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TESE DE DOUTORADO

Avaliação do Efeito da administração aguda pregabalina na ativação do córtex somatosensitivo e motor esquerdo de fibromialgicas por meio da Espectroscopia Infravermelha funcional (fNIRS)

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“Caminante, son tus huellas, el camino y nada más;

Caminante, no hay camino, se hace camino al andar.

Al andar se hace el camino, y al volver la vista atrás

se ve la senda que nunca, se ha de volver a pisar.

Caminante no hay camino, sino estelas en la mar.”

Antonio Machado

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RESUMO

A Fibromialgia (FM) é uma síndrome que se caracteriza por dor crônica difusa, fadiga, transtornos do sono e alterações de humor. Embora sua fisiopatologia não esteja totalmente elucidada, o processo neurobiológico parece envolver alterações do córtex sensitivo e motor e de suas conexões com estruturas subcorticais que constituem a neuromatriz da dor, assim como alterações neuropáticas periféricas. Sabe-se que o aumento do cálcio intracelular acima de certo limiar, pode ser parte do processo dependente de atividade que leva à sensibilização central, por aumento do influxo de cálcio por meio de canais NMDA, AMPA, canais dependentes de voltagem, e por liberação de reservas intracelular microssomais. A sensibilização central pode ser também interpretada como um processo de plasticidade mal-adaptativa que sustenta circuitos da dor e de seus correlatos. Por tanto, o córtex sensitivo e motor tem sido alvo diagnóstico e terapêutico para o estudo e tratamento da dor crônica.

Dentre as múltiplas estratégias farmacológicas tem sido preconizado o uso da pregabalina, aprovado pela *Food and Drug Administration* (FDA) dos EUA para uso no tratamento de fibromialgia em 2007. A pregabalina age inibindo os canais de cálcio pré sinápticos dependentes de voltagem por se ligar à proteína auxiliar alfa-2-delta. *In vitro*, este fármaco reduz a liberação de neurotransmissores cálcio-dependentes incluindo glutamato, norepinefrina, calcitonina e substância P, estes neurotransmissores têm sido associados à sensibilização do sistema nervoso. Portanto, considerando o potencial neuromodulador da pregabalina baseado no seu mecanismo de ação, é um fármaco atrativo para avaliar o papel da modulação do córtex sensitivo e motor na fisiopatolgia da FM. No entanto, para que se avance no conhecimento do efeito dos

fármacos na função encefálica, necessitamos utilizar recursos de neuroimagem que sejam exequíveis em contextos diversos, e que permitam mensurar o efeito dos fármacos dinamicamente.

Dentre os recursos de imagem existe a *Functional Near Infrared Spectroscopy* (fNIRS) que permite avaliar ativação cortical por meio da mudança no consumo local de oxigênio que acompanha o disparo neuronal regional, mensurado pelas mudanças na concetração da oxi- e desoxi-hemoglobina. Com estas considerações, hipotetizamos que a modulação farmacológica induzida pela pregabalina poderia ser mensurada, clinicamente por meio de testes psicofísicos da dor (que avaliam vias nociceptivas associadas a termoreceptores e barorreceptores) e à nível de neuroimagem por meio do fNIRS. Por se tratar de um mesmo sistema com potencial de ser avaliado de forma virtualmente simultânea, hipotetizamos também que existirá uma associação entre as modulações clínicas (testes psicofísicos) e neurológicas (fNIRS do córtex sensitivo e motor primários). Desta forma, neste estudo avaliou-se o efeito de pregabalina (150 mg) em dose única em fibromiálgicas e controles saudáveis. Em ambos os grupos a pregabalina foi comparada ao placebo, num desenho de estudo randomizado, duplo-cego, cruzado. Avaliou-se o efeito das intervenções, intra e inter-grupos, na ativação cortical de maneira indireta, pela concetração da oxi-hemoglobina durante testes psicofísicos da dor por meio meio do *Quantitative Sensory Testing (QST)* e algometria de pressão, que foram comparados com a ativação cortical durante uma tarefa motora de percussão dos dedos da mão (*left hand finger tapping*). Foram estudadas mulheres com idade entre 18 e 65 anos, 17 fibromiálgicas e 10 controles saudáveis. Os parâmetros do QST foram avaliados uma hora após dose única de 150 mg de pregabalina.

Resultados: Na linha de base, as fibromiálgicas apresentaram alterações no QST sugestivas de lesão de fibras finas: o limiar de detecção de calor (HDT, do inglês *Heat Detection Threshold*) foi maior que nas controles (35,53 ºC ± 3,22 vs. 33,33 ºC ± 0,85; p<0,05), enquanto o limiar de dor por pressão (PPT, do inglês *Pain Pressure Threshold*) foi menor (2,44 kg/cm^2 ± 1,08 vs. 4,32 kg/cm^2 ± 1,45; P<0,01). Não foram observadas diferenças nos outros componentes do QST, nem mudanças com a pregabalina. Quando comparados com as saudáveis, nas fibromiálgicas o HDT, limiar de dor por calor (HPT) e a tolerância ao calor (HT) evocaram activação nos giros frontal médio, precentral e póscentral, porém, de menor amplitude do que as controles. Depois da administração da pregabalina, aumentou a ativação em responsta ao HDT, mas não teve correlação com o valor do limiar. Já o HPT mostrou se correlacionar de forma inversa com a ativação nos giros frontal superior (r_s=-0,552, p=0,033) e precentral (r_s=-0,545, p=0,036) na linha de base e após pregabalina (r_s=-0,52, p=0,047). A HT também apresentou uma correlação inversa com os giros frontal superior (r_s=-0,645, p=0,032) e precentral (r_s=-0,655, p=0,029), mas neste caso, esta correlação desapareceu após ter recebido pregabalina. A ativação cortical pelo PPT não detectou diferenças entre fibromiálgicas e controles.

Conclusões: O perfil nos testes psicofísicos nas pacientes apresenta correlação com sua ativação cortical. As alterações nos testes sugerem alterações de fibras finas nociceptivas, o que é explicado por um componente de neuropatia periférica, que na fibromialgia é acompanhado por diminuição da ativação em áreas sensitivas e motoras, e aumento da ativação em áreas associadas com processamento cognitivo da dor, cuja atividade foi elevada com a pregabalina. Quando comparadas às controles, nas fibromiálgicas a HT recrutou mais áreas associada ao processamento cognitivo da dor, o

que fortalece a hipótese a favor da existência do componente de sensibilização central na fibromialgia. Desta forma, estes achados reforçam a provável coexistência de alterações periféricas e centrais na fisiopatologia da fibromialgia.

Palavras chave: fibromialgia, pregabalina, espectroscopia infravermelha funcional, teste sensitivo quantitativo.

ABSTRACT

Fibromyalgia is a syndrome characterized by presenting chronic diffuse pain, fatigue, mood and sleep disturbances. Although its pathophysiology has not been totally elucidated yet, the neurobiological processes seems to involve funciontal alterations of the sensorimotor cortex and its conections with subcortical structures (related to the pain neuronal matrix), and also, with quantitative and qualitative alterations in fine sensitive fibers from the peripheral nervous system. It is known that increased intracellular calcium above certain threshold might be part of a process activity-dependant that leads to central sensitization, due to elevated calcium influx through NMDA and AMPA channels, as well as voltage-dependent channels, and also due to relase of intracellular microsomal reserves. Central sensitization can also be interpreted as a maladaptive plasticity that sustains pain circuits and its correlated. Thus, the sensorimotor cortex has been a diagnostic and therapeutic target for the study and treatment of chronic pain.

Among the multiple pharmacological strategies, the use of pregabalin has been recommended and approved by the Food and Drug Administration (FDA) of the United States of America for treatment of patients with fibromyalgia since 2007. Pregabalin acts by inhibiting voltage-dependant pre-synaptic calcium channels by binding to the auxiliary protein alfa-2-delta. *In vitro*, this drug reduces the liberation of neurottransmissors that depend on calcium, and that include glutamate, norepinephrine, calcitonin and P-substance. All the latter mentioned neurotransmitters are associated with the central nervou system sensitization. Thus, considering its potential as neuronal modulation, taking into accout tis mechanisms of action, the pregabalin is an appealing

drug to study the role of the modulation fo the sensorimotor cortex in the pathophysiology of fibromyalgia. Nevertheless, to incrase the knowledge about the effect of drugs on the cortical function, we need to use feasible neuroimaging resources able to be applied in diverse contexts, and that allow to measure the effect of the drugs in real time.

Among the neuroimaging resources, there is the *Functional Near Infrared Spectroscopy* (fNIRS), which allows to assess cortical activation estimating the uptake of regional oxygen, that accompanies local neuronal firing. The fNIRS measures changes in the concentration of oxy and desoxy hemoglobin. Given these considerations, we hypothezise that the pharmacological modulation induced by pregabalin could be measured, clinically through psychophysical pain testing, and at the neuroimaging level using fNIRS. Given that it is about the same system with the potential to be assessed in complementary and virtually simultaneous ways, we also hypothesize that there still could exist an association between the clinical modulation (psychophysical tests) and cortical sensorimotor activation (assessed by fNIRS). In this way, this study appraised the effect of a single dose of pregabalin (150 mg) in the cortical activation and psicophysical pain testing in fibromyalgic and in healthy subjects. In both groups, pregabalin was compared to placebo, in a randomized, double-blinded, cross-over trial design. We assessed the effect of pregabalin, within and between-groups, on the cortical activity in an indirect way via the changes in oxy-hemoglobin upon heat and pressure stimuliation inside a protocol of QST, and also compared the psychophysical pain tests results with the performance during a Left Hand Fingertapping Task. We studied women aging 18 to 65, 17 of them with fibromyalgia

and 10 healthy controls. QST parameters were assessed one hour after a single dose of 150 mg of pregabalin.

Results: At baseline, patients with fibromyalgia presented QST alterations suggestive of fine nerve fibers lesion: baseline HDT was higher in fibromyalgia (35.53±3.22 vs. 33.33±0.85, P<0.05), while PPT was lower (2.44±1.08 vs. 4.32±1.45, P<0.01) than healthy volunteers, but did not change with pregabalin. When compared to healthy subjects, HDT, HPT, and HT evoked smaller activation in the middle frontal, pre- and post-central gyri in fibromyalgia, that increased after pregabalin (only for HDT-induced activation), but that was not correlated to the HDT. HPT was inversely correlated to the activation in the superior frontal (r_s=-0.552, p=0.033) and precentral gyri (r_s=-0.545, p=0.036), remaining unchanged after pregabalin (r_s=-0.52, p=0.047). HT was inversely correlated to the middle frontal (r_s=-0.645, p=0.032) and precentral gyri activation (r_s=-0.655, p=0.029), but was no longer correlated after pregabalin. PPT cortical activation did not differ between fibromyalgia and healthy volunteers.

Conclusions: The psychophysical pain testing profile in fibromyalgia has a cortical correlate. Alterations in tests for small fibers support its probable peripheral neuropathic component, and was accompanied by decreased activation in sensorimotor areas but increased in pain-related cognitive processing cortexes, and whose activity is increased by pregabalin. Also, upon HT fibromyalgia patients recruited more areas related to pain cognitive processing, which could favor the hypothesis of a component of central sensitization in fibromyalgia, and which was poorly modulated by pregabalin.

Taken together, these findings support the co-existence of both, peripheral and central alterations in fibromyalgia.

Keywords: fibromyalgia, fNIRS, pain testing, pregabalin.

LISTA DE TABELAS DO ARTIGO CIENTÍFICO

LISTA DE FIGURAS DA TESE

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LISTA DE SIGLAS E ABREVIATURAS

°C – Graus Celcius

μs – microssegundos

μV – Microvolt

^{1}H-MRS – Espectroscopia por ressonância de prótons, do inglês *^{1}H Magnetic Ressonance Spectroscopy*)

ANOVA – Análise de variância, do inglês *Analysis of Variance*

AUC – Área sob a curva, do inglês *Area Under the Curve*

BDI-II – Inventário de depressão de Beck – II, do inglês *Beck Depression II*

BDNF – Fator neurotrófico derivado do cérebro, do inglês *Brain-derived Neurotrophic Factor*

B-PCP:S – Escala funcional da dor, do inglês *Brazilian Profile of Chronic Pain: Screen*

CNS – Conselho Nacional de Saúde

CPC – Centro de Pesquisas Clínicas

CPE – Centro de Pesquisa Experimental

CPM – Modulação condicionada da dor, do inglês *Conditioned Pain Modulation*

CPT – Tarefa de pressão sob frio (do inglês *Cold Pressor Task*)

EA – Efeitos Adversos

EAV – Escala análoga visual

ELISA – Ensaio de imunoabsorção ligado a enzima, do inglês *Enzyme Linked Immuno Sorbent Assay*

FM – Fibromialgia

fNIRS – Espectroscopia infravermelha funcional, do inglês *functional near infrared spectroscopy*

GABA – Ácido gama-aminobutírico, do inglês *gamma-aminobutyric acid*

Glx – glutamato/glutamina

HbO – Hemoglobina oxigenada, Oxi-hemoglobina

HbR – Hemoglobina desoxigenada, Desoxi-hemoglobina

HCPA – Hospital de Clínicas de Porto Alegre

HDT – Limiar de detecção de calor, do inglês *Heat Detection Threshold*

^{1}H-MRS (do inglês ^{1}*H Magnetic Ressonance Spectroscopy*).

HPT – Limiar de dor por calor, do inglês *Heat Pain Threshold*

HT- Tolerância ao calor, do inglês *Heat Tolerance*

IDATE – Inventário de ansiedade traço-estado

LTD – Depressão ao longo prazo, do inglês *Long Term Depression*

LTP – Potenciação ao longo prazo, do inglês *Long Term Potentiation*

M1 – Córtex motor primário

mA – Milliampere

min – Minutos

MINI – Entrevista neuropsiquiátrica internacional reduzida, do inglês *Mini-international neuropsychiatric interview*

nm - nanometro

PSQI – Indicador de qualidade do sono de Pittsburgh, do inglês *Pittsburgh Sleep Quality Index*

QIF – Questionário de impacto da fibromialgia

REDCap – do inglês *Research Electronic Data Capture*

seg – segundos

PPT – Limiar de dor à pressão, do inglês *Pressure Pain Threshold*.

TCLE – Termo de consentimento livre e esclarecido

QST – Teste sensorial quantitativo, do inglês *Quantitative Sensory Testing*

UAMP – Unidade de Análises Moleculares e de Proteínas

UFRGS – Universidade Federal do Rio Grande do Sul

INDICE

APRESENTAÇÃO

Esta tese sera estruturada em seis capitulos

Capítulo I – Introdução

Capítulo II – Revisão sistematizada da literatura

Capítulo III – Jutificativa, marco conceitual e objetivos

Referências da revisão da literatura (não constitui um capítulo)

Capítulo IV – Artigo científico

Capítulo V – Considerações finais e perspectivas futuras

Capítulo VI - Anexos

CAPÍTULO I – INTRODUÇÃO

A fibromialgia (FM) é uma síndrome de dor crônica que atinge aproximadamente 2% da população dos Estados Unidos, sendo responsável pelo impacto negativo ao indivíduo e à sociedade [1,2], pois além das alterações na percepção da dor, os pacientes apresentam sono não reparador, cansaço, e alterações cognitivas, o que fortalece a linha de pensamento moderna da doença como sendo decorrente de alterações do sistema nervoso central [3]. Na FM, as redes neurais responsáveis pelo processamento da dor sofrem mudanças consideradas mal adaptativas [4], as quais apresentam potencial de serem modificadas por meio de estratégias multimodais [5], que incluem fármacos [6], mudanças comportamentais [7], atividade física e meios físicos (calor terapêutico) [8]. De forma consistente, as estratégias terapêuticas são multifatoriais, objetivando o alívio sintomático, e portanto, o alvo é modificar a plasticidade mal adaptativa nas vias de processamento da dor. Associadas a estas, tem sido proposto intervenções não farmacológicas para revertê-las, incluindo terapia cognitivo comportamental, técnicas de relaxamento, estimulações periféricas como eletroacupuntura e estimulações transcranianas de corrente contínua e magnética [7]. Também, anticonvulsivantes e antidepressivos tem sido preconizados, alguns deles com suporte científico suficiente, como a pregabalina, aprovada pela *Food and Drug Administration* (FDA) dos EUA para uso no tratamento de fibromialgia em 2007.

A pregabalina demonstrou ser capaz de reduzir a liberação de diversos neurotransmissores cálcio-dependentes, incluindo glutamato, norepinefrina, calcitonina e substância P [9]. Diferentes estudos experimentais tem demostrado o seu efeito modulador duradouro ao longo do tempo na fibromialgia [10,11], no entanto, são escassos os estudos sobre o mecanismo de ação deste fármaco *in vivo*, num contexto de vida real

[12–14]. Dentre os poucos estudos existentes, um deles comparou o efeito da pregabalina na ativação cortical em resposta ao estimulo doloroso, usando ressonância magnética funcional (*f*MRI) em mulheres fibromialgias, comparadas com saudáveis sem tratamento. O estudo demonstrou efeito no giro supramarginal, giro frontal superior e inferior, giro temporal médio, tálamo, cerebelo e córtex calcarino[14]. Outro estudo experimental, *crossover,* com pacientes fibromiálgicas avaliou o efeito de 450 mg de pregabalina diários, durante 14 dias. O efeito foi avaliado com técnicas de neuroimagem combinadas *f*MRI, espectroscopia de prótons e análise de conectividade funcional, mostrou que a pregabalina diminui os níveis de glutamato+glutamina na ínsula posterior, assim como da conectividade funcional nas áreas de funcionamento em repouso do cérebro [13]. Usando estes métodos de avaliação é possível observar *in vivo* os mecanismos de ação de um fármaco em sistemas modificados pela doença, destacando que a fMRI fornece informações detalhadas sobre a localização da estrutura envolvida nas respostas, mas com uma baixa precisão na avaliação da função da estrutura estudada, além do elevado custo para sua realização, fazendo deste um exame de acesso restrito.

Na busca por alternativas mais acessíveis na avaliação do córtex cerebral, devido ao seu melhor balanço entre acesso à área cortical e sua respectiva função, a fNIRS, ou espectroscopia com método óptico de neuroimagem funcional, surge como candidata por preencher os critérios de dinamicismo e practicidade desejáveis. Esta técnica permite o mapeamento funcional do córtex do cérebro em tempo real, *in vivo*, em humanos e em animais, fornecendo informação sobre atividade neuronal, que é inferida por mudanças hemodinâmicas que às acompanham [15]. A fNIRS utiliza níveis seguros de luz, que podem ser emitidas por laser ou por LED, mas com comprimentos de onda no espectro próximo ao infravermelho, entre 650 e 950 nm, e que penetra (até

2cm) no tecido biológico, permitindo atingir o córtex cerebral e interagir com oxi- e deoxi-hemoglobina do sangue regional. Medindo-se a intensidade da luz que emerge do crânio por meio de detectores colocados sobre o escalpo, pode-se inferir, de forma não invasiva, a variação do nível de oxigenação do tecido cerebral, permitindo a confirmação da localização das regiões de ativação.

Embora a FM seja uma patologia com grande impacto na vida dos pacientes, sua fisiopatologia não está totalmente elucidada, evidências recentes sugerem que o processo neurobiológico inclui alterações funcionais do córtex motor e de suas conexões com estruturas subcorticais que constituem a neuromatriz da dor[16], assim como alterações quantitativas e qualitativas em fibras finas sensitivas do sistema nervoso periférico[17]. Além das alterações periféricas existe um processo de desinibição central. Sabe-se que o aumento de cálcio intracelular é um mecansimo central no desencadeamento e na manutenção da hiperexcitilidade neuronal, que se sustenta pelo desequilíbrio nos mecanismos de excitação e de inibição, os quais induzem processos de plasticidade mal-adaptativa que sustenta circuitos reverberantes da dor e de seus correlatos[18]. Então, o córtex sensitivo e motor tem sido alvo diagnóstico e terapêutico no tratamento da dor.

Dentre as múltiplas estratégias farmacológicas tem sido preconizado o uso da pregabalina, aprovado pela *Food and Drug Administration* (FDA) dos EUA para uso no tratamento de fibromialgia. A pregabalina age inibindo os canais de cálcio pre sinápticos dependentes de voltagem, com ligação à proteína auxiliar alfa-2-delta. *In vitro*, este fármaco reduz a liberação de neurotransmissores cálcio-dependentes incluindo glutamato, norepinefrina, calcitonina e substância P, tendo sidos estes neurotransmissores associados à sensibilização do sistema nervoso[9]. Portanto, considerando seu potencial neurmodulador baseado neo seu mecanismo de ação, ela é

um fármaco atrativo para estudar o papel da modulação do córtex sensitivo e motor na fisiopatolgia da FM.

No entanto, para que se avance no conhecimento do efeito dos fámarcos na função cortical, se faz necessário utilizar recursos de neuroimagem que sejam acessíveis em diversos contextos, e que permitem mensurar o efeito dos fármacos em tempo real. Dentre os recurso de imagem existe a *Functional Near Infrared Spectroscopy* (fNIRS) que permite avaliar ativação cortical por meio da mudança no consumo local de oxigênio que acompanha o disparo neuronal regional, mensurado pelas mudanças na concentração da oxi- e desoxi-hemoglobina[15,19]. Com estas considerações, hipotetizamos que a modulação farmacológica induzida pela pregabalina, poderia ser mensurada clinicamente por meio de testes psicofísicos da dor, e à nível de neuroimagem por meio do fNIRS. Por se tratar de um mesmo sistema com potencial de ser avaliado de forma virtualmente simultânea, hipotetizamos também uma associação entre os parâmetros clínicos (testes psicofísicos) e neurológicas (fNIRS no córtex sensitivo e motor primários). Desta forma, neste estudo avaliou-se o efeito de pregabalina (150 mg) em dose única em fibromiálgicas e controles saudáveis. Em ambos os grupos a pregabalina foi comparada ao placebo, num estudo randomizado, duplo-cego, cruzado. Avaliou-se o efeito das intervenções, intra e inter-grupos, na ativação cortical de maneira indireta, pela concentração da oxi- hemoglobina durante testes psicofísicos da dor por meio meio do *Quntitative Senory Testing (QST)* e algometria de pressão, que foram comparados à ativação cortical durante uma tarefa motora de percussão dos dedos da mão (*left hand finger tapping*). Esta tese originou um artigo que esta formatado para ser submetido a *Neuroimage*.

CAPÍTULO II - REVISÃO SISTEMATIZADA DA LITERATURA

1. ESTRATÉGIAS PARA LOCALIZAR E SELECIONAR AS INFORMAÇÕES

Na busca literária, ressaltou-se os principais aspectos relacionados à FM, Pregabalina e a espectroscopia infravermelha funcional (fNIRS). A estratégia de busca envolveu as seguintes bases de dados: LILACS, PubMed e EMBASE, sem restrição de data de publicação. Abaixo encontra-se o fluxograma (Figura 1) representando o número de artigos disponíveis nas bases de dados pesquisadas.

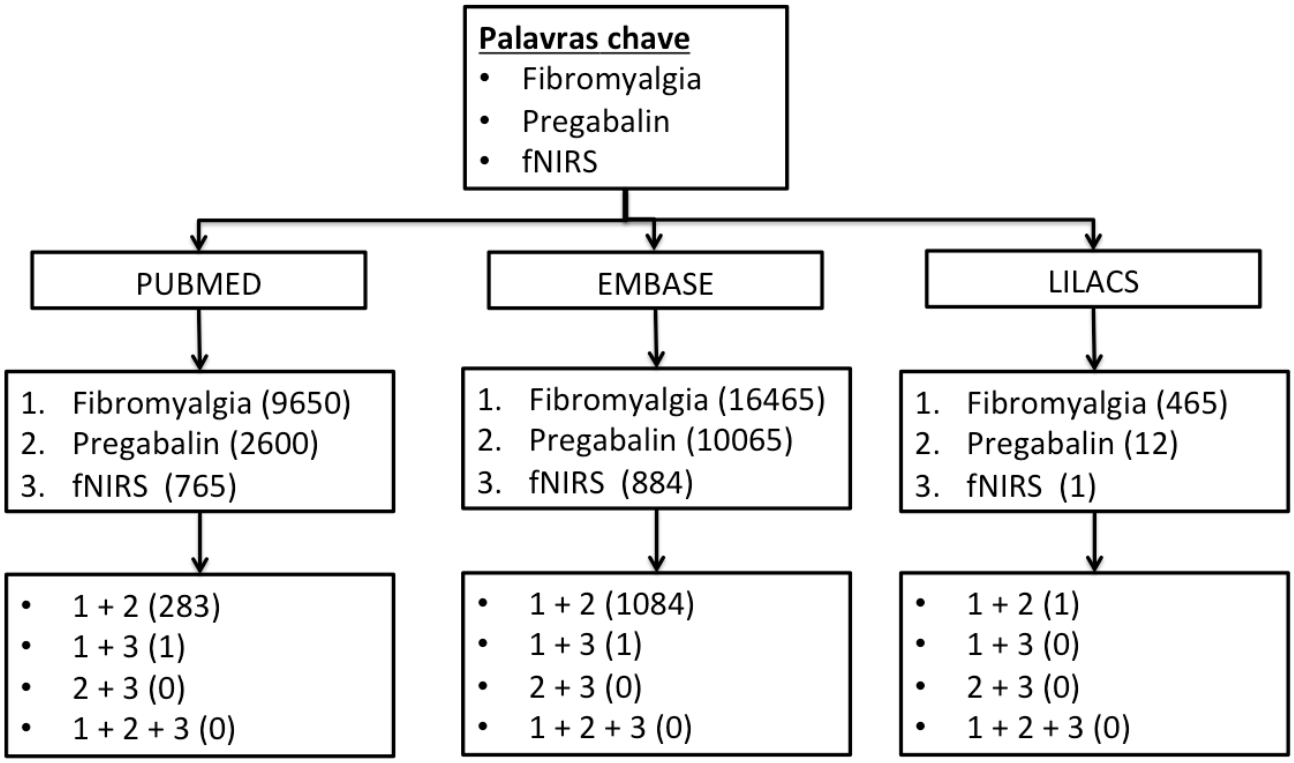

Figura 1: Fluxograma da busca na literatura. Abreviaturas: fNIRS – Espectroscopia infravermelha funcional (tradução livre do inglês *functional near infrared spectroscopy).*

2. FIBROMIALGIA

A fibromialgia (FM) é uma síndrome que envolve múltiplas queixas, entre as quais se destaca a presença de dor crônica generalizada, cansaço, sono não reparador, sintomas depressivos e cognitivos [20,21]. Acomete cerca de 2% da população nos Estados Unidos, sendo diagnosticada com maior frequência em mulheres [1]. A dor crônica é um processo mal adaptativo do sistema nervoso central, tornando o sistema mais vulnerável às demandas dos meios interno e externo, com impacto negativo ao indivíduo e à sociedade. A FM afeta mais de 3% da população mundial, onera o sistema de saúde ao considerar as incapacidades, afastamentos e aposentadorias precoces associadas[2,22]. Por se tratar de uma patologia crônica que concorre com incapacidade para as atividades da vida diaria. Embora existam mecanismos fisiopatogênicos plausíveis que expliquem o desencadeamento e sustentação dos processos neurobiológicos disfuncionais [8,23], ainda não existe consenso pleno sobre os mecanismos fisiopatogênicos da FM, nem sobre as estratégias terapêuticas que permitam reverter ou conter os processos de neuroplasticidade mal adaptativa já instaurados na neuromatriz da dor e no sistema de aferente periférico.

As queixas de hiperalgesia, alodinia e dor generalizada, fortalecem a argumentação sobre a presença de amplificação do *input* nociceptivo, hipótese que tem sido sustentada por meio de estudos de neuroimagem que mostram alteração em regiões corticais associadas ao processamento da dor [13,24]. Além disto, alterações periféricas entendidas como mudanças musculares, fasciais e nervosas periféricas, estão apresentando relevância na explicação da fisiopatologia da fibromialgia, pois estudos da última década que tem mostrado alterações na utilização do oxigênio em nível muscular [25], no tamanho e densidade capilar muscular[26]. A nível de fibras periféricas, tem sido

observado que fibromiálgicas comparadas com pacientes com depressão e com hígidas, apresentam maior limiar de detecção ao frío e ao calor, menores potenciais evocados porém com maior latência, que é característico de lesão de nervo periférico. Esta observação foi confirmada com biopsias de pele, que evidenciaram menor número de fibras nervosas finas não mielinizadas[27]. É também característico nas pacientes fibromialgicas o menor limiar de dor à pressão, que é generalizado, com pouca interferência de fatores cognitivos[28,29].

Estudo meta-analítico que comparou controles sem dor e pacientes com FM, observou que na FM existem diferentes padrões de atividade na ínsula, amígdala, córtex cingulado anterior/médio, giro temporal superior, córtex somatosensitivo primário e secundário, e giro lingual[30], que correspondem às áreas tradicionalmente descritas como neuromatriz da dor. Portanto, uma melhor compreensão do efeito das terapêuticas nesta neuromatriz, poderia proporcionar embasamento científico ao processo diagnóstico e terapêutico destas doenças.

O tratamento da FM objetiva contra-regular o processo neuroplástico disfuncional, por meio de técnicas multimodais. As intervenções farmacológicas incluem analgésicos simples, sedativos e relaxantes musculares promovendo o controle sintomático, atingindo algum nível de sucesso terapêutico, embora com resultados heterogêneos e de magnitude variável [6,31]. Associadas a estas, tem sido propostas algumas intervenções não farmacológicas, incluindo terapia cognitivo comportamental, técnicas de relaxamento, estimulações periféricas como eletroacupuntura, e estimulação transcraniana com campos magnéticos e com corrente contínua[7].

3. PREGABALINA

A pregabalina é estruturalmente semelhante ao neurotransmissor inibitório ácido gama-aminobutírico (GABA), sendo estrutura química o S-enatiômero do ácido 3-aminometil-5-metilhexanóico [9,32]. Possui elevada afinidade à proteína alfa-2-delta dos canais de cálcio dependentes de voltagem no sistema nervoso central. A ligação da pregabalina à proteína alfa-2 delta é o que confere o efeito analgésico, ansiolítico e anticonvulsivante [32,33]. A pregabalina é um dos medicamentos de primeira linha para o tratamento da fibromialgia, com favorável perfil de efeitos adversos. Devido a sua farmacocinética, doses únicas são capazes de atingir 90% da sua biodisponibilidade em até uma hora, com pouca interação com outros medicamentos. No entanto, para conseguir efeitos clínicos na fibromialgia objetivando a diminuição de efeitos adversos, é necessária sua titulação durante pelo menos 12 a 26 semanas, até atingir doses entre 300 a 600 mg[34].

Os efeitos adversos (EA) da pregabalina tem sido descritos em vários ensaios clínicos randomizados[10,25,35–38], são frequentes, mas a taxa de EA graves são semelhantes ao placebo. A taxa de descontinuação do uso do medicamento também é baixa e está relacionada à dose. Dentre os EA mais frequentes estão tontura, sonolência, ganho de peso e edema periférico[32].

In vitro, este fármaco demonstrou ser capaz de reduzir a liberação de diversos neurotransmissores cálcio-dependentes, incluindo glutamato, norepinefrina, calcitonina e substância P[9]. Estudo em voluntários saudáveis usando 600 mg de pregabalina e estimulação magnética demostrou que o fármaco pode agir em diferentes circuitos inibitórios no córtex motor humano, o que sugere um efeito ativador de receptores GABA-B[39]. Vários ensaios clínicos randomizados tem demostrado o efeito modulador

da pregabalina ao longo do tempo [10,34,40], no entanto, são escassos estudos sobre o mecanismo de ação deste fármaco *in vivo,* em humanos.

Poucos ensaios clínicos tem estudado as alterações que a pregabalina exerce no cérebro humano. Um deles, que investigou as alterações cerebrais usando *f*NIRS, comparou o efeito da pregabalina no estimulo doloroso em mulheres fibromialgias, estratificando segundo a resposta ao tratamento, e comparando com saudáveis (sem pregabalina). O estudo demonstrou que o fármaco exerce efeitos nos giros supramarginal, frontal superior e inferior, temporal médio, tálamo cerebelo e córtex calcarino[14], sendo eles parte da neuromatriz da dor. O estudo demonstrou efeito no giro supramarginal, giro frontal superior e inferior, giro temporal médio, tálamo cerebelo e córtex calcarino. Outro estudo experimental, *crossover,* com pacientes fibromiálgicas avaliou o efeito de 450 mg de pregabalina diários, durante 14 dias. O efeito foi avaliado com técnicas de neuroimagem combinadas (*f*MRI, espectroscopia de prótons, e análise de conectividade funcional), onde foi demonstrado que a pregabalina diminuiu os níveis de neurotransmissores excitatórios (glutamato e glutamina) na ínsula posterior, e redução da conectividade funcional nas áreas de funcionamento em repouso do encéfalo[13]. Usando estes métodos de avaliação é possível observar *in vivo* os mecanismos de ação de um fármaco em sistemas modificados pela doença.

Mesmo com estes avanços na tecnologia de neuroimagem (*i.e.* fMRI, H-MRS) que auxiliam de forma significativa o entendimento de processos fisiopatológicos, estas tecnologias são ainda incapazes de resolver a necessidade clínica de levar estes achados à beira do leito do paciente. A realidade clínica precisa tecnologias de menor custo, menos invasiva, que dispense o uso de contrastes ou meios endovenosos, menos restritas, mas igualmente confiáveis. Nesta perspectiva, o fNIRS representa uma alternativa com potencial de satisfazer estas necessidades, pois trata-se de uma

tecnologia de relativo menor custo e manutenção, que dispensa acessos venosos (favorecendo sua aplicação em pediatria, população sensível e psiquiátrica), e interpretação que envolve vários processos complexos, porém com potencial de ser automatizado, favorecendo seu uso clínico. Do ponto de vista de ciências básicas, fNIRS também oferece versatilidade para ser usado de forma simultânea com eletroencefalografia, estimulação magnética transcraniana, estimulação elétrica transcraniana, ou outras terapias farmacológicas e não farmacológicas. Constituindo-se por tanto, em elo de grande importância para encurtar as distâncias entre os cenários clínicos e pesquisa básica. Particularmete na fibromialgia, é relevante o aprimoramento de técnicas clínicas, pois não existe modelo animal aceito com unanimidade que consiga reproduzir a doença para o seu estudo.

4. ESPECTROSCOPIA INFRAVERMELHA FUNCIONAL (fNIRS)

O emprego de uma técnica de avaliação funcional do córtex cerebral que ofereça portabilidade, facilidade de aplicação e interpretação, é fundamental quando o objetivo é levar o conhecimento para contexto clínico aplicado. Sendo assim, é desejável investigar marcadores complementares, úteis na previsão de resposta terapêutica, que permitam de forma accessível escolher os melhores candidatos à uma terapia. É sabido que não existe técnica única que substitua o ^{1}H-MRS, existem alterativas de menor custo e maior disponibilidade, que de forma complementar se aproximam ao ^{1}H-MRS, como a espectroscopia infravermelha funcional (fNIRS, do inglês *Functional Near Infrared Spectroscopy*)[15] e a estimulação magnética transcraniana (EMT) [41].

O uso da fNIRS permite avaliar a ativação cortical de modo inferencial, por meio do estudo das mudanças na sua hemodinâmica. Esta técnica emprega feixes de luz no espectro próximo ao infravermelho que são projetados sobre o escalpo para inferir concentrações corticais dos estados da hemoglobina, cujas mudanças obedecem ao nível de ativação das diferentes regiões corticais, permitindo assim a geração de mapas funcionais [15] (Figura 2).

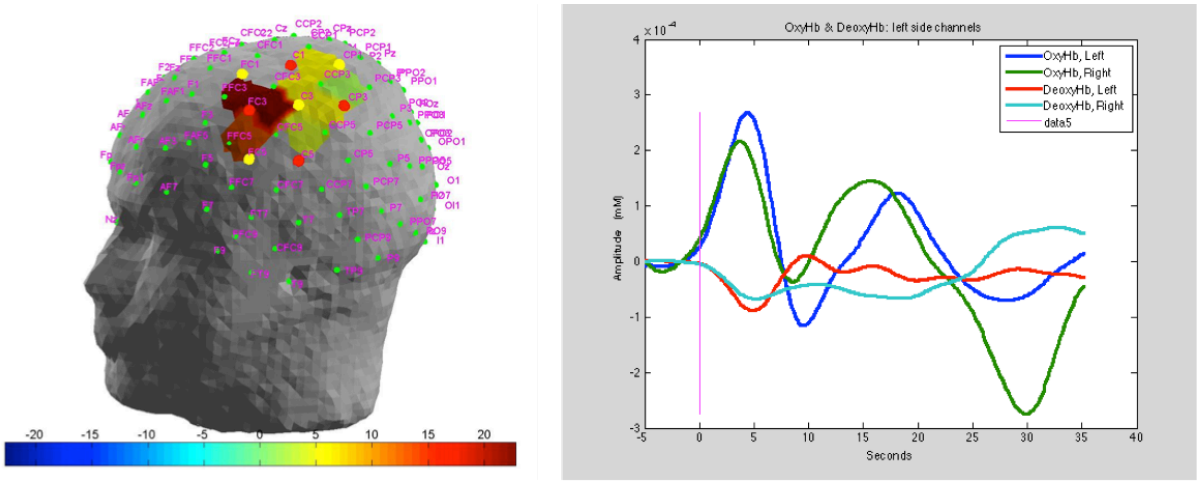

Figura 2. Mapas topográficos e ativação cortical usando fNIRS. Esquerda: Demonstração de mapas de ativação no M1 durante uma tarefa motora. Direita: Exemplo de curvas médias de Hemoglobina oxigenada (OxyHb) e desoxigenada (DesoxyHb) após tarefa motora com a mão direita (Right) e esquerda (Left).

A fNIRS é um recurso com maior viabilidade para mensurar os processos fisiológicos e as respostas corticais em ambientes mais naturais. De acordo com o contexto, a mudança de estado da hemoglobina permite fazer um nexo de causalidade nos estados subsequentes à determinadas testes ou tratamentos. Por esta razão a fNIRS é uma técnica com maior viabilidade para uso nos cenários de pesquisa e também clinico.

No presente estudo mensurar-se-á o efeito da pregabalina em redes neurais corticais de regiões de interesse, particularmente zonas topográficas somatossensitivas, onde ocorre grande parte do processamento neurobiológico em circuitos que constituem a neuromatriz da dor. A resposta das vias sensório discriminativas será avaliada por estímulos nociceptivos de pressão com intensidade crescente[17,42], e por estímulos nociceptivos térmicos [43]. Ambos tipos de estimulação nociceptiva induzem resposta cortical dependente da magnitude, que é passível de ser detectada pelo fNIRS, portanto, poderia ser empregada como marcador objetivo de resposta. Estudos de neuroimagem em voluntários saudáveis e em pacientes com diferentes etiologias de dor crônica, tem destacado o papel de regiões encefálicas no processamento da dor, a saber: córtex sensitivo primário (S1), sedundário (S2), córtex insular, cingulado anterior e prefrontal, assim como tálamo e cerebelo[44]. O fato de algumas destas regiões pertencer a áreas subcorticais, ou não expostas à bóveda craniana, pode gerar dúvidas sobre a aptidão da fNIRS para sua avaliação. Entretanto, já têm sido realizados estudos usando fNIRS para explorar o potencial de avaliar a utilidade de assessar áreas corticais superficias preferencialmente, com intuito de analisar a resposta nociceptiva. Em voluntários saudáveis, tem sido mostrado que a avaliação do lobo frontal, S1 e S2 pode ser suficiente para identificar a "assinatura" da ativação por estímulo táctil [45], calor [43], frío[46,47], pressão mecânica[48,42,49], elétrica gengival [50], elétrica muscular[51], elétrica periférica neuronal[52,53], e visceral sob sedação moderada[54,55].

Na FM ocorre um processamento anormal da resposta nociceptiva, expresso pela hiperalgesia, alodinia e somação temporal à dor (ausência de mudança na função cortical a estímulos de intensidade variada)[56]. No entanto, a avaliação integrada do sistema neurobiológico da dor em tempo real, utilizando recursos técnicos modernos e portáteis como a fNIRS, criaram possibilidades de acesso que auxiliarão na

compreensão do efeito terapêutico nos mecanismos de neuroplasticidade, os quais determinam a LTP (potencial de longa duração, do inglês *Long term potentiation*) e a LDP (potencial de curta duração, do inglês *long term depression*). Este método de acessar a função cerebral por parâmetros de ativação cortical por meio da fNIRS, representa grande avanço no campo da neurociência clínica. A compreensão integrada da neurobiologia dos pacientes com FM através de métodos acessíveis, mais econômicos e maior disponibilidade ao clínico, tem o potencial para impactar em decisões clínicas. O entendimento da neurobiologia do paciente com FM antes e após a intervenção terapêutica, contribuiria na busca de marcadores à resposta terapêutica, aproximando a prática clínica ao ideal da medicina personalizada.

CAPÍTULO III - JUSTIFICATIVA, MARCO CONCEITUAL E OBJETIVOS

1. JUSTIFICATIVA

Fibromialgia (FM) é uma síndrome que se caracteriza por dor crônica difusa, fadiga, transtornos do sono e alterações de humor. Embora sua fisiopatologia não esteja totalmente elucidada, o processo neurobiológico parece envolver alterações funcionais do córtex motor e de suas conexoes com estruturas subcorticais que constituem a neuromatriz da dor, assim como alterações quantitativas e qualitativas em fibras finas sensitivas do sistema nervoso periférico. Sabe-se que o aumento do cálcio intracelular é um mecansimo central no desencadeamento e na manutenção da excitilidade neuronal, que é mantido pelo desequilibrio nos mecanismos de excitação e de inibição, os quais induzem processos de plasticidade mal-adaptativa que sustenta circuitos reverberantes da dor e de seus correlatos. Sendo assim, o córtex sensitivo e motor tem sido alvo diagnóstico e terapêutico no tratamento da dor. No entanto, para que se avance no conhecimento do efeito dos famarcos na função cortical, necessitamos utilizar recursos de neuroimagem que sejam acessíveis nos diversos contextos, e que permitem mensurar o efeito dos fármacos em tempo real. Dentre os recurso de imagem existe a *functional Near Infrared Spectroscopy* (fNIRS), que permite avaliar a ativação cortical por meio da mudança no consumo local de oxigênio que acompanha o disparo neuronal regional, mensurado pelas mudanças na concetração da oxi-hemoglobina.

2. MARCO CONCEITUAL

A fibromialgia constitui uma síndrome de dor crônica que cursa alterações corticais decorrentes da neuroplasticidade mal adaptativa. A mesma tem sido alvo para estudos diagnósticos e terapêuticos visando a compreensão das alterações na neuromatriz da dor, dentre as quais se destacam o córtex somatossensitivo e a ínsula. Adiciona-se a essas o córtex motor, que tem sido um ponto de modulação *top-down* no tratamento da dor crônica, incluindo a fibromialgia.

Os mecanismos mais aceitos para o entendimento fisiopatológico da fibromialgia no momento envolvem o desequilíbrio entre a percepção dolorosa e os mecanismos de modulação dessas vias aferentes. Níveis elevados de substância P em líquor e níveis reduzidos de serotonina e seus precursores em líquor, soro e plaquetas são sugestivos desses desequilíbrios, uma vez que a substância P é mediadora das vias aferentes enquanto a serotonina medeia a inibição da dor. Outra explicação para a alteração da atividade da serotonina seria o polimorfismo dos receptores de serotonina, o que pode explicar também o agrupamento familiar desses pacientes. Alterações cerebrais em porções rostrais ao tálamo poderiam ser responsáveis pela percepção elevada de estímulos ambientais, com a consequente alterações de informações proprioceptivas, térmicas e táteis ou pressórica em sensações dolorosas. Finalmente, os mecanismos reducionistas de explicação fisiopatológica da fibromialgia não têm encontrado respaldo na literatura e explicações multicausais são as mais aceitas. Dentre os fármacos aprovados para o tratamento da FM esta a pregabalina, fármaco que está envolvido em processos de neuromodulação em regiões encefálicas que participam da neuromatriz da dor. O efeito cortical de fármacos como a pregabalina pode ser avaliado usando recursos de imagem como o fNIRS, a qual permite realizar avaliações em ambientes naturais (dinâmicas), com alta resolução temporal, sendo portátil, e

possibilitando explorar assinaturas corticais frente a eventos. Sendo assim, a hipótese deste estudo é que a ativação do córtex motor e somatosensitivo esquerdos de pacientes com fibromialgia está associada com testes psicofísicos da dor e que podem ser modulados pela pregabalina. A seguir apresenta-se de forma sucinta e representativa, o mapa conceitual (Figura 3) para facilitar o entendimento do racional teórico deste estudo

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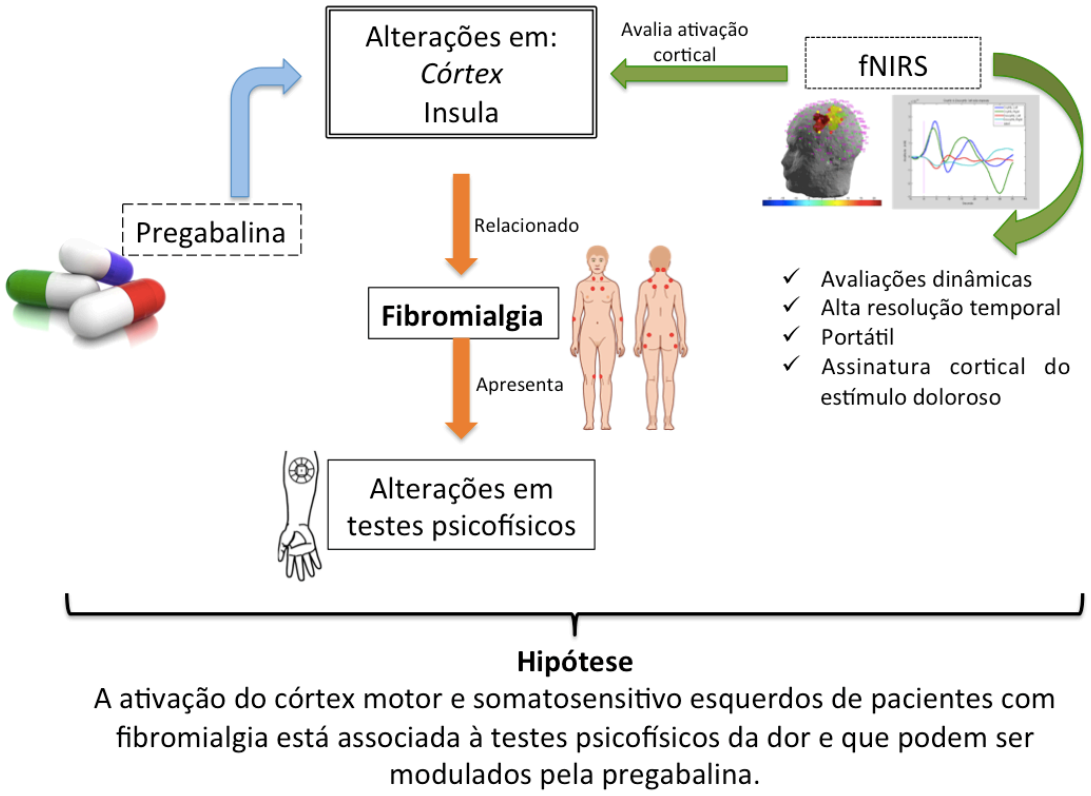

Figura 3: Marco Conceitual. Abreviaturas: fNIRS – Espectroscopia infravermelha funcional (tradução livre do inglês *functional near infrared spectroscopy).*

3. OBJETIVOS

Objetivo Geral

Avaliar as alterações corticais no processamento nociceptivo na fibromialgia, comparada a individuos saudáveis e a sua modulação induzida pela pregabalina.

Objetivos especificos

- Comparar a ativação – excitabilidade do córtex somatossensitivo de maneira inferencial, pelas mudanças relatias na concentração da oxi-hemoglobina, em fibromiálgicas e sujeitos saudáveis.

- Descrever o efeito da pregabalina em dose única (150 mg) na ativação cortical, em comparação ao placebo, e o impacto das características de base do sistema modulado pelo fármaco, comparando estas respostas entre ambos os grupos (fibromiálgicas e saudáveis), intra e inter-grupos. Para este fim, serão considerados os seguintes desfechos:

- Comparação entre atividade motora e estímulo sensitivo: Características topográficas e amplitude de ativação cortical em resposta a atividade motora e à estímulo sensitivo com diferentes modalidades: calor e pressão.
- Topografia e amplitude da ativação cortical em resposta aos estímulos, caracterização segundo a modalidade.
- Correlação da resposta aos testes psicofísicos da dor, e sua ativação cortical regional evocada.
- Padrões de ativação que possam refletir processos fisiopatológicos – assinaturas de alterações corticais.

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CAPITULO IV – ARTIGO CIENTÍFICO

Sensorimotor cortical activation correlates to psychophysical pain testing and its modulation by pregabalin in patients with fibromyalgia. A functional near infra-red spectroscopy, cross-over, randomized, blinded, placebo-controlled trial

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Conflict of Interest: There was no financial relationship between any of the authors or any commercial interest in the outcome of this study.

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ABSTRACT

Pathophysiological mechanisms possibly involved in fibromyalgia include peripheral and central sensitization of the nervous system. **Aim**: To measure the correlate between left sensorimotor cortex using functional near infra-red spectroscopy (fNIRS) after different experimental acute pain modalities (i.e. motor task, heat and pressure), in fibromyalgia patients and healthy subjects and their response upon an approved pharmacological therapy (pregabalin). **Methods**: We designed a cross-over, randomized, blinded, placebo-controlled trial. Seventeen adult women with fibromyalgia and ten healthy subjects under 65 years old were recruited and randomized to a single dose of pregabalin 150 mg or placebo. One hour after the intervention, we studied the cortical activation using fNIRS, measured during a left-hand finger tapping task (LHFT), and upon Heat detection (HDT), heat pain (HPT), heat tolerance (HT) and pressure pain thresholds (PPT). **Results**: baseline HDT was higher in fibromyalgia compared to healthy subjects (35.53±3.22 vs. 33.33±0.85, $P<0.05$), while PPT was lower (2.44±1.08 vs. 4.32±1.45, $P<0.01$), but did not change with pregabalin. When compared to healthy subjects, HDT, HPT, and HT evoked smaller activation in the middle frontal, pre- and post-central gyri in fibromyalgia, that increased after pregabalin (only for HDT-induced activation), but that was not correlated to the HDT. HPT was inversely correlated to the activation in the superior frontal ($r_s=-0.552$, $p=0.033$) and precentral gyri ($r_s=-0.545$, $p=0.036$), remaining unchanged after pregabalin ($r_s=-0.52$, $p=0.047$). HT was inversely correlated to the middle frontal ($r_s=-0.645$, $p=0.032$) and precentral gyri activation ($r_s=-0.655$, $p=0.029$), but was no longer correlated after pregabalin. PPT cortical activation did not differ between fibromyalgia and healthy volunteers. **Conclusions**: The psychophysical pain testing profile in fibromyalgia has a

cortical correlate. Alterations in the HDT, which is a test for small fibers, support its probable peripheral neuropathic component. Also, it was accompanied by decreased activation in sensorimotor areas but increased in pain-related cognitive processing cortexes, and whose activity is elevated by pregabalin. Additionally, upon the HT fibromyalgia patients recruited more areas related to pain cognitive processing, which is a characteristic that speaks in favor of a component of central sensitization in fibromyalgia, and which was poorly modulated by pregabalin. Taken together, these findings suggest that the sensorimotor cortex activation assessed by fNIRS may be a useful measure to assess the influence of peripheral changes on the pain pathways and its influence on cortical pain processing in fibromyalgia.

Keywords: fibromyalgia, fNIRS, pain testing, pregabalin.

Trial registration in clinicaltrials.gov Identifier: NCT02639533

INTRODUCTION

Fibromyalgia is a generalized chronic pain syndrome that is usually accompanied by conditions with decreased pain thresholds, fatigue, sleep and mood disturbances [21,57]. Although its pathophysiological cause has not been completely elucidated yet, the most accepted models tend to explain the syndrome in light of peripheral and central sensitization processes [23]. Likewise, fibromyalgia treatment is usually multifactorial, as it aims to modulate bot peripheral and central nervous system alterations. For such purpose, both pharmacological and non-pharmacological therapies are usually employed [6,8,31].

One of the pharmacological interventions approved for used in fibromyalgia, is pregabalin. Although structurally similar to the neurotransmitter gamma-amino-butyric-acid (GABA), pregabalin binds with high affinity to the alpha-2-delta subunit of the voltage-dependent calcium channels, which are more prevalent in the pre-synaptic nerve endings [9]. It is thought to aid reducing sensitization symptoms as it reduces neurotransmitters' liberation, including glutamate, norepinephrine, calcitonin and P-substance [9], with proven long-lasting modulatory effect in fibromyalgia [10,11].

To improve understanding of the components of the pathophysiological processes involved in fibromyalgia, and how they are modulated by pregabalin, different human study modalities have been used. Extensive neuroimaging research support the existence of a brain network for acute pain perception among healthy volunteers, having as main components the primary (S1) and secondary (S2) somatosensory, insular, anterior cingulate, and prefrontal cortices and thalamus (for review, see [44]). As with functional near infra-red spectroscopy (fNIRS), other authors had already shown in healthy volunteers its ability to detect and discern S1, S2, and frontal cortex activity upon different stimulation modalities: tactile [45], heat [43], cold [46,58], pressure [48,42,49], electrical

gingival [50], electrical muscular [51], electrical peripheral neuronal [52,53]; and visceral pain under sedation [54,55]. However, the use of fNIRS to study the cortical activation upon experimental pain among fibromyalgia patients had been poorly explored. A previous study by other authors [17] compared cortical activation upon painful pressure stimuli between fibromyalgia, major depression patients, and healthy controls, and found greater cortical activation among fibromyalgia patients, particularly in the dorsolateral prefrontal cortex (DLPFC), S1 and S2, but did not find correlations between the stimuli and the cortical signal. Besides not having compared the pressure stimulation to other modalities. Particularly for pregabalin in fibromyalgia, neuroimaging studies have shown that the drug might modulate activation in the supramarginal, superior and inferior frontal, medial temporal, thalamus, cerebellum, and calcarine cortex[14], reduced excitatory neurotransmitters in the posterior insula, and reduced functional connectivity in the default mode network [13].

Although there is no established pathognomonic feature or biomarker characterizing the disease, recent research is moving forward identification of functional cerebral alterations [30], and brain signatures associated to the pathophysiological, symptom-related features of the disease [59]. As single modalities have not been enough to address this endeavor, and would not be sufficient considering the dynamic nature of biological systems, it is reasonable to consider using different tools to improve the understanding of the disease. Combining different modalities of psychophysical pain testing with fNIRS is an appealing method to study pain processing in fibromyalgia, because it allows to study in an extended way the systems hypothesized to be involved in the pathophysiology of the disease. On one side of the nociceptive volley, there are several reports of reduced number of epidermal nerve fibers in skin biopsies in fibromyalgia patients compared to healthy volunteers [27,60]. And on the other side, there is evidence of

morphological and functional alterations in the brain pain matrix, including areas involved in anticipation, attention and emotional processing of pain, as well as motor control [61]. Different modalities of stimulation would thus allow to assess the integrity of peripheral nociceptors depending on the type of stimulus, and to study how abnormal cortical processing occurs (for the signal received, irrespective of its integrity and of whether it was corrupted in its detection itself). Psychophysical pain testing also accounts for the known abnormal cognitive process of the pain, because it also relays on the subjective perception of the stimuli. Thus, we hypothesize that the pharmacological modulation of pregabalin on the psychophysical pain testing among fibromyalgia patients can be quantified at the sensorimotor cortex with a signature of the stimulation modality, and that it will differs to the one induced in healthy subjects, providing new insights regarding the pathophysiological processes involved in fibromyalgia.

To test our hypothesis, different objectives were set. First, we aimed to assess whether a single dose of pregabalin could modulate the experimental pain experience (i.e. psychophysical pain testing) among fibromyalgia patients, and how it would differ from healthy subjects. Then, to probe the left somatosensory cortex for regional activation after different experimental acute pain modalities (i.e. heat and pressure), in fibromyalgia patients and healthy subjects. After that, to determine whether the sensorimotor cortical activation induced by different experimental acute pain modalities differs from the cortical activation due to a motor task. In other words, test whether the signal is specific for pain. And if so, to assess if it is modality-dependent. Finally, to study the correlation between the psychophysical pain tests and its evoked regional cortical activation.

METHODS

Study Design

This randomized, double-blind, placebo-controlled crossover study was conducted according to the Declaration of Helsinki at Hospital de Clínicas de Porto Alegre (Rio Grande do Sul, Brazil). The protocol was approved by the IRB from Hospital de Clínicas de Porto Alegre (Approval number: 14-0624), and registered in a Brazilian registry (Plataforma Brasil, CAAE: 40234514.4.0000.5327), and U.S. National Institute of Health public registry (ClinicalTrials.gov NCT02639533), too. All subjects and patients provided written informed consent before participating in this study. None of the patients neither the pain-free subjects received monetary or any other compensation for participating in this study. The Experimental cross-over design, assessments and interventions in each visit are presented in figure 1. The present manuscript is presented according to the CONSORT guidelines [62].

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

Participants

Healthy (pain-free) subjects. Subjects were recruited from the general population by advertising posts in universities, internet, and public places in the great Porto Alegre area. Subjects were considered eligible to participate if they were female, right-handed, and aging 19 to 65 years old. Initial screening for eligibility was performed by phone using a structured questionnaire. Subjects were ineligible if experiencing acute or chronic pain, using analgesics in the past week, having any: rheumatologic, clinically significant or unstable medical or psychiatric disorder, history of alcohol or substance abuse in the past 6 months, neuropsychiatric disease, or using psychotropic drugs.

Further screening was performed in a personal interview, confirming the information gathered via telephone, and further assessing the presence of depressive symptoms using the Beck Depression Inventory (BDI) II [63,64]. Scores equal or higher than 13 were also excluded.

Fibromyalgia patients. Patients' list was gathered from the institutional chronic pain clinic at HCPA. Telephone contact was established for initial screening. Subjects were also recruited by referrals from other clinic units, and through media advertising. Diagnoses were confirmed by a personal interview with an experienced pain physician, who was independent from the trial conduction, but adhered to the 2010 American College of Rheumatology criteria [57]. Literate females, with pain score on the numerical pain score (zero to ten) equal to or greater than six in most of the days during the last three months, right-handed and aging 19 to 65 were considered eligible. Subjects were excluded if: having neurological, neurosurgical, or oncologial disease (current or past); history of alcohol or drugs abuse; pregnancy or breastfeeding; current or past use of pregabalin. All interviews and data collection visits were performed in the Center for Clinical Research, at HCPA, Porto Alegre, RS.

Interventions

Research subjects received both, pregabalin and placebo according to their randomization sequence. Pregabalin was acquired from ZODIAC as Prebictal®, in solid capsules containing pregabalin 150 mg and excipients. Placebo was manufactured with identical solid capsules containing starch. Intervention was administered per oral with a cup of water during visit 2 and 3, after initial VAS for pain assessment.

Outcomes

Primary outcome: cortical activation upon different stimulation modalities

Cortical activation evoked by psychophysical pain testing, as inferred by the amplitude of the relative changes in the concentration of oxygenated hemoglobin ([HbO]) in the left somatosensory cortex. Data were collected using the commercially available device NIRScout by NIRx® (nirx.net), with 4 sources (LEDs at 2 wave-lengths 760 nm and 850 nm), and 4 detectors placed over the scalp using the caps provided by the manufacturer (EASYCAP®, http://www.easycap.de), in a customized position to create 10 channels (source – detector combination) able to capture the left somatosensory cortex activation at a sampling rate of 7.81 Hz (Figure 2). fNIRS data acquisition was performed at baseline after applying standardized questionnaires, and during visit 2 and 3, one hour after receiving the allocated intervention (Figure 1). Subjects were sit in a comfortable chair with arm rest, and lights turned off during data acquisition to minimize contamination from other light sources. After verifying correct positioning and adequate signal capture, we proceed to data gathering. The evoking tests were performed while recording the cortical activation, for posterior offline analysis. After explaining, demonstrating and solving doubts about each evoking test, subjects were asked to remain with their eyes closed, and to reduce any kind of motor activity not related to the experiment. Each evoking test (*i.e.* finger tapping task, heat detection threshold, heat pain threshold, heat tolerance and pressure pain threshold) will be explained in the following section.

Briefly, offline fNIRS data was initially processed using the NIRSlab tool provided by NIRx®. Raw data was checked for adequacy, and channels with gain setting >8 and/or

coefficient of variation > 7.5% were excluded. Band-pass filters were applied with roll off width 15%-15%, low cutoff frequencies of 0.01 Hz, and high cutoff frequencies of 0.2 Hz. Differential Pathlength Factors were adjusted for each subject and wavelength according to age [65], and hemodynamic states were calculated. Given the few repetitions for each evoking test, a non-parametric (and very conservative) approach was adopted (avoiding statistical parametrical mapping due to violation of assumptions). Relative changes in oxygenated hemoglobin ([HbO]) were later exported for analysis in a custom R-project script (R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.). Briefly, given a temporal reference (marker), the script calculated the average of the trials for each subject (adjusting for the baseline), for further gathering subjects' averages and calculating the mean amplitude of the response (maximus minus minimum value).

--- Insert Figure 2 ---

Secondary outcomes: stimulation modalities.

Motor task: Left hand finger-tapping task (LHFT)

To address the question regarding specificity of the cortical activation upon painful stimulation, first we strived to clarify whether the cortical signal could belong to a motor response (*i.e.* activation of the digital pen marker) and not due to the painful stimuli. To use a standardized approach, we opted for a finger-tapping task. The left-hand finger tapping task (LHFT) was combined with a right-hand finger tapping task, to avoid the effect of conditioning. While sit in a comfortable position, subjects were asked

to tap their finger over the surface of the chair arm rest, in a specified order: thumb, middle, pinky, index and ring finger, during 20 seconds (see Figure 1, A). After allowing several practice trials (as many as needed for the subject to memorize the sequence), data gathering began. Preceded by 20 seconds of rest (sit, eyes-closed, no movement), ten periods of task (either right or left hand finger tapping) that lasted 20 seconds, interleaved by 20 seconds of rest, were performed in a previously randomized order. Subjects were told when to begin and to finish each task and rest period. A researcher did the respective marks in the NIRScout® for the offline analysis when each task period began.

Heat-induced pain: Quantitative Sensory Testing

A quantitative sensory testing protocol was used to evoke thermal pain. First, to assess the heat detection threshold, a Peltier-based device thermode (30x30mm) [66] was applied to the ventral face of the right forearm., which increased temperature at a rate of 1ºC/sec, starting at 30ºC, to a maximal temperature of 52ºC (Figure 1, B). Participants were asked to press a button in a digital marker pen as soon as they felt the first heat sensation (heat detection threshold – HDT). The corresponding mark was placed on the NIRScout® system as soon as the subject pressed the digital marker pen of the Peltier-based device thermode. After marking, the thermode rapidly reduced the temperature back to 30°C. A single training session was offered before, so participants could familiarize with the test. Three measures were taken, with a minimum of 20 seconds' interval between trials, and although the thermode remained in the ventral face of the forearm, its position was slightly changed for each trial to avoid response suppression or cutaneous sensitization of heat nociceptors. Following the same paradigm (one practice trial, three repetitions), patients were later asked to mark when perceiving the first pain

sensation (Heat pain detection threshold). The corresponding mark was also placed on the NIRScout® system. Finally, as a single trial (without practice neither repetitions), patients were asked to press the digital marker pen when experiencing the maximal heat perception they could tolerate (Heat tolerance – HT). Mark was also placed on the NIRScout® as soon as the subject marked the HT on the digital marker pen. For safety reasons, the Peltier-based device was programed to cool down automatically if 52ºC were reached before the subject pressed the digital marker pen.

Pressure evoked pain: Pressure Pain Threshold (PPT)

Pressure pain threshold (PPT): the PPT was assessed using a digital algometer device (JTECH Medical Industries, Salt Lake City, UT). The algometer's 1 cm^2 hard-rubber probe was pressed against the right ventral face of the forearm with constant increasing pressure (Figure 1, C). The procedure stopped as soon as the subject reported the begin of an uncomfortable painful pressure (when the sensation of pressure changed to one of pain), and the PPT was recorded. This was repeated three times, with a minimum interval of 20 seconds inter stimuli. The average of the three measures was calculated and used as a PPT.

Other instruments and assessments

An independent researcher, trained in questionnaires administration, was responsible for collecting data using the following instruments. As a screening tools, patients' depressive symptoms were evaluated using the Beck Depression Inventory II [67], sleep quality symptoms with the Pittsburgh Sleep Quality Index [68], while anxiety characteristics were assessed with the reduced version of the State-Trait Anxiety

Inventory (STAI)[69]. Psychiatric morbidity was screened using the Mini International Neuropsychiatric Interview (MINI) Brazilian Version [70]. We used out laboratory standardized questionnaire to assess demographic data. Quality of life was evaluated among patients using the Fibromyalgia Impact Questionnaire (FIQ) [71]. The worst pain intensity during the last month (at baseline), and in the last week (for visits 2 and 3) was asked using a visual analog scale for pain (0 to 10 cm). The VAS scores ranged from no pain (zero) to worst pain possible (10 cm). The catastrophizing thinking related to pain was evaluated using the Brazilian Portuguese version of the Catastrophizing Scale (B-PCS)[72].

Sample size calculation

Using data extracted from a study exploring the use of fNIRS to detect the cortical signal after thermal stimulation [43], an effect size f≈0.28 was estimated. Considering an alpha error=0.05, beta=0.95, for two groups and three repeated measures, with expected correlation among repeated measures=0.75, and nonsphericity correction=1, a sample size of 10 subjects per group was calculated using G*Power, calculation for a-priori power analysis, using a model of ANOVA for repeated measures, and expecting within-between comparisons [73]. To increase the experience and understanding of the effect of the therapy among fibromyalgia patients we opted for recruiting 1.5 times the estimated required sample of patients, and to account for higher dropouts and loss to follow-up in this population, an additional recruitment of 20% was calculated. Final sample size estimated was 18 fibromyalgia patients, and 10 healthy volunteers. Unequal allocation can be scientifically and ethically advantageous. On one side, a larger sample size in the active treatment group confers a gain in statistical power for the primary outcome,

and for monitoring certain adverse events. On the other side, a smaller control sample reduces the exposure of healthy volunteers to potential adverse events [74].

Randomization, allocation concealment and blinding

Randomization table was generated using the online free tool at www.sealedenvole.com, using simple randomization. A research assistant not involved in the present study prepared two plastic pots for each patient, each with the allocated intervention per the randomization list. The plastic pots were identified with a unique id and visit number for each subject. After enrollment, researchers asked this research assistant to provide the plastic pot corresponding to the current id number and visit.

To control for possible measurement bias participants were instructed to discuss all aspects related to their treatment only with their treating physician (rather than the research staff). Given that the plastic pots were prepared beforehand, and were identical (as well as the capsules containing the drug or the placebo), the chances of predicting the intervention before administration were extremely low. Further, to assess whether blinding was adequate, at the end of each visit we applied a questionnaire requiring participants to guess whether they had received pregabalin or placebo, and to rate on a Likert scale their confidence on the answer (five categories: no confidence to completely confident). Neither the clinician confirming diagnoses, nor the researchers applying questionnaires, fNIRS or psychophysical pain tests were aware of the allocated interventions. Randomization was only open after statistical analyses of the data. Although clinicians taking care of the patients could ask for opening the randomization for clinical reasons, it was not requested for any subject.

Statistical methods

Baseline characteristics of the sample will be first compared using ANOVA (for continuous variables) or chi-square tests between groups (healthy subjects vs. patients) and within the randomized intervention (placebo or pregabalin first) to determine comparability within-between them. Then, to assess the potential existence of a carry-over effect, psychophysical tests at the end of the experiment (end of visit 2) were compared using paired t-tests between the randomized allocation (placebo vs. pregabalin first), stratifying for group (healthy subjects and patients) and adjusting the statistical significance using Bonferroni's correction. To study whether the cortical activation observed upon painful stimulation was due to the nociception and not because of the activation of the digital pen mark, we compared the amplitudes of the signals during the psychophysical tests with the one during the LHFT using paired t-tests, channel by channel, with the corresponding adjustment for multiple comparisons. After that, primary and secondary outcomes (cortical activation upon stimulation) were compared within-between groups and randomized intervention using mixed models, where the amplitude of each channel during an evocative test was modeled as dependent variable that considered the repeated measures nature of the test (including an identification variable), while the randomization (pregabalin or placebo) and group (healthy volunteer or fibromyalgia patient) were used as independent variables. Bonferroni's correction was used to adjust for multiple comparisons. Further, to study the correlation between psychophysical pain tests and the amplitude of the cortical activation, a spearman correlation analysis was performed.

Results

Sample characteristics

Participants assessed for eligibility, inclusion and randomization, are presentment in the flow diagram (Figure 3 A and B Fibromyalgia and Healthy subjects flow diagrams). Subjects were recruited from May 2014 to January 2016, and stopped when the pre-specified required sample size was reached. Baseline characteristics of the sample per the randomization order are presented in table 1. Fibromyalgia and healthy subjects were comparable, although fibromyalgia patients had higher body mass index, less year of formal education, and more cases with hypertension. Besides that, as expected, fibromyalgia patients also screened positive for diverse psychiatric disorders according to the MINI, and presented higher scores (*i.e.* more symptomatic) for anxiety, sleep disturbances, pain catastrophizing, and B-PCP:S scores in all domains.

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

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

There was no carry-over effect, as there was no difference in the pain scores (P=0.72), neither psychophysical pain tests (HDT, P=0.34; HPT, P=0.06, HT, P=0.23; PPT, P=0.18), when compared within randomization arms. Compared to controls, fibromyalgia patients demonstrated higher pain scores in the VAS, higher HDT, and lower pressure pain thresholds (PPT). The groups presented comparable HDT, HPT and HT. Neither pregabalin or placebo altered any of the psychophysical pain tests (table 2). Regional cortical activation was inferred from the relative changes in [HbO] assessed by

fNIRS. The normalized amplitude of the hemodynamic response (maximum minus minimum value adjusted for the mean within the temporal window assessed) was calculated for each channel and test, as described in the methods section.

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

Is the cortical activation the same for motor task and nociception? *Testing the somatosensory cortex activation upon different stimulation modalities: motor task vs. heat and pressure.*

To address whether the cortical activation during nociceptive stimulation differed to the one during the use of the digital pen mark, we compared the psychophysical tests' signal with the one of a left-hand finger tapping task (LHFT) (table 3, Supplementary Figure 1, parts A, B and C). Heat detection threshold: The amplitude of the signal during the HDT was not different to the one of the LHFT among controls, at any assessment time. On the counter side, the activation during HDT among patients with fibromyalgia had lower amplitude than the activation during LHFT in the baseline in the precentral, postcentral, and middle frontal gyrus (Ch 2, 6, 10). These differences were no longer observed after placebo or pregabalin. Heat Pain Threshold: During HPT, healthy subjects' activation was of smaller amplitude than during LHFT in the precentral and middle frontal gyrus in baseline (Ch 6), and after placebo (Ch 5). For patients, significant differences were observed in the precentral and middle frontal gyrus (Ch 2, 6) during HPT at baseline and after pregabalin, but not after placebo. Heat tolerance: During HT, healthy subjects showed differences in baseline and placebo in the precentral gyrus (Ch 2, 5) ($p=0.032$), and after pregabalin in a wider area, expanding from the precentral, to the postcentral

and inferior parietal gyri (Ch 5, 8, 9, 10). Still, at baseline, patients presented smaller amplitudes during HT in the superior frontal, precentral and postcentral gyri (Ch 1, 2, 3), and after pregabalin in precentral and postcentral gyri (Ch 3, 6), but not after placebo. Pressure Pain Threshold: Among healthy subjects the cortical activation during PPT was smaller in the precentral, postcentral and inferior parietal gyri than during LHFT after receiving placebo and pregabalin (Ch 7 and 6, respectively). However, the amplitude of the activation was not different among patients at any assessment time. Having shown that the amplitude of the activation due to nociceptive stimuli differed to the one of a LHFT in some regions, and was influenced by the experimental interventions, we proceed to study the amplitudes during the psychophysical tests, themselves.

---- Insert Table 3 ---

Does the somatosensory cortex activation differ between fibromyalgia patients and healthy subjects? *Differences in the activation upon stimulation modalities between groups, and their modulation by pregabalin*

The amplitude of the cortical activation upon each psychophysical pain test were compared between patients and healthy subjects, for the intervention. Heat detection threshold: The activation during the HDT was greater in healthy subjects than in fibromyalgia patients at baseline (but not after the interventions), in almost all the areas assessed (Ch 1, 2, 3, 4, 8, 9, 10) (mean differences= 16.97, p=0.005; 11.59, p=0.025; 12.33, p=0.047; 15.93, p=0.011; 11.94, p=0.027; 11.73, p=0.031; 17.49, p=0.01; respectively). Heat pain threshold: HPT induced higher amplitude of the signal among

healthy subjects in the baseline (but not after the interventions), particularly in the pre- and postcentral gyri (Ch 3 5 9 10; mean differences= 16.97, p=0.04; 15.51, p=0.04; 11.20, p=0.038; 18.61, p=0.042; respectively). Heat Tolerance: Cortical activity did not differ between patients and controls at any assessment time. Pressure Pain Threshold: Cortical activation did not differ between patients and controls during baseline nor placebo, but pregabalin increased the difference in the amplitude of the signals among patients in postcentral gyrus (Ch 5 6, 9) (mean difference=27.3, p=0.034; 30.58, p=0.01; 40.08, p=0.003).

--- Insert Figure 4 ---

Was the response to pregabalin the same among fibromyalgia patients and healthy subjects?

The amplitude of the cortical activation upon psychophysical pain test were compared by intervention (i.e. baseline, placebo and pregabalin) within subjects, stratifying for patients and healthy subjects. Healthy subjects: During the HDT and the HT the amplitudes of the activation did not differ by intervention. Now, the HPT cortical activation reduced after pregabalin in the pre- and postcentral giry (Ch 10, mean difference -36.37, p=0.001). Further, the PPT-induced signal was also reduced by pregabalin when compared to baseline in virtually all the cortical areas assessed (Ch 3, 4, 5, 6, 8, 9, 10; mean differences 32.79, p=0.012; 24.19, p=0.038; 42.10, p=0.001; 53.57, p<0.0001; 29.22, p=0.04; 53.40, p<0.0001; 36.54, p=0.033; respectively).

Fibromyalgia patients: Compared to baseline, pregabalin increased the cortical activation during HDT on the pre- and postcentral gyri (Ch 2, 3, 7; mean differences= 15.35, p=0.003; 15.69, p=0.007; and 12.29, p=0.013, respectively), and placebo did not.

Now, the amplitude of the activation did not change with the interventions upon HPT, HT, or PPT.

Were the psychophysical pain tests correlated to the amplitude of the cortical activation?

Having demonstrated that the amplitudes of the cortical activation differed between psychophysical tests and a motor task, for both patients and healthy subjects, we explored its potential correlation with the results of the psychophysical pain tests (Table 4).

Healthy subjects: the HDT only had a strong inverse correlation with the amplitude of the cortical activation in the postcentral gyrus after pregabalin (Ch 8, 9; r_s =-0.81, p<0.001; r_s =-0.83, p=0.01, respectively). HPT showed a strong correlation with the precentral gyrus (Ch 2; r_s=0.712, p=0.03) at baseline. After pregabalin, the strong correlation expanded to the postcentral gyrus, too (Ch 3, 8; r_s=0.778, p=0.039; r_s=0.821, p=0.023). HT had a strong correlation with the pre- and postcentral gyri activation (Ch 2, 3, 8, 10, r_s=0.875, p=0.001; r_s=0.687, p=0.028; r_s=0.687, p=0.028; r_s=0.644; p=0.044, respectively), which remained after placebo (Ch 3; r_s=0.719, p=0.045) and pregabalin (Ch 2, 9; r_s=-0.775, p=0.041; 0.786, p=0.036, respectively) in a smaller, more concentrated area towards postcentral gyrus. PPT at baseline was strongly correlated with the precentral gyrus (Ch 1, 3; in the baseline (r_s=0.732, p=0.039; r_s=0.766, p=0.027, respectively), but changed direction of the association after pregabalin (Ch 3; r_s=-0.786, p=0.036).

Fibromyalgia patients: HDT activation only had a moderate correlation with cortical activation after pregabalin in the postcentral gyrus (Ch 9, r_s =-0.55, p=0.04). HPT was

moderately correlated with the precentral gyrus at baseline (Ch 1, 2; r_s=-0.552, p=0.033; r_s=-0.545, p=0.036, respectively), and after pregabalin (Ch 1; r_s=-0.52, p=0.047). HT had inverse and strong correlation after placebo in the middle frontal / precental gyri (Ch 4, 6; r_s=-0.645, p=0.032; r_s=-0.655, p=0.029, respectively). PPT: was correlated to the middle frontal and precentral gyri (Ch 4, 6, 10; r_s=0.673, p=0.025; r_s=0.697, p=0.033; 0.673, p=0.033, respectively) after placebo only.

--- Insert Table 4 ---

Adverse effects

All the adverse effects observed were transient, lasting less than 12 hours, and resulted in no permanent harm. Placebo: *headache,* three subjects reported mild headache, and five with moderate. *Somnolence,* eight mild somnolence, five moderate, three severe. *Dizziness*: one mild and one moderate intensity. *Others*: one subject referred "feeling drunk-like", one with flares, and one with mild nausea. Pregabalin: *headache*, four subjects referred mild headache, and one moderate headache. *Somnolence*: five mild somnolence, ten moderate, nine severe. *Dizziness*: three mild, five moderate, two with severe intensity. *Others*: five with mild dry mouth, four with nausea, one with severe vomiting, and one with blurry vision.

Blinding

In both, fibromyalgia and healthy subjects, pregabalin induced significantly more sedation than placebo (mean difference=1.48, SEM=0.69, P=0.033 for healthy subjects;

mean difference=3.14, SEM=0.53, P<0.0001 for patients). Testing blinding among patients, an effect of the sequence allocated (pregabalin or placebo first), had a significant effect. Although those randomized to receive pregabalin first guessed better the intervention received (chi-square, P=0.002), about half of them had declared not knowing what they had receive. On the other hand, those randomized to receive placebo first were unable to guess the intervention (chi-square, P=0.193), and about three quarters of them declared not knowing what they had receive.

Discussion

After corroborating that it is possible to detect the sensorimotor cortical activation due to different modalities of nociception and motor tasks using fNIRS in healthy subjects and in fibromyalgia, we showed how it correlates to psychophysical pain testing, and how it is modulated by a single dose of pregabalin. Apart from the study [17], that addressed PPT and fNIRS without studying its response to treatment, to the best of our knowledge, the present is the first report exploring the cortical activation to different nociceptive modalities using fNIRS in patients with fibromyalgia, and its response to a pharmacological intervention.

Heat Detection Threshold

Psychophysical pain testing should be interpreted considering the underlying processes involved. In our protocol, temperature was increased at a rate of 1 ºC/sec to assess the HDT, which is considered to activate preferentially C fibers when used to achieve sub-threshold limits. This type of unmyelinated, multimodal, small diameter, low-threshold, slow conduction fibers, are responsible for the dull, badly-localized, “secondary pain”

[75]. In our experiment, the activation during HDT recruited a wide area that was comparable to the LHFT among healthy subjects, and that remained unchanged after the interventions, thus, confirming that actual cortical activation was detected. The relatively ample area of activation evoked by the HDT is in accordance with the dull, poorly localized thermal perception referred after activation of C-fibers.

When compared to healthy subjects, the HDT evoked smaller activation amplitudes in all the assessed areas in fibromyalgia patients, with accentuated differences in the middle frontal, pre- and post-central gyri. From the psychophysical perspective, there was a higher detection threshold among fibromyalgia, too. The smaller activation area together with a higher threshold can be interpreted as reduced nociceptive input from the periphery (neuropathy, to some extent), which is agreement with the reported small fiber abnormalities in patients with fibromyalgia [27]. Further, the accentuated differences in the middle frontal gyrus can be attributed to the already documented increased activation in this area, associated with the anticipation of pain [76]. This pattern of cortical activation in fibromyalgia was modulated (increased) by a single dose of pregabalin, and expanded to the inferior parietal gyrus. Our finding is consistent with a previous report [14] that used fMRI to study the effects of the clinical use of pregabalin in patients with fibromyalgia using PPT, in which the drug increased activation in the thalamus, postcentral, calcarine, middle frontal, and middle cingulate gyri, and in the inferior parietal lobe, precuneous and insula. However, in our study, although the amplitude of the cortical activation using fNIRS was not associated with the HDT at baseline, after receiving pregabalin patients and healthy subjects presented an inverse correlation between the HDT and the activation in the postcentral gyrus, which could be attributed to an increased activation induced by the pregabalin, as had been also observed by other authors before [14]. Although the effect of pregabalin was not enough to

induce statistically significant changes in the psychophysical pain tests, a functional cortical change occurred in areas related to pain perception (e.g. inferior parietal gyrus), which has been associated with catastrophizing thinking in fibromyalgia [77].

Heat Pain Threshold

In both groups (patients and healthy subjects), the HPT induced smaller activation than the LHFT that was more evident in the middle frontal, pre- and post-central gyri, and tended to remain unaltered after the interventions. The reduced activation area could be explained if considering that increasing temperatures tend to stimulate lightly myelinated A-δ fibers, whose threshold usually lays around 43ºC, and correspond to a more localized, "faster" and acute pain [78]. When compared to healthy subjects, like the phenomena observed with HDT, the HPT-induced activation had smaller amplitude in fibromyalgia, but in the HPT tended to remain unchanged after pregabalin. Our finding is line with the demonstrated reduction in intraepidermal innervation and regeneration sparing myelinated nerve fibers in patients with fibromyalgia [27], in which even having less fibers, the HPT also did not differ to their controls (healthy and depressed). Thus, the reduced activation could be associated with the also reduced nociceptive small-medium fibers innervation in fibromyalgia.

Among the healthy, the HPT was directly correlated with the amplitude of the activation in the postcentral gyrus, which is consistent with the known sensory function of the SI area. Such correlation expanded to the inferior parietal gyrus after pregabalin.

Fibromyalgia patients presented a different pattern, having inverse correlation between HPT and the superior frontal and precentral gyri, remaining unchanged after the intervention. Such pattern could be interpreted as the weight of the cognitive and

inhibitory modulation in the assessed pain threshold, in the sense that lower thresholds ("more sensitive patients") would have higher frontal activation, as this area is related to different pain modulation elements, such as anticipation, and triggering of the descending inhibitory pain modulation system [61], which is currently considered as relevant part of the pathophysiology of fibromyalgia. As the areas activated during the HPT are related to such relevant systems for the pathophysiology of the disease, it is understandable that big changes did not occur with the dosage of pregabalin that we used.

Heat Tolerance

The HT also activates C and A-δ fibers because of the heating speed [75]. In our experiment, like the HPT, the HT also induced smaller amplitudes of activation compared to the LHFT in both, healthy and fibromyalgia, in the pre- and post-central gyri (sensorimotor area). Once more, the pregabalin extended the difference in the activation area compared to the LHFT to the middle frontal gyrus in fibromyalgia. In our sample, we observed a similar phenomenon as the one observed in another study [27], were HT did not differ between samples and controls. Even so, the HT correlated directly with the amplitude of the activation in the pre- and postcentral gyri among the healthy. In fibromyalgia, the HT was inversely correlated to the middle frontal and precentral gyri activation, though. This finding supports the growing body of evidence showing the relevance of the frontal areas in the altered cognitive pain processing in fibromyalgia [61], which is also accompanied by the S2 disinhibition that is associated with the central sensitization [16].

Pressure Pain Threshold

The PPT only presented differences with the LHFT among healthy volunteers, evoking smaller amplitude of the activation after placebo and after pregabalin in the middle frontal, precentral and postcentral gyri. The activation during PPT did not differ between patients and healthy subjects in baseline or after placebo, but pregabalin did reduce the amplitude of the signal among the healthy in the same areas, extending its effect to the postcentral gyrus, too, but with little effect among fibromyalgia patients. Further, the inferences of the PPT correlation with these areas was limited by the borderline statistical significance. Although the reduced tolerance to pressure among fibromyalgia patients is a salient feature of the disease, the brain activity alterations seem to lay in subcortical areas, which limits the ability of fNIRS to detect them. A proton magnetic resonance spectroscopy (H-MRS) study showed that levels of glutamate and glutamine in the right posterior insula were elevated among patients with fibromyalgia when compared to healthy volunteers [79]. Classical studies showing altered cortical activation used supra-threshold stimuli to elicit such response, which explains de divergence with our results, as we assessed the first pain sensation (*i.e.* pain threshold), only [76]. Thus, to use fNIRS to study the activation during pressure pain, future studies should strongly consider using supra-threshold stimuli.

Limitations

Our study should be interpreted with consideration of its limitations. Fibromyalgia patients probably have multiple dysfunctional neuronal circuits where the defective pain modulatory function stands out. Given that pregabalin is a ligand to the alpha-2-delta subunit on voltage-gated calcium channels; it reduces calcium influx at nerve terminals

resulting in a decreased release of several neurotransmitters, like glutamate, norepinephrine, and substance P. (Micheva KD, et al., 2003; Spaeth M 2008). Because voltage-gated calcium channels are not exclusive of the neuronal terminals, a potential effect on astroglia, that could also modulate neurovascular coupling and consequently mislead fNIRS inferences could hypothetically exist. Nevertheless, as we have comparisons with baseline and controls, and our observations agree with other authors experiments, if is such effect exist, it is would be of minor relevance in the present report.

Although in agreement with some scientific literature, the fact that we could not demonstrate the increased activation induced by pregabalin in fibromyalgia for all the psychophysical tests, but for the HDT only, could not only be related with the disease, but also be a consequence of the dosage used. In the fMRI reports studying the effects of pregabalin on fibromyalgia, the dosages were superior, and lasted longer than ours [14,79]. Nevertheless, as previously exposed, exposing healthy volunteers to higher doses of pregabalin and for longer periods of time would be ethically reprehensible, and could seriously compromise blinding.

It is worth noting that the non-parametric approach employed for analyzing fNIRS curves has the limitation of not addressing the contour of the curves, neither the latency of the response, which are relevant parameters when trying to discriminate the nature of the activation, in other words, when searching for “brain activation signatures” of pain, as other authors have previously addressed [45,43,52,54,55]. Also, although fNIRS offers an approximate anatomical correlate, it is worth noting that the channels are created by combining sources and light detectors placed about 3 cm apart, thus the cortical areas activity that is inferred has a modest spatial resolution (1 to 3 cm^2)[80]. Thus, the reader

must remind that the areas where significant activation was detected, are educated approximations of the underlying cortical processing.

Conclusions

The psychophysical pain testing profile of fibromyalgia has a cortical correlate that is supported by its probable underlying pathophysiological mechanisms. The subtle but characteristic alterations in tests assessing small fibers with below-threshold stimulation support its probable peripheral neuropathic component. Such trait was accompanied by an activation that is decreased in sensorimotor areas but increased in pain-related cognitive processing cortexes, and whose activity is increased by pregabalin. When facing stronger stimulation, fibromyalgia patients also recruited more areas related to pain cognitive processing, having higher activation among those with reduced tolerance, which is a characteristic that speaks in favor of a component of central sensitization in fibromyalgia, and which is poorly modulated by the doses of pregabalin that we used. Taken together, these findings support the co-existence of both, peripheral and central alterations in fibromyalgia.

Future directions

Due to its versatility, relative low cost and portability, the fNIRS has great potential to enter clinical grounds and change the way it is understood and practiced. As shown here, fNIRS in combination with other techniques to assess human physiology, has the potential to improve the understanding of pathophysiological mechanisms. Also, cortical activity in response to certain stimuli could potentially be used as ancillary marker for nociception and pain processing in the future. Because of the traditional type

of fNIRS analysis using parametrical models, applying its inferences to the single subject would not be suitable. However, using non-parametrical analyses that compare against the subject itself, could prove usefulness in the future, for both diagnostic and follow-up purposes, as well as to identify potential responders to therapy. Also, they could be used to test the nociceptive system response to a single intervention (non-invasive brain stimulation, for instance), as an effort to study the odds to response or not to a given intervention.

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Declaration of interests

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TABLES

Table 1. Characteristics of the sample. Data are presented as mean ± SD or frequency (n=27).

Variable	Healhty subjects (n=10)		Fibromyalgia (n=17)	
	Placebo First (n=3)	Pregabalin First (n=7)	Placebo First (n=9)	Pregabalin First (n=8)
Age	43.00±14.73[a]	44.00 ± 7.79[a]	51.56 ± 8.25 [a]	49.38 ± 9.71 [a]
Body Mass Index (Kg/m^2)	27.80±3.62 [a]	22.85±3.08 [a]	31.09±6.58 [c]	31.55±8.48 [b,c]
Retired (yes/no)	0/3 [a]	0/7[a]	4/5[a]	3/5[a]
Civil status				
Single / lives alone (yes/no)	1/2	0/7	1/8	0/8
Married (yes/no)	2/1	7/0	4/5	6/2
Stable relationship (yes/no)	0/3	0/7	4/5	2/6
Years of formal education	15.00±3.61 [c,d]	18.29±2.98 [a,c]	11.00±3.87 [b,d]	9.00±3.70 [b,d]
Smoking (yes/no)	1/2 [a]	2/5 [a]	7/2[a]	5/3 [a]
Hypertension (yes/no)	0/33[e]	0/7 [a]	6/3[d]	4/4[c]
Diabetes (yes/no)	0/3 [a]	0/7 [a]	0/9[a]	1/7 [a]
Number of months dealing with continuous pain			158.67±78.41 [b]	145.50±118.21 [b,c]
Psychoactive drugs in regular use**				
Amitriptyline			4	2
SSRI			5	3
Benzodiazepine			1	1
Antoconvulsant (non-gabapentinoids)			1	0
Dual antidepressant			0	1
Psychiatric Diagnosis according to the MINI***				
Major Depression Disorder - Current (yes/no)			5/4	3/5
Major Depression Disorder - Past (yes/no)			3/6	2/6
Melancolic Depression (yes/no)			5/4	2/6
Bipolar Mood Disorder - Type I (yes/no)			0/9	3/5
Bipolar Mood Disorder - Type II (yes/no)			2/7	0/8
Hypomaniac episode - Past (yes/no)			4/5	0/8
Maniac episode - Current (yes/no)			2/7	1/7
Maniac episode - Past (yes/no)			2/7	2/6
Panic disorder without agoraphobia (yes/no)			2/7	2/6
Panic disorder with agoraphobia (yes/no)			2/7	1/7
Obsessive Compulsive Disorder (yes/no)			1/8	0/8
Post-traumatic Stress Disorder (yes/no)			2/7	0/8
Substances abuse disorder (including alcohol)			0/9	0/8
Generalized Anxiety Disorder (yes/no)			3/6	1/7
Suicidal Risk (yes/no)			5/4	1/7
Low			2/7	1/7
Moderate			0/9	0/8
High			3/6	0/8

Fibromyalgia Impact Questionnaire.				
State Anxiety (State-Trait Anxiety Inventory)	17.67±8.08 [a]	18.00±3.78 [a]	27.56±6.22 [b]	27.00±4.53 [b]
Trait-Anxiety (State-Trait Anxiety Inventory)	16.67±5.68 [a,b]	16.71±2.49 [a]	29.11±9.58 [b]	29.63±6.80 [b]
Pittsburgh Sleep Quality Index	4.00±2.00 [a]	4.29±2.81 [a]	10.44±4.47 [b]	15.00±4.07 [b]
Beck Depression Inventory (BDI) II	7.00 (12.12 [a]	2.43±3.04 [a]	23.67±15.29 [b]	27.38±10.11 [b]
Worst pain during the last month (VAS-10cm)			8.00±1.95 [b]	7.85±1.60 [b]
B-PCP:S, Brazilian Portuguese Profile for Chronic Pain: Screen				
Brazilian Portuguese Pain Catatrophizing Scale (BP-PCS)			33.56±13.86 [b]	34.25±10.54 [b]

** Significant difference at two-tailed alpha=0.05.*

Different letters denote significant differences after adjusting for multiple comparisons.

*** Patients could be using more than one drug at the same time.*

**** Patients could have more than one disorder at the same time.*

Abbreviations. MINI, Mini Neuropsychiatric Interview. SSRI, Selective Serotonin Reuptake Inhibitor. PSQI, Pittsburgh Sleep Quality Index. BDI II, Beck Depression Inventory II. VAS, Visual Analog Scale. B-PCP:S, Brazilian Portuguese Profile for Chronic Pain: Screen. FIQ, Fibromyalgia Impact Questionnaire.

Table 2. Psychophysical pain testing by group and intervention. Data area presented as mean (SD) (n=27).

	Healhty subjects (n=10)			**Fibromyalgia (n=17)**		
	Baseline	**Placebo**	**Pregabalin**	**Baseline**	**Placebo**	**Pregabalin**
VAS for pain (cm)	0.08±0.14	0.16±0.31	0.48±1.24	4.55±2.38	4.13±2.38	3.98±2.62
Heat Detection Threshold (ºC)	33.33±0.85[a]	33.55±1.44	34.55±3.70	35.53±3.22[b]	35.04±3.73	35.14±3.38
Heat Pain Threshold (ºC)	42.51±3.92	43.09±3.24	42.45±4.07	42.28±4.32	41.00±4.83	41.77±4.50
Heat Tolerance (ºC)	48.74±1.93	48.60±2.06	48.15±1.09	46.93±3.79	45.66±4.51	46.04±4.83
Pressure Pain Threshold (kg/cm^2)	4.32±1.45[a]	4.48±2.09[a]	4.55±1.65[a]	2.44±1.08[b]	2.29±1.11[b]	2.63±1.16[b]

Different letters denote significant differences after adjusting for multiple comparisons.

Table 3. Differences in the amplitude of the cortical activation during psychophysical pain testing and the LHFT. Data are presented as mean difference (test amplitude – LHFT amplitude) ± SD (n=27).

	Healthy subjects (n=10)			Fibromyalgia (n=17)		
Channel	**Baseline**	**Placebo**	**Pregabalin**	**Baseline**	**Placebo**	**Pregabalin**
Heat Detection Threshold						
1	9.14±39.30	-6.04±48.32	-5.65±59.08	-16.04±44.03*	-6.90±60.79	-8.27±61.23
2	-0.39±45.22	3.24±68.67	-4.22±37.68	-17.82±32.76***	-12.10±62.16	-5.99±37.57
3	-7.99±60.41	4.84±52.93	-12.90±37.03	-15.45±41.15*	-1.12±53.75	-4.24±39.86
4	0.16±58.02	1.18±58.57	-10.21±43.86	-15.62±39.91*	-10.32±54.03	-11.66±39.20
5	-14.04±72.04	-14.84±59.56	8.87±57.13	-14.95±36.64*	1.05±51.56	-7.69±35.39
6	-20.30±60.71	0.83±44.94	-17.33±43.02	-14.81±25.48***	-7.33±48.37	-12.40±31.31*
7	-1.34±31.02	-18.41±68.05	-6.72±40.52	-12.72±32.01*	-15.03±45.93*	-2.58±35.34
8	2.30±49.04	-9.87±52.80	-13.11±38.31	-14.89±36.51*	-10.50±62.75	-5.31±38.98
9	-5.91±47.92	4.31±38.99	-4.97±33.59	-11.65±28.31*	-4.66±41.43	-3.03±36.17
10	-5.39±67.71	3.61±38.96	-6.73±39.48	-16.56±33.16***	-11.45±53.64	-8.08±31.11
Heat Pain Threshold						
1	-2.88±36.48	-11.01±53.63	-33.98±76.92	-7.57±48.11	-0.56±46.89	-15.55±49.45*
2	-6.33±45.60	-4.31±58.80	-6.89±37.27	-13.17±37.83*	-0.60±53.94	-16.71±37.63***
3	1.35±58.60	-7.74±32.30	-8.99±42.65	-15.57±42.35*	2.34±47.96	-16.54±46.09*
4	-4.81±49.95	-13.12±56.75	-16.08±62.69	-14.31±41.27*	-11.80±33.92*	-14.41±38.06*
5	-12.66±54.49	-15.02±36.62*	-2.10±74.65	-16.91±41.91**	0.68±49.03	-15.68±41.66*
6	-20.19±50.87*	-8.22±36.00	-6.63±65.24	-14.88±28.19***	-9.56±43.58	-15.90±36.53***
7	-2.04±54.64	-0.87±53.79	-6.65±30.38	-4.68±35.99	-4.97±34.24	-8.39±30.18
8	-4.19±48.69	-24.76±79.59	-14.60±47.29	-8.61±38.70	-5.55±48.73	-13.78±39.16
9	-4.38±39.21	-6.11±38.42	-5.82±46.42	-9.57±33.61	2.91±43.42	-7.00±36.51*
10	-0.09±64.41	-4.20±48.90	-14.47±50.47	-10.35±33.27*	-12.72±44.67	-10.48±46.55
Heat Tolerance						
1	-21.17±28.24*	-9.66±47.26	-8.50±35.93	-38.51±37.61***	-4.94±81.06	-16.62±43.72
2	-3.60±81.58	4.77±65.98	-15.24±35.76	-29.22±44.74**	-22.94±95.68	-16.61±25.42
3	-26.52±44.69	-15.69±33.78	-22.24±46.50	-31.11±38.23**	-3.23±56.45	-22.52±23.99*
4	-17.30±42.22	17.47±53.38	-11.63±15.95	-20.17±44.21	-9.21±57.74	-22.94±35.44
5	-25.07±56.88	-28.64±29.93*	-25.85±20.83*	-5.59±46.59	-17.16±66.06	-18.14±29.81
6	-19.35±44.08	-0.062±40.83	-17.55±46.83	0.47±38.71	-8.53±51.94	-29.19±24.32***
7	13.39±84.12	7.90±30.29	-21.16±33.79	-6.07±58.15	-6.16±90.44	-1.76±32.58
8	2.59±48.56	-24.67±30.82	-42.09±24.62***	-16.59±59.28	-16.96±76.03	-3.48±25.81
9	-9.91±47.99	-8.10±31.92	-23.04±14.47***	4.46±56.09	3.62±53.23	-3.42±24.36
10	-3.93±42.05	-0.41±38.12	-18.37±19.27*	10.86±71.40	-10.72±52.62	-6.73±29.56

Pressure Pain Threshold						
1	-2.83±51.06	-35.25±93.11	8.31±86.88	-7.14±51.84	10.54±78.71	-13.86±67.46
2	-17.45±66.19	-29.79±74.64	-14.56±91.88	-13.96±43.52	-0.81±62.07	-13.10±61.92
3	-1.81±60.91	-30.22±61.36	-20.43±88.54	-10.78±51.76	10.10±64.64	-12.42±98.54
4	-14.75±47.35	-13.50±55.53	-33.05±80.30	-16.49±76.23	12.10±73.77	-14.46±67.26
5	-15.60±74.40	-25.82±53.06	-32.22±101.99	-11.56±52.49	8.78±59.12	-7.74±107.27
6	-18.79±66.06	-16.41±32.11	-52.75±107.99	-6.21±55.74	6.49±58.10	-16.45±60.81
7	-7.46±54.72	-21.08±51.08	-12.02±82.92*	-6.80±44.32	-8.30±51.29	-9.59±75.99
8	-3.03±65.85	-34.54±61.14*	-34.42±88.57	-11.04±51.46	6.05±82.14	-14.06±113.45
9	-2.05±54.60	-4.88±26.53	-36.67±84.73	-11.26±52.58	4.97±51.02	-6.57±81.41
10	-4.99±77.63	1.11±32.42	-42.87±100.34	1.37±72.41	-4.78±53.35	-18.03±68.51

** Significant difference at two-tailed alpha=0.05, ** at two-tailed alpha=0.01. *** at two-tailed alpha=0.005.*

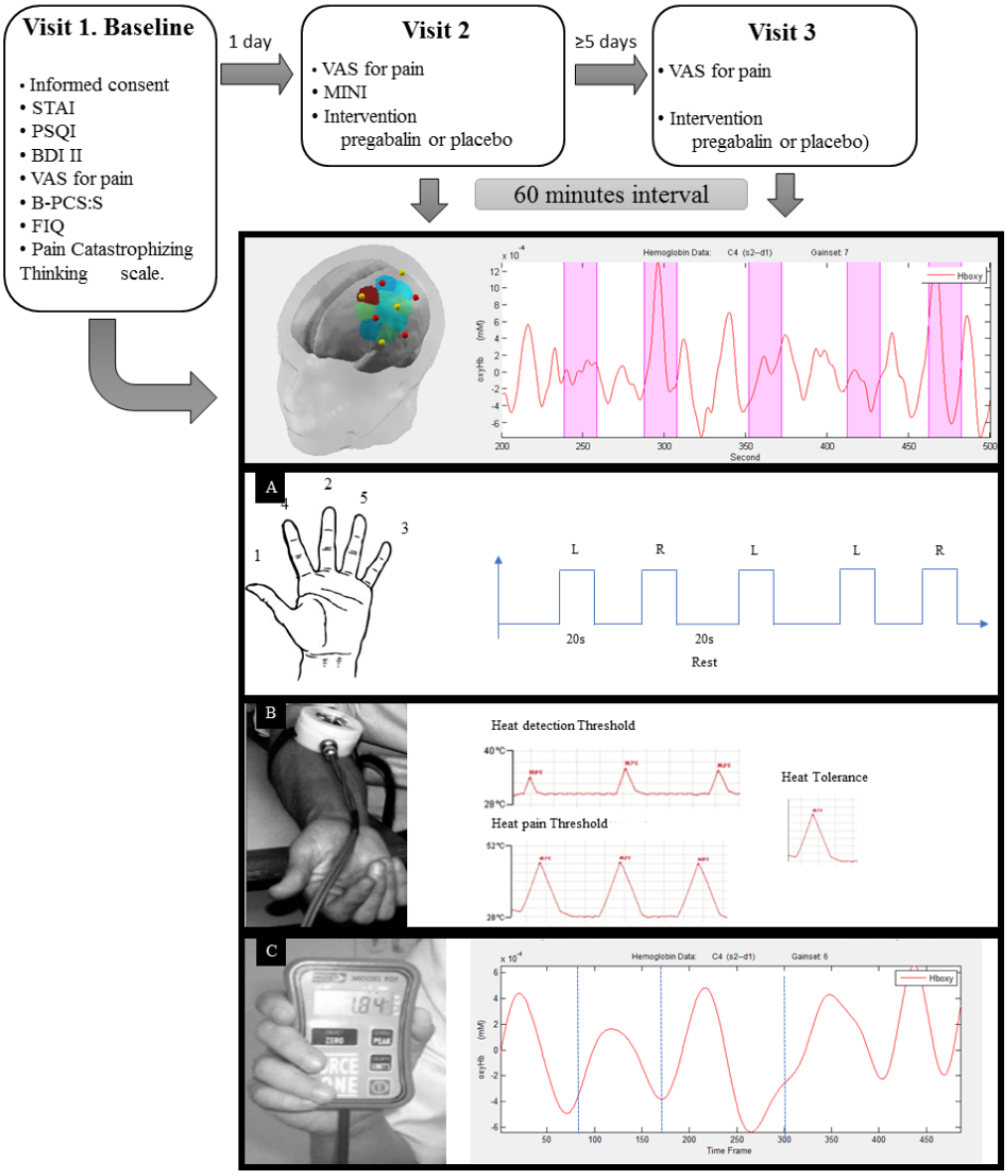
Table 4. Correlation between psychophysical pain testing and amplitude of the hemodynamic response

	Healthy subjects (n=10)			Fibromyalgia (n=17)		
Channel	**Baseline**	**Placebo**	**Pregabalin**	**Baseline**	**Placebo**	**Pregabalin**
Heat Detection Threshold						
1	-0.438	-0.593	-0.683	-0.474	0.291	0.254
2	-0.635	0.054	-0.342	-0.398	-0.126	-0.042
3	-0.200	-0.074	-0.304	-0.457	0.051	0.011
4	-0.600	-0.607	-0.381	-0.294	0.086	0.254
5	-0.450	-0.429	-0.238	-0.129	0.119	-0.360
6	-0.633	-0.429	-0.690	-0.059	-0.053	0.024
7	-0.653	0.185	-0.190	-0.380	-0.427	-0.236
8	-0.400	0.054	-0.144	-0.284	-0.361	-0.810**
9	-0.017	-0.179	-0.833*	0.296	-0.029	-0.554*
10	0.000	-0.072	-0.429	-0.364	-0.515	-0.174
Heat Pain Threshold						
1	0.183	0.237	0.523	-0.552*	-0.345	-0.520*
2	0.712*	0.050	0.468	-0.545*	-0.051	-0.314
3	0.200	-0.134	0.778*	-0.368	-0.020	-0.172
4	0.400	0.033	0.536	0.134	-0.095	-0.238
5	0.267	0.167	0.429	-0.064	0.187	-0.080
6	0.000	-0.283	0.613	-0.246	-0.367	-0.256
7	0.293	-0.167	0.357	0.125	-0.053	-0.124
8	0.150	-0.367	0.821*	-0.152	0.106	-0.286
9	0.317	-0.133	0.607	-0.407	0.297	0.145
10	0.300	-0.283	-0.107	0.196	-0.323	-0.025
Heat Tolerance						
1	0.486	-0.012	-0.482	0.386	-0.582	0.164
2	0.875**	0.024	-0.775*	0.408	-0.473	0.370
3	0.687*	0.719*	0.090	0.064	-0.373	0.200
4	0.559	0.071	0.036	0.236	-0.645*	-0.042
5	0.529	0.548	0.286	0.043	-0.518	0.479
6	0.109	0.429	-0.143	0.079	-0.655*	0.224
7	0.578	0.263	0.036	0.259	-0.509	0.492
8	0.687*	0.333	0.571	-0.080	-0.527	0.285
9	0.419	0.690	0.786*	0.182	-0.373	0.188
10	0.644*	0.310	0.107	0.121	-0.127	0.261
Pressure Pain Threshold						
1	0.732*	-0.359	0.214	-0.102	0.430	0.308
2	0.143	-0.103	-0.500	-0.003	0.152	-0.095
3	0.766*	-0.103	-0.786*	0.143	0.539	0.130
4	0.238	0.100	-0.571	0.341	0.697*	0.182
5	0.381	0.400	-0.536	0.313	0.188	0.134
6	0.000	0.000	-0.179	0.286	0.673*	0.134
7	0.000	-0.100	-0.541	-0.011	0.091	0.218
8	0.643	-0.700	-0.464	0.000	0.236	0.284
9	0.476	0.100	-0.679	-0.016	0.442	0.165
10	0.476	0.500	-0.321	0.027	0.673*	-0.455

* Significant difference at two-tailed alpha=0.05, ** at two-tailed alpha=0.01, *** at two-tailed alpha=0.005.

FIGURES

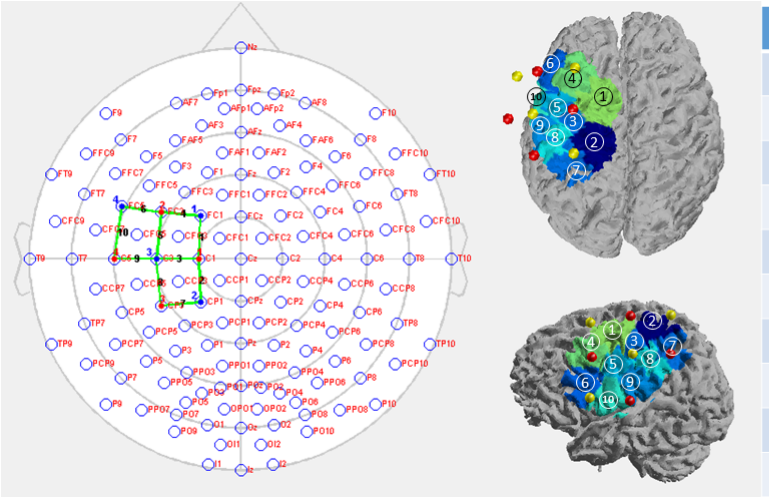
Figure 1. Experimental cross-over design visits.



The wash-out period was at least five days. The first left vignette represents the cortical areas assessed by the fNIRS montage, and at its right, the oxygenated hemoglobin changes during a task (shaded period), and during rest. Vignette A, left-side:

representation of the fingers' movement order for the left-hand finger-tapping task (LHFT); right-side: the boxcar paradigm of the finger-tapping task, with order of right (R) and left (L) hand task randomized. Vignette B, left-side: Peltier-based device thermode placed over the right forearm volar face; right-side: representation of the temperature changes according to the psychophysical test. Vignette C, left-side: Pressure Pain digital algometer; right-side: oxygenated hemoglobin changes during the task. Note that for both thermal and pressure stimulation the duration of the stimuli varies according the patients' response. Abbreviations: STAI, state-trait anxiety inventory; PSQI, Pittsburgh sleep quality index; BDI II, Beck depression inventory; VAS, visual analog scale; B-PCS:S, Brazilian Portuguese profile for chronic pain screening; FIQ, fibromyalgia impact questionnaire; fNIRS, functional near infra-red spectroscopy; LHFT, left hand finger tapping task; HDT, heat detection threshold; HPT, heat pain threshold; HT, heat tolerance; PPT, pressure pain detection threshold; MINI, mini neuropsychiatric interview.

Figure 2. Experimental probes montage.



fNIRS channel	EEG electrode	Approximate cortical area
1	FC1-C1	Superior Frontal - Precentral Gyrus
2	C1-CP1	Precentral - postcentral gyrus
3	C3-C1	Postcentral - Precentral gyrus
4	FC3-C1	Middle frontal - Precentral gyrus
5	FC3-C3	Middle frontal -postcentral gyrus
6	FC3-FC5	Middle frontal - Precentral gyrus
7	CP3-CP1	Inferior parietal - postcentral gyrus
8	C3-CP3	postcentral - inferior parietal gyrus
9	C5-C3	Postcentral - postcentral gyrus
10	FC5-C5	Precentral - postcentral gyrus

Left: light sources (red dots) and detectors (blue dots) placement in relation to the 10-20 electroencephalography system, and their corresponding acquisition channels (green lines). Center, axial (superior) and sagittal (inferior) representation of the cortical region captured by the montage, with light sources (red globes), detectors (yellow globes), and channels (circled numbers). Right: Relationship between fNIRS channels, 10-20 EEG system electrodes positioning, and approximate correlation with cortical areas.

Figure 3. Patients flow chart according to the CONSORT statement.

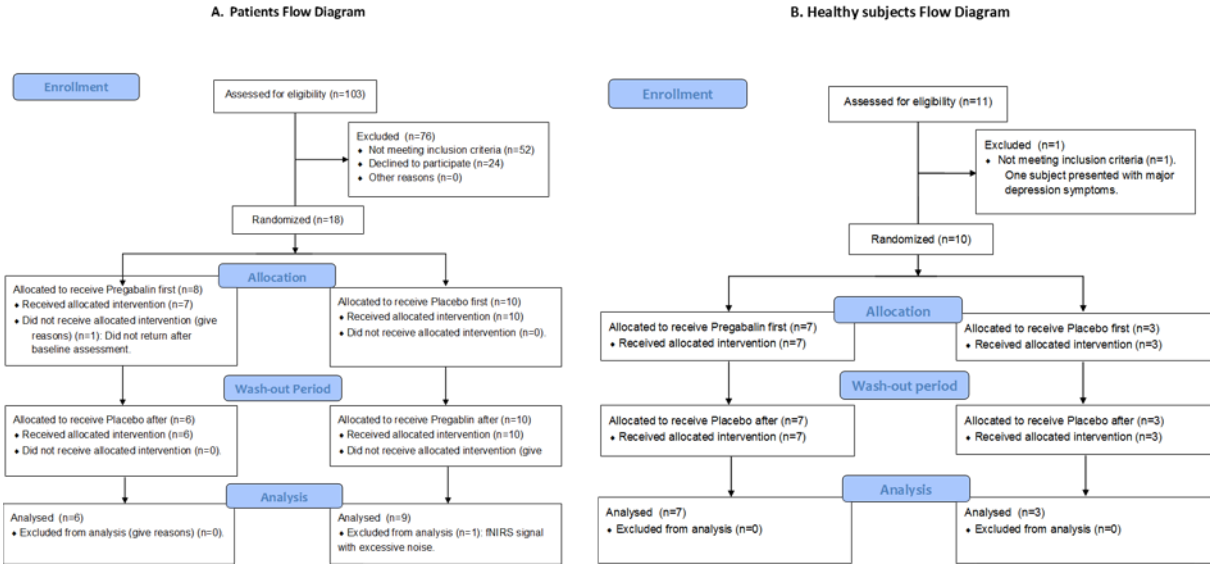
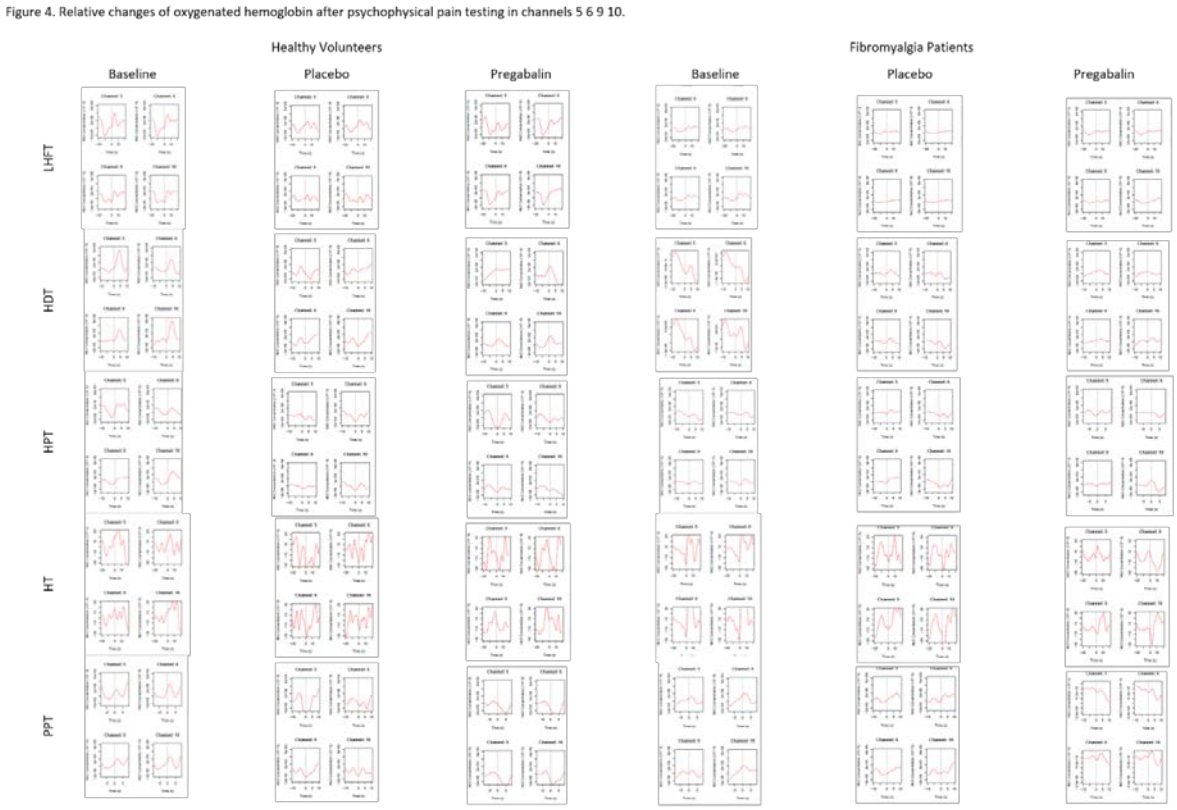
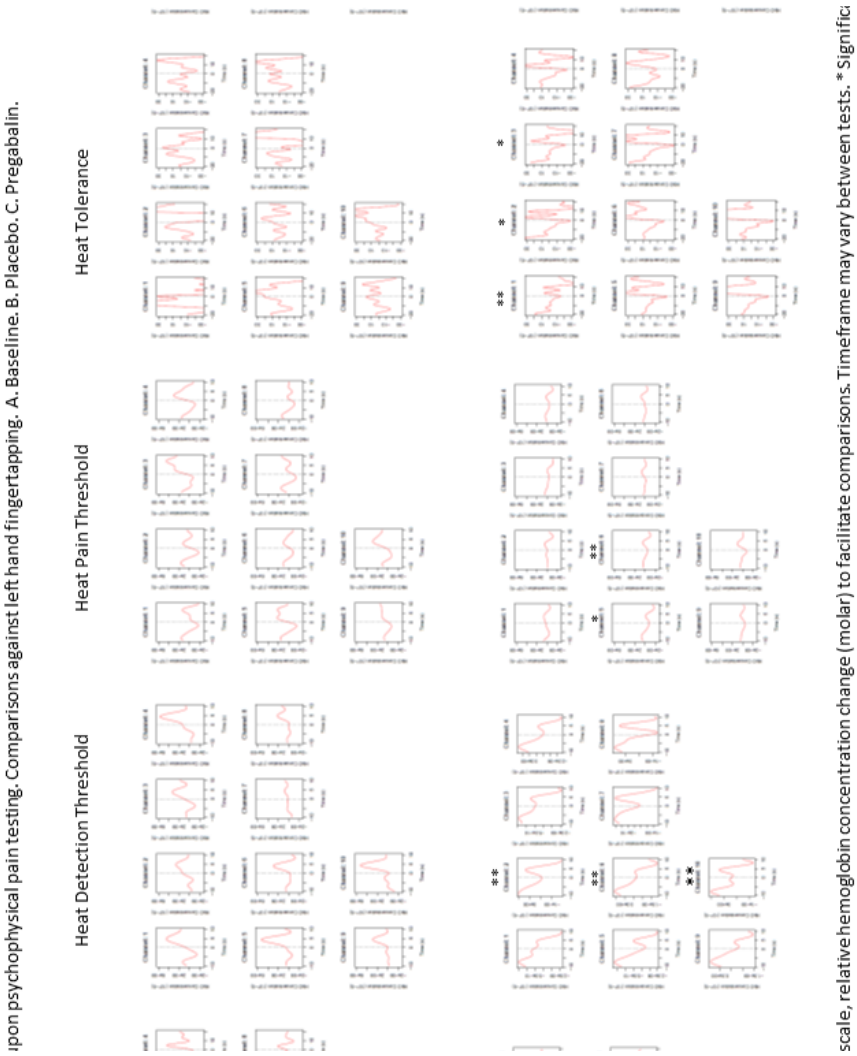


Figure 4. Relative changes of oxygenated hemoglobin after psychophysical pain testing in channels 5 6 9 and 10.



SUPPLEMENTARY MATERIAL

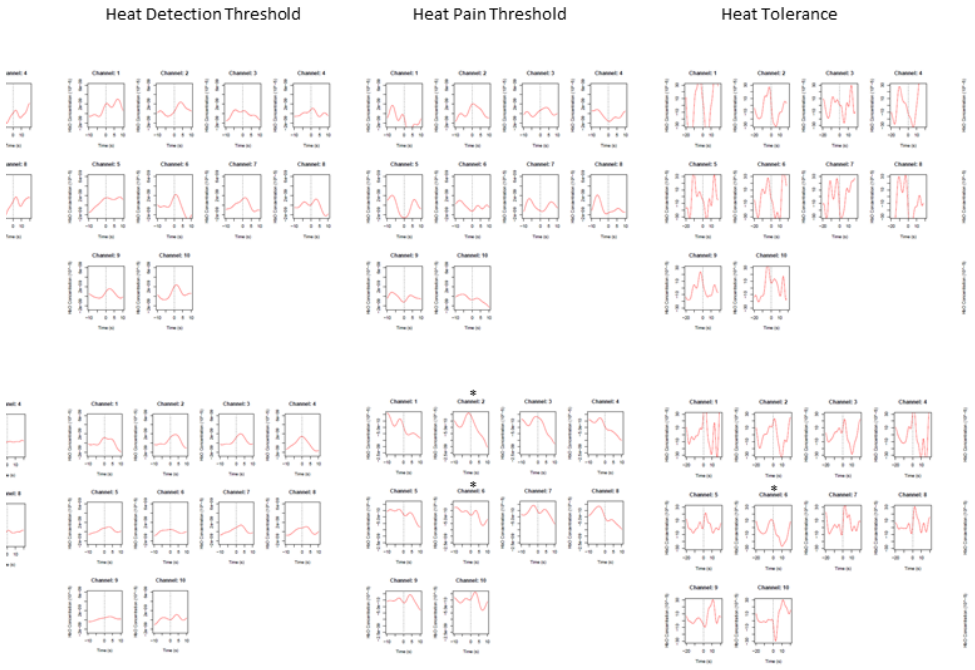
Appendix 1. Cortical activation upon psychophysical pain testing.



ıpon psychophysical pain testing. Comparisons against left hand fingertapping. A. Baseline. B. Placebo. C. Pregabalin.

scale, relative hemoglobin concentration change (molar) to facilitate comparisons. Timeframe may vary between tests. * Significa
two-tailed alpha=0,01; and *** at two-tailed alpha=0,005.

ance) share the same y-axis scale, relative hemoglobin concentration change (molar) to facilitate comparisons. Timeframe may va
hand fingertapping activation, at two-tailed alpha=0,01; and *** at two-tailed alpha=0,005.



ance) share the same y-axis scale, relative hemoglobin concentration change (molar) to facilitate comparisons. Timeframe may va
hand fingertapping activation, at two-tailed alpha=0,01; and *** at two-tailed alpha=0,005.

CAPÍTULO V - CONSIDERAÇÕES FINAIS E PERSPECTIVAS FUTURAS

1. CONSIDERAÇÕES FINAIS

Os resultados obtidos com esta tese de doutorado permitem emitir as seguintes conclusões:

- As características da resposta a estímulos doloros padronizados em pacientes com fibromialgia apresentam uma correlação com a atividade cortical motora, somática e sensitiva, que é consistente com os mecanismos patofisiológicos mais aceitos para entender esta doença.
- Por um lado, a apresentação de alterações sutis nos testes psicofísicos da dor, reforçam as hipóteses que explicam o quadro clínico da doença e sua resposta às terapias, por mecanismos associados a neuropatia periférica. Esta característica, foi acompanhada por diminuição na ativação de áreas sensitivas e motoras, e aumento nas áreas relacionadaso ao processamento cognitivo do impulso nociceptivo, sendo ésta modulada (no sentido de aumentar ativação) pela pregabalina.
- Pacientes fibromialgias apresentam maior recrutamento de áreas corticais responsáveis pelo processamento cognitivo do estímulo, em resposta a eventos nociceptivos de maior amplitude. De maneira consistente, o limiar de tolerância está correlacionado de forma inversa com a ativação, em outras palavras, as pacientes menos tolerantes (mais sensíveis) apresentam maior ativação cortical. Estes achados reforçam a presença de mecanimos de sensibilização ao nível de sistema nervoso central (encéfalo) na doença.

- Na dosagem empregada no nosso estudo, a pregabalina não foi capaz de mudar a ativação cortical frente a estímulos de maior amplitude. É plausível pensar que por se tratar de um processo patológico instaurado de longa data, mesmo agindo em elos chaves do processo fisiológico alterado, uma intervenção única não conseguiria levar o sistema de volta ao funcionamento normal.

2. PERSPECTIVAS FUTURAS

Esta tese de doutorado corrobora para o fortalecimento e solidificação de uma linha de pesquisa que estuda processos de neuromodulação da dor. Além disso, abre a possibilidade de introduzir em nosso meio o uso da fNIRS como ferramenta para auxiliar na avaliação de pacientes com síndromes dolorosas. Os resultados obtidos serão divulgados para a comunidade científica afim de possibilitar melhora na assistência de pacientes que sofrem de patologias que demandam fartas quantias do sistema público de saúde brasileiro.

Além disso a confirmação de “assinaturas hemodinâmicas” no córtex, nos permite avaliação “objetiva” de mudanças neuroplásticas associadas à dor. Com os resultados desse estudo pretende-se no futuro, identificar características corticais de pacientes respondedores e não respondedores às terapias utilizadas.

CAPÍTULO VI - ANEXOS

1. COLABORAÇÕES EXTERNAS

Prof. Dr. Rickson Mesquita – Unicamp, colaborações com NIRS.

Prof. Dr. João Ricardo Sato – Universidade Federal do ABC, colaborações com NIRS.

2. PRODUÇÃO CIENTÍFICA DURANTE O PERÍODO DE DOUTORAMENTO (2013-2017).

2.1 Artigos completos publicados em periódicos

1. BRIETZKE, ALINE P.; ROZISKY, JOANNA R.; **DUSSAN-SARRIA, JAIRO A.**; DEITOS, ALICIA; LASTE, GABRIELA; HOPPE, PRISCILA F.T.; MULLER, SUZANA; TORRES, IRACI L.S.; ALVARES-DA-SILVA, MÁRIO R.; DE AMORIM, RIVADAVIO F.B.; FREGNI, FELIPE; CAUMO, WOLNEI. Neuroplastic Effects of Transcranial Direct Current Stimulation on Painful Symptoms Reduction in Chronic Hepatitis C: A Phase II Randomized, Double Blind, Sham Controlled Trial. Frontiers in Neuroscience, v. 9, p. 498, 2016.

frontiers in Neuroscience

CLINICAL TRIAL
published: 11 January 2016
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Neuroplastic Effects of Transcranial Direct Current Stimulation on Painful Symptoms Reduction in Chronic Hepatitis C: A Phase II Randomized, Double Blind, Sham Controlled Trial

*Aline P. Brietzke [1], Joanna R. Rozisky [1], Jairo A. Dussan-Sarria [1], Alicia Deitos [1], Gabriela Laste [1], Priscila F. T. Hoppe [1], Suzana Muller [1], Iraci L. S. Torres [1], Mário R. Alvares-da-Silva [2], Rivadavio F. B. de Amorim [3], Felipe Fregni [3] and Wolnei Caumo [1]**

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Introduction: Pegylated Interferon Alpha (Peg-IFN) in combination with other drugs is the standard treatment for chronic hepatitis C infection (HCV) and is related to severe painful symptoms. The aim of this study was access the efficacy of transcranial direct current stimulation (tDCS) in controlling the painful symptoms related to Peg-IFN side effects.

Materials and Methods: In this phase II double-blind trial, twenty eight ($n = 28$) HCV subjects were randomized to receive either 5 consecutive days of active tDCS ($n = 14$) or sham ($n = 14$) during 5 consecutive days with anodal stimulation over the primary motor cortex region using 2 mA for 20 min. The primary outcomes were visual analogue scale (VAS) pain and brain-derived neurotrophic factor (BDNF) serum levels. Secondary outcomes were the pressure-pain threshold (PPT), the Brazilian Profile of Chronic Pain: Screen (B-PCP:S), and drug analgesics use.

Results: tDCS reduced the VAS scores ($P < 0.003$), with a mean pain drop of 56% ($p < 0.001$). Furthermore, tDCS was able to enhance BDNF levels ($p < 0.01$). The mean increase was 37.48% in the active group. Finally, tDCS raised PPT ($p < 0.001$) and reduced the B-PCP:S scores and analgesic use ($p < 0.05$).

Conclusions: Five sessions of tDCS were effective in reducing the painful symptoms in HCV patients undergoing Peg-IFN treatment. These findings support the efficacy of tDCS as a promising therapeutic tool to improve the tolerance of the side effects related to the use of Peg-IFN. Future larger studies (phase III and IV trials) are needed to confirm the clinical use of the therapeutic effects of tDCS in such condition.

Trial registration: Brazilian Human Health Regulator for Research with the approval number CAAE 07802012.0.0000.5327.

Keywords: Hepatitis C, chronic pain, Hepatitis C virus, PEG-IFN, transcranial direct current stimulation

2. CAUMO, WOLNEI; DEITOS, ALÍCIA; CARVALHO, SANDRA; LEITE, JORGE; CARVALHO, FABIANA; **DUSSÁN-SARRIA, JAIRO ALBERTO**; LOPES TARRAGÓ, MARIA DA GRAÇA; SOUZA, ANDRESSA; TORRES, IRACI LUCENA DA SILVA; FREGNI, FELIPE. Motor Cortex Excitability and BDNF Levels in Chronic Musculoskeletal Pain According to Structural Pathology. Frontiers in Human Neuroscience, v. 10, p. 357, 2016.

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ORIGINAL RESEARCH
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Motor Cortex Excitability and BDNF Levels in Chronic Musculoskeletal Pain According to Structural Pathology

Wolnei Caumo[1,2,3,4], Alicia Deitos[1,2], Sandra Carvalho[5], Jorge Leite[5], Fabiana Carvalho[1,2], Jairo Alberto Dussán-Sarria[1,2], Maria da Graça Lopes Tarragó[1,2], Andressa Souza[6], Iraci Lucena da Silva Torres[1,7] and Felipe Fregni[8]*

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The central sensitization syndrome (CSS) encompasses disorders with overlapping symptoms in a structural pathology spectrum ranging from persistent nociception [e.g., osteoarthritis (OA)] to an absence of tissue injuries such as the one presented in fibromyalgia (FM) and myofascial pain syndrome (MPS). First, we hypothesized that these syndromes present differences in their cortical excitability parameters assessed by transcranial magnetic stimulation (TMS), namely motor evoked potential (MEP), cortical silent period (CSP), short intracortical inhibition (SICI) and short intracortical facilitation (SICF). Second, considering that the presence of tissue injury could be detected by serum neurotrophins, we hypothesized that the spectrum of structural pathology (i.e., from persistent nociception like in OA, to the absence of tissue injury like in FM and MPS), could be detected by differential efficiency of their descending pain inhibitory system, as assessed by the conditioned pain modulation (CPM) paradigm. Third, we explored whether brain-derived neurotrophic factor (BDNF) had an influence on the relationship between motor cortex excitability and structural pathology. This cross-sectional study pooled baseline data from three randomized clinical trials. We included females (n = 114), aged 19–65 years old with disability by chronic pain syndromes (CPS): FM (n = 19), MPS (n = 54), OA (n = 27) and healthy subjects (n = 14). We assessed the serum BDNF, the motor cortex excitability by parameters the TMS measures and the change on numerical pain scale [NPS (0–10)] during CPM-task. The adjusted mean (SD) on the SICI observed in the absence of tissue injury was 56.36% lower than with persistent nociceptive input [0.31(0.18) vs. 0.55 (0.32)], respectively. The BDNF was inversely correlated with the SICI and with the change on NPS (0–10) during CPM-task. These findings suggest greater disinhibition in the motor cortex and the descending pain inhibitory system in FM and MPS than in OA and healthy subjects.

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3. DE SOUZA, ANDRESSA; SILVA, R.B.; **DUSSAN-SARRIA, JAIRO ALBERTO**; LAZZAROTTO, A. R.. Dor neuropática em pacientes com HIV/AIDS em uso de terapia antirretroviral. Clinical and Biomedical Research, v. 36, p. 156-164, 2016.

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Dor neuropática em pacientes com HIV/AIDS em uso de terapia antirretroviral

Neuropathic pain in HIV/AIDS patients using antiretroviral therapy

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RESUMO

A infecção pelo vírus da imunodeficiência humana (HIV) representa um dos maiores problemas de saúde da atualidade em virtude de seu caráter pandêmico e gravidade. O uso da terapia antirretroviral está associado ao desenvolvimento de diferentes tipos de dor, sendo a dor neuropática uma delas. A dor neuropática ocorre pela lesão do sistema nociceptivo, que envolve mudanças moleculares, fisiológicas e anatômicas. Considerando que a infecção por HIV gera custos elevados para o sistema de saúde, e que suas comorbidades elevam ainda mais esses custos, a compreensão dos mecanismos da dor nessa população possibilita uma melhor assistência e uma redução da carga para o sistema. A dor neuropática pode apresentar diferentes possíveis mecanismos fisiopatológicos envolvidos, tornando-se um desafio para o tratamento de pacientes com HIV. A compreensão sobre a neurofisiologia da dor neuropática e o HIV pode promover melhores abordagens aos pacientes e a redução de comorbidades associadas, com impacto na qualidade de vida.

Palavras-chave: *Dor neuropática; HIV; terapia antirretroviral*

ABSTRACT

Human immunodeficiency virus (HIV) infection is a major health problem nowadays due to its pandemic character and severity. The use of antiretroviral therapy is associated with the development of different types of pain, and neuropathic pain is one of them. Neuropathic pain is caused by an injury to the nociceptive system, which involves molecular, physiological and anatomical changes. Considering that HIV infection generates high costs for the health system, and that its comorbidities raise such costs even more, understanding the mechanisms of pain in this population can result in better care practices and reduction of burden to the system. Neuropathic pain may present different pathophysiological mechanisms becoming a challenge for HIV treatment. The understanding of the neurophysiology of neuropathic pain and the HIV can promote better patient approaches and the reduction of associated comorbidities with an impact on quality of life.

Keywords: *Neuropathic pain; HIV; antiretroviral therapy*

O vírus da imunodeficiência humana (HIV) pertence à família Retroviridae, subfamília Lentiviridae. Ele causa efeitos citopáticos em curto prazo e uma infecção longitudinal persistente que culmina em um quadro clínico de imunodeficiência, que corresponde à síndrome da imunodeficiência adquirida (AIDS). A sua principal característica é a supressão profunda da imunidade mediada por células T, que torna o indivíduo suscetível a infecções oportunistas, neoplasias secundárias e doenças neurológicas que, se não forem combatidas, levam-no inevitavelmente ao óbito[1].

A Organização Mundial da Saúde (OMS) informou que, ao final de 2013, havia 35 (33,1-37,2) milhões de pessoas vivendo com a infecção no mundo[2]. Embora a incidência global tenha sido estabilizada em 2,1 milhões em 2013, o número de pacientes que recebem a terapia antirretroviral combinada (TARV)

4. VERGARA-DIAZ, GLORIA; **DUSSAN-SARRIA, JAIRO ALBERTO**; DOMINGUEZ-IGLESIA, MASSIEL; O?BRIEN, ANNE; ADANS-DESTER, CATHERINE; LUI, SIEW KWAON; BONATO, PAOLO . Retrospective Analysis of

Clinical Practice Data of Robot-Assisted Gait Training in Patients with Spinal Cord Injury. Archives of Physical Medicine and Rehabilitation (Print), v. 97, p. e136, 2016.

Research Poster 815

Forearm Endurance Training in a Woman With Kearns-Sayre Syndrome: Case Report

Kevin McCully (University of Georgia), Brad Willingham, Hannah Bossie, Fran Kendall

Research Objectives: To measure clinical symptoms, muscle-specific endurance, and muscle mitochondrial capacity in a person with KSS before and after one month of endurance training.
Design: Before-after case control.
Setting: University setting.
Participants: Participant was a 48-year-old female with a diagnosis of KSS, confirmed by an abnormal biopsy indicating a complex 1 defect, a genetically confirmed mitochondrial DNA deletion mutation, and elevated lactate levels.
Interventions: The non-dominant forearm was trained for 18 sessions of 20-30 minutes using 3-5 pounds over 34 days.
Main Outcome Measure(s): Muscle-specific endurance was measured with surface mechanomyography and electrical stimulation at 2, 4, and 6 Hz sequentially. Mitochondrial capacity was measured using the rate of recovery of oxygen consumption after a short bout of electrical stimulation.
Results: Contractions per training session increased from 240 to 525. Symptoms of muscle fatigue and weakness following training sessions decreased markedly throughout the training program. Grip strength increased from 19.1 to 20.7 kg. Muscle endurance index at 6 Hz increased from 67% to 83%. Muscle mitochondrial capacity (recovery rate constant) increased from 1.4/min to 2.2/min.
Conclusions: A patient with mitochondrial disease, specifically KSS, was able to significantly improve her muscle function with endurance training. The 57% increase in mitochondrial capacity is consistent with training adaptations reported for non-diseased controls using a similar training protocol. This suggests her muscles have the ability to upregulate mitochondrial biogenesis despite her genetic disease. This study also demonstrates the potential of accelerometry-based assessments of muscle endurance and muscle mitochondrial tests to noninvasively evaluate patients with mitochondrial diseases.
Key Words: Exercise, mitochondrial, fatigue
Disclosures: None disclosed.

Research Poster 816

Retrospective Analysis of Clinical Practice Data of Robot-Assisted Gait Training in Patients with Spinal Cord Injury

Gloria Vergara-Diaz (Department of Physical Medicine and Rehabilitation, Harvard Medical School Spaulding Rehabilitation Hospital), Jairo Alberto Dussan-Sarria, Massiel Dominguez-Iglesia, Anne O'Brien, Catherine Adans-Dester, Siew Kwaon Lui, Paolo Bonato

Research Objectives: To study training settings and outcomes of robot-assisted gait training in patients with spinal cord injury (SCI).
Design: Retrospective cohort.
Setting: Rehabilitation hospital (inpatient).
Participants: Adults with stable SCI who received robot-assisted gait training between June 2009 and June 2014.
Interventions: Robot-assisted gait training.
Main Outcome Measure(s): Demographic and clinical data: type of injury, ASIA Impairment Scale (AIS) level; number of sessions and Lokomat® therapy settings.
Results: The data of 84 patients 47.1 [28 - 63.7] years of age (median and interquartile range) were included. 70.2% were male subjects. 84% had acute SCI; 52.4% with traumatic etiology. Only 14% were complete injuries. AIS grade was predominantly D (31%) with 57.7% of the injuries at the cervical level. A median of 14[10.75-17.25] patients per year underwent robot-assisted gait training. The median number of training sessions was 5 [3-8]. 39 patients performed more that 6 sessions. The settings (median and interquartile range) of the first session were: 50 % [36.5-50] Body-Weight Support, 85 % [70-100] guidance force, and treadmill speed 2.0 km/h [1.8-2.1]. We intend to compare our data with data extracted from the ARTIC database, a multi-site initiative aiming to evaluating correlations among the type of injury, therapy plan used, and functional outcomes in patients undergoing Lokomat® therapy.
Conclusions: The median number of robot-assisted gait training sessions was lower than we anticipated. However, considering our clinical setting, these results could be related to the short duration of inpatient stay when therapy time has to be allocated for other functional goals besides ambulation. We suggest that it would be advisable to establish standard protocols for robot-assisted gait training in order to assess the effectiveness of robot-assisted training in patients with SCI.
Key Words: Spinal Cord Injuries, Robotic Exoskeleton, Biomedical Technology, Rehabilitation
Disclosures: None disclosed.

Research Poster 817

Impact of Biopsychosocial Factors on Quality of Participation in Persons with Spinal Cord Injury: Multisite Cross-Sectional Study

Brent Walter (Washington University in St. Louis), Jess Dashner, Alex Wong, Allen Heinemann

Research Objectives: To assess the role of biological, psychological, and socio-environmental factors in persons with spinal cord injury (SCI).
Design: Multisite cross-sectional study. Participants completed patient-reported measures of health, personal and environmental factors, and participation. We used the biopsychosocial model to guide the selection of study variables. We used exploratory factor analysis to obtain a factor score to represent the quality of participation. Variables reduction method ($p<0.1$) was used to select variables for the final regression model.
Setting: Three medical institutions located in Chicago, Michigan and St. Louis.
Participants: 150 community-dwelling adults with SCI.
Interventions: Not applicable.
Main Outcome Measure(s): PROMIS, NIH Toolbox, NeuroQoL, Community Participation Indicators, Economic Quality of Life, SCI Quality of Life Measurement System, Environmental Factors Item Banks and other legacy measures of environmental factors.
Results: One single-factor solution was obtained to represent quality of participation (Only 1 factor with eigenvalue >1.0 and explained 69.5% of variances). Biological factors including pain ($p=0.045$), upper extremity function ($p<0.001$), psychological factors including loneliness ($p=0.016$ and meaning ($p=0.048$), as well as socio-environmental factors including the use of assistive technology for mobility were associated with the quality of participation. The final model explained for 53.2% of variances in participation ($F(11, 138)=16.424$, $p<0.001$).
Conclusions: Pain, upper extremity function, loneliness, life meaning, and assistive technology use of adults with SCI were associated with the quality of participation. These results can provide targets for interventions to improve participation for SCI.
Key Words: Outcome Assessment (Health Care), Participation, Spinal Cord Injury
Disclosures: None disclosed.

Research Poster 820

Community Reintegration in Veterans with Traumatic Brain Injury: Perspectives from Community Event Observations

Christine Melillo (VA), Karen Besterman-Dahan, Gail Powell-Cope, Christina (Tina) Dillahunt-Aspillaga, Bridget Hahm, Jason Lind, Kiersten Downs, Nicole Antinori

Research Objectives: The objective of this poster is to report preliminary data describing: (a) public events supporting community reintegration (CR), and (b) contextual factors that facilitate and impede CR.

5. KNIJNIK, LEONARDO M.; **DUSSÁN-SARRIA, JAIRO A.**; ROZISKY, JOANNA R.; TORRES, IRACI L. S.; BRUNONI, ANDRE R.; FREGNI, FELIPE; CAUMO, WOLNEI. Repetitive Transcranial Magnetic Stimulation for Fibromyalgia:

Systematic Review and Meta-Analysis. Pain Practice (Print), v. 1533, p. n/a-n/a, 2015.

ORIGINAL ARTICLE

Repetitive Transcranial Magnetic Stimulation for Fibromyalgia: Systematic Review and Meta-Analysis

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■ **Abstract**

Background: Fibromyalgia (FM) is a prevalent chronic pain syndrome with few effective therapeutic options available. Repetitive transcranial magnetic stimulation (rTMS) is an emerging therapeutic alternative for this condition; however, results have been mixed.

Objectives: To evaluate the efficacy of rTMS on FM, a comprehensive systematic review and meta-analysis were performed.

Methods: Relevant published, English and Portuguese language, randomized clinical trials (RCT) comparing rTMS (irrespective of the stimulation protocol) to sham stimulation for treating FM pain intensity, depression, and/or quality of life (QoL) were identified, considering only those with low risk for bias. Trials available until April 2014 were searched through MEDLINE, EMBASE, the Cochrane Library Databases, and other 26 relevant medical databases covering from every continent. The outcomes for pain, depression, and QoL assessed closest to the 30th day after rTMS treatment were extracted, and changes from baseline were calculated to compare the effects of rTMS vs. placebo.

Results: One hundred and sixty-three articles were screened, and five with moderate to high quality were included. rTMS improved QoL with a moderate effect size (Pooled SMD = −0.472 95%CI = −0.80 to −0.14); it showed a trend toward reducing pain intensity (SMD = −0.64 95% CI = −0.31 to 0.017), but did not change depressive symptoms.

Conclusion: In comparison with sham stimulation, rTMS demonstrated superior effect on the QoL of patients with FM 1 month after starting therapy. However, further studies are needed to determine optimal treatment protocols and to elucidate the mechanisms involved with this effect, which

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Pain Practice, Volume ••, Issue •, 2015 ••–••

6. DEITOS, ALÍCIA; **DUSSÁN-SARRIA, JAIRO ALBERTO**; DE SOUZA, ANDRESSA; MEDEIROS, LICIANE; TARRAGÔ, MARIA DA GRAÇA; SEHN, FRANCISLEA; CHASSOT, MÔNICA; ZANETTE, SIMONE; SCHWERTNER,

ANDRÉ; FREGNI, FELIPE; TORRES, IRACI L.S.; CAUMO, WOLNEI. Clinical Value of Serum Neuroplasticity Mediators in Identifying the Central Sensitivity Syndrome in Patients with Chronic Pain with and Without Structural Pathology. The Clinical Journal of Pain, v. na, p. 1, 2015.

ORIGINAL ARTICLE

Clinical Value of Serum Neuroplasticity Mediators in Identifying the Central Sensitivity Syndrome in Patients With Chronic Pain With and Without Structural Pathology

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Maria da Graça Tarragô, MD,†§ Francislea Sehn, MSc, PT,*†*
Mônica Chassot, MSc,† Simone Zanette, MD, PhD,*†§*
André Schwertner, MD,† Felipe Fregni, MD, PhD,‖¶#*
Iraci L. S. Torres, PhD,†** and Wolnei Caumo, MD, PhD*†††‡‡*

Background and Objectives: Central sensitivity syndrome (CSS) encompasses disorders with overlapping symptoms in a spectrum of structural pathology from persistent somatic nociception (eg, osteoarthritis) to absence of tissue injury such as in fibromyalgia, chronic tension-type headache, and myofascial pain syndrome. Likewise, the spectrum of the neuroplasticity mediators associated with CSS might present a pattern of clinical utility.

Methods: We studied the brain-derived neurotrophic factor (BDNF), tumor necrosis factor-α (TNF-α), and interleukins 6 (IL-6) and IL-10 in female patients with CSS absent of structural pathology (chronic tension-type headache [n = 30], myofascial pain syndrome [n = 29], fibromyalgia [n = 22]); with CSS due to persistent somatic/visceral nociception (osteoarthritis [n = 27] and endometriosis [n = 32]); and in pain-free controls (n = 37).

Results: Patients with CSS absent of structural pathology presented higher serum TNF-α (28.61 ± 12.74 pg/mL) and BDNF (49.87 ± 31.86 ng/mL) than those with persistent somatic/visceral nociception (TNF-α = 17.35 ± 7.38 pg/mL; BDNF = 20.44 ± 8.30 ng/mL) and controls (TNF-α = 21.41 ± 5.74 pg/mL, BDNF = 14.09 ± 11.80 ng/mL). Moreover, CSS patients absent of structural pathology presented lower IL levels. Receiver operator characteristics analysis showed the ability of BDNF to screen CSS (irrespective of the presence of structural pathology) from controls (cutoff = 13.31 ng/mL, area under the curve [AUC] = 0.86, sensitivity = 95.06%, specificity = 56.76%); and its ability to identify persistent nociception in CSS patients when experiencing moderate-severe depressive symptoms (AUC = 0.81; cutoff = 42.83 ng/mL, sensitivity = 56.80%, specificity = 100%). When the level of pain measured on the visual analog scale was <5 and moderate-severe depressive symptoms were observed TNF-α discriminated structural pathology in the chronic pain conditions (AUC = 0.97; cutoff = 22.11 pg/mL, sensitivity = 90%, specificity = 91.3%).

Conclusion: Neuroplasticity mediators could play a role as screening tools for pain clinicians, and as validation of the complex and diffuse symptoms of these patients.

Key Word: central sensitivity syndrome, chronic pain, plasticity, BDNF, TNF, cytokines

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A.D. and J.A.D.-S. contributed equally.

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Central sensitivity syndrome (CSS) is an empirical concept proposed to improve the understanding and management of a group of disorders with overlapping features including psychological distress, sleep disturbances, fatigue, pain, allodynia, hyperalgesia, expansion of receptive field, and hypersensitivity to noise and chemical substances. In some of these disorders, such as fibromyalgia (FM), chronic fatigue syndrome, chronic tension-type headache (CTTH), myofascial pain syndrome (MPS), and primary dysmenorrhea[1] there is scarce evidence of structural pathology. In contrast, in other conditions like osteoarthritis (OA)[2] or endometriosis,[3] there is anatomic structural pathology (which accounts for persistent somatic or visceral nociceptive input, respectively) and demonstrated hyperalgesia outside of the sensitized areas. In OA, low pressure pain thresholds over the affected joints reflect local sensitization due to local inflammation, whereas reduced pain thresholds at sites remote from the affected joint support the hypothesis of central sensitization (CS).[4–10] Similarly, in endometriosis, the hyperalgesia has also been described distant from the pelvis, in the first dorsal interosseus muscle

7. MEDEIROS, LICIANE F.; CAUMO, WOLNEI; **DUSSÁN-SARRIA, JAIRO**; DEITOS, ALICIA; BRIETZKE, ALINE; LASTE, GABRIELA; CAMPOS-

CARRARO, CRISTINA; DE SOUZA, ANDRESSA; SCARABELOT, VANESSA L.; CIOATO, STEFANIA G.; VERCELINO, RAFAEL; DE CASTRO, ALEXANDRE L.; ARAÚJO, ALEX SANDER; BELLÓ-KLEIN, ADRIANE; FREGNI, FELIPE; TORRES, IRACI L.S.. Effect of Deep Intramuscular Stimulation and Transcranial Magnetic Stimulation on Neurophysiological Biomarkers in Chronic Myofascial Pain Syndrome. Pain Medicine (Malden, Mass.), 2015.

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OXFORD

NEUROMODULATION SECTION

Original Research Article

Effect of Deep Intramuscular Stimulation and Transcranial Magnetic Stimulation on Neurophysiological Biomarkers in Chronic Myofascial Pain Syndrome

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Abstract

Objective. The aim was to assess the neuromodulation techniques effects (repetitive transcranial magnetic stimulation [rTMS] and deep intramuscular stimulation therapy [DIMST]) on pain intensity, peripheral, and neurophysiological biomarkers chronic myofascial pain syndrome (MPS) patients.

Design. Randomized, double blind, factorial design, and controlled placebo-sham clinical trial.

Setting. Clinical trial in the Laboratory of Pain and Neuromodulation at Hospital de Clínicas de Porto Alegre (NCT02381171).

Subjects. We recruited women aged between 19- and 75-year old, with MPS diagnosis.

Methods. Patients were randomized into four groups: rTMS + DIMST, rTMS + sham-DIMST, sham-rTMS + DIMST, sham-rTMS + sham-DIMST; and received 10 sessions for 20 minutes each one (rTMS and DIMST). Pain was assessed by visual analogue scale (VAS); neurophysiological parameters were assessed by transcranial magnetic stimulation; biochemical parameters were: BDNF, S100β, lactate dehydrogenase, inflammatory (TNF-α, IL6, and IL10), and oxidative stress parameters.

Results. We observed the pain relief assessed by VAS immediately assessed before and after the intervention ($P < 0.05$, $F_{(1,3)} = 3.494$ and $F_{(1,3)} = 4.656$, respectively); in the sham-rTMS + DIMST group and both three active groups in relation to sham-rTMS + sham-DIMST group, respectively. There was an increase in the MEP after rTMS + sham-DIMST ($P < 0.05$). However, there was no change in all-peripheral parameters analyzed across the treatment ($P > 0.05$).

Conclusion. Our findings add additional evidence about rTMS and DIMST in relieving pain in MPS

8. VERGARA-DIAZ, GLORIA; DOMINGUEZ-IGLESIA, MASSIEL; **DUSSAN-SARRIA, JAIRO A.**; CRANDELL, DAVID; BONATO, PAOLO. Poster 272 Risk

Factors of Reamputation or Amputation of the Contralateral Lower Limb in Amputees with Dysvascular Disease. PM & R (Philadelphia, 2009): the journal of injury, function and rehabilitation, v. 7, p. S181, 2015.

Risk Factors of Reamputation or Amputation of the Contralateral Lower Limb in Amputees with Dysvascular Disease

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Disclosures: G. Vergara-Diaz: I Have No Relevant Financial Relationships To Disclose.

Objective: To identify risk factors leading to reamputation or amputation of the contralateral lower limb in patients with a major lower-extremity amputation (MLEA) due to dysvascular disease.

Design: Retrospective study

Setting: We examined the medical records and ancillary files of adult patients who underwent a lower-limb amputation and were admitted at Spaulding Rehabilitation Hospital (SRH) for inpatient care from August 2013 to August 2014.

Participants: We selected medical records of adults with a dysvascular MLEA, and excluded those of subjects who had an amputation due to complications following unrelated surgical procedures, joint disease, trauma, or cancer. Hence, the medical records of 59 subjects were included in the study.

Interventions: None

Main Outcome Measures: Ipsilateral reamputation or major amputation of the contralateral lower limb.

Results or Clinical Course: Patients were 61.0 ± 13.4 years old, mostly male (68%), white Caucasian (45%), with high prevalence of hypertension (85%), diabetes (81%), dyslipidemia (63%), smoking habit or history (69%), depression (69%) and evidence of coronary artery disease (40%), who underwent a below-knee amputation (83%). 27% of the patients were reamputated, 81% contra-lateral, 60% within a year from the first MLEA. Acute vascular event (P=.01), perioperative infection of the stump (P=.05), anxiety (P=.05), and evidence of peripheral disease in the contralateral limb (P=.05), were associated with a new amputation in univariate analysis.

Conclusion: About 25% of the patients who are admitted to the Amputees Clinic are there due to a second reamputation, 80% of which after undergoing bilateral amputation. In about one third of the cases, the reamputation or amputation of the contralateral limb occurred within a year. Acute vascular event at the moment of the first MLEA was identified as the main risk factor associated with a new amputation.

9. CHASSOT, MÔNICA; **DUSSAN-SARRIA, JAIRO ALBERTO**; SEHN, FRANCISLEA CRISTINA; DEITOS, ALÍCIA; DE SOUZA, ANDRESSA;

VERCELINO, RAFAEL; TORRES, IRACI LS; FREGNI, FELIPE; CAUMO, WOLNEI. Electroacupuncture analgesia is associated with increased serum brain-derived neurotrophic factor in chronic tension-type headache: a randomized, sham controlled, crossover trial. BMC Complementary and Alternative Medicine (Online), v. 15, p. 144, 2015.

Chassot et al. BMC Complementary and Alternative Medicine (2015) 15:144
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BMC Complementary & Alternative Medicine

RESEARCH ARTICLE Open Access

Electroacupuncture analgesia is associated with increased serum brain-derived neurotrophic factor in chronic tension-type headache: a randomized, sham controlled, crossover trial

Mônica Chassot[1,3], Jairo Alberto Dussan-Sarria[1,2,3], Francislea Cristina Sehn[1,3], Alícia Deitos[1,3], Andressa de Souza[3,4], Rafael Vercelino[1,3], Iraci LS Torres[1,5], Felipe Fregni[6] and Wolnei Caumo[1,2,3,7*]

Abstract

Background: Chronic tension-type headache (CTTH) is characterized by almost daily headaches and central sensitization, for which electroacupuncture (EA) might be effective. The central nervous system (CNS) plasticity can be tracked in serum using the brain-derived neurotrophic factor (BDNF), a neuroplasticity mediator. Thus, we tested the hypothesis that EA analgesia in CTTH is related to neuroplasticity indexed by serum BDNF.

Methods: We enrolled females aged 18–60 years with CTTH in a randomized, blinded, placebo-controlled crossover trial, comparing ten EA sessions applied for 30 minutes (2–10 Hz, intensity by tolerance) in cervical areas twice per week vs. a sham intervention. Treatment periods were separated by two washout weeks. Pain on the 10-cm visual analog scale (VAS) and serum BDNF were assessed as primary outcomes.

Results: Thirty-four subjects underwent randomization, and twenty-nine completed the protocol. EA was superior to sham to alleviate pain (VAS scores 2.38 ± 1.77 and 3.02 ± 2.49, respectively, $P = 0.005$). The VAS scores differed according to the intervention sequence, demonstrating a carryover effect ($P < 0.05$). Using multiple regression, serum BDNF was adjusted for the Hamilton depression rating scale (HDRS) and the VAS scores (r-squared = 0.07, standard β coefficients = –0.2 and –0.14, respectively, $P < 0.001$). At the end of the first intervention period, the adjusted BDNF was higher in the EA phase (29.31 ± 3.24, 27.53 ± 2.94 ng/mL, Cohen's $d = 0.55$).

Conclusion: EA analgesia is related to neuroplasticity indexed by the adjusted BDNF. EA modulation of pain and BDNF occurs according to the CNS situation at the moment of its administration, as it was related to depression and the timing of its administration.

Keywords: Electroacupuncture, Brain derived neurotrophic factor, Chronic tension type headache, Neuroplasticity

Background

According to the International Classification of Headache Disorders, chronic tension type headache (CTTH) is characterized by daily (or almost daily) headaches that last several hours per day [1]. Its prevalence in the general population ranges from 2-5% [2,3], leads to a negative impact on both functionality and quality of life, and increases the risk for excessive analgesic consumption [4]. As with some other chronic pain conditions, patients suffering CTTH present amplification of afferent signals [5], central sensitization phenomena and inadequate descendent inhibitory control [6]. The nervous system changes induced by chronic pain are performed by different actors, including the brain-derived neurotrophic factor (BDNF).

BDNF has been identified as a key player in the sensitization of the system, altering the excitatory/inhibitory balance in the central nervous system (CNS), and in the amplification of pain transmission modulation of the nociceptive sensory inputs in the spinal cord [7,8].

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10. ZANETTE, SIMONE AZEVEDO; **DUSSAN-SARRIA, JAIRO ALBERTO**;

SOUZA, ANDRESSA; DEITOS, ALICIA; TORRES, IRACI SILVA; CAUMO, WOLNEI. Higher serum S100B and BDNF levels are correlated with a lower pressure-pain threshold in fibromyalgia. Molecular Pain, v. 10, p. 46, 2014.

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MOLECULAR PAIN

RESEARCH Open Access

Higher serum S100B and BDNF levels are correlated with a lower pressure-pain threshold in fibromyalgia

Simone Azevedo Zanette[1,2,3,4†], Jairo Alberto Dussan-Sarria[2,3,4†], Andressa Souza[2,3,4,5], Alicia Deitos[2,3], Iraci Lucena Silva Torres[2,3,6] and Wolnei Caumo[2,3,4,6*]

Abstract

Background: Fibromyalgia (FM) is conceptualized as a central sensitization (CS) condition, that presents high serum brain-derived neurotrophic factor (BDNF) and neuroglia activation. Although the S100B protein regulates neuroglia functions, it has been traditionally used as a proxy of central nervous system damage. However, neither BDNF nor S100B association with the clinical picture of FM has been elucidated. To explore their association with the pressure-pain threshold (PPT) in FM, we performed a cross-sectional study, including 56 females with confirmed FM aged 18–65 years. Linear regression models were used to adjust for potential confounding factors between serum BDNF, S100B and PPT.

Results: Serum BDNF and S100B were correlated (Spearman's Rho = 0.29). Serum BDNF (*log*) and S100B (*log*) were correlated with the PPT (*log*) (Partial $\eta^2 = 0.129$, $P = 0.012$ for the BDNF (*log*), and Partial $\eta^2 = 0.105$, $P = 0.025$ for the S100B (*log*)). Serum BDNF (*log*) was inversely associated with PPT (*log*) ($\beta = -1.01$, SE = 0.41), age ($\beta = -0.02$, SE = 0.15) and obsessive compulsive disorder ($\beta = -0.36$, SE = 0.15), while serum S100B (*log*) was inversely associated with PPT (*log*) ($\beta = -1.38$, SE = 0.50), only.

Conclusions: Both neuroglia key mediators in the CS process were inversely correlated with the PPT. Serum assessment of BDNF and S100B deserve further study to determine its potential as a proxy for the CS spectrum in FM.

Keywords: Fibromyalgia, S100B, BDNF, Pain-pressure threshold, Central sensitization

Background

Fibromyalgia (FM) is estimated to affect between 1.6% [1] and 6.4% [2] of the population, and is characterized by widespread chronic pain accompanied by fatigue, non-satisfying sleep and cognitive symptoms [3]. Although its pathophysiology is not fully understood, abnormalities in pain processing [4] related to central sensitization (CS) and a reduced descending pain modulation with sensory amplification [5,6] have been recognized as components of FM. In the CS model, pain hypersensitivity and enhanced receptive field characteristics of the disease [7] may be explained as a consequence of increased neuronal membrane excitability, synaptic facilitation and nociceptive pathway disinhibition mediated at the molecular level by the modification of receptor kinetics (*e.g.*, N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)) [8] and at the cellular level by the interaction of both neurons and microglia interchanging neurotransmitters and inflammatory cytokines (*e.g.*, substance P, tumor necrosis factor alpha, and brain-derived neurotrophic factor (BDNF)), which results in enhanced neuronal and nociceptive pathway functions [8-10].

BDNF is a neurotrophic factor that is widely distributed in the central nervous system (CNS) and is capable of strengthening excitatory (glutamatergic) synapses as well as weakening inhibitory (GABAergic) synapses [11]. In the context of CS, microglial cells activated by astrocytes can

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11. SOUZA, ANDRESSA; **DUSSAN-SARRIA, JAIRO ALBERTO**; MEDEIROS, LICIANE FERNANDES; SOUZA, ANA CLÁUDIA; OLIVEIRA, CARLA; SCARABELOT, VANESSA LEAL; ADACHI, LAUREN NAOMI; WINKELMANN-DUARTE, ELISA CRISTIANA; PHILIPPI-MARTINS, BÁRBARA BEATRIZ; NETTO, CARLOS ALEXANDRE; CAUMO, WOLNEI; TORRES, IRACI L.S. Neonatal hypoxic-ischemic encephalopathy reduces c-Fos activation in the rat hippocampus: evidence of a long-lasting effect. International Journal of Developmental Neuroscience, v. 14, p. S0736-5748, 2014.

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Neonatal hypoxic–ischemic encephalopathy reduces *c-Fos* activation in the rat hippocampus: evidence of a long-lasting effect

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ABSTRACT

The effect of neonatal hypoxic–ischemic encephalopathy (HIE) on maturation of nociceptive pathways has been sparsely explored. To investigate whether neonatal HIE alters neuronal activity, nociceptive behavior, and serum neuroplasticity mediators (brain-derived neurotrophic factor [BDNF] and tumor necrosis factor-α [TNF]) in the short, medium, and long term. Neonate male Wistar rats were randomized to receive a brain insult that could be either ischemic (left carotid artery ligation [LCAL]), hypoxic (8% oxygen chamber), hypoxic–ischemic (LCAL and hypoxic chamber), sham-ischemic, or sham-hypoxic. Neuronal activity (*c-Fos* activation at region CA1 and dentate gyrus of the hippocampus), nociceptive behavior (von Frey, tail-flick, and hot-plate tests), neuroplasticity mediators (BDNF, TNF), and a cellular injury marker (lactase dehydrogenase [LDH]) were assessed in blood serum 14, 30, and 60 days after birth. Neonatal HIE persistently reduced *c-Fos* activation in the ipsilateral hippocampal region CA1; however, contralateral *c-Fos* reduction appeared only 7 weeks after the event. Neonatal HIE acutely reduced the paw withdrawal threshold (von Frey test), but this returned to normal by the 30th postnatal day. Hypoxia reduced serum LDH levels. Serum neuroplasticity mediators increased with age, and neonatal HIE did not affect their ontogeny. Neonatal HIE-induced reduction in neuronal activity occurs acutely in the ipsilateral hippocampal region CA1 and persists for at least 60 days, but the contralateral effect of the insult is delayed. Alterations in the nociceptive response are acute and self-limited. Serum neuroplasticity mediators increase with age, and remain unaffected by HIE.

1. Introduction

The development of the nociceptive system in humans occurs during the second and third gestational trimesters, and continues during the first 2 years of life (Anand and Hickey, 1987). Nociceptive afferents are myelinated in the 30th gestational week (Kostovic and Rakic, 1990), thalamocortical fibers in the 37th (Deshpande and Anand, 1996), and inhibitory pathways during the 3rd postnatal week (Boucher et al., 1998; Van Praag and Frenk, 1991). Although the maturation of the nociceptive system in rats occurs in an order similar to that seen in humans, a newborn rat has a nociceptive system that resembles that of a human fetus at 24 gestational weeks (Dobbing and Sands, 1979; Andrews and Fitzgerald, 1997; Marsh et al., 1997), while that of a 7-day-old rat pup (P7) corresponds to that of a full-term human neonate (Tuor et al., 1996). Thus, any insult to the central nervous system (CNS) occurring in this period

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BMC Neuroscience

RESEARCH ARTICLE Open Access

Association of anxiety with intracortical inhibition and descending pain modulation in chronic myofascial pain syndrome

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Abstract

Background: This study aimed to answer three questions related to chronic myofascial pain syndrome (MPS): 1) Is the motor cortex excitability, as assessed by transcranial magnetic stimulation parameters (TMS), related to state-trait anxiety? 2) Does anxiety modulate corticospinal excitability changes after evoked pain by Quantitative Sensory Testing (QST)? 3) Does the state-trait anxiety predict the response to pain evoked by QST if simultaneously receiving a heterotopic stimulus [Conditional Pain Modulation (CPM)]? We included females with chronic MPS (n = 47) and healthy controls (n = 11), aged 19 to 65 years. Motor cortex excitability was assessed by TMS, and anxiety was assessed based on the State-Trait Anxiety Inventory. The disability related to pain (DRP) was assessed by the Profile of Chronic Pain scale for the Brazilian population (B.PCP:S), and the psychophysical pain measurements were measured by the QST and CPM.

Results: In patients, trait-anxiety was positively correlated to intracortical facilitation (ICF) at baseline and after QST evoked pain ($\beta = 0.05$ and $\beta = 0.04$, respectively) and negatively correlated to the cortical silent period (CSP) ($\beta = -1.17$ and $\beta = -1.23$, respectively) ($P < 0.05$ for all comparisons). After QST evoked pain, the DRP was positively correlated to ICF ($\beta = 0.02$) ($P < 0.05$). Pain scores during CPM were positively correlated with trait-anxiety when it was concurrently with high DRP ($\beta = 0.39$; $P = 0.02$). Controls' cortical excitability remained unchanged after QST.

Conclusions: These findings suggest that, in chronic MPS, the imbalance between excitatory and inhibitory descending systems of the corticospinal tract is associated with higher trait-anxiety concurrent with higher DRP.

Keywords: Transcranial magnetic stimulation, Chronic pain, Noninvasive brain stimulation, Neuromodulation, Anxiety, Myofascial pain syndrome

Background

Pain is not simply determined by the intensity of the nociceptive stimulus but also by orchestrated mechanisms that work together, including psychological factors [1]. As one of these psychological factors, anxiety involves both physiological and psychological aspects that affect the way sensory interpretation occurs [2]. Anxiety is considered adaptive because a threatening situation induces body changes that increase the state of arousal. Anxiety can be presented as a state-anxiety (i.e., referred to acute situation-driven episodes that fluctuate over time) or as a trait-anxiety (i.e., a lifelong pattern, in the form of a personality feature) [3]. Maladaptive anxiety can take over and have negative effects on people's lives [4].

These descriptions are consistent with the State-Trait Anxiety theory, which predicts that individuals with high trait-anxiety are generally hypersensitive to stimuli and are psychologically more reactive [3,4]. Anxious patients present signs of restlessness, sympathetic overactivity, and resistance to sedation [5]. Thus, similarly to pain mechanisms, it is conceivable that anxiety is associated with alterations in brain excitability [5]. Previous reports

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13. HENRIQUES, ALEXANDRE ANNES; **DUSSÁN-SARRIA, JAIRO A.**; BOTELHO, LEONARDO M.; CAUMO, WOLNEI. Multidimensional Approach to Classifying Chronic Pain Conditions-Less Is More. The Journal of Pain (Print), v. 15, p. 1199-1200, 2014.

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Letters to the Editor

Multidimensional Approach to Classifying Chronic Pain Conditions—Less Is More

To the Editor:

Our team received with enthusiasm the proposal from the ACTTION-APS Pain Taxonomy (AAPT),[1] which claims an evidence-based and multidimensional approach to classifying chronic pain conditions. Attempting to approach an ideal diagnostic system that aims to systematize, organize, and standardize the biopsychosocial assessment of chronic pain patients, a 5-dimension taxonomy was proposed. This perspective drew inspiration from the *Diagnostic and Statistical Manual of Mental Disorders* (DSM), *Research Diagnostic Criteria for Temporomandibular Disorders* (RDC/TMD), and International Classification of Headache Disorders diagnostic systems.[1] Of these, the DSM is probably the most widely used system in multidisciplinary settings and an essential requirement in the care of pain patients. The evolution of the taxonomies is both necessary and inherent to the progress of science, which develops together with patients', clinicians', researchers', and third-party payers' needs.

However, the DSM-5 (the latest version) has significantly changed its multiaxial diagnostic system in favor of a less complicated nonaxial documentation of diagnoses.[2,3] The multiaxial approach was not universally informative or helpful for patient care.[3] The major goals for this transformation were to improve compatibility between the DSM-5 and WHO's *International Classification of Diseases 11th Revision*[3] (other nonpsychiatry specialties prioritize clinically relevant findings without using an axial distribution); to stimulate a dimensional (more process- and pathogenesis-oriented) rather than a categorical diagnostic approach[5]; to minimize neglecting application and registry of some axes; to be more feasible for both pediatric and adult settings[4]; and to avoid segregation and reduction of therapeutic efforts toward patients with "less treatable" disorders (eg, personality disorders).

In the same way, we believe that this pentadimensional approach[1] for classifying chronic pain might face significant limitations in its applicability for clinicians and researchers. In the practice setting, excessive categories in the analysis of a clinical case tend to create confusion and negligence of some of these categories. Also, this could turn into an obstacle for fluid communication between multidisciplinary professionals at the expense of health care team synchronization and integration. Further, adopting an exclusive multidimensional diagnostic system for chronic pain may hinder the harmonization with acute pain care teams.

We support the systematic use of assessment, follow-up, and prognostic tools that have been validated in the various dimensions of a biopsychosocial approach, as long as their relationships with the mechanisms of disease are clear and evidence based. Likewise, it should be required that these tools present an established association with hard and clinically relevant outcomes, and sanctioned consensus by clinicians, researchers, and third-party payers. In this way, we suggest considering the field experience and evolution of the DSM system.[4] The use of 2 or 3 dimensions may represent a more feasible and practical solution, reducing the complexity and improving the clinical decision making for all involved actors in the pain patients' integrated care pathway, through primary to quaternary health care settings.

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2.2. Resumos publicados em anais de congressos

1. GAMERMANN, P.W.; STEFANI, L.P.C.; MACHADO, V.R.; **DUSSAN-SARRIA, JAIRO ALBERTO**. PROJETO DE REALOCAÇÃO DE RECURSOS HUMANOS PARA AUMENTAR A REALIZAÇÃO DE ANALGESIA DE PARTO EM HOSPITAL UNIVERSITÁRIO. In: 63 Congresso Brasileiro de Anestesiologia, 2016, Brasilia. REVISTA BRASILEIRA DE ANESTESIOLOGIA, 2016. v. 66. p. S54.

2. COMIRAN, H.H.; FISCHER, F.; GAMERMANN, P.W.; **DUSSAN-SARRIA, JAIRO ALBERTO**. AVALIAÇÃO DO BLOQUEIO NEUROMUSCULAR RESIDUAL EM SALA DE RECUPERAÇÃO PÓS-ANESTÉSICA: ESTUDO DE CASOS E CONTROLES. In: 63 Congresso Brasileiro de Anestesiologia, 2016, Brasília. REVISTA BRASILEIRA DE ANESTESIOLOGIA, 2016. v. 66. p. S106.

3. STEFANI, L.P.C.; NOVELO, B.; FREITAS, L.A.; EVALDT, C.A.; STAHLSCHIMIDT, A.; **DUSSAN-SARRIA, JAIRO ALBERTO**. EPIDEMIOLOGIA, FATORES DE RISCO E DESFECHOS DE PACIENTES SUBMETIDOS A CIRURGIAS NÃO ELETIVAS. In: 63 Congresso Brasileiro de Anestesiologia, 2016, Brasília. EPIDEMIOLOGIA, FATORES DE RISCO E DESFECHOS DE PACIENTES SUBMETIDOS A CIRURGIAS NÃO ELETIVAS, 2016. v. 66. p. S188.

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7. DUSSAN-SARRIA, JAIRO ALBERTO; ZANETTE, SIMONE AZEVEDO; SOUZA, ANDRESSA; DEITOS, ALICIA; TORRES, I.L.S.; CAUMO, WOLNEI. HIGHER SERUM S100B AND BDNF LEVELS ARE CORRELATED WITH LOWER PRESSURE-PAIN THRESHOLD IN FIBROMYALGIA. In: 15th World Congress on Pain, 2014, Buenos Aires. Annals of the 15th World Congress on Pain, 2014.

8. DEITOS, A.; DALLAGNOL, L.; MEDEIROS, L.F.; VIDOR, L.P.; D**USSÁN-SARRIA, JAIRO ALBERTO**; BRIETZKE, A.; ROZISKY, J.R.; TORRES, I.L.S.; FREGNI, F.; CAUMO, W. Eficácia da estimulação magnética transcraniana no tratamento da dor crônica miofascial: ensaio clínico fase II, randomizado, cego, em paralelo, controlado com placebo-sham. In: V Simpósio Internacional em Neuromodulação, 2013, São Paulo. Anais do V Simpósio Internacional em Neuromodulação, 2013. v. 1. p. 1-37.

9. VIDOR, LILIANE PINTO; TORRES, I.L.S.; MEDEIROS, LICIANE FERNANDES; **DUSSAN-SARRIA, JAIRO ALBERTO**; DALL'AGNOL, LETIZZIA; DEITOS, ALICIA; BRIETZKE, ALINE; LASTE, GABRIELA; ROZISKY, JOANNA R; QUEVEDO, A.; FREGNI, FELIPE; CAUMO, WOLNEI. Associação da ansiedade com Inibição intracortical e modulação descendente de dor na Síndrome de dor crônica miofascial.. In: V Simpósio Internacional em Neuromodulação, 2013, São Paulo. Anais do V Simpósio Internacional em Neuromodulação. São Paulo: Instituto Escala, 2013. v. 1. p. 1-37.

3. 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	51
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	53
Introduction5			
Background and objectives	2a	Scientific background and explanation of rationale	55
	2b	Specific objectives or hypotheses	55-57
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	56
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	58
	4b	Settings and locations where the data were collected	58
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	59
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	60-64
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	64

	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	64-65
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	64-65
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	64-65
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	64-65
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	64-65
	11b	If relevant, description of the similarity of interventions	64-65
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	65
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	65
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	66
	13b	For each group, losses and exclusions after randomisation, together with reasons	66
Recruitment	14a	Dates defining the periods of recruitment and follow-up	66
	14b	Why the trial ended or was stopped	NA

Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	67
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	67
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)	67-72
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	NA
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	NA
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	72
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	77
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	73-77
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	73-77
Other information			
Registration	23	Registration number and name of trial registry	54
Protocol	24	Where the full trial protocol can be accessed, if available	NA
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	79-80