

# Potency of descending pain modulatory system is linked with peripheral sensory dysfunction in fibromyalgia

## An exploratory study

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### Abstract

Fibromyalgia (FM) is characterized by chronic widespread pain whose pathophysiological mechanism is related to central and peripheral nervous system dysfunction. Neuropathy of small nerve fibers has been implicated due to related pain descriptors, psychophysical pain, and neurophysiological testing, as well as skin biopsy studies. Nevertheless, this alteration alone has not been previously associated to the dysfunction in the descending pain modulatory system (DPMS) that is observed in FM. We hypothesize that they associated, thus, we conducted a cross-sectional exploratory study.

To explore small fiber dysfunction using quantitative sensory testing (QST) is associated with the DPMS and other surrogates of nociceptive pathways alterations in FM.

We run a cross-sectional study and recruited 41 women with FM, and 28 healthy female volunteers. We used the QST to measure the thermal heat threshold (HTT), heat pain threshold (HPT), heat pain tolerance (HPT), heat pain tolerance (HPT<sub>o</sub>), and conditional pain modulation task (CPM-task). Algometry was used to determine the pain pressure threshold (PPT). Scales to assess catastrophizing, anxiety, depression, and sleep disturbances were also applied. Serum brain-derived neurotrophic factor (BDNF) was measured as a marker of neuroplasticity. We run multivariate linear regression models by group to study their relationships.

Samples differed in their psychophysical profile, where FM presented lower sensitivity and pain thresholds. In FM but not in the healthy subjects, regression models revealed that serum BDNF was related to HTT and CPM-Task (Hotelling Trace = 1.80,  $P < .001$ , power = 0.94,  $R^2 = 0.64$ ). HTT was directly related to CPM-Task ( $B = 0.98$ ,  $P = .004$ , partial- $\eta^2 = 0.25$ ), and to HPT ( $B = 1.61$ ,  $P = .008$ , partial  $\eta^2 = 0.21$ ), but not to PPT. Meanwhile, BDNF relationship to CPM-Task was inverse ( $B = -0.04$ ,  $P = .043$ , partial- $\eta^2 = 0.12$ ), and to HPT was direct ( $B = -0.08$ ,  $P = .03$ , partial- $\eta^2 = 0.14$ ).

These findings high spot that in FM the disinhibition of the DPMS is positively correlated with the dysfunction in peripheral sensory neurons assessed by QST and conversely with serum BDNF.

**Abbreviations:** ACR = American College of Rheumatology, BDI-II = Beck Depression Inventory, BDNF = brain-derived neurotrophic factor, BP-PCS = Brazilian Portuguese Catastrophizing Scale, CPM-task = conditional pain modulation task, DPMS = descending pain modulatory system, ELISA = Enzyme-Linked Immunosorbent Assay, FIQ = Fibromyalgia Impact Questionnaire, FM = Fibromyalgia, HCPA = Hospital de Clínicas de Porto Alegre, HPT = heat pain threshold, HPT<sub>o</sub> = heat pain tolerance, HTT = thermal heat threshold, LTD = long term depression, NGF = neural growth factor, NMDA = N-methyl-D-aspartate, NPS = numerical pain scale, NRM = nucleus raphe magnus, PKC = Protein kinase C, PPT = pain pressure threshold, PSQI = Pittsburgh Sleep Quality Index, QST = quantitative sensory testing, STAI = State-Trait Anxiety Inventory, STT = spinothalamic tract, tDCS = transcranial direct

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current stimulation, TRK = tyrosine kinase, TTD = thermal thresholds determination, UFRGS = Universidade Federal do Rio Grande do Sul, VAS = Visual Analog scale.

**Keywords:** algometry, brain-derived neurotrophic factor, conditional pain modulation, fibromyalgia, pain threshold, quantitative sensory testing

## 1. Introduction

Fibromyalgia (FM) is a syndrome that comprises chronic widespread pain, usually described as occurring in deep somatic tissues (i.e., in muscles and joints), accompanied by fatigue, circadian rhythm disturbances, anxiety, depression, and pain catastrophizing.<sup>[1]</sup> Patients present high distress, functional disability, bowel/bladder irritability, and abnormal pain sensitivity for pressure and thermal stimuli,<sup>[2]</sup> often described as burning or prickling sensations. Recently, a growingly accepted pathophysiological model for fibromyalgia attributes central and peripheral nervous system alterations (both functional and anatomical) as responsible for the wide spectrum of the clinical picture.<sup>[3]</sup>

To understand the potential role of the peripheral nervous system involvement in fibromyalgia, it is relevant to address the nociception physiology. Sensitivities to warmth and cold involve specific fibers, which has a range of average resting skin temperatures (25–35°C). These fibers are activated by threshold stimuli that fire low-threshold afferent fibers, which converges to nociceptive spinothalamic tract (STT) neurons. These convergent stimuli are associated with nerve injury, and can induce selective apoptosis of inhibitory GABAergic inter-neurons.<sup>[4]</sup> This processes decreased the inhibitory receptors expression (on primary afferent terminals and postsynaptic neurons)<sup>[5]</sup> and it culminates with a higher perception of repetitive nociceptive stimuli, that is, temporal and spatial summation.<sup>[6,7]</sup> Both phenomena comprise a primary mechanism of the pathophysiology of neuropathic pain syndromes, but it also has been pointed out as a possible mechanism in FM.

A sizeable multicenter cohort study compared sensory symptoms of fibromyalgia with painful diabetic neuropathy and it found that both conditions share similar descriptors to characterize the sensorial perceptions (i.e., the pain of burning quality, a prickling sensation, and the existence of touch-evoked).<sup>[8]</sup> Although in FM there are signs of “small fiber neuropathy,” the relationship between nerve conduction findings or biopsies and the severity of pain-related symptoms is incongruent.<sup>[9]</sup> In fact, the peripheral nociception modulates the central nervous system (CNS), and the level of dysfunction in peripheral sensorial fibers could be linked to the imbalance of excitability/inhibitory pain pathways. Also, this process involves the high-affinity of brain-derived neurotrophic factor (BDNF) to the trkB receptor, which enhances C fiber-evoked responses, strengthening excitatory (glutamatergic), and weakening inhibitory (GABAergic) synapses.<sup>[10]</sup> Accordingly, a study in rats under persistent pain, at nucleus raphe magnus (NRM), the BDNF-K<sup>+</sup>-Cl<sup>-</sup>-cotransporter (KCC2)-GABA induced a shift in the potential gradient chlorine currents, such that the inhibitory GABA-A currents become excitatory.<sup>[11]</sup>

This neurotrophic factor contributes to decreased pain threshold in FM patients and central sensitization augmentation.<sup>[12,13]</sup> It is involved in the disinhibition process related to the loss of the descending pain inhibitory pathways and with the

increase in intracortical facilitation (as indexed by transcranial magnetic stimulation).<sup>[12,13]</sup> Additionally, it is associated with severity and clinical course of mood disorders<sup>[14]</sup> and it predicted effect of therapeutic approaches involving changes in the neuroplasticity state, as the transcranial direct current stimulation (tDCS) in cognitive in FM.<sup>[15]</sup>

Taking this into account; we hypothesize that the dysfunction of small peripheral fibers could be related to the dysfunction of descending pain modulatory system in FM. Thus, we conducted this exploratory study to examine if small fiber dysfunction in FM is linked to a malfunction in the descending pain modulatory system. To test this hypothesis, we run 3 different tests: we used the QST to measure the thermal heat threshold (HTT), the heat pain threshold (HPT), and the heat pain tolerance (HPT). Also, the QST was used to assess the change in Numerical Pain Scale (NPS0–10) during a conditional pain modulation task (CPM-task). While the algometry was used to determine the pain pressure threshold (PPT).

## 2. Methods

### 2.1. Study design

This exploratory study was conducted at one single center in Hospital de Clínicas de Porto Alegre (Rio Grande do Sul, Brazil) during 2016 to 2017, and recruited patients with fibromyalgia, and controls with no pain. The protocol was reviewed and approved by the Ethics Committee Board of the Hospital de Clínicas de Porto Alegre (Institutional Review Board IRB 0000921) in accordance with the Declaration of Helsinki. All subjects gave their written informed consent before participation.

**2.1.1. Design overview, setting, and participants.** In this cross-sectional study, women, aged between 18 and 60 years, were recruited from local community care units, an institutional chronic pain clinic, by referrals from other hospital units, and by phone and newspaper. FM sample selection considered the American College of Rheumatology criteria for fibromyalgia.<sup>[16]</sup> A physician with >15 years of experience in pain clinic, and highly skilled at diagnosing chronic pain conditions, re-examined patients and then re-confirmed their diagnosis. FM patients were included if they had experienced pain scored in the numerical pain scale (NPS 0–10)  $\geq 5$  (i.e., moderate or severe pain) in the last 3 months. Additionally, the pain should be associated with disability, as assessed by an affirmative answer to dichotomous questions (yes/no) of a structured questionnaire. The queries inquired if their pain had interfered with work; enjoyable activities; responsibilities at home; relationships; personal goals; and thinking clearly, problem-solving, concentrating, or recall. We excluded patients with a rheumatic or neurologic condition such as stroke or Parkinson disease; the habitual use of corticosteroids; or other uncompensated chronic disease. We excluded patients with any malignancy diagnosis, human immunodeficiency virus, or if they were illiterate.

**2.1.2. Healthy controls.** The volunteers were recruited from the general population by advertisement postings in the universities, on the internet, and in public places in the Porto Alegre area. Subjects were considered eligible to participate if they were women, ranging between 19 and 60 years age. They answered a structured questionnaire that assessed the following variables: current acute or chronic pain conditions, use of analgesics in the past week, rheumatologic disease, clinically significant or unstable medical or psychiatric disorder, history of alcohol or substance abuse in the past 6 months, neuropsychiatric comorbidity, and use of psychotropic drugs. They were excluded if answered positively to any of these questions, or if they presented scores  $\geq 13$  in the Beck Depression Inventory (BDI-II).<sup>[17]</sup> Unlike the patient sample, none of the control volunteers underwent a physical examination.

## 2.2. Instruments and assessments

**2.2.1. Outcomes and measurements.** The primary outcome was the score on NPS (0–10) during the conditioned pain modulated (CPM-Task). The secondary outcomes were the temperature in the quantitative sensory testing (QST) to provoke pain score 6 on the NPS (0–10); the pain pressure threshold (PPT). The primary factor of interest was the thermal thresholds determination induced by the QST. It was assessed as surrogate for small fibers dysfunction involved in pain and thermal processing, and is described in detail below.

- (a) Quantitative Sensory Testing (QST): all subjects underwent a standardized protocol performed in a single session, by the same researcher. A single training session was offered before so participants could get familiar with the device. All subjects were oriented to keep the eyes closed during the examination and to be concentrated in the stimuli applied. The QST was used to measure the thermal thresholds determination (TTD) and heat pain threshold (HPT). The assessment used the method of limits with a computer Peltier-based device thermode (30X30 mm)<sup>[18]</sup> in a computer Peltier-based device using the method of limits. It was attached to the skin on the ventral aspect of the mid-forearm. The QST was set at 32°C and temperature was increased at a rate of 1°C/s to a maximum of 52°C. To define the TTD and HPT on each patient, the mean of three assessments was calculated (inter-stimuli interval of 40 seconds).<sup>[18]</sup> The thermode position was slightly altered between trials to avoid sensitization and response suppression of cutaneous heat nociceptors. The QST was used to determine the temperature needed to induce a mild pain (6/10 on the NPS), and was defined as the mean of three assessments with an inter-stimuli interval of 40 seconds.<sup>[18]</sup> The maximum tolerated temperature was also determined, and named Heat Pain Tolerance (HPTO), with a single trial (no previous training neither repetitions allowed). Patients were asked to press the digital indicate when experiencing the maximal heat perception they could tolerate.
- (b) To measure the CPM-task we evaluated the pain intensity in two tonic HPT test stimuli separated by an intense heterotopic pain stimulation. We used the HPT as conditioning pain stimulus to elicit a prolonged pain sensation to trigger CPM. The CPM-task consisted of three steps. First, we stimulated the dominant forearm with augmenting temperature until previously determined to obtain moderate pain (6/10 in the NPS), and asked again the NPS attributed to it. This temperature was maintained constant during the task.

Then (more precisely, 30 s after), the non-dominant hand was immersed in cold water (temperature of 0–1°C) during 1 minute. After 30 seconds of applying the cold stimulus, subjects were asked to provide the NPS (0–10) attributed to the heat stimulus. To maintain the water temperature at 0 to 1°C we used a thermostat to control the temperature variation. To determine the CPM we subtracted the NPS (0–10) after cold stimuli to the one before it (in other works,  $NPS_{after} - NPS_{before}$  cold). If the patients did not report a reduction or report an increase in their pain score during the CPM-task, the descendent modulatory systems was considered to have failed to modulate the nociceptive response.<sup>[12]</sup>

- (c) The pressure pain threshold (PPT) is defined as the minimum force applied which induces pain. Pressure algometry measurements were performed to evaluate PPTs in the right forearm region. The PPT was measured with a digital algometer (Somedic, Stockholm, Sweden). The pressure was always manually applied by the same researcher at a rate of increase of 50 kPa/s through a circular rubber-coated pressure probe (1 cm<sup>2</sup>). Then, subjects were asked to verbalize when perceiving the first pain. The pressure pain threshold for each subject was determined with the mean of three right forearm single-point algometry.
- (d) The laboratory outcome measured was the serum level of BDNF. We collected the 69 blood samples before starting the assessment. Samples were centrifuged for 10 minutes at 4500 × g at 4°C, and we stored at –80°C. Serum BDNF was determined using an Enzyme-Linked Immunosorbent Assay (ELISA) using a ChemiKine BDNF Sandwich ELISA Kit, CYT306 (Chemicon/Millipore, Billerica, MA). The lower detection limit of the kit for BDNF is 7.8 pg/mL.

**2.2.2. Other instruments and assessments.** All the instruments used to assess psychological state were validated in the Brazilian population. Two independent medical examiners that were blinded to the aim of the study were trained to conduct the psychological tests and to administer the pain scales. Depressive symptoms were assessed using the Beck Depression Inventory (BDI-II).<sup>[17,19]</sup> Pain catastrophizing was assessed using the Brazilian Portuguese Catastrophizing Scale (BP-PCS)<sup>[20]</sup> and, sleep quality with the Pittsburgh Sleep Quality Index (PSQI).<sup>[21]</sup> To assess the anxiety, we used the refined version of the State-Trait Anxiety Inventory (STAI) obtained using the Rasch model, which was reduced from the state-trait STAI-Form X scales.<sup>[22]</sup> The scores in the state- and trait-scales ranges from 13 to 52, and from 12 to 36, respectively. To evaluate the quality of life we used the Fibromyalgia Impact Questionnaire (FIQ).<sup>[23]</sup> The pain intensity was measured with a 100-mm VAS. The VAS scores ranged from no pain (0) to worst possible pain (100 mm). We used a standardized questionnaire to assess demographic data and medical comorbidities.

## 2.3. Sample size

A sample size of 61 subjects would detect an effect size for correlations of 0.2, with a power of 95% at a 0.05 alpha level for an effect size ( $f^2 = \text{determination coefficient} = 0.2$ ) for the multiple hierarchical regression analysis, allowing for 3 predictors. It was calculated using the Post hoc statistical power calculator for hierarchical multiple regression at <https://www.danielsoper.com/statcalc/calculator.aspx?id=17>. Finally, considering the likely attrition rate and other unexpected factors, the required sample size was determined to be 69 patients.

## 2.4. Statistical analysis

Descriptive statistics were used to summarize the main socio-demographic features the sample. *t* tests for independent samples and chi-squared and Fisher exact tests were used to compare continuous and categorical variables between groups, respectively. To test for normality we used the Shapiro-Wilk test. Because the BDNF is not normally distributed, we performed a logarithmic transformation. After verifying the corresponding assumptions, the Pearson correlation coefficient was used to assess bivariate relationship between covariates (catastrophizing, state-trait anxiety, and depressive symptoms) with the outcomes related to psychophysical measures (CPM-task, HPT, PPT, and HPTO). To address imbalance between groups (FM and healthy subjects), that could interfere with the HTT (main interest factor), we generated a propensity score to reduce or eliminate these effect in further analysis of the relationship between the HTT and outcomes. To create the adjusted index of HTT, we run a regression analysis using the stepwise method to enter the covariates. The criteria to include the covariates in the model was to present a *P*-value <.05. The dependent variable was the HTT, and the independent variables were years of education, age, the number of chronic disorders, use of psychotropic medications, state-anxiety, depressive symptoms, and pain catastrophizing. The adjusted HTT-index (main interest factor) was used together with the serum BDNF as independent variables in multivariate analysis models. To assess their relationship with the dependent measures (CPM-task—primary outcome) and HPT, PPT, and HPTO (secondary outcomes), we created a multivariate model for each group (FM and healthy subjects). Bonferroni correction for multiple comparisons was applied. Spearman correlation was used to study the relationship between CPM-task and BDNF, and HTT. Data were analyzed using SPSS software version 22.0 (SPSS, Chicago, IL).

## 3. Results

### 3.1. Demographic and clinical characteristics of the subjects

We screened 75 potential participants with a diagnosis of FM, and included 41 subjects. We screened 41 potential participants for the control group (healthy volunteers) and enrolled 28 subjects. The reasons for exclusion were: not fulfilling the diagnostic criteria for FM, lacking disability as defined in the protocol, and the presence of another relevant comorbidity (i.e.,

**Table 1**

**Demographic and clinical characteristic of the sample.**

	Control (n=28)	Fibromyalgia (n=41)	<i>P</i> -value*
Demographic			
Age, y	32.1 (4.56)	49.1 (8.32)	<.001
BMI, kg/m <sup>2</sup>	22.9 (3.3)	29.6 (±6.6)	<.001
Level of education, y	16 (2.9)	11.4 (±4.0)	<.001
Clinical			
Beck Depression Inventory—BDI-II	2.3 (4.12)	24.8 (6.34)	<.001
Pain Catastrophizing Scale—PCS	6.1 (4.31)	31.9 (12.02)	<.001
State-Trait Anxiety Inventory—STAI			
STAI—state	19.0 (3.9)	29.0 (6.4)	<.001
STAI—trait	15.2 (3.9)	26.0 (6.6)	<.001
Pittsburgh Sleep Quality Index—PSQI	5.57 (3.76)	10.6 (4.91)	<.011

Data area presented as mean (standard deviation) (n=69).

\* Compared using *t*-test for independent samples.

rheumatoid arthritis, previous stroke). The clinical and demographic characteristics of the subjects are presented in Table 1.

### 3.2. Univariate analysis

**3.2.1. Assessment of relationship between psychological measures and psychophysical parameters.** The Pearson correlation coefficient was used to identify potential confounding factors in the bivariate relationships between the psychological and psychophysical measures which comprised our outcomes (CPM-task, PPT, HPT, HPTO, and HTT, see Table 2).

### 3.3. Psychophysical measures and BDNF by group (FM vs healthy subjects)

As expected, psychophysical tests presented differences between the studied groups, see Table 3.

Taking into account that there are differences between FM and healthy subjects in some factors that can interfere with the HTT (main factor of interest), we generated a propensity score to reduce this effect in further analysis, as described in the statistical analysis section. The adjusted determination coefficient of the model was  $R^2=0.13$ , explaining 13% of the variance in the HTT (standard  $\beta$  coefficient for the state-anxiety =  $-0.46$ ,  $t=-3.02$ ; standard  $\beta$  coefficient for the pain catastrophizing =  $0.55$ ,  $t=3.33$ , both  $P < .01$ ).

**Table 2**

**Pearson (*r*) correlation between potential confounding factors and outcomes (N=69).**

	STAI-S	STAI-T	BDI-II	BP-PCS	HTT	PPT	HPT	HPTOL
STAI-T	$r=0.74^{**}$							
BDI-II	$r=0.78^{**}$	$r=0.86^{**}$						
BP-PCS	$r=0.68^{**}$	$r=0.72^{**}$	$r=0.80^{**}$					
HTT	$r=-0.25^*$	$r=0.03$	$r=-0.26^*$	$r=-0.05$				
PPT	$r=-0.23$	$r=-0.17$	$r=-0.27^*$	$r=-0.12$	$r=0.05$			
HPT	$r=-0.32^{**}$	$r=-0.16$	$r=-0.24^*$	$r=-0.03$	$r=0.19$	$r=0.19$		
HPTOL	$r=-0.023^*$	$r=-0.15$	$r=-0.27^*$	$r=-0.04$	$r=0.19$	$r=0.28^*$	$r=0.70^{**}$	
CPM-task	$r=0.33^{**}$	$r=0.42^{**}$	$r=0.43^{**}$	$r=0.53^{**}$	$r=-0.14$	$r=-0.05$	$r=-0.09$	$r=-0.17$

State-Trait Anxiety (STAI-S and STAI-T); BDI-II = Beck Depression Inventory II (BDI-II); BP-PCS = Brazilian Portuguese Pain Catastrophizing Scale; HTT = heat thermal threshold; HPT = heat pain tolerance; CPM-task = change on NPS (0–10) during the conditioned pain modulated; PPT = pain pressure threshold.

\* Correlation is significant at the 0.05 level (2-tailed).

\*\* Correlation is significant at the 0.01 level (2-tailed).



**Table 3****Psychophysical tests (HTT, PPT, HPT, HPTO, CPM-task) and BDNF by group (n=69).**

	FM (n=41)	Healthy controls (n=28)	P-value*
Heat thermal thresholds (HTT), °C	33.62 (1.44)	33.82 (1.17)	.54
Heat thermal thresholds (HTT-adjusted)	33.81 (0.56)	34.12 (0.34)	.01
Pain pressure threshold (PPT), °C	2.44 (0.74)	3.51 (1.11)	.001
Heat pain threshold (HPT), °C	39.87 (4.77)	42.24 (3.10)	.01
Heat pain tolerance (HPTO), °C	45.49 (3.81)	47.84 (2.20)	.002
Change on NPS (0–10) during the (CPM-task)	−0.46 (1.92)	−3.54 (1.93)	.001
Brain-derived neurotrophic factor (BDNF), ng/mL	38.08 (20.78)	16.21 (7.85)	.001

Celsius degree (°C); Numerical Pain Scale (NPS 0–10) equal to 6; change on NPS (0–10) during the conditioned pain modulated (CPM-task). Heat thermal threshold (HTT) (adjusted index generate by a regression analysis).

\* Comparisons of mean using *t* test for independent samples.

### 3.4. Multivariate analysis of the relationship between the HTT-adjusted and psychophysical measures (HPT, HPTO, HPP) by group

Taking into account that FM and healthy subjects are different in many aspects, we ran separate multivariate analysis for each group. Tables 4 and 5 present the result of the multivariate regression analysis in FM (Table 4) and healthy subjects (Table 5). The dependent variables were the psychophysical measures (PPT, HPT, HPTO, CPM-task). The independent variables were the HTT-adjusted and the BDNF. The multivariate model revealed a significant relationship between the HTT-adjusted, BDNF, and CPM-Task in FM (Hotelling Trace=1.80,  $F [34]=16.23$ ,  $P < .001$ ), with a power of 0.94, and  $R^2=0.64$ ; thus, explaining 64% of the variance in the outcome variables (Table 4). The HTT-adjusted was positively correlated with the

CPM-task and with the HPT. Indeed, an increase in their pain score during the CPM-task indicates the worst function of descending pain modulatory system. However, the HTT was correlated neither with the PPT nor with the HPTOL (see Table 4). On the other hand, these associations were not present in the healthy control sample (Table 5).

The multiple regression analysis confirmed a positive correlation between the HTT-adjusted with the CPM-task in FM, but not in the healthy subjects (Table 4). For illustrative purposes the scatter plot of the HTT-adjusted and CPM-task in fibromyalgia is presented in Figure 1. The Spearman correlation coefficient ( $\rho$ ) was 0.35 (confidence interval 95% = 0.05–0.59).

Scatter plots of the raw change on NPS (0–10) during CPM-task and BDNF by group (FM and healthy subjects) are presented in Figure 2A and B, respectively. We observed an inverse

**Table 4****The relationship between BDNF and HTTP-adjusted index with the psychophysical outcomes (HPT, HPP, HPT, and CPM-task) in fibromyalgia (n=41).****B. Fibromyalgia group**

Dependent variable	Type III sum of squares	df	Mean square	F	Sig.	Partial- $\eta^2$
Change on NPS (0–10) during the CPM-task	52.900 <sup>a</sup>	2	26.450	5.885	0.008	0.250
Pain pressure threshold	0.100 <sup>b</sup>	2	0.050	0.080	0.923	0.005
Heat pain threshold	295.406 <sup>c</sup>	2	147.703	8.610	0.001	0.357
Heat pain tolerance	47.196 <sup>d</sup>	2	23.598	1.791	0.184	0.104
Parameter	B	Std. error	t	Sig.	CI 95%	Partial- $\eta^2$
Change on NPS (0–10) during the CPM-task						
Intercept	−29.81	10.924	−2.729	0.010	(−52.09 to −7.53)	0.194
Brain derivate neural factor (BDNF) (log)	−0.04	0.020	−2.068	0.043	(−0.08 to −0.01)	0.121
Heat thermal threshold (adjusted index)	0.98	0.318	2.777	0.004	(0.23–1.69)	0.250
Pain pressure threshold						
Intercept	4.043	3.696	1.094	0.283	(−3.49–11.58)	0.037
Brain derivate neural factor (BDNF) (log)	0.001	0.007	0.09	0.929	(−0.02–0.02)	0.000
Heat thermal threshold (adjusted)	−0.04	0.10	−0.40	0.691	(−0.26–0.18)	0.005
Heat pain threshold (HPT)						
Intercept	−18.73	19.446	−0.963	0.343	(−58.39–20.95)	0.029
Brain derivate neural factor (BDNF) (log)	0.080	0.036	2.240	0.03	(0.07–0.15)	0.139
Heat thermal threshold (adjusted)	1.609	0.566	2.842	0.008	(0.45–2.76)	0.207
Heat pain tolerance						
Intercept	23.445	17.040	1.376	0.179	(−11.30–58.19)	0.06
Brain derivate neural factor (BDNF) (log)	0.03	0.03	1.109	0.276	(−0.03–0.09)	0.04
Heat thermal threshold (adjusted)	0.60	0.49	1.217	0.233	(−0.40–1.61)	0.05

BDNF = brain-derived neurotrophic factor, CPM = conditional pain modulation, NPS = numerical pain scale.

<sup>a</sup> R-squared = 0.24 (adjusted R-squared = 0.19).

<sup>b</sup> R-squared = 0.005 (adjusted R-squared = −0.06).

<sup>c</sup> R-squared = 0.35 (adjusted R-squared = 0.32).

<sup>d</sup> R-squared = 0.10 (adjusted R-squared = 0.05).

**Table 5**

**The relationship between BDNF and HTT-adjusted with the psychophysical outcomes (HPT, HPP, HPT, and CPM-task) in healthy subjects (n=28).**

Dependent variable	Type III sum of squares	df	Mean square	F	Sig.	Partial- $\eta^2$
Change on NPS (0–10) during the CPM-task	17.951 <sup>a</sup>	2	8.976	2.736	0.08	0.18
Pain pressure threshold	5.740 <sup>b</sup>	2	2.870	2.597	0.09	0.17
Heat pain threshold	24.728 <sup>c</sup>	2	12.364	1.308	0.29	0.09
Heat pain tolerance	8.384 <sup>d</sup>	2	4.192	0.854	0.43	0.06

	B	Std. error	t	Sig.	CI 95%	Partial- $\eta^2$
Change on NPS (0–10) during the CPM-task						
Intercept	–17.29	13.552	–1.276	0.21	(–45.20–10.62)	0.061
Heat thermal threshold (adjusted index)	0.35	0.39	0.90	0.37	(–0.45–1.15)	0.032
Brain derivate neural factor (BDNF) (log)	0.10	0.04	2.28	0.03*	(0.01–0.20)*	0.173
Pain pressure threshold						
Intercept	–12.16	7.865	–1.547	0.134	(–28.36–4.03)	0.087
Heat thermal threshold (adjusted index)	0.438	0.226	1.934	0.065	(–0.03–0.90)	0.130
Brain derivate neural factor (BDNF) (log)	0.040	0.026	1.535	0.137	(–0.02–0.09)	0.086
Heat pain threshold						
Intercept	51.219	23.003	2.227	0.035	(3.84–98.59)	0.165
Heat thermal threshold (adjusted index)	–0.313	0.662	–0.473	0.640	(–1.68–1.05)	0.009
Brain derivate neural factor (BDNF) (log)	0.110	0.077	1.436	0.163	(–0.05–0.27)	0.076
Heat pain tolerance						
Intercept	27.449	16.582	1.655	0.110	(–6.70–61.59)	0.099
Heat thermal threshold (adjusted index)	0.598	0.477	1.252	0.222	(–0.38–1.58)	0.059
Brain derivate neural factor (BDNF) (log)	–0.008	0.055	–0.144	0.887	(–0.12–0.10)	0.001

BDNF=brain-derived neurotrophic factor, CPM=conditional pain modulation, NPS=numerical pain scale.

<sup>a</sup>  $R$ -squared=0.180 (adjusted  $R$ -squared=0.114).

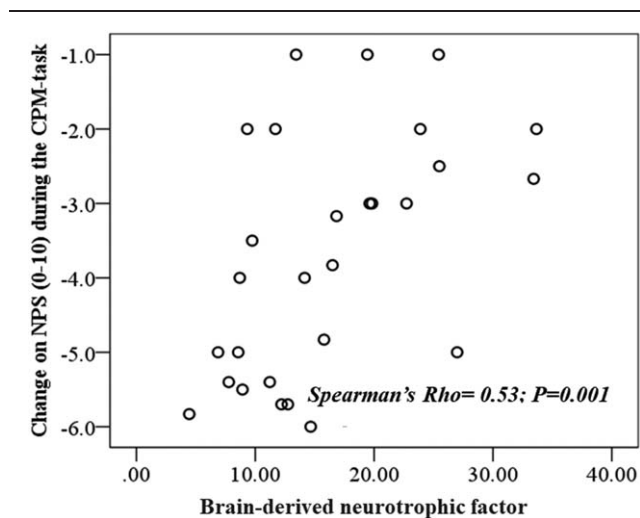
<sup>b</sup>  $R$ -squared=0.172 (adjusted  $R$ -squared=0.106).

<sup>c</sup>  $R$ -squared=0.095 (adjusted  $R$ -squared=0.022).

<sup>d</sup>  $R$ -squared=0.064 (Adjusted  $R$ -squared=–0.011).

\*  $P < .05$ .

nonparametric correlation between BDNF and CPM-Task in FM group ( $Rho = -0.32$ , Confidence Interval 95% = 0.05–0.59), suggesting that in FM, the increase of serum BDNF is associated with stronger function of the descending pain modulatory system (DPMS), given that higher values in the CPM-Task suggests less function of the DPMS. In the FM group, 43.9% presented CPM-task values  $>0$ , suggesting a summation effect. Although in the healthy group no subjects exhibited this phenomenon, we had observed a positive correlation between the BDNF and CPM-task.

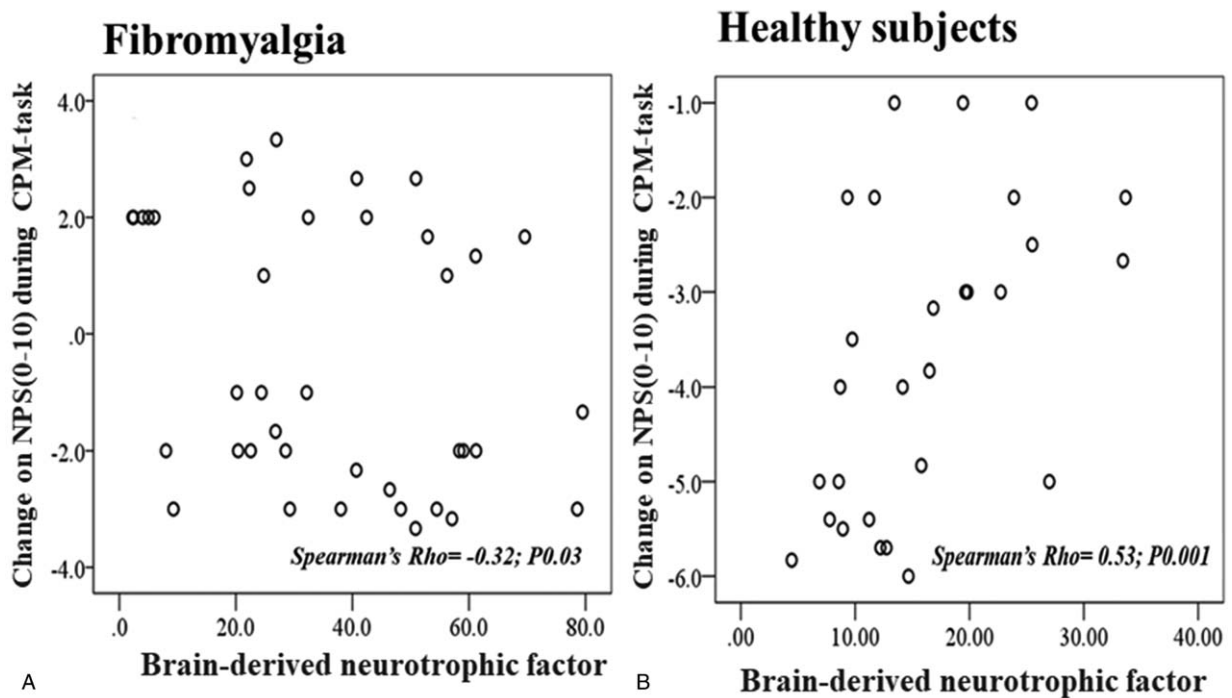


**Figure 1.** Scatterplot of the heat thermal threshold (HTT) and the change on NPS (0–10) during CPM-task (n=41) in fibromyalgia. CPM=conditional pain modulation, NPS=numerical pain scale.

#### 4. Discussion

This study confirmed our hypothesis that the peripheral sensory dysfunction is associated with the disengagement of the descending pain modularity system (DPMS) in FM, but not in healthy subjects. The HTT-adjusted was positively correlated with the CPM-task. Indeed, an increase in the pain score during the CPM-task indicates worst function of the DPMS. Further, the HTT was positively correlated with the BDNF, but not with the PPT. Also, the FM sample presented lower pain thresholds, for both heat and pressure.

The current study expanded available data in the scientific literature showing that the peripheral sensory dysfunction in FM was associated with the disengagement of the DPMS. This finding is aligned with previous reports that suggested that in FM exist greater disinhibition at the cortical level compared with those in which the primary mechanism of pain was persistent and intense nociceptive input (i.e., OA).<sup>[13]</sup> Given the exploratory nature of our study, we could infer that: either the dysfunction in small fibers leads to distinct plastic changes in pain modulatory pathways; or the alterations of the modulatory system induce small fibers dysfunction; or both processes are consequences of different phenomena keeping their functioning correlated. Independently of the mechanisms, our findings speak in favor of a cross-talk between the peripheral afferent and the neural network of the spine-bulbospinal loop along with changes in the BDNF secretion. Thus, this set of changes reinforce the hypothesis that the disinhibition process is a feature of FM, as the dysfunctions in excitatory/inhibitory systems in pain pathways are nothing but the biological grounds of the clinical picture known as central sensitization syndrome. Even though this relationship is complex, our results give neurophysiological support to link the peripheral dysfunction of small fibers, and the



**Figure 2.** Scatterplot of the brain-derived neurotrophic factor (BDNF) and the change on the NPS (0–10) during CPM-task. (A) Fibromyalgia ( $n = 41$ ), and (B) healthy subjects ( $N = 28$ ). CPM=conditional pain modulation, NPS=numerical pain scale.

activity of the descending inhibitory system with the central sensitization<sup>[24]</sup> and higher activation in the brainstem.<sup>[25]</sup>

The downward negative spiral of pain and the DPMS have significant implications regarding the nosological status of FM: small fiber pathology may be a peripheral nervous system contributor to the complex pathophysiology of pain in fibromyalgia. Hence, the combination of “lesion of the somatosensory nervous system” (i.e., fiber loss) and pain defines “neuropathic pain.”<sup>[26]</sup> Thus, the pain described by fibromyalgia patients appears to be closely related to neuropathic pain; which by definition comprise an identifiable group of signs and symptoms and a small fiber impairment, which would qualify fibromyalgia as a disease that deserves to be recognized accordingly. The HTT assessed by the QST and the heat-pain thresholds are considered the most useful and specific for evaluation of a small fiber neuropathy.

Although the QST measures abnormalities in either the central or peripheral nervous system, both alterations can result in the same deficit. The QST has been used in many longitudinal studies and clinical trials to identify neuropathy and is widely available.<sup>[27]</sup> Finally, QST is unable to distinguish between feigned and real loss of sensation.<sup>[28]</sup> However, the reliability of the QST is in question to require conscious integration from the patient and cognitive conditions to comprehend the test (due to disease or medication).

In the present study patients with FM showed presented with lower HPT and PPT compared with healthy subjects. Our finding is in line with previous reports that displayed higher mechanical pain sensitivity and higher cold pain sensitivity,<sup>[29]</sup> and lower heat pain thresholds<sup>[30]</sup> in FM compared with healthy controls, using QST, and using CO<sub>2</sub>-laser stimulation.<sup>[31]</sup> Assessment of laser-evoked potentials in patients with fibromyalgia gave hints for C-fibers sensitization, and ultra-late laser-evoked potentials

could be recorded in the majority of patients.<sup>[32]</sup> In fact, these set of findings showed conflicting results by not precise reasons, but on the overall, give support for the concept of the peripheral neuropathy in the fibromyalgia syndrome.

The small sample size is a limitation of this study, although significance values and statistical methods were kept within the conventionally accepted by the scientific literature. The design of our study limits the ability to determine causality, but still provides interesting insights that might motivate further research as it strengthens the notion of peripheral small fiber dysfunction in FM, effect. The HTT assesses the function in small fibers indirectly, but still it has been shown to have a relatively precise measure of the C-fibers function.<sup>[26]</sup> However, the QST measures are a useful tool for neurophysiological assessment in using a non-invasive method, and that showed a great sensibility to assess peripheral neuropathy. In this study, only women were evaluated, taking into account that sex differences in pain perception and modulation are a controversial topic.<sup>[33]</sup> This limitation restricts the possibility of a direct comparison with other studies but has the advantage of avoiding possible contamination of the data. Because FM is more prevalent in women,<sup>[34]</sup> these results might have more considerable clinical implications. Although we recruited healthy volunteers to assess the relationship between the cortical inhibitory function and the PDMS, it is worth noting that our control sample was younger on average.

According to our results, patients with FM has dysfunctional pain and increase BDNF levels that may be involved in the pathophysiology of pain.<sup>[35]</sup> In the dorsal horn, activation of the receptors by endogenous BDNF seems to contribute to hyperalgesia, suggesting that BDNF contributes to the sensitization of the dorsal neurons (wind up), facilitating the activation of NMDA receptors. Therefore, acute or chronic stimulation of pain

improves the phosphorylation of several receptor subunits (NMDAR) in the spinal cord.<sup>[36,37]</sup> The release of BDNF during pain modulation is mediated by the activation of NMDA receptors by glutamate, along with substance P, acting as rapid neurotransmitters and slow modulators at primary pain synapses.<sup>[38]</sup> In sensory neurons, the concentration of BDNF and substance P depends on the availability of neurogenic growth factor (NGF), which is produced in the periphery, reaching the dorsal ganglia after binding to the trkA receptors of the terminal fibers containing substance P and BDNF. Studies have shown that increased NGF in peripheral tissues in inflammatory processes promote expression of BDNF and substance P in sensory neurons.<sup>[38,39]</sup> And inhibition of Trk-B receptor activity can completely abolish allodynia and thermal hyperalgesia of the sciatic nerve in neuropathic pain models.<sup>[40]</sup> Inhibitors of TRK tyrosine kinase activity and protein kinase C (PKC) inhibitors also blocked neuropathic pain. Thus, BDNF plays an important role in the regulation of inflammatory pain and secondary hyperalgesia, but BDNF released only from nociceptors has no importance in the development of neuropathic pain.<sup>[41]</sup>

We found that FM patients presents an increased pain response to painful mechanical stimuli, similar to others studies.<sup>[42,43]</sup> Comparing healthy subjects and FM patients in heat pain threshold and tolerance to the heat, FM group have been shown reduced heat pain threshold and tolerance to heat. Authors suggested central sensitization as a contributing pathophysiologic factor.<sup>[44,45]</sup> Accordingly, wind-up as an experimental correlate for central sensitization can be evoked in controls and in FM patients, but clear differences between controls and patients can be observed. The magnitude of the sensory response to the first stimulus within a series is greater in FM, as in the amount of temporal summation within a series.<sup>[46]</sup>

Our results add to the growing evidence that abnormalities of cutaneous fiber C pain are related to specific aspects of central sensitization in FM patients<sup>[42]</sup> supported by QST, CPM-task, and PPT. With FM pain, there is increased excitation of the central neurons that induces rearrangements of the dorsal horn neurons. All changes are collectively “central sensitization.”<sup>[47]</sup> A previous consequence of the peripheral lesion is a spinal microglial activation leading to a release of brain-derived neurotrophic factor (BDNF).<sup>[48]</sup> However, the mechanisms involved in “central sensitization” remain poorly understood and the link between pain and BDNF remains unclear. Indeed, BDNF engaging in acute pain remains controversial. Some data are consistent with an anti-nociceptive role, while others are consistent with a pro-nociceptive effect.<sup>[49]</sup> These authors shown that blocking signaling between BDNF and the receptor TrkB reverses the allodynia. And that BDNF is a crucial signaling molecule between microglia and neurons. Blocking this microglia-neuron signaling pathway may represent a therapeutic strategy for treating neuropathic pain.<sup>[50]</sup>

Our results showed that the perception of referred pain when providing a conditioning stimulus at the same time (CPM task) applied in the FM group has a loss of inhibitory system function, as demonstrated in a similar study.<sup>[51]</sup> To increase the modulation system of endogenous inhibitory pain during the CPM task, one study administered melatonin in FM patients and the authors found positive results.<sup>[43]</sup> Another study showed that the CPM-task applied in patients with knee osteoarthritis had decreased activation of the endogenous pain modulation system.<sup>[51]</sup> However, when administered CPM-task in healthy individuals, the observed response is based on the principle of pain inhibiting pain.<sup>[52]</sup> Thus, we observe that in healthy

individuals, the system of downward modulation of pain is preserved, maintaining a basal state of sensory processing. And data support the apparent loss of downward net inhibition as a mechanism that promotes chronic pain, while inhibition engagement protects against chronic pain.<sup>[53]</sup>

## 5. Conclusion

These findings high spot that in FM the disinhibition of the DPMS is positively correlated with the dysfunction in peripheral sensory neurons assessed by QST and conversely with serum BDNF.

## Author contributions

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**Writing – original draft:** Wolnei Caumo, Luciana Conceição Antunes, Jairo Alberto Dussán-Sarria, Iraci Lucena da Silva Torres.

**Writing – review & editing:** Wolnei Caumo, Felipe Fregni.

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