primary outcomes					
Motor evocate potential (MEP) mV					
Sham (n=13)		a-EIMS (n=13)			
Mean (SD) (pre vs post)	Change mean (%)†	Mean (SD) (pre vs post)	Change mean (%)†	Difference on mean change (CI 95%)	*P**
1.10 (0.68) vs 1.23 (0.77)	8.77	1.31 (0.88) vs 1.01 (0.67)	−22.90	31.67 (2.34 to 60.89)	0.03*
Secondary outcomes					
Pain pressure threshold (kgf/cm^2)					
6.05 (1.58) vs 6.40 (1.46)	0.69	5.93 (1.64) vs 8.40 (2.40)	28.76	28.07 (7.22 to 49.92)	0.01*
Pain score on VAS†					
4.92 (1.93) vs 4.32 (1.23)	−16.18	5.29 (1.91) vs 3.11 (1.54)	−84.26	−68.08 (−104 to −31.45)	0.001*
Change in NPS (0–10) during the CPM-task‡					
7.92 (1.65) vs 6.73 (1.71)	−21.75	7.53 (1.39) vs 4.84 (1.99)	−78.92	−57.18 (−104.14 to −10.21)	0.01*
Intracortical facilitation					
0.95 (0.26) vs 1.02 (0.26)	2.42	0.82 (0.35) vs 0.64 (0.22)	- 34.0	−37.32 (−69.93 to - 5.00)	0.02*
Short intracortical inhibition					
0.65 (0.41) vs 0.70 (0.47)	−6.42	0.47(0.28) vs 0.58 (0.29)	2.70	−9.10 (−52.51 to 34.12)	0.66
Cortical silent period (CSP) (ms)					
59.27 (12.70) vs 57.01 (13.41)	−6.10	51.34 (17.13) vs 61.65 (19.66)	16.75	22.85 (10.90 to 34.79)	0.001*

PPT, pressure pain threshold in the knee area (L1–L5 and the S1–S2 dermatome); Short intracortical facilitation (amplitude/MEP amplitude ratio = ICF); short intracortical inhinibition (amplitude/MEP amplitude ratio = SICI). The cortical silent period (CSP) expressed in milliseconds (ms). Change in NPS (0-10) during the CPM-TASK [(NPS10-10 s CPM) minus (NPS00-10 first CPM)]; thus, more negative values indicate higher functioning of the descending modulatory system. CI, confidence interval. Linear regression model – Adjusted $R^2 = 0.43$.

* MANCOVA. Mean difference of group. Significant differences among treatment groups according to the Bonferroni test.

† Percent change = [(post-treatment values minus pretreatment values/post-treatment values) × by 100.

‡ Means in NPS (0–10) obtained after intervention.

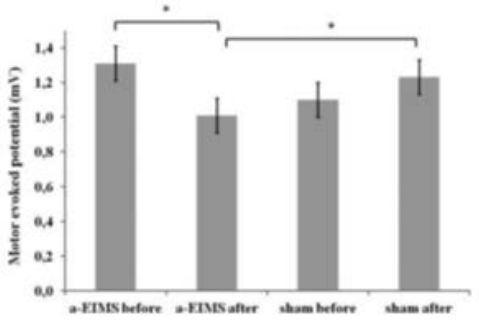

Figure 3 Mean of MEP (mV) at 1 h before intervention and immediately after in the two experimental groups. The error bars indicate standard error of the mean. Asterisk (*) indicates differences between the sham and a-EIMS groups. All comparisons were performed by a mixed analysis of variance model, followed by the Bonferroni test for post hoc multiple comparisons.

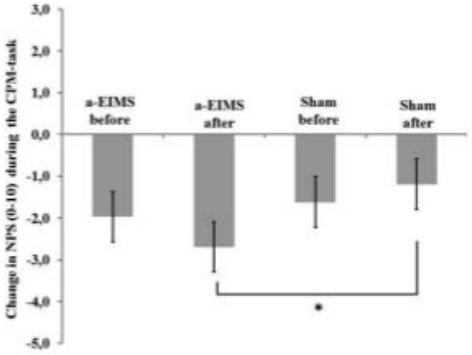

Figure 4 The change in NPS (0–10) during CPM-task, at 1 h before intervention and immediately after in the two experimental groups. The error bars indicate standard error of the mean. Asterisk (*) indicates differences between the sham and a-EIMS groups. All comparisons were performed by a mixed analysis of variance model, followed by the Bonferroni test for post hoc multiple comparisons. Numerical Pain Scale (NPS0-10).

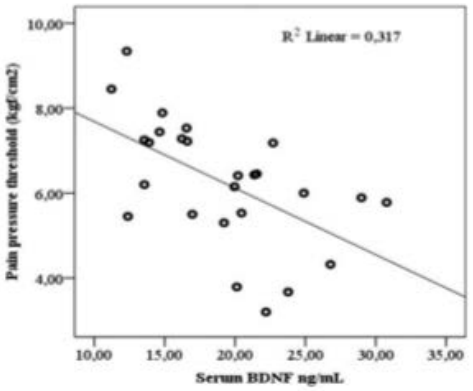

Figure 5 Scatter plot of serum BDNF (ng/mL) and PPT.

Table 3 Multivariate linear regression of the interaction between motor evocate potential and pain pressure threshold compared with treatment group considering the serum BDNF ($n = 26$)

Parameter	B	T	*P*	95% (CI)
Dependent variable: motor evocate potential (MEP) mV				
a-EIMS vs. sham[a]	−35.89	−2.48	0.02*	−65.81 to −5.98
Serum BDNF	21.09	1.23	0.23	−14.42 to 56.61
Interaction				
Serum BDNF vs. a-EIMS	15.10	0.88	0.38	−20.10 to 50.30
Serum BDNF vs. sham	26.99	1.47	0.15	−10.89 to 64.89
Dependent variable: pain pressure threshold (kgf/cm²)				
a-EIMS vs. sham[a]	32.87	3.36	0.003*	12.62 to 53.13
Serum BDNF	−23.68	−2.08	0.05	−47.71 to 0.36
Interaction				
Serum BDNF vs a-EIMS	−18.79	−1.74	0.09	−41.20 to 3.61
Serum BDNF vs Sham	−31.37	−2.69	0.01*	−55.49 to −7.52

Pressure pain threshold in the knee area (L1–L5 and the S1–S2 dermatome).
BDNF, brain-derived neurotrophic factor; CI, confidence interval.
* $P < 0.05$.
[a]Reference category is the sham group; hence, the *B* value is the difference in the mean of a-EIMS minus sham.

References

1 Doherty MJ. Pain in osteoarthritis. In: Giamberardino MA, ed. Pain 2002: An Updated Review: Refresher 845 Course Syllabus IASP Press; 2002: 51–7.

2 Arendt-Nielsen L, Nie H, Laursen MB, et al. Sensitization in patients with painful knee osteoarthritis. Pain 2010;149:573–81.

3 Kosek E, Ordeberg G. Abnormalities of somatosensory perception in patients with painful

osteoarthritis normalize following successful treatment. Eur J Pain 2000;4: 229–38.

4 Gwilym SE, Keltner JR, Warnaby CE, et al. Psycho-physical and functional imaging evidence supporting the presence of central sensitization in a cohort of osteoarthritis patients. Arthritis Rheum 2009; 61: 857 1226–34.

5 Gwilym SE, Filippini N, Douaud G, Carr AJ, Tracey I. Thalamic atrophy associated with painful osteoarthritis of the hip is reversible after arthroplasty: a longitudinal voxel-based morphometric study. Arthritis Rheum 2010;62:2930–40.

6 Deitos A, Dussan-Sarria JA, de Souza A, et al. Clinical value of serum neuroplasticity mediators in identifying the central sensitivity syndrome in patients with chronic pain with and without structural pathology. Clin J Pain 2014;30.

7 Liepert J, Hallett M, Samii A, et al. Motor cortex excitability in patients with cerebellar degeneration. Clin Neurophysiol 2000; 111:1157–64.

8 Malcolm M, Triggs W, Light K, et al. Reliability of motor cortex transcranial magnetic stimulation in four muscle representations. Clin Neurophysiol 2006; 117:1037–46.

9 McKay D, Ridding M, Thompson P, Miles T. Induction of persistent changes in the organization of the human motor cortex. Exp Brain Res 2003; 143: 879 342–9.

10 Ridding M, McKay D, Thompson P, Miles T. Changes in corticomotor representations induced by prolonged peripheral nerve stimulation in humans. Clin. Neurophysiol 2001; 112:1461–9.

11 Marconi B, Filippi G, Koch G, et al. Long-term effects on motor cortical excitability induced by repeated muscle vibration during contraction in healthy subjects. J Neurol Sci 2008; 275:51–9.

12 Vidor LP, Torres IL, Medeiros LF, et al. Association of anxiety with intracortical inhibition and descending pain modulation in chronic myofascial pain syndrome. BMC

Neurosci 2014;19:42.

13 Zanette SA, Dussan-Sarria JA, Souza A, et al. Higher serum S100B and BDNF levels are correlated with a lower pressure-pain threshold in fibromyalgia. Mol Pain 2014;10:46.

14 Mamounas LA, Blue ME, Siuciak JA, Altar CA. Brain-derived neurotrophic factor promotes the survival and sprouting of serotonergic axons in rat brain. J Neurosci 1995; 15:7929–39.

15 Sommer C, Kress M. Recent findings on how proinflammatory cytokines cause pain: pheripheral mechanisms in inflammatory and neuropathic hyperalgesia. Neurosci Lett 2004; 361:184–7.

16 Kawasaki Y, Zhang L, Cheng JK, Ji RR. Cytokine mechanisms of central sensitization: distinct and overlapping role of interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha in regulating synaptic and neuronal activity in the superficial spinal cord. J Neurosci 2008; 28:5189–94.

17 Sheng WS, Hu S, Kravitz FH, Peterson PK, Chao CC. Tumor necrosis factor alpha upregulates human microglial cell production of interleukin-10 in vitro. Clin Diagn Lab Immunol 1995; 2:604–8.

18 Lijun B, Richard EH, Jian K, et al. Neurobiological mechanisms of acupuncture 2014. Evid Based Complement Alternat Med 2014; 765068.

19 Han JS. Acupuncture analgesia: areas of consensus and controversy. Pain 2011; 152:S41–8.

20 Couto C, de Souza IC, Torres IL, Fregni F, Caumo W. Paraspinal stimulation combined with trigger point needling and needle rotation in treating myofascial pain: randomized sham-controlled clinical trial. Clin J Pain 2014; 30: 214–23.

21 Nunes G, de Castro LV, Wageck B, et al. Translation into Portuguese of questionnaires to assess knee injuries. Acta Ortop Bras 2013; 21: 288–94.

22 Ravaud P, Giraudeau B, Auleley GR, et al. Radiographic assessment of knee osteoarthritis: reproducibility and sensitivity to change. J Rheumatol 1996; 929 23:1756–64.

23 Gunther KP, Scharf HP, Puhl W, et al. Reproducibility of radiologic diagnosis in gonarthrosis. Z Orthop Ihre Grenzgeb 1997; 135:197–202.

24 Ravaud P, Auleley GR, Amor B. Radiographic assessment of progression in knee osteoarthritis. Rheumatol Eur 1995; 24:129–31.

25 Dall‟Agnol L, Medeiros LF, Torres IL, et al. Repetitive transcranial magnetic stimulation increases the corticospinal inhibition and the brain-derived neurotrophic factor in chronic myofascial pain syndrome: an explanatory double-blinded, randomized, sham-controlled trial. J Pain 2014;15: 942 845–55.

26 Warmenhoven F, van Rijswijk E, Engels Y, et al. The Beck Depression Inventory (BDI-II) and a single screening question as screening tools for depressive disorder in Dutch advanced cancer patients. Support Care Cancer 2012;20:319–24.

27 Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res 1989;28:193–213.

28 Sehn F, Chachamovich E, Vidor LP, et al. Cross-cultural adaptation and validation of the Brazilian Portuguese version of the pain catastrophizing scale. Pain Med 2012;13:1425–35.

29 Rossini PM, Narici L, Martino G, et al. Analysis of interhemispheric asymmetries of somatosensory evoked magnetic fields to right and left median nerve stimulation. Electroencephalogr Clin Neurophysiol 1994;91:476–82.

30 Rothwell JC, Hallett M, Berardelli A, et al. Magnetic stimulation: motor evoked potentials. The International Federation of Clinical Neurophysiology. Electroencephalogr Clin Neurophysiol Suppl 1999; 52:97–103.

31 Fuhr P, Agostino R,MH. Spinal motor neuron excitability during the silent period after cortical stimulation. Electroencephalogr Clin Neurophysiol 1991;81: 257–62.

32 Kujirai T, Caramia MD, Rothwell JC, et al. Cortico-cortical inhibition in human motor cortex. J Physiol 1993Nov;501–19.

33 Tokimura H, Ridding MC, Tokimura Y, Amassian VE, Rothwell JC. Short latency facilitation between pairs of threshold magnetic stimuli applied to human motor cortex. Electroencephalogr Clin Neurophysiol 1996; 101:263–72.

34 Sanger TD, Garg RR, Chen R. Interactions between two different inhibitory systems in the human motor cortex. J Physiol 2001; 530:307–17.

35 Yarnitsky D, Arendt-Nielsen L, Bouhassira D, et al. Recommendations on terminology and practice of psychophysical DNIC testing. Eur J Pain 2010; 14:339.

36 Tousignant-Laflamme Y, Page S, Goffaux P, Marchand S. An experimental model to measure excitatory and inhibitory pain mechanisms in humans. Brain Res 2008; 1230:73–9.

37 von Baeyer CL, Piira T, Chambers CT, Trapanotto M, Zeltzer LK. Guidelines for the cold pressor task as an experimental pain stimulus for use with children. J Pain 2005; 6:218–27.

38 Willer JC, De Broucker T, Le Bars D. Encoding of nociceptive thermal stimuli by diffuse noxious inhibitory controls in humans. J Neurophysiol 1989; 62: 1028–38.

39 Marchand S, Arsenault P. Spatial summation for pain perception: interaction of inhibitory and excitatory mechanisms. Pain 2002;95:201–6.

40 Berrada K, Wariaghli G, Idrissi Z, et al. FRIO133- normative values for the Western Ontario and MCMaster universities in the general population compared with knee osteoarthritis patients. Ann Rheum Dis 2011;70:88.

41 Diggle P, Heagerty P, Liang K-Y, Zeger S. Analysis of Longitudinal Data. Clarendon Press; 1994.

42 Staud R. Abnormal endogenous pain modulation is a shared characteristic of many chronic pain conditions. Expert Rev Neurother 2012;12:577–85.

43 Becker R, Doring C, Denecke A, Brosz M. Expectation, satisfaction and clinical outcome of patients after total knee arthroplasty. Knee Surg Sports Traumatol Arthrosc 2011;19:1433–41.

44 Beswick AD, Wylde V, Gooberman-Hill R, Blom A, Dieppe P. What proportion of patients report long-term pain after total hip or knee replacement for osteoarthritis? A systematic review of prospective studies in unselected patients. BMJ Open 2012;2:1017 e000435.

45 Nakamura H, Kitagawa H, Kawaguchi Y, Tsuji H.Intracortical facilitation and inhibition after transcranial magnetic stimulation in conscious humans. J Physiol 1997;498: 817–23.

46 Ziemann U, Lonnecker S, Steinhoff BJ, Paulus W. Effects of antiepileptic drugs on motor cortex excitability in humans: a transcranial magnetic stimulation study. Ann Neurol 1996; 40: 367–78.

47 Volz MS, Medeiros LF, Tarrago M da G, et al. The relationship between cortical excitability and pain catastrophizing in myofascial pain. J Pain 2013; 14:1140–7.

48 Fedi M, Berkovic SF, Macdonell RA, et al. Intracortical hyperexcitability in humans with a GABAA receptor mutation. Cereb Cortex 2008; 18:664–9.

49 Lo YL, Cui SL. Acupuncture and the modulation of cortical excitability. Neuroreport 2003;14: 1229–31.

50 Maioli C, Falciati L, Marangon M, Perini S, Losio A. Short- and long-term modulation of upper limb motor-evoked potentials induced by acupuncture. Eur J Neurosci 2006; 23:1931–38.

51 Knijnik LM, Dussan-Sarria JA, Rozisky JR, et al. Repetitive transcranial magnetic

stimulation for fibromyalgia: systematic review and meta-analysis. Pain Practice 2015 (in press).

52 Kumru H, Soler D, Vidal J, et al. The effects of transcranial direct current stimulation with visual illusion in neuropathic pain due to spinal cord injury: an evoked potentials and quantitative thermal testing study. Eur J Pain 2013; 17: 55–66.

53 Baldry P. Management of myofascial trigger point pain. Acupunct Med 2002; 20:2–10.

54 Hong CZ, Chen YC, Cindy P, Yu J. Immediate effects of various physical medicine modalities on pain threshold of an active myofascial trigger point. J Musculoskel Pain 1993; 1:37–53.

55 Le Bars D, Dickenson AH, Besson JM. Diffuse noxious inhibitory controls (DNIC). II. Lack of effect on non-convergent neurones, supraspinal involvement and theoretical implications. Pain 1979;6:305–27.

56 Spezia Adachi LN, Quevedo AS, de Souza A, et al. Exogenously induced brain activation regulates neuronal activity by top-down modulation: conceptualized model for electrical brain stimulation. Exp Brain Res 2015; 233:1377–89.

57 Coull JA, Beggs S, Boudreau D, et al. BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. Nature 2005;438: 1017–21.

58 Yajima Y, Narita M, Usui A, et al. Direct evidence for the involvement of brain-derived neurotrophic factor in the development of a neuropathic pain-like state in mice. J Neurochem 2005; 93:584–94.

59 Nahman-Averbuch H, Martucci KT, Granovsky Y, et al. Distinct brain mechanisms support spatial vs temporal filtering of nociceptive information. Pain 2014;155:2491–501.

60 Moont R, Crispel Y, Lev R, Pud D, Yarnitsky D.Temporal changes in cortical activation during conditioned pain modulation (CPM), a LORETA study. Pain 2011;152: 1469–1477.

61 Irnich D, Beyer A. Neurobiological mechanisms of acupuncture analgesia. Schmerz 2002; 16:93–102.

9 Terceiro artigo – Submetido à Brain Stimulation

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Transcranial Direct Current Stimulation Potentiates the effects of Electrical Intramuscular Stimulation at Relieving Pain and Improving the Descending Pain Modulatory System in Patients with Knee Osteoarthritis: A Randomized, Double-Blinded, Factorial Design and Sham-Controlled Study

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RUNNING TITLE: tDCS potentiates the IMS effect on pain.

DECLARATION OF CONFLICT OF INTEREST:

The authors declare that there are no relationships, financial or otherwise, 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 speaker's bureau or any other form of financial compensation.*

ABSTRACT (maximum 250 words)

Background: The transcranial direct current stimulation (tDCS) and the electrical intramuscular stimulation (IMS), both have been effective to improve pain.

Objective: To test the hypothesis that the active(a)-tDCS/ active-(a)-IMS compared to the control condition-sham(s)-tDCS/ sham(s)-IMS and the a-tDCS/s-IMS or s-tDCS/a-IMS would be better at improving pain measures and the descending pain modulatory system (DPMS). Also, we assessed whether the brain-derivate-neural-factor (BDNF) could predict the effect of therapy.

Methods: Sixty women with knee osteoarthritis (OA), 50 to 75 years old, were randomized to receive five sessions of one of these four interventions (a-tDCS/a-IMS; s-tDCS/s-IMS; a-tDCS/s-IMS; s-tDCS/a-IMS) applied over the M1. We assessed the pain score in the Visual Analogue Scale (VAS), the pressure pain threshold (PPT), the function of the DPMS and severity of disability from OA.

Results: A GEE model revealed a main effect of interventions on the VAS score ($P<0.03$). The cumulative mean (SD) on the VAS at the end of treatment was 0.46(0.04) in the a-tDCS/a-IMS group, and 1.79(2.53) and 1.81(2.98) in the a-tDCS/s-IMS and s-tDCS/a-IMS groups, respectively. Whereas in the s-tDCS/s-IMS this value was 2.98(1.17). The relative risk to use analgesia was 75% greater in patients receiving s-tDCS/s-IMS compared to a-tDCS/a-IMS, also the a-tDCS/a-IMS showed a greater effect in the DPMS. The BDNF effect in the PPT was independent of the intervention.

Conclusion: The patients receiving a-tDCS/a-IMS produced a greater and sustained improvement on pain measures as well as in the DPMS function, whereas the relationship of BDNF with the PPT was independent of the intervention group.

Keywords: osteoarthritis, intramuscular stimulation, pain pressure threshold, conditioned pain modulation (CPM), transcranial direct current stimulation.

***Registration in Clinical trials.gov**: NCT01747070.*

Highlights:

The combined effect (a-tDCS/a-IMS) produced a greater effect in the pain measures and PPT in KOA.

The combined treatment (a-tDCS/a-IMS) showed an additive effect to improve the DPMS function.

The relationship between the BDNF and the PPT treatment was independent of interventions effect.

INTRODUCTION

The disability associated with osteoarthritis (OA) is estimated to occur in 85% of those over 65 years old [1, 2] and it is the most common cause of disability in older women [2]. The persistent inflammatory processes [1] lead to segmental [3] and central sensitization (CS) [4] . The CS is characterized by pain threshold reduction, hyperalgesia inside and outside the sensitized area, expansion of the receptive field, as well as diffuse pain [5]. These symptoms and the severity of referred pain in the KOA are associated with the number of trigger points (TPs) [6]. These findings, together with changes in the neurophysiological measures of the primary motor cortex observed in chronic pain [i.e. larger motor evoked potential (MEP) and shorter current silent period (CSP)] indicates that it induces a cortical and spinal hyperexcitability [7]. These findings also point to an imbalance between excitatory/inhibitory systems within the central nervous system (CNS) [8]. Therefore, it makes sense to explore the potential benefits of therapeutic approaches to reduce the excitability of the corticospinal system, such as the peripheral and central modulatory techniques.

The electrical intramuscular stimulation (IMS) is a peripheral technique that may reduce the intracortical facilitation (ICF) and increase the CSP [9]. This effect showed that the IMS improves the inhibitory function by a bottom-up mechanism, and its effect on pain and disability has been demonstrated in different chronic pain conditions, such as myofascial pain syndrome [10] and chronic tensional headache [11]. Whereas, transcranial direct current stimulation (tDCS) applied on the primary motor cortex (M1) can modulate the descending pain control systems in a top-down manner. It has been effective at treating several chronic pain syndromes (i.e. fibromyalgia; phantom pain; chronic migraine; etc.) [12-14], trigeminal neuralgia [15] and low back pain [16]. Besides the effects of IMS and tDCS on clinical complaints, we need to know if their combined effect can improve the outcomes related to pain, disability, and the descending pain modulatory system. In addition, we need to improve our comprehension of the neuroplasticity mechanism that induces, maintains and sustains CS in OA. The brain-derived neurotrophic factor (BDNF) has a leading role

in this process of CS, because it increases the strength of the glutamatergic synapses, while it weakens GABAergic synapses, and it promotes a disinhibition state in the pain pathways [17]. The serum BDNF has been negatively correlated with both the SICI (short intracortical inhibition) [18] and with the pain threshold [19]. Additionally, higher serum BDNF has been associated with a lower function in the descending pain modulatory system [7]. Considering these pools of results, it is plausible to hypothesize that we could optimize their analgesic effects if we combine therapies (tDCS /IMS) that could induce a modulation in two ways, top-down and bottom-up manner.

Therefore, in this explanatory trial, we tested the hypothesis that the active(a)-tDCS/active-(a)-IMS compared to the control condition(s)-tDCS/sham(s)-IMS and the a-tDCS/s-IMS or s-tDCS/a-IMS would be better at improving pain scores on the Visual Analogue Scale (VAS) and the descending pain modulatory system function supported by changes on the Numerical Pain Scale (NPS 0-10) during the conditioned pain modulation (CPM)-task (primary outcomes). We also tested whether is the a-tDCS/a-IMS would be better than other groups at reducing analgesic use, to improve Pain Pressure Threshold (PPT) and the functional capacity assessed by the Western Ontario and McMaster Universities Index (WOMAC) (secondary outcomes). Also, we assessed whether the brain-derivate-neural-factor (BDNF) could predict the effect of therapy.

PATIENTS AND METHODS

Design Overview, Setting, and Participants

The Research Ethics Committee at the Hospital de Clínicas de Porto Alegre (HCPA) (Institutional Review Board IRB- number 11-0013) approved the protocol of this study following the Declaration of Helsinki. All patients provided their formal consent before participating in this randomized, double-blind, factorial design, four-groups parallel, clinical trial. Our patients were recruited from the general population through the referrals from physicians of Chronic Pain and from

Physiatrists of the Physiatry Service, as well as by public posters in different hospital services and mass dissemination newspapers. Our sample consisted of 60 women, 50 -75 years old, right handed, with moderate to severe pain and stiffness of the knee, and functional impairment without significant relief with medication. The symptoms must be present for at least six months. At baseline, a physiatrist with over ten years of experience in treating OA evaluated the radiographs of knees and the degree of OA by the Kellgren-Lawrence (K-L) grading scale [20]. This scale is already validated and has high reproducibility in the grading of knee osteoarthritis (KOA) [21]. Graduates in 3-4 K-L have been included. The interview at baseline consisted of the WOMAC Index, which is a validated and widely used instrument to assess pain, stiffness and functional limitation in KOA [22]. Eligible patients were those who answered pain moderate, severe or extreme in at least one of five issues: pain when lying down, sitting, standing, walking or climbing stairs. They had stiffness in the morning or late in the day. They also reported moderate, severe or extreme difficulty in at least one of seventeen questions. Exclusion criteria were: use of current corticosteroids, other neurological, orthopedic or rheumatologic diseases, previous surgery on the affected area in the last six months, previous experience with acupuncture or other uncompensated chronic diseases. We did not include patients who received physical therapy in the last 30 days, with a body mass index (BMI) greater than 35 kg / m2 or who presented inability to read or write.

----------Insert figure 1----------

Interventions

The patients were randomized in four groups of interventions: a-tDCS/a-IMS (n=15), a-tDCS/s-IMS (n=15), s-tDCS/a-IMS (n=15), s-tDCS/s-IMS (n=15). They received in consecutive days five sessions of treatment. Immediately after they received one session of a-tDCS or s-tDCS all patients received one session of the a-IMS or the s-IMS during 30 minutes, with a frequency of 2Hz (figure 1).

Active-(a)-tDCS

The a-tDCS was applied with the anode in the primary motor cortex (M1) contralateral to the treated knee, and the cathode in the contralateral supraorbital region. Five sessions were held, lasting 30 minutes, with an intensity of 2mA [23], ramp up and ramp down duration of 30 seconds, with a current density of 0.057 mA/cm2. The rubber electrodes used were attached a sponge soaked in 0.9% saline and had a size of 35cm2 (5cm vs. 7cm). The authors use the method 10 - 20 electroencephalogram to locate M1. The distance maintained between the electrodes was 7-8 cm. To minimize the adverse effects, sponges were changed with every patient [24].

Sham(s)-tDCS

The sham stimulation was performed in the same way as the active, but the tDCS device was prepared to turn off after 30 seconds of ramp-up, mimicking the feel of active stimulation to the patient.

Active Electrical Stimulation (a-IMS)

This study uses acupuncture needles with guide tubes (DongBang Acupuncture Incorporation, Korea), with 40mm in length and 0.25mm in diameter. Electrical stimulation was applied to the needles with an electroacupuncture device (Sikuro, São Paulo, Brazil). The location of the IMS is based on sensitized roots by chronic pain in KOA and the affected muscles [25]. Needles were deeply inserted alongside the spinous processes L1, L2, L3, L4, L5, S1 and S2 (nerve roots involved in the knee) and to the corresponding dermatomes at the following correspondent's anatomic sites: vast medial, rectus femoris, vast lateral, and tibialis anterior muscles; and the pes anserine bursae [9].

Sham-IMS (s-IMS)

Patients also received five sessions of 30 min. The electro-acupuncture device (Sikuro, São Paulo, Brazil) was prepared so that no electrical stimulation passes to the patient, but it remained on with the diode blinking; promoting visible and audible electrical stimulation. The needles were replaced by rubber electrodes placed on the same sites of active stimulation.

Instruments and Assessments

All questionnaires that were applied are validated for the Brazilian population. The questionnaires were used at baseline and at the end of interventions. The sleep quality was assessed by Pittsburgh Sleep Quality Index [26], depressive symptoms through Beck Depression Inventory [27] and the catastrophizing of pain was assessed with the Brazilian Portuguese Catastrophising Scale (BP-PCS) [28].

Outcomes

The primary outcomes were the pain score on the Visual Analogue Scale before and after each intervention session and the descending pain modulatory system function assessed by the changes on the NPS(0-10) during the CPM-task. The secondary outcomes were the WOMAC index, the PPT, and analgesic consumption.

Outcomes assessment

a) Pressure Pain Threshold (PPT): We use an electronic algometer (J-Tech Medical Industries, USA) to do the tests. Before performing the test, patients are advised to differentiate the sensations of pressure and pain. Patients are instructed to alert verbally when noticing the onset of pain. The researcher that assesses the pain threshold was trained. The systematic evaluation of the superficial and profound hyperalgesia was made by an experienced physiatrist. We carried out three successive

measurements at 3-5 minute intervals, an average kg / cm2 was used. The most sensitive areas corresponding to sclerotomes and myotomes of L1-L5 and S1-S2 were tested with an algometer with a rubber probe with a diameter of 1 cm2, in the knee or leg to be treated.

b) CPM: To test the CPM, the protocol used is outlined by Tousignant-Laflamme et al. [29] and for experimental pain stimulus the guidelines of cold-pressor task (CPM task) was used [30]. The CPM task was used as a conditioning stimulus; it consists of immersing the non-dominant hand in cold water (0 -1° C) for 1 min. In the 30 final seconds, we measured the initial TPP1 in the dominant arm (test stimulus), considering the sensation of pain 6 in 10 by the patient. Before the CPM task, we measure TPP0, obtaining the value of referred pain as 6 in 10 by the patient. The CPM task activates the diffuse noxious inhibitory control-like effect [31], and its efficiency is quantified by subtracting the TPP1 from the TPP0, where negative values indicate inhibitory CPM.

c) The WOMAC index was used to assess the functional capacity related to pain, stiffness, and difficulties during daily activities. The WOMAC is a reliable and valid instrument, widely used in research on the knee (KOA). The answers refer to the symptoms of the last 48 hours. It consists of 24 questions that seek to evaluate the impact of osteoarthritis on daily function of the patient. It is available in two formats: 5-points Likert - type and 100mm VAS. The 24 items are divided into three subscales: pain (5 items), stiffness (2 items) and physical function (17 items). The score is obtained as follows: pain (0-20), stiffness (0-8) and physical function (0-68). The final score is obtained by adding the three subscales; the maximum score is 96 [32].

d) The VAS was used to assess the pain intensity, ranging from 0mm (no pain) to 100mm (intolerable pain, the worst possible). The pain has been evaluated by two questions, one before the intervention (what was the level of your knee pain for most of the time in the last 24 hours?); and one after (what level is your knee pain right now?).

e) The use of analgesics was computed from the average weekly intake in the last three months. In data analysis, the analgesic intake was treated as a dichotomous variable, where analgesic intake for

less than four days in a week was considered zero (reference value), and analgesic use for more than four days considered one. This approach was adopted because patients with chronic pain take rescue analgesics erratically and the frequency of use vary weekly depending on the level of pain.

f) Serum BDNF: We used standard procedures for biological samples. The serum BDNF was gathered in fasting patients (for at least eight hours) early in the morning. The samples were gathered before the first and after the last intervention. Plastic tubes were centrifuged for 10 min at 5000 x g at 4°C. Serum was frozen at -80°C until the assays were performed. Serum neuroplasticity mediator concentrations were determined using BDNF (Chemicon/Milipore, catalog no. CYT306, lower detection limit of the kit=7.8 pg/ml). The personnel involved in sample handling and analysis were unaware of the trial's results.

Sample Size

The sample size was defined by the primary outcome: pain score on the VAS (0-10). The sample calculation was based on one of our previous studies [33]. The sample size calculation was done using a mean difference between groups of 0.6 [pool of standard deviation (0.25)]. We assumed a type I error of 5%, a type II error of 20%, and a power of 80%. Assuming a normal distribution, we determined that a sample size of 54 patients would be necessary. Finally, considering other unexpected factors like attrition rate the required sample size was determined to be 60 patients.

Randomization

Randomization was generated in blocks of 4, seeking a digital equalization of patients between the groups. Before the recruitment phase, the randomization was generated using a computer system by researchers who do not administer the intervention. They put the sequence in separately sealed envelopes. After the patient agrees on participation and signs the informed consent form, the next envelope was delivered to the clinician administering the intervention. The envelope was opened

only by the physician that would apply the intervention and only at the time of administering it to the patient. The patients were assigned for one of four groups: 1) a-tDCS/a-IMS; 2) a-tDCS/s-IMS; 3) s-tDCS/a-IMS, 4); s-tDCS/s-IMS.

Blinding

The authors have taken the following measures to avoid bias: The same physician applied all the interventions, who had ten years of experience in acupuncture and extensive training in transcranial direct current stimulation. Participants were instructed to clarify their doubts regarding interventions only with the doctor who was applying the interventions, and they had no opportunity to meet each other. The scales were administered by two evaluators blind to randomization.

Statistical analysis

The mean differences between the groups (a-IMS/a-tDCS, a-tDCS/s-IMS, s-tDCS/a-IMS and s-tDCS/s-IMS) at baseline were assessed using ANOVA, and categorical variables were examined using chi-square or Fisher's exact and Kruskal-Wallis tests. The values are presented as the mean (standard deviation) or frequency. Continuous variables were tested for normality using Shapiro-Wilks test.

To examine the changes in the outcome measures (pain score in the VAS, PPT, WOMAC, and analgesic doses) between experimental groups (a-tDCS/a-IMS, a-tDCS/s-IMS, s-tDCS/a-IMS and s-tDCS/s-IMS), we applied generalized estimating equations (GEE). The GEE analyses were conducted with an exchangeable working correlation structure to account for correlation between the five sessions from a single participant [34]. The factors were the intervention type (a-tDCS/a-IMS, a-tDCS/s-IMS, s-tDCS/a-IMS and s-tDCS/s-IMS) and the time. In the final models, interactions among the factors and sequence were also examined. For pairwise comparison of the predicted marginal means, multiple comparison tests were performed for each dependent variable separately. In these

analyses, we used Bonferroni's Test to adjust the differences for multiple comparisons. The Cramer's V was used as a measure of effect size for Wald qui-square test. The size effect was interpreted as follows: Standards for interpreting Cramer's V as proposed by Cohen (1988) [35].

To compare intervention group's NPS score and serum BDNF levels during the CPM-task at baseline and at the end of treatment we used the Kruskal-Wallis Test with Dunn's Multiple Comparison Test. All analyses were performed assuming intention-to-treat with the last observation carried forward. The data were analyzed using SPSS version 24.0 (SPSS, Chicago, IL).

RESULTS

3.1. Patients characteristics

The patient's clinical and demographic characteristics are shown in Table 1. Patients were allocated into four groups: a-tDCS/a-IMS (n=15), a-tDCS/s-IMS (n=15), s-tDCS/a-IMS (n=15) and s-tDCS/s-IMS (15). Fifty-nine patients completed the study; one patient in the a-tDCS/a-IMS group withdrew because of fear of needles. The groups were balanced by the randomization; there was no significant difference between them. We did not observe any serious or moderate side effects from the intervention. The incidence of side effects due to tDCS was reported by < 12% of patients, and it was similar between groups. Itching was reported in 6 patients (10%) with a similar incidence between groups. However, the frequency of these adverse effects was not statistically different between groups. The confidence that the treatment received was higher than 78.6% across all groups.

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

3.2. Analysis of the treatment effect on the primary outcomes: pain on VAS and descending modulatory system on CPM

The GEE model revealed that compared to s-tDCS/s-IMS all interventions showed lower pain scores at the end of the last session. There is a main effect of interventions on the VAS ($P < 0.05$) [Wald $\chi2=8.69$; Df=3 = $P<0.03$)]. Also, there is a significant effect of time (Wald $\chi2=533.07$; $P<0.001$) and a significant interaction between group and time Wald $\chi2=$ (30.71; $P<0.01$). The mean VAS (0-10) is presented in Figure 2. The group with the largest effect size estimated by the Cohen's d based on the change in the VAS score before and after intervention was observed in the group that received two active interventions (a-tDCS/a-IMS) (see Table 2). The cumulative mean (SD) on the VAS at the baseline vs. the pain scores after the last session in the a-tDCS/a-IMS group was 6.32 (1.97) vs. 0.46 (0.04). The estimate of effect size by the Cohen's d within the group was 2.97. For the a-tDCS/s-IMS group, these values were 6.52 (2.63) vs. 1.79 (2.53), the Cohen's d was 1.79, and for the s-tDCS/a-IMS group these values were 6.75 (2.62) vs. 1.81 (2.98), the Cohen's d was 1.88, respectively. While in the s-tDCS/s-IMS these values were 7.17 (2.47) vs. 2.98 (1.17) and the Cohen's d was 1.69.

----------Insert figure 2----------

----------Insert table 2----------

To compare intervention group's NPS (0-10) during the CPM task at the baseline, we used the Kruskal-Wallis Test (P=0.94). The median and quartile (Q25-75) in each one group was the following: a-tDCS/a-IMS was -2 (Q25-75= -4 to 3), a-tDCS/s-IMS was -2.25 (Q25-75= -4 to 3), s-tDCS/a-IMS was -2 (Q25-75= -4 to 1) and in the s-tDCS/s-IMS was -2 (Q25-75= -4 to 1). The change on the NPS (0-10) during the CPM-task at the end of treatment is presented in figure 3.

There is a statistically significant difference between the a-tDCS/a-IMS group compared with all other three groups. However, there are not differences statistically significant between the other groups.

----------Insert figure 3----------

3.3. Analysis of the treatment effect on the secondary outcomes: WOMAC score, PPT, and analgesic consumption

A GEE model revealed an effect of group (Wald $\chi2$=8.99, Df=3), P=0.03, an effect of time (Wald $\chi2$=51.48, Df=1), P=0.00 and an interaction between group and time (Wald $\chi2$=2.72, Df=3), P=0.01. The active groups (a-tDCS/a-IMS, s-tDCS/a-IMS and a-tDCS/s-IMS) showed a statistically significant increase in the PPT compared with sham. This result indicates a modulation of the nociceptive threshold by treatments, probably acting in the perception of pain.

A GEE model revealed an effect of group (Wald $\chi2$=8.48, Df=3), P=0.03, an effect of time (Wald $\chi2$=86.80, Df=1), P<0.001 and an interaction between group and time (Wald $\chi2$=27.02, Df=3), P<0.001. A significant improvement in the WOMAC scores was observed in the group with a-tDCS/a-IMS, a-tDCS/s-IMS and s-tDCS/a-IMS compared to s-tDCS/s-IMS (table 2). The WOMAC scores decreased significantly in the groups that received a-IMS, regardless if they were combined with a-tDCS or not.

The incidence of analgesic use throughout the treatment period was 74% in the group that received the s-tDCS/s-IMS compared to 45% in the group that received a-tDCS/a-IMS. Thus, when we compared these two groups, the relative risk (RR) for the s-tDCS/s-IMS to use analgesia was 1.75, confidence interval [(CI) 95% 1.29 to 2.35). In patients that received a-tDCS/s-IMS the incidence of analgesic use was 69.2%. Hence the RR to use the painkillers was 1.13 (CI 95% 0.78 to 1.64) compared to s-tDCS/s-IMS. In patients who received s-tDCS/a-IMS the rate of painkiller use was 49.3%, compared to s-tDCS/s-IMS the RR to use analgesia was 1.60 (CI 95% 1.17 to 2.18).

3.4. Relationship between the serum BDNF and the PPT at the end of treatment

The comparisons between groups are presented in Table 3. No statistically significant differences in serum BDNF was observed among the intervention groups, neither at baseline nor after end of treatment.

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

One prominent issue is whether the level of baseline BDNF can predict the PPT after the intervention. It was demonstrated that a higher level of serum BDNF at baseline is correlated negatively with the PPT at the end of treatment independently of the intervention group. The scatter plot of the negative correlation between the PPT and BDNF is presented in Figure 4. The Spearman correlation coefficient was r2=-0.37, and its confidence interval (CI) 95% was (-0.57 to -0.13); P<0.001. That is, patients with higher PPT presented a lower level of serum BDNF at the baseline or vice-versa.

----------Insert figure 4----------

DISCUSSION

The most relevant finding of this study was to show that five sessions of a-tDCS over the M1 combined with the a-IMS produced a greater reduction in the pain scores compared to the other three groups. In patients that received a-tDCS/a-IMS the effect size on pain scores within the group was notably larger. There was a reduction in the pain scores immediately after intervention in all groups. However, this effect persisted until the next session only in those receiving a-tDCS/a-IMS (figure 2). Similarly, the a-tDCS/a-IMS group presented a lower risk to use additional analgesics compared to s-

tDCS/s-IMS, and it also induced an effect with a higher impact in the improvement of the descending pain modulatory systems. The present findings showed an increase in the PPT in all groups that received active treatment, whereas the enhancement of the disability related to pain was observed in a comparable manner in the two groups, which received the a-IMS. The BDNF levels before starting treatment predicted a higher PPT at the end of treatment independently of the intervention group.

The improvement in pain scores using two approaches to modulate the pain pathways in top-down and bottom–up manner suggest that they induced a strengthening of the descending inhibitory system and a homeostasis of the excitatory system involved in pain processing. This hypothesis found a biological plausibility based on previous findings when the combined treatment produced a better effect on pain scores. According to previous studies, the superiority of combined interventions was better than each one of them alone (i.e. chronic low back pain (tDCS/ peripheral electrical stimulation) [36], in myofascial pain (tDCS/trigger point injection) [37] and the (tDCS/aerobic exercises for fibromyalgia) [38]. Our findings suggest that the use of the combined therapies in sequence promoted a synergistic effect. This synergistic effect suggests the occurrence of priming, a phenomenon by which one intervention favors responsiveness to another [36, 38]. The a-tDCS increased the excitability promoting LTP at the cortical level [36], based on default effects it may facilitate the effect of IMS to induce a reduction of excitability (LTD) at the spinal level [39]. That is, the subsequent treatments followed the principle of homeostatic metaplasticity, where the little neural activity promotes synaptic changes for long-term potentiation (LTP, increased excitability), and high activity levels favor long-term depression (LTD decreased excitability) [36]. The novelties of our research were to test a treatment of KOA, which is a cheap and safe alternative (a-tDCS/a-IMS). This approach shows that this combined intervention to modulate a top-down and bottom-up manner also was more efficient to improve the descending inhibitory pain system.

One hypothesis to explain our results is the capacity of IMS to reduce the cortical excitability through several neurobiological processes, such as decreases in NMDA receptor and GABA-A

receptor activity, reducing the imbalance between inhibition and excitation [40]. Another possibility is the modulation of intracortical excitability and transmission efficiency of corticospinal neurons [41]. Additionally, it is possible that the cortical reorganization of the neural pain circuits occurs via strengthening of the peripheral modulation centers such as corticospinal pathways and thalamic structures [8]. According to previous evidence, the IMS may increase the PPT through myelinated A-δ fibers and unmyelinated C-fibers. The insertion of a needle promotes a stimulation throughout 72h of the A δ fibers, promoting activation of enkephalinergic, serotonergic, and noradrenergic inhibitory systems [42]. Also, the electro-acupuncture (IMS) stimulate the K+/Na+ pumps furthermore prolonging the effect[43]. Some effects of IMS occur far from the treatment field, like the accelerating release and synthesis of serotonin (5HT) and norepinephrine in the central nervous system improving the function of the deficient pain modulatory pathway [44]. Another explanation comes from the increment of BDNF secretion induced by IMS [11]. Its action promotes a reduction of NMDA-receptor(r) function and glutamate excitotoxicity [45]. The BDNF has a neuromodulatory activity to induce neurogenesis and synaptic strengthen [46]. Also, it produces free radicals' reduction by superoxide dismutase regulation [47]. Besides that, BDNF maintains intracellular calcium homeostasis via calcium channels protein's cellular membrane control [48]. It also regulates the expression of neuronal genes involved in the central sensitization process, through transcription factors such as *c-fos* and *c-jun* [49].

The effective analgesic effect on pain scores using anodal tDCS stimulation on M1 has been reported as effective in chronic pain. In a systematic review, they find similar analgesic effects compared to FDA-approved pharmaceuticals for fibromyalgia [50]. Evidence suggest the effect of controlling the excitability of perpendicular intracortical fibers about the electrode [51]. The process also involves opioidergic, glutamatergic, GABAergic and serotoninergic neurotransmissions [50]. tDCS changes the excitability of synaptic inputs and the frequency of spontaneous firing. It modifies the synaptic microenvironment, altering the synaptic force through NMDA receptors or GABAergic

activity. The mechanism of action with long-lasting neuroplastic effects has as one of its primary elements the calcium-dependent synaptic plasticity of glutamatergic neurons [51]. Also, it modulates intracortical and corticospinal neurons and has non-synaptic effects due to temporary changes in the density of the protein channels [52]. Another explanation for our long-lasting effects with tDCS may be dependent on protein synthesis and accompanied by changes in intracellular cyclic AMP and calcium levels, dividing characteristics with long-term potentiation (LTP) [53, 54]. Another mechanism would be its ability to restore the inhibitory (GABAergic) system that is deficient in these patients [54]. Also, the tDCS can promote prolonged neurochemical changes, which modulates the conduction in the spinal cord and segmental reflexes [52]. Thus, its effect on the neural networks can interfere in functional connectivity, synchronization, and oscillatory activities in cortical and subcortical networks [51]. The electrotaxis generated by electric fields might contribute to neuromodulation as well as changes in BDNF expression [54]. Besides, it influences several different tissues and in multiple cellular structures, as non-neuronal components of the CNS [52]. These non-neuronal effects could be involved in the therapeutic action [51].

We observed that the intervention did not change the serum levels of BDNF. However, the BDNF levels at baseline were correlated negatively with the PPT at the end of treatment independently of the intervention group. Although the explanation for this finding is not clear, according to the previous study, the BDNF levels are higher in situations of inflammation and persistent pain [55], where it might play a pro-nociceptive role [46]. According to a previous study, serum levels of BDNF were higher in KOA compared to healthy patients the same age [56]. In other chronic pain conditions, such as fibromyalgia, our group already had demonstrated an inverted correlation between BDNF and PPT [19]. Possibly, the increased BDNF promotes the disinhibition of the descending inhibitory system through its binding to the trkB receptor by enhancement of the evoked responses of the C fibers, activating the signaling pathways in the spinothalamic pathway and enhancing the excitation [17]. Our hypothesis is that the more dysfunctional patients have higher

levels of BDNF, a more disinhibited descending inhibitory system, a lower PPT, and more pain with less capacity to respond to treatment.

Several issues concerning the design of our study must be addressed: first, even though the tDCS is an efficient technical solution to conduct blinded studies with blinding of both patients and experimenters [57], the efficacy of patient blinding has been questioned, especially when we applied a stimulation intensities of 2 mA [58]. However, the rate of all side effects observed in the present study was less than 12%, with a similar incidence between groups, and 78.6% of them reported to have confidence in the treatment that they were receiving. Thus, it is improbable that unblinding could change the directions of our conclusions. Second, a limitation of this study was the number of treatment sessions, although we found a sustained improvement in the pain of these patients from the fourth session we believed that a greater number of courses would lead to better and more lasting effects. As our goal was to verify the possibility of a summation of force through a bottom-up (IMS) and a top-down (tDCS) approach, this did not hamper our findings. Third, another limitation was the fact that our sample was of only women, not allowing to extrapolate our findings for men. Our choice was because the prevalence of KOA is higher among women and to avoid a confounding bias since there is a difference in pain measurements related to the gender [59, 60]. This study opened new therapeutic possibilities for a very common pathology and with few effective conservative therapy, with the hope of gaining quality of life through low-cost treatments with minimal adverse effects. Future studies with a larger number of sessions and long-term follow-up will still be needed to sediment these treatment possibilities in KOA patients.

In conclusion, a-tDCS/a-IMS produced a greater and sustained improvement on pain measures, functional capacity as well as improvement in the descending pain modulatory system function. The effect of BDNF in the PPT was independent of the intervention group. These findings suggest that the combined treatments are more effective at reducing pain and improving the functional capability

of KOA patients. Also, these results showed that a higher serum BDNF level predicted lower treatment effects in pain threshold independently of the intervention group.

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Author's contribution

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.

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REFERENCES

1. Goldring MB, Goldring SR. Osteoarthritis. J Cell Physiol 2007; 213(3): 626-34.

2. Mascarin NC, Vancini RL, Andrade MS, Magalhães EP, Lira CAB, Coimbra IB. Effects of kinesiotherapy, ultrasound and electrotherapy in management of bilateral knee osteoarthritis: prospective clinical trial. BMC Musculoskel Dis 2012; 13(182). doi: 10.1186/1471-2474-13-182.

3. Gwilym SE, Keltner JR, Warnaby CE, Carr AJ, Chizh B, Chessell I, Tracey I. Psychophysical and functional imaging evidence supporting the presence of central sensitization in a cohort of osteoarthritis patients. Arth Rheum 2009; 61(9): 1226-34. doi: 10.1002/art.24837.

4. Skou ST, Graven-Nielsen T, Rasmussen S, Simonsen OH, Laursen MB, Arendt-Nielsen L. Facilitation of pain sensitization in knee osteoarthritis and persistent post-operative pain: A cross-sectional study. Eur J Pain 2014; 18(7): 1024-31. doi: 10.1002/j.1532-2149.2013.00447.x.

5. Graven-Nielsen T, Wodehouse T, Langford RM, Arendt-Nielsen L, Kidd BL. Normalization of widespread hyperesthesia and facilitated spatial summation of deep-tissue pain in knee osteoarthritis patients after knee replacement. Arthritis Rheum 2012; 64: 2907-2916. doi: 10.1002/art.34466.

6. Alburquerque-García A, Rodrigues-de-Souza DP, Fernández-de-las-Peñas C and Alburquerque-Sendín F. Association between muscle trigger points, ongoing pain, function, and sleep quality in elderly women with bilateral painful knee osteoarthritis. J Manipulative Physiol Ther 2015; 38(4): 262-8. doi: 10.1016/j.jmpt.2014.10.018.

7. Botelho LM, Morales-Quesada L, Rozisky JR, Brietzke AP, Torres ILS, Deitos A, Fregni F, Caumo W. A Framework for Understanding the Relationship between Descending Pain Modulation, Motor Corticospinal, and Neuroplasticity Regulation Systems in Chronic Myofascial Pain. Front Hum Neurosci 2016;27(10): 308. doi: 10.3389/fnhum.2016.00308.

8. Dall'Agnol L, Medeiros LF, Torres IL, Deitos A, Brietzke A, Laste G, de Souza A, Vieira JL, Fregni F, Caumo W. Repetitive transcranial magnetic stimulation increases the corticospinal inhibition and the brain-derived neurotrophic factor in chronic myofascial pain syndrome: an explanatory double-blinded, randomized, sham-controlled trial. J Pain 2014; 15(8): 845-55. doi: 10.1016/j.jpain.2014.05.001.

9. Tarragó MGL, Deitos A, Brietzke AP, Vercelino R, Torres ILS, Fregni F, Caumo W. Descending control of nociceptive processing in knee osteoarthritis is associated with intracortical disinhibition: An exploratory study. Medicine (Baltimore) 2016; 95(17): e3353. doi: 10.1097/MD.0000000000003353.

10. Couto C, de Souza IC, Torres IL, Fregni F, Caumo W. Paraspinal stimulation combined with trigger point needling and needle rotation in treating myofascial pain: randomized sham-controlled clinical trial. Clin J Pain 2014; 30(3): 214-23. doi: 10.1097/AJP.0b013e3182934b8d.

11. Chassot M, Dussan-Sarria J, Sehn FC, Deitos A, de Souza A, Vercelino R, Torres IL, Fregni F, Caumo W. Electroacupuncture analgesia is associated with increased serum brain-derived neurotrophic factor in chronic tension-type headache: a randomized, sham controlled, crossover trial. BMC Complement Altern Med 2015; 7(15): 144. doi: 10.1186/s12906-015-0664-x.

12. Valle A, Roizenblatt S, Botte S, Zaghi S, Riberto M, Tufik S, Boggio PS, Fregni F. Efficacy of anodal transcranial direct current stimulation (tDCS) for the treatment of fibromyalgia: results of a randomized, sham-controlled longitudinal clinical trial. J Pain Manag 2009; 2(3): 353–361.

13. Bolognini N, Olgiati E, Maravita A, Ferraro F, Fregni F. Motor and parietal cortex stimulation for phantom limb pain and sensations. Pain 2013; 154(8): 1274-80. doi: 10.1016/j.pain.2013.03.040.

14. Antal A, Kriener N, Lang N, Boros K, Paulus W. Cathodal transcranial direct current stimulation of the visual cortex in the prophylactic treatment of migraine. Cephalalgia 2011; 31(7): 820–8. doi: 10.1177/0333102411399349.

15. Hagenacker T, Bude V, Naegel S, Holle D, Katsarava Z, Diener HC, Obermann M. Patient-conducted anodal transcranial direct current stimulation of the motor cortex alleviates pain in trigeminal neuralgia. J Headache Pain 2014; 25: 15:78. doi: 10.1186/1129-2377-15-78.

16. O'Connell NE, Cossar J, Marston L, Wand BM, Bunce D, De Souza LH, Maskill DW, Sharp A, Moseley GL. Transcranial Direct Current Stimulation of the Motor Cortex in the Treatment of

Chronic Nonspecific Low Back Pain: A Randomized, Double-blind Exploratory Study. Clin J Pain 2013; 29(1): 26–34. doi: 10.1097/AJP.0b013e318247ec09.

17. Latremoliere A, Woolf CJ. Central sensitization: a generator of pain hypersensitivity by central neural plasticity. J Pain 2009; 10(9): 895-926. doi: 10.1016/j.jpain.2009.06.012.

18. Caumo W, Deitos A, Carvalho S, Leite J, Carvalho F, Dussán-Sarria JA, Lopes Tarragó Mda G, Souza A, Torres IL, Fregni F. Motor Cortex Excitability and BDNF Levels in Chronic Musculoskeletal Pain According to Structural Pathology. Front Hum Neurosci 2016; 15(10): 357. doi: 10.3389/fnhum.2016.00357.

19. Zanette SA, Dussan-Sarria JA, Souza A, Deitos A, Torres ILS, Caumo W. Higher serum S100B and BDNF levels are correlated with a lower pressure-pain threshold in fibromyalgia. Mol Pain 2014; 10: 46. doi: 10.1186/1744-8069-10-46.

20. Ravaud P, Giraudeau B, Auleley GR, Chastang C, Poiraudeau S, Ayral X, Dougados M. Radiographic assessment of knee osteoarthritis: reproducibility and sensitivity to change. J Rheumatol 1996; 23(10): 1756-64.

21. Ravaud P, Auleley GR, Amor B, Dougados M. Radiographic assessment of progression in knee osteoarhritis. Rheumatol Eur 1995; 24(2): 129-31.

22. Nunes G, de Castro LV, Wageck B, Kume V, Chiesa GS, de Noronha M. Translation into Portuguese of questionnaires to assess knee injuries. Acta Ortop Bras 2013; 21(5): 288–94. doi: 10.1590/S1413-78522013000500010.

23. Vaseghi B, Zoghi M, Jaberzadeh S. How Does Anodal Transcranial Direct Current Stimulation of the Pain Neuromatrix Affect Brain Excitability and Pain Perception? A Randomised, Double-Blind, Sham-Control Study. PLoS One 2015 Mar 4;10(3):e0118340. doi: 10.1371/journal.pone.0118340.

24. Medeiros LF, de Souza IC, Vidor LP, de Souza A, Deitos A, Volz MS, Fregni F, Caumo W, Torres IL. Neurobiological effects of transcranial direct current stimulation: a review. Front Psychiatry 2012; 28(3): 110. doi: 10.3389/fpsyt.2012.00110.

25. Imamura M, Imamura ST, Kaziyama HH, Targino RA, Hsing WT, de Souza LP, Cutait MM, Fregni F, Camanho GL. Impact of nervous system hyperalgesia on pain, disability, and quality of life in patients with knee osteoarthritis: a controlled analysis. Arthritis Rheum 2008; 59(10): 1424-31. doi: 10.1002/art.24120.

26. Buysse, Reynolds CF 3rd, Monk TH, Berman SR, Kuupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res 1989; 28(2): 193-213.

27. Warmenhoven F, van Rijswijk E, Engels Y, Kan C, Prins J, van Weel C, Vissers K. The Beck Depression Inventory (BDI-II) and a single screening question as screening tools for depressive disorder in Dutch advanced cancer patients. Support Care Cancer 2012; 20(2): 319-24. doi: 10.1007/s00520-010-1082-8.

28. Sehn F, Chachamovich E, Vidor LP, Dall-Agnol L, de Souza IC, Torres IL, Fregni F, Caumo W. Cross-cultural adaptation and validation of the Brazilian Portuguese version of the pain catastrophizing scale. Pain Med 2012; 13(11): 1425–1435. doi: 10.1111/j.1526-4637.2012.01492.x.

29. Tousignant-Laflamme Y, Pagé S, Goffaux P, Marchand S. An experimental model to measure excitatory and inhibitory pain mechanisms in humans. Brain Res 2008; 1230: 73-79. doi: 10.1016/j.brainres.2008.06.120.

30. von Baeyer CL, Piira T, Chambers CT, Trapanotto M, Zeltzer LK. Guidelines for the cold pressor task as an experimental pain stimulus for use with children. J Pain 2005; 6(4): 218-27.

31. Willer JC, De Broucker T, Le Bars D. Encoding of nociceptive thermal stimuli by diffuse noxious inhibitory controls in humans. J Neurophysiol 1989; 62(5): 1028-38.

32. Berrada K, Wariaghli G, Idrissi Z, Hmamouchi I, Abouqal R, Allali F, Hajjaj-Hassouni N. FRIO133-Normative Values for the Western Ontario and MCMaster Universities in the General Population Compared with Knee Osteoarthritis Patients. Ann Rheum Dis 2011; 70(3): 88.

33. Tarrago MdaG, Deitos A, Brietzke AP, Torres ILS, Stefani LC, Fregne F, Caumo W. Electrical Intramuscular Stimulation in Osteoarthritis Enhances the Inhibitory Systems in Pain Processing at Cortical and Cortical Spinal System. Pain Med 2015; Sep 23. doi: 10.1111/pme.12930.

34. Zeger SL, Liang KY, Albert PS. Models for longitudinal data: a generalized estimating equation approach. Biometrics 1988; 44(4): 1049-60.

35. Cohen J. How can I intepret the effect sizes of Cramer's V when DF >3?. Cohen's book. In: Statistical power analysis for the behavioral sciences (2nd ed.). Hillsdale, New Jersey: Lawrence Erlbaum Associates; 1988.

36. Schabrun SM, Jones J, Elqueta Cancino EL, Hodges PW. Targeting Chronic Recurrent Low Back Pain From the Top-down and the Bottom-up: A Combined Transcranial Direct Current Stimulation and Peripheral Electrical Stimulation Intervention. Brain Stimul 2014; 7(3):451-9. doi: 10.1016/j.brs.2014.01.058.

37. Choi YH, Jung S, Lee CH, Lee SU. Additional Effects of Transcranial Direct-Current Stimulation and Trigger-Point Injection for Treatment of Myofascial Pain Syndrome: A Pilot Study with Randomized, Single-Blinded Trial. J Alt Compl Med 2014; 20(9): 698–704. doi: 10.1089/acm.2013.0243.

38. Mendonça ME, Simis M, Grecco LC, Battistella LR, and Fregni F. Transcranial direct current stimulation combined with aerobic exercise to optimize analgesic responses in fibromyalgia: a randomized placebo-controlled clinical trial. Fron. Hum. Neurosci 2016; 10: 68. Doi: 10.3389/fnhum.2016.00068.

39. Zhang R, Lao L, Ren K, Berman BM. Mechanisms of Acupuncture-Electroacupuncture on Persistent Pain. Anesthesiol 2014; 120(2): 482-503. doi: 10.1097/ALN.0000000000000101.

40. Vidor LP, Torres IL, Medeiros LF, Dussán-Sarria JA, Dall'agnol L, Deitos A, Brietzke A, Laste G, Rozisky JR, Fregni F, Caumo W. Association of anxiety with intracortical inhibition and descending pain modulation in chronic myofascial pain syndrome. BMC Neurosci 2014; 19(15): 42. doi: 10.1186/1471-2202-15-42.

41. Maioli C, Falciati L, Marangon M, Perini S, Losio A. Short- and long-term modulation of upper limb motor-evoked potentials induced by acupuncture. Eur J Neurosci 2006; 23(7): 1931-8.

42. Baldry P. Management of myofascial trigger point pain. Acupunt Med 2002; 20: 2-10.

43. Hong CZ, Chen YC, Pon CH, Yu J. Immediate effects of various physical modalities on the pain threshold of active myofascial trigger points. J Musculoskelet Pain 1993; 1: 89-101.

44. Le Bars D, Dickenson AH, Besson JM. Diffuse noxious inhibitory controls (DNIC). II. Lack of effect on non-convergent neurones, supraspinal involvement and theoretical implications. Pain 1979; 6(3): 305-27.

45. Polyakova M, Stuke K, Schuemberg K, Mueller K, Schoenknecht P, Schroeter ML. BDNF as a biomarker for successful treatment of mood disorders: a systematic & quantitative meta-analysis. J Affect Disord 2015;174:432-40. doi: 10.1016/j.jad.2014.11.044.

46. Merighi A, Salio C, Ghirri A, Lossi L, Ferrini F, Betelli C, Bardoni R. BDNF as a pain modulator. Prog Neurobiol 2008; 85: 297–317. doi: 10.1016/j.pneurobio.2008.04.004.

47. Tremblay R, Hewitt K, Lesiuk H, Mealing G, Morley P, Durkin JP. Evidence that Brain-Derived Neurotrophic Factor Neuroprotection Is Linked to Its Ability to Reverse the NMDA-Induced Inactivation of Protein Kinase C in Cortical Neurons. J Neurochem 1999; 72(1): 102-11.

48. Ikeda O, Murakami M, Ino H, Yamazaki M, Koda M, Nakayama C. Effects of brain-derived neurotrophic factor (BDNF) on compression-induced spinal cord injury: BDNF attenuates down-regulation of superoxide dismutase expression and promotes up-regulation of myelin basic protein expression. J Neuropathol Exp Neurol 2002; 61: 142–53.

49. Blanquet PR, Mariani J, Derer P. A calcium/calmodulin kinase pathway connects brain-derived neurotrophic factor to the cyclic AMP-responsive transcription factor in the rat hippocampus. Neurosc 2003; 118(2):477–90.

50. DosSantos MF, Ferreira N, Toback RL,Carvalho AC and DaSilva AF. Potential Mechanisms Supporting the Value of Motor Cortex Stimulation to Treat Chronic Pain Syndromes. Front Neurosci. 2016;10:18. doi: 10.3389/fnins.2016.00018.

51. Lefaucher JP, Antal A, Ayache SS, Benninger DH, Brunelin J, Cogiamanian F, Cotelli M, De Ridder D, Ferrucci R, Langguth B, Marangolo P, Mylius V, Nitsche MA, Padberg F, Palm U, Poulet E, Priori A, Rossi S, Schecklmann M, Vanneste S, Ziemann U, Garcia-Larrea L, Paulus W. Evidence-based guidelines on the therapeutic use of transcranial direct current stimulation (tDCS). Clin Neurophysiol 2017;128(1):56-92. doi: 10.1016/j.clinph.2016.10.087.

52. Brunoni AR, Nistche MA, Bolognini N, Bikson M, Wagner T, Merabet L, et al. Clinical research with transcranial direct current stimulation (tDCS): challenges and future directions. Brain Stimul 2012;5(3):175-95. doi: 10.1016/j.brs.2011.03.002.

53. Nitsche MA, Cohen LG, Wassermann EM, Priori A, Lang N, Antal A, Paulus W, Hummel F, Boggio PS, Fregni F, Pascual-Leone A. Transcranial direct current stimulation: State of the art 2008. Brain Stimul 2008; 1(3): 206-23. DOI:10.1016/j.brs.2008.06.004.

54. Pelletier SJ, Cicchetti F. Cellular and molecular mechanisms of action of tDCS: evidence from in vitro and in vivo models. Int J Neuropsychopharmacol 2014; 31:18(2). DOI: 10.1093/ijnp/pyu047.

55. Klein K, Aeschlimann A, Jordan S, Gay R, Gay S, Sprott H. ATP Induced Brain-Derived Neurotrophic Factor Expression and Release from Osteoarthritis Synovial Fibroblasts Is Mediated by Purinergic Receptor P2X4. PLoS One 2012;7(5):e36693. doi: 10.1371/journal.pone.0036693.

56. Simão AP, Mendonça VA, de Oliveira Almeida TM, Santos SA, Gomes WF, Coimbra CC, Lacerda AC. Involvement of BDNF in knee osteoarthritis: the relationship with inflammation and clinical parameters. Rheumatol Int 2014;34(8):1153-7. doi: 10.1007/s00296-013-2943-5.

Código de campo alterado

57. Gandiga PC, Hummel FC, Cohen LG. Transcranial DC stimulation (tDCS): a tool for double-blind sham-controlled clinical studies in brain stimulation. Clin Neurophysiol 2006; 117(4): 845-50. doi: 10.1016/j.clinph.2005.12.003.

58. O'Connell NE, Cossar J, Marston L, Wand BM, Bunce D, Moseley GL, De Souza LH. Rethinking clinical trials of transcranial direct current stimulation: participant and assessor blinding is inadequate at intensities of 2mA. PLoS One 2012;7(10):e47514. doi: 10.1371/journal.pone.0047514.

59. Imamura M, Ezquerro F, Alfieri FM, Vilas Boas F, Tozetto-Mendoza TR, Chen J, Özçakar L, Arendt-Nielsen L, Battistella LR. Serum Levels of Proinflammatory Cytokines in Painful Knee Osteoarthritis and Sensitization. Int J Inflam 2015;2015:329792. doi: 10.1155/2015/329792.

60. Fillingim RB, King CD, Ribeiro-Dasilva MC, Rahim-Williams B, Riley JL 3rd. Sex, gender, and pain: a review of recent clinical and experimental findings. J Pain 2009; 10(5): 447-85. doi: 10.1016/j.jpain.2008.12.001.

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Legend of figures

Figure 1: Flow chart is showing recruitment and progress through the study. Pain pressure threshold (PPT); conditioned pain modulation (CPM); Western Ontario and McMaster Universities Index (WOMAC); Pittsburgh Sleep Quality Index (PSI); Numerical Pain Scale (NPS 0-10); Functional Pain Scale (FPS); Pain Catastrophizing Scale (BP-PCS); Pittsburgh Sleep Quality Index, Beck Depression Inventory (BDI).

Figure 2: Mean VAS scores before and after treatments and standard error of the mean (SEM). The a-tDCS+ a-IMS showed a significant difference from the first treatment session and had sustained effect from the fourth session. All active groups showed a significant difference after the last

treatment session. Groups numbers above the bars indicate the statistically significant differences between them.

Figure 3: Median and Q (25-75) of change on Numerical Pain Scale (0-10) during CPM-task. The comparison was made using the Kruskal-Wallis Test with Dunn's Multiple Comparison Test to identify changes between groups. Although active treatments changed the NPS, only a-tDCS/a-IMS modified the descending modulatory system function (*). The difference statically significant is showed by the asterisk.

Figure 4. Scatter plot of the correlation between BDNF and pain pressure threshold (n=60).

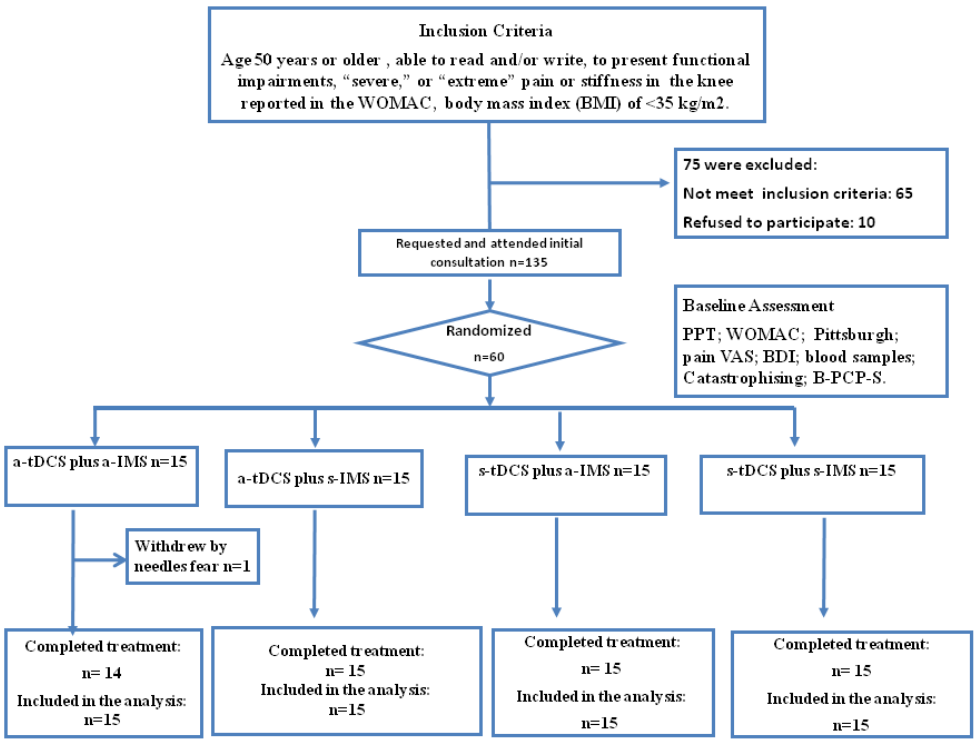
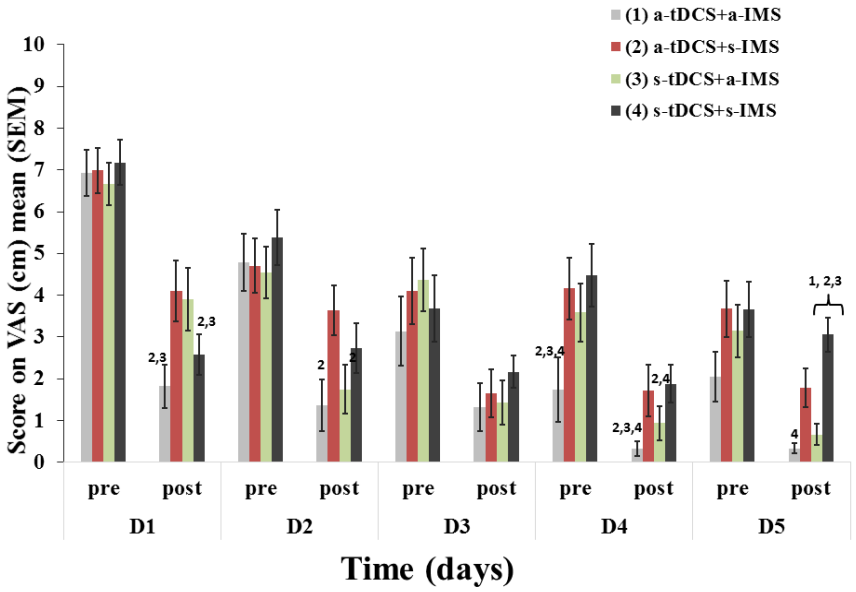


Figure 1


(1) a-tDCS+a-IMS
(2) a-tDCS+s-IMS
(3) s-tDCS+a-IMS
(4) s-tDCS+s-IMS
Score on VAS (cm) mean (SEM)
10
9
8
7
6
5
4
3
2
1
0
pre
post
D1
D2
D3
D4
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Time (days)
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4
1, 2,3

Figure 2.

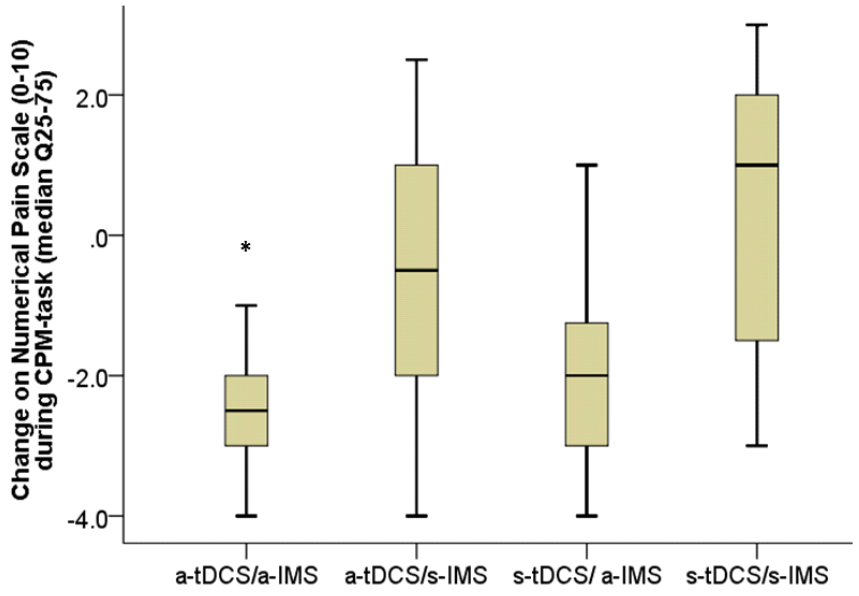


Figure 3

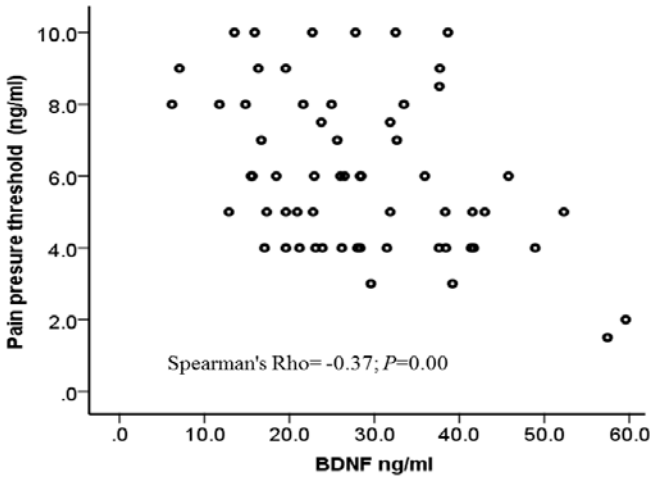


Figure 4

Table 1: Demographic and clinical characteristics of the study sample. Values are given as the mean (SD) or frequency (n=60).

Variable	a-tDCS/aIMS (n=15)	a-tDCS/sIMS (n=15)	s-tDCS/aIMS (n(=15)	s-tDCS/s-IMS (n=15)	P
Age (years)	66.00 (9.08)	64.14 (9.82)	64.40 (6.02)	63.87 (7.07)	0.88
Body Mass Index (kg/m2)	29.27(4.15)	29.47(3.69)	30.85(4.82)	28.83(3.85)	0.58
Employed (yes/no) (%)	2/14 (27.1%)	3/10 (22.0%)	1/14 (25.4%)	3/12(25.4%)	0.60
School years	7.75 (4.19)	8.32 (4.15)	9.13 (4.63)	6.20 (3.84)	0.28
Pain during the most part of days (VAS)	6.31 (1.14)	6.57 (0.51)	6.07 (1.39)	6.73 (0.46)	0.26
Psychiatric disease diagnosis (yes/no)	6/10(26.7)	6/8(23.3)	3/12(25.0)	5/10(25.0)	0.59
Psychotropic medication Yes/No (%)ǂ	8(53.3)	8(53.3)	3(20)	7 (46.7)	0.41
Smoking (yes/no) (%)	2/14(26.7)	0/14(23.3)	0/15(25.0)	1/14(25.0)	0.32
Alcohol (yes/no)	4/12(27.1)	2/12(23.7)	2/13(25.4)	1/13(23.7)	0.58
Number of chronic disease	1(0.89)	1.5 (1.5)	1.8 (1.74)	1.4(0.83)	0.39
Type of chronic disease					
Diabetic (yes/no)	2 (13.3)	2 (13.3)	3 (20)	4 (26.7)	
Hypothyroidism (yes/no)	2 (13.3)	3 (20)	1(6.7)	1 (6.7)	
Hypertension (yes/no)	8 (53.3)	7 (46.7)	10 (66.7)	13 (86.7)	
Other (yes/no)	1(6.7)	0	2(13.3)	1(6.7)	
WOMAC (total score) ¥	54.75(19.80)	52.36(20.35)	54.13(18.56)	50.47(13.75)	0.95
W- stifness	4.93 (2.91)	4.50 (2.50)	4.93 (2.74)	4.33 (1.76)	0.88
W- daily activities	25.60 (10.87)	24.07 (10.55)	23.53 (10.68)	22.33 (9.72)	0.86
W- pain	10.93 (4.43)	10.64 (5.11)	11.80 (3.36)	10.87 (3.56)	0.88
Pain score in the VAS in the last 24 hours	5.59 (2.63)	6.07 (2.42)	5.27(1.91)	5.50 (2.77)	0.84
Beck Depression Inventory	14.75 (11.22)	12.64 (9.47)	11.07 (12.07)	8.53 (4.70)	0.36
Brazilian Portuguese Pain Catastrophizing	29.37(10.51)	28.86 (9.80)	22.27(13.22)	26.73(11.36)	0.30
Pittsburgh Sleep Quality Index Score	18.94 (13.73)	23.71 (14.08)	22.73 (12.25)	16.80 (12.72)	0.45
PPT in (kg/cm2/second)	2.81(1.41)	3.56(2.54)	3.71(2.81)	3.41(1.85)	0.72

¥ To compare median was used the Kruskal Wallis Test.

Table 2. Outcomes – pain measures and descending pain control system function. Data present as mean (SD) (n=60).

	Mean (SD)						
	After intervention	**Before intervention**	**CI 95%**	**Wald χ^2**	**Df**	**Effect size**	**P**
Primary outcomes:* The cumulative *pain scores on VAS (0 -10cm) after intervention throughout the treatment							
(1) a-tDCS/a-IMS [a] (n= 15)	0.46 (0.04) [2,3,4]	vs. 6.32 (1.97)	-5.42 (-8.24 to -4.36)	8.69	3	0.57	0.03
(2) a-tDCS/ s-IMS [b] (n= 15)	1.79 (2.53) [1,4]	vs. 6.52 (2.63)	-4.73 (-7.65 to -3.11)				
(3) s-tDCS/a-IMS [c] (n= 15)	1.81 (2.98) [1,4]	vs. 6.75 (2.62)	-4.6 (-7.84 to -2.38)				
(4) s-tDCS/s-IMS [d] (n= 15)	2.98 (1.17) [1,2,3]	vs. 7.17 (2.47)	-4.19 (-8.10 to -2.93)				
Secondary outcomes:							
b. Pressure pain threshold (PPT) in (kg/cm2/second)							
(1) a-tDCS/a-IMS [a] (n= 15)	6.00 (1.76)	vs. 3.24 (1.40)	2.96 (0.94 to 4.54) [a]	8.99	3	0.60	0.02
(2) a-tDCS/ s-IMS [b] (n= 15)	6.53 (3.27)	vs. 3.86 (2.53)	2.67 (0.92 to 4.63) [a]				
(3) s-tDCS/a-IMS [c] (n= 15)	6.43 (3.38)	vs. 3.76 (2.80)	2.67 (0.28 to 5.82) [a]				
(4) s-tDCS/s-IMS [d] (n= 15)	4.73 (1.37)	vs. 2.95 (1.38)	1.78 (0.55 to 3.02) [b]				
WOMAC score							
(1) a-tDCS/a-IMS [a] (n= 15)	27.31 (23.19)	vs. 54.75 (19.80)	-27.44 (-38.41 to -16.46)[a,b]	8.48	3	0.56	0.03
(2) a-tDCS/ s-IMS [b] (n= 15)	32.50 (21.61)	vs. 52.36 (20.35)	-19.14 (-28.28 to -10.00)a				
(3) s-tDCS/a-IMS [c] (n= 15)	22.13 (13.22)	vs. 54.13 (18.56)	-30.00 (-39.29 to -20.70)[a,b]				
(4) s-tDCS/s-IMS [d] (n= 15)	46.60 (16.42)	vs. 50.47 (13.74)	-5.20 (-15.03 to 4.63)				

*Df = degrees of freedom; * P<0.05 indicates significant differences between treatment in the estimated marginal means adjusted for multiple comparisons by Bonferroni test.*

Pairwise comparisons of predicted marginal means were performed according to the concept of least-squares means. Significant P-values are indicated in bold. CI, confidence interval.

The Cramer's V was used as a measure of effect size for Wald qui-square tests. The size effect was interpreted as follows: Standards for interpreting Cramer's V as proposed by Cohen (1988) are the following:

DF=1 (0.10 = small effect); (0.30 = medium effect); (0.50=large effect)

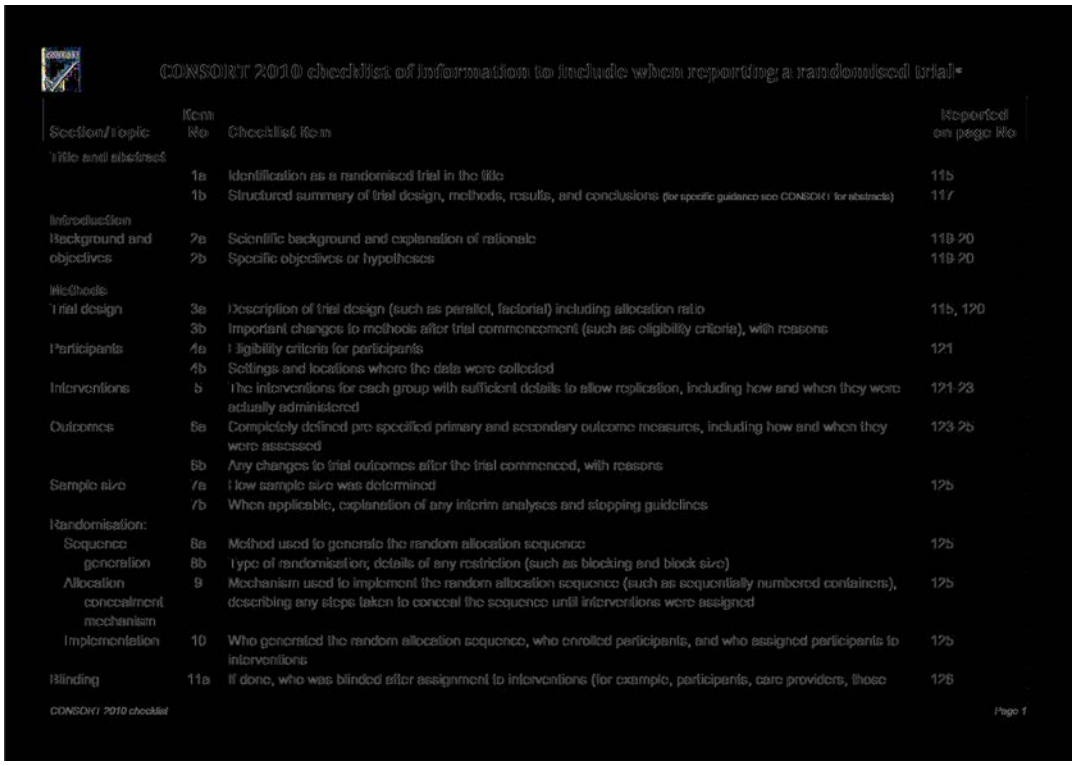
DF=2 (0.07 = small effect); (0.21= medium effect); (0.35 = large effect)

DF=3 (0.06 = small effect); (0.17 = medium effect); (0.29 = large effect)

Table 3. Comparisons between the serum BDNF between groups before at the end of treatment. Data are presented as mean (SD) and median interquartile (n=60).

	Median	Quartile (25 -75)	P	Median	Quartile (25 -75)	P
(1) a-tDCS /a-IMS (n= 15)	27.89	(14.83 ; 59.57)	0.56	18.69	(4.47 ; 35.00)	0.58
(2) a-tDCS / s-IMS (n= 15)	30.15	(6.18 ; 57.43)		19.96	(10.18 ; 42.00)	
(3) s-tDCS / a-IMS (n= 15)	24.95	(13.52 ; 38.68)		21.70	(5.99 ; 50.00)	
(4) s-tDCS /s-IMS (n= 15)	23.87	(11.76 ; 42.69)		18.11	(5.20 ; 49.68)	

**Median were compared using the Kruskal Wallis Test*



CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	115
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	117
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	118-20
	2b	Specific objectives or hypotheses	118-20
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	115, 120
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	
Participants	4a	Eligibility criteria for participants	121
	4b	Settings and locations where the data were collected	
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	121-23
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	123-25
	6b	Any changes to trial outcomes after the trial commenced, with reasons	
Sample size	7a	How sample size was determined	125
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	125
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	
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	125
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	125
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	126

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	126-27
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	126-27
Results			
Participant 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	127, 143
	13b	For each group, losses and exclusions after randomisation, together with reasons	127, 143
Recruitment	14a	Dates defining the periods of recruitment and follow-up	
	14b	Why the trial ended or was stopped	
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	146
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	
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)	127-29, 147
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	130, 148
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	134
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	134
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	
Other information			
Registration	23	Registration number and name of trial registry	118
Protocol	24	Where the full trial protocol can be accessed, if available	
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

10 Considerações finais

Os resultados mostraram que as pacientes com dor crônica devida à osteoartrite de joelhos apresentam sistema inibitório descendente de dor enfraquecido, observados através do potencial evocado motor aumentado e do período silente reduzido. Bem como, ativação reduzida do sistema endógeno de modulação da dor, demonstrado através da pequena mudança na modulação condicionada de dor.

No segundo estudo observamos a possibilidade de reforço do sistema inibitório corticoespinhal no processamento da dor cortical e infracortical através da estimulação intramuscular. Obtivemos redução do potencial evocado motor, da facilitação intracortical e aumento do período silente. Além de aumento do limiar de dor á pressão e redução da escala visual analógica de dor. A relação do BDNF foi inversa ao limiar de dor á pressão.

O terceiro estudo encontrou melhora significativa e sustentada durante o seguimento de três meses com a utilização de estimulação intramuscular e estimulação transcraniana de corrente contínua. As cinco sessões de tratamento com abordagens central (estimulação transcraniana de corrente contínua) e periférica (estimulação intramuscular) mostraram uma boa capacidade de reforço do sistema inibitório descendente de dor e da homeostase do sistema excitatório.

11 Perspectivas futuras

A osteoartrite de joelhos é altamente prevalente. Com o envelhecimento da população a incidência tende a aumentar. É uma patologia incapacitante, prejudicando a funcionalidade dos portadores. Multifatorial, tendo o processo inflamatório como principal agente de piora progressiva. Embora existam várias alternativas de tratamento conservador, ainda não há uma possibilidade realmente eficaz na melhora dos sinais e sintomas.

O primeiro estudo evidenciou o sistema inibitório descendente de dor enfraquecido, achado até então não demonstrado nessa patologia. Corroborando os resultados encontrados em outas patologias de dor crônica. Também de forma inédita obtivemos a evidência de reforço do sistema inibitório descendente de dor através da estimulação elétrica intramuscular. Por fim, através de abordagem central e periférica nossos resultados mostraram melhora álgica e funcional significativa e duradoura. Os resultados são bastante animadores, abrindo a possibilidade de um tratamento conservador eficaz, seguro e de baixo custo.

A partir dos resultados desses estudos criaram-se subsídios para novas pesquisas com um tempo de tratamento e seguimento mais prolongados, visando melhora ainda mais expressiva e permitindo às pacientes um tempo maior livre de dor. A possibilidade de avaliação e tratamento prévios ao tratamento cirúrgico, visando resultados melhores de alívio de dor e ganho de funcionalidade no pós operatório também é uma possibilidade a ser investigada. Possibilidades essas alinhadas com o Laboratório de Dor e Neuromodulação do HCPA-UFGRS.

Os estudos desta tese inseriram-se no projeto temático deste Laboratório, cujo alvo das investigações é a integração dos diversos mecanismos neurobiológicos, diagnósticos e terapêuticos usando técnicas de neuromodulação, e que **visam gerar conhecimento** para alicerçar novas políticas de assistência e treinamento no manejo da dor aguda e crônica.

Essa abordagem integra a pesquisa à assistência envolvendo uma equipe de saúde multidisciplinar e permite estabelecer as interfaces da assistência ao paciente com dor e com a educação da equipe de saúde, tendo como prioridade o diagnóstico e tratamento aplicados. Colabora assim, para a concretização da aplicação do conhecimento aos pacientes, cria uma estrutura funcional para que o conhecimento gerado seja transferido ao paciente e à sociedade e auxilia na elaboração de protocolos clínicos de diagnóstico e de tratamento. Este conjunto de ações visa qualificar o sistema

de saúde com terapêuticas eficazes, baseadas no processo fisiopatológico e suportadas por desfechos clínicos relevantes aos pacientes e à sociedade. Desta forma, buscou-se contribuir com o desenvolvimento dos grupos de pesquisa envolvidos, colaborando tanto qualitativa quanto quantitativamente com o desempenho científico e tecnológico. Com isto, auxiliar o fortalecimento da pesquisa brasileira no cenário nacional e internacional.

12 Apêndices

12.1. Apêndice I

PROTOCOLO DO PRIMEIRO ESTUDO

DOR & NEUROMODULAÇÃO - HCPA/CNPq (subárea 2.10.08.00-0) PROJETO 11-0013

Nome: _____N° Banco:_____ Data:_____Fone residencial:_____Celular:_____	
Demográfico:	
WOMAC:	
WHOQOL-BREF:	
Beck:	
Pittsburgh:	
Catastrófico:	
EAV dor:	
EAV sono:	
EAV humor:	
EAV ansiedade:	
BDNF:	
Excitabilidade cortical: MT:	
	MEP:
	CSP:
	ICF:
	SICI:
Algometria de pressão:	
CPM:	
Cegamento:	

12.2. Apêndice II

PROTOCOLO DO SEGUNDO ESTUDO

DOR & NEUROMODULAÇÃO - HCPA/CNPq (subárea 2.10.08.00-0) PROJETO 11-0013

Nome:_____N° banco:_____ Data:_____ Fone residencial: _____ Celular:_____
Demográfico : WOMAC: WHOQOL BREF: BECK: PITTSBURGH: Catastrófico:

EAV dor: EAV sono: EAV humor: EAV ansiedade: BDNF: Excitabilidade cortical: MT: MEP: CSP: ICF: SICI: Algometria de pressão: CPM:
Tratamento: Excitabilidade cortical: MT: MEP: CSP: ICF: SICI: Algometria de pressão: CPM: EAV dor: Cegamento:

12.3. Apêndice III

PROTOCOLO DO TERCEIRO ESTUDO

DOR & NEUROMODULAÇÃO - HCPA/CNPq (subárea 2.10.08.00-0) -PROJETO 11-0013

Nome:_____N° banco:_____ Data:_____ Fone residencial: _____ Celular:_____ Demográfico : WOMAC: WHOQOL BREF: BECK:

PITTSBURGH:					
Catastrófico:					
EAV dor:					
EAV sono:					
EAV humor:					
EAV ansiedade:					
Funcional de dor:					
BDNF:					
Cortisol:					
CPM:					
Algometria de pressão:					
Tratamento	Dia 1	Dia 2	Dia 3	Dia 4	Dia 5
EAV dor inicial:					
Efeitos adversos:					
EAV dor final:					
WOMAC:					
WHOQOL BREF:					
BECK:					
PITTSBURGH:					
Catastrófico:					
EAV dor:					
EAV sono:					
EAV humor:					
EAV ansiedade:					
Funcional de dor:					
BDNF:					
Cortisol:					
CPM:					
Algometria de pressão:					

12.4. Apêndice IV

Termo de Consentimento Informado Livre e Esclarecido

Nome do estudo: EFEITO DA ESTIMULAÇÃO TRANSCRANIANA DE CORRENTE CONTÍNUA E DA ELETROESTIMULAÇÃO INTRAMUSCULAR NA DOR, NA CAPACIDADE FUNCIONAL E NA EXCITABILIDADE CORTICAL DE PACIENTES COM OSTEOARTRITE

Número do protocolo:_____

Instituição: Universidade Federal do Rio Grande do Sul.

Pesquisador Responsável: Professor Dr. Wolnei Caumo, F. 99813977.

Comitê de pesquisa e Ética em saúde do HCPA: (51) 3359-8304.

Nome do participante:_____

Você está sendo convidada a participar de um estudo que utilizará dois tipos de tratamento. Um cujo estímulo é realizado na cabeça, denominado Estimulação Transcraniana de Corrente Contínua e o outro é um tipo de acupuntura chamada Estimulação intramuscular. Ambos serão utilizados para tratar a dor da osteoartrite de joelhos.

1- Objetivos deste estudo:

Avaliar se o uso de uma técnica de estimulação na cabeça ou na região lombar baixa e nos músculos doentes podem reduzir a dor da osteoartrite de joelho.

2- Explicação dos procedimentos:

A senhora terá que responder perguntas a respeito de sua dor, atividades, qualidade de vida e de sono.

Após a assinatura deste termo a senhora será sorteada para um dos tratamentos:

Grupo 1: 5 sessões consecutivas de: estimulação intramuscular e estimulação no crânio.

Grupo 2: 5 sessões consecutivas de: estimulação intramuscular sem estímulo ativo e estimulação do crânio sem estímulo ativo.

Grupo 3: 5 sessões consecutivas de: estimulação no crânio e estimulação intramuscular sem estímulo ativo.

Grupo 4: 5 sessões consecutivas de: estimulação no crânio sem estímulo ativo e estimulação intramuscular.

As aplicações na cabeça serão realizadas por meio de aparelho cujo estímulo é indolor, sem choques, cortes ou cirurgias, o aparelho causa uma vibração ativando o cérebro e promovendo alívio da dor, a aplicação dura 30 minutos. Na acupuntura, será aplicado um estímulo por meio de aparelho conectado às agulhas, que serão colocadas na região da coluna lombar e nos músculos doentes. Estas sessões também terão duração de 30 min. As aplicações serão realizadas diariamente até completar cinco sessões. A senhora poderá usar paracetamol para dor caso necessite. As perguntas sobre o nível de dor, qualidade de vida, sono e sintomas depressivos serão realizadas durante o tratamento e até três meses depois. Também serão coletadas duas amostras de sangue: antes do começar o tratamento e após a última sessão. O sangue será usado para medirmos marcadores que poderão avaliar o efeito do tratamento. Este material será utilizado nesta pesquisa ou em outras que eventualmente venha a se necessitar (segundo item 9).

As amostras podem ficar congeladas por até 5 anos no laboratório de Pesquisa Clínica do Hospital de Clínicas de Porto Alegre.

3- Possíveis riscos e desconfortos:

Responder ás perguntas dos questionários.

Leve dor da picada das agulhas para coleta de sangue e de acupuntura.

Eventual inchaço ou pequena mancha roxa no local da coleta do sangue.

Leve dor na região lombar onde serão colocadas as agulhas.

Leve coceira, formigamento, dor na cabeça por alguns minutos após aplicação do tratamento.

Alteração de humor após a aplicação na cabeça.

Sonolência após as aplicações.

4- Possíveis benefícios deste estudo:

Melhora de sua dor por um tempo ainda não bem conhecido, mas por pelo menos três meses.

Caso algum ou ambos os tratamentos funcionem, isto poderá trazer benefício à senhora e a outras pacientes com quadro similar.

5- Direito de desistência:

A senhora pode desistir de participar a qualquer momento. Sua decisão de não participar ou de deixar a pesquisa depois de iniciada, não afetará qualquer atendimento médico posterior na presente instituição.

6- Privacidade:

Todas as informações obtidas no presente estudo poderão ser publicadas com finalidade científica, preservando os dados de identificação. Para manutenção do sigilo relacionado ao material coletado será criado um código para sua identificação.

Caso deseje, você poderá ter acesso a seus dados, assim como tem direito de retirar o seu consentimento a qualquer momento. Sendo necessário apenas informar ao pesquisador sua vontade.

7- Contato dos Pesquisadores:

Caso a Sra. tenha alguma dúvida ou queira algum esclarecimento poderá entrar em contato com os pesquisadores através dos telefones: Prof ° Wolnei F 9981-3977 (2° andar do HCPA Laboratório de Cronobiologia – sala 2201 E- f. 3359-8083) e MD Maria da Graça Lopes Tarragó, f. 9917-8023, Serviço de Fisiatria do HCPA, zona 2, f. 3359-8430/3359-8556 ou ainda o Comitê de Ética do Hospital de Clínicas- f. 3359-8304.

8- Ressarcimento de despesas:

A Sra. não terá despesas com a sua participação na pesquisa.

9- Armazenamento de Materiais Biológicos:

O material biológico coletado, como sangue e saliva será armazenado sob a responsabilidade da instituição depositária, a qual deverá ter norma ou regulamento aprovado pelo CEP dessa instituição. Este material poderá ser utilizado em pesquisas futuras, se houver necessidade. Desta forma, se faz necessária a obtenção de seu consentimento para uso em novo projeto de pesquisa. Caso a Sra. Tenha interesse em saber informações como, por exemplo, resultados de exames para acompanhamento clínico, estes lhe serão fornecidos.

10- Consentimento:

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

_____

Assinatura do entrevistado

_____

Assinatura da testemunha

_____

Assinatura do pesquisador responsável

Porto Alegre, de 20 .