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BDNF as a potential biomarker for the fibromyalgia-like model in male Wistar rats

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ABSTRACT

Introduction: Fibromyalgia syndrome is characterized by central sensitization, with imbalance between the descending pain inhibition pathways and the ascending pain signaling pathways, including changes in the serotonergic, dopaminergic and catecholaminergic circuits. The aim was to evaluate the nociceptive response, depression-like behavior, and central and peripheral biomarkers levels (BDNF and TNF-α) in male adult Wistar rats submitted to a fibromyalgia-like model induced by reserpine.

Methods: Sixteen male adult Wistar rats were allocated by weight in control and fibromyalgia groups. Mechanical and thermal hyperalgesia were evaluated by Von Frey test and Hot Plate test respectively, and depression-like behavior was evaluated by Forced Swim test. Also BDNF and TNF-α were measured in serum and central structures.

Results: Rats in the fibromyalgia group presented a lower thermal and mechanical threshold, and an increased immobility time. also reduced serum BDNF levels, without changes in TNF-α levels. There was a positive correlation between mechanical and thermal nociceptive threshold and serum BDNF levels, and a negative correlation between thermal nociceptive threshold and spinal cord BDNF levels. Also, there was a negative correlation between immobility time and serum BDNF levels.

Conclusion: In summary, this study provides confirmation that the fibromyalgia-like model induced by reserpine effectively replicates the symptoms of fibromyalgia. Additionally, it provides evidence supporting the involvement of brain-derived neurotrophic factor (BDNF) in the development of this pain model.

Keywords: *Fibromyalgia; Rats, BDNF; TNF-α*

INTRODUCTION

Fibromyalgia syndrome is characterized by chronic widespread pain, fatigue, non-restorative sleep, and cognitive symptoms[1], afflicting mainly women and affecting nearly 2% of the world population. The main pathophysiological phenomenon of fibromyalgia is the central sensitization, characterized by a limitation of descending pain inhibition pathways and by the improvement of the ascending pain signaling pathways[2]. Several pathophysiological hypotheses have been proposed to elucidate this syndrome, which the most accepted considers that there is an imbalance between nociception and physiological pain control[3]. According to this hypothesis, there is a global decrease in inhibitory pathways related to the pain, thereby allowing low-intensity or non-nociceptive stimuli to be processed in pre-cortical and cortical structures involved in the affective and cognitive pain process, resulting in an increase of painful perception[4]. Therefore, therapeutic strategies aim to modulate neuroplasticity processes in the pain neuromatrix (multiple brain areas implied in affective, cognitive, and evaluative responses to pain).

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Animal models of disease allow us to evaluate hypotheses through experiments that cannot be tested on humans. Due to the serotonergic, dopaminergic and catecholaminergic described circuit changes, in fibromyalgia patients, a model of this disease has been proposed in rats, using the drug reserpine[5]. The repeated injection of reserpine can induce depletion of biogenic amines in rats, inducing generalized allodynia, a finding that is characteristic of this disease. In this way, this model of the disease offers a plausible neurobiological substrate, with a consistent disease behavioral correlation which thus offers subsidies to study the combination of pharmacological and non-pharmacological intervention, their behavioral, neuroanatomical, and neurobiological effects.

In face of this, the aim was to evaluate the nociceptive response, depression-like behavior, and central and peripheral biomarkers levels (BDNF and TNF-α) in male adult Wistar rats submitted to a fibromyalgia-like model induced by reserpine.

MATERIALS AND METHODS

Animals

Sixteen male Wistar rats [55 – 65 days old (≃ 250g) at the beginning of treatment were used, which was provided by the Animal Reproduction and Experimentation Center (CREAL) through the Institute of Basic Health Sciences (ICBS) of Federal University of Rio Grande do Sul (UFRGS). Rats were assigned or weight-matched (in grams) and housed in groups of 2 to 3 rats per polypropylene cage (49 cm × 34 cm × 16 cm). Rats were kept at a 12-hour light/dark standard cycle (lights on at 7am and lights off at 7pm), in a controlled temperature environment (22 ± 2° C) having free access to water and to Nuvital® feed.

All experiments and procedures were approved by the Institutional Committee for Animal Care and Use (GPPG-HCPA protocol Nº 2015-0272) and according to the Guide for the Care and Use of Laboratory Animals 8th ed. 2011 and law 11.794 (Brazil). The experimental protocol complied with the ethical and methodological standards of the ARRIVE guidelines[6]. The sample size was calculated to detect the statistical significance between means considering an alpha = 0.05 and power of 90%[7].

Experimental Design

Rats were assigned or weight-matched and divided into 2 experimental groups: Control (vehicle) and fibromyalgia (reserpine). The mechanical hyperalgesia threshold was evaluated by the electronic von Frey test before the administration of reserpine and five days after the last dose of the drug. Also, forced swim and hot plate tests were performed five days after the last dose of the drug (Figure 1).

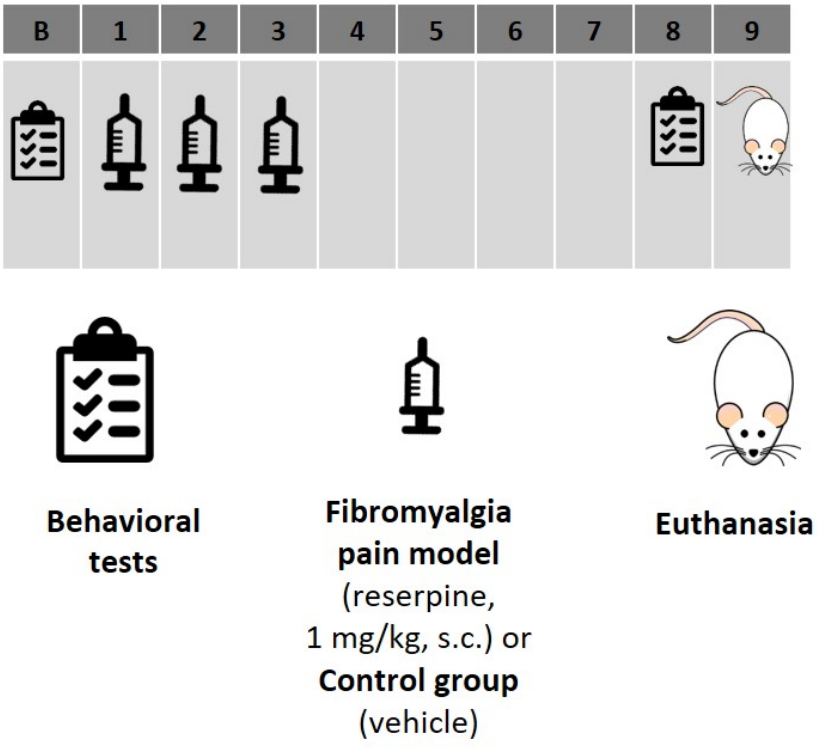


Figure 1: Experimental design.

Fibromyalgia Model

To induce a fibromyalgia-like model, the rats received reserpine (1 mg/kg, s.c.) diluted in 0.5% acid acetic glacial (vehicle) for three consecutive days. In this way, there were two experimental groups, one received vehicle and the other, reserpine. Reserpine can induce depletion of biogenic amines in rats, inducing generalized hyperalgesia, a finding that is characteristic of the disease[7]. Five days after the last reserpine administration (8th day), the rats were assessed for behavioral tests (von Frey, hot plate and forced swim). For weight maintenance of the rats subjected to the reserpine model, it was needed to add sunflower seed, because of significant weight loss, since 20% means in euthanasia of rats. This sunflower seed was added only during 3 days from 8th to 10th day after first reserpine injection. The amount added was 3 to 5 g per cage per day.

Von Frey Test

Mechanical hyperalgesia was assessed by the electronic von Frey test[8]. First, rats were allowed to acclimate in acrylic boxes on a wire metal grid for 15 minutes 24h before testing to avoid stress-induced novelty. Then, in the next day rats were allowed to the boxes again, and after 5 minutes a plastic tip was perpendicularly probed under the animal's right rear paw. Flick, biting or shaking responses were taken as nociceptive measures and recorded in grams (g) throughout the mean of 3 trials.

Hot Plate Test

For thermal hyperalgesia, the rats were also acclimated 24h before testing for allowing them to explore the apparatus for 5 minutes in the switched off mode. On day testing the temperature was set up to 55° C. After that, rats were lowered on a warmed surface circumvented by a transparent polypropylene funnel. The time between the placement, and the first aversive response to thermal noxious stimulus understood as paw shaking or biting was timed in a single trial and expressed in seconds (s). A 20 second cutoff was established to avoid tissue damage[9].

Forced Swim Test

This test was performed to assess depressive-like behavior. The animals were placed into a round glass tank (dimensions 40 cm x 40 cm x 52 cm), the tank was filled with water (22-25° C) until a depth of 35 cm, in such a way that the tail of the rats could not touch the bottom of the tank. The animals were placed into water for five minutes, the immobility time of the rat was recorded in seconds, considering the total of immobility and/or movements to keep the head out of the water with no intention of escaping. After the test, the rats were dried with cotton pads and a hairdryer[10]. The tests were filmed and analyzed by two trained researchers, with the goal of subsequently performing a double check of obtained results to guarantee their reliability and was carried out five days after the last dose of reserpine.

Tissue collection

Rats were killed by decapitation 24 hours after the last behavioral evaluation; and the cerebral cortex, spinal cord, hippocampus, and brainstem were harvested and immediately stored in the - 80° C freezer. Also, the serum was collected and stored in the - 80° C freezer for further analysis.

Biochemical assays

TNF-α and BDNF levels were determined by enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies for each cytokine and neurotrophin (R&D Systems, Minneapolis, United States) using the manufacturer's protocol. Optical density was measured using an ELISA reader at a wavelength of 450 nm. Total protein was measured by the Bradford method using bovine serum albumin as the standard[11]. Data were expressed in picograms per milligram (pg/mg) of protein.

Statistical analysis

For behavioral and biochemical data, a t test for independent samples (Student t test) was used by comparing the control group and fibromyalgia-like model. Spearman's correlation was made between behavioral tests and BDNF levels. A $P < 0.05$ was taken as significant. All data are expressed as mean ± standard error of the mean (S.E.M.) and were run in the SPSS 26.0 statistical program.

RESULTS

Behavioral measures

Regarding mechanical hyperalgesia, it was found to be an interaction between group vs time (repeated measures ANOVA, $P < 0.001$, $F^{1,12} = 0.660$). At baseline, there was no difference between groups upon the mechanical hyperalgesia (Figure 2 Panel A). However, on the 8th day, after the reserpine-induced fibromyalgia-like model, there was a difference between groups; rats that received reserpine displayed a decrease in the mechanical nociceptive threshold compared to those that received vehicle (Figure 2, Panel A, $n = 7 – 8$ per group).

Regarding thermal sensitivity, we found differences between groups by showing that reserpine-induced

fibromyalgia-like model group displayed a marked thermal hyperalgesia compared to the vehicle group five days after the last injection (independent Student t test, P = 0.033; Figure 2, Panel B; n = 8 per group).

Concerning the forced swimming test, we also noted that a reserpine-induced fibromyalgia model triggered an increased immobility time compared to vehicle groups (independent Student t test, P < 0.001, Figure 2, Panel C, n = 5 per group).

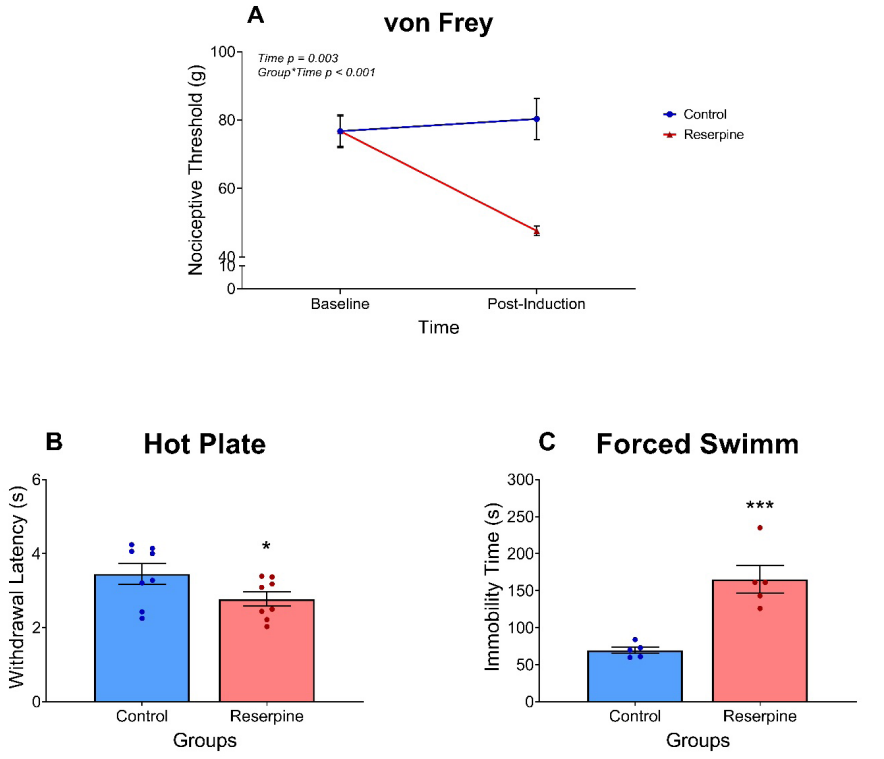


Figure 2: Behavioral assessments.

A: Mechanical hyperalgesia threshold assessed in the Von Frey test at baseline and after induction of fibromyalgia model (8th day). *Significant difference from control group (repeated measures ANOVA, P < 0.001). The vertical lines indicate the mean ± S.E.M of paw withdrawal threshold in grams (g); B: Thermal hyperalgesia assessed by the Hot Plate test. The vertical lines indicate the mean ± S.E.M of paw withdrawal threshold in seconds (s) *Significant difference from control group (Student t test, P = 0.033); C: Immobility time assessed by the Forced Swim test. The vertical lines indicate the mean ± S.E.M of immobility time in seconds (s). *Significant difference from control group (Student t test, P < 0.001).

Biochemical Analysis

Regarding cerebral cortex, brainstem, and spinal cord BDNF levels, we found no statistical difference between groups (independent Student t test, P = 0.453, P = 0.507, P = 0.576, respectively; n = 7 – 8 per group, Table 1). However, the serum BDNF levels were decreased in rats subjected to reserpine-induced fibromyalgia rat model in relation to the control group (independent Student t test; P < 0.001; n = 7 – 8 per group, Table 1).

Regarding TNF-α, no significant differences were found in all evaluated structures (hippocampus, brainstem, cerebral cortex, and serum) (independent Student t test, P = 0.213, P = 0.0.262, P = 0.120, P = 0.459, respectively; n = 7 – 8 per group, Table 1).

Table 1: Biomarkers profile after reserpine-induced fibromyalgia rat model in serum and central nervous system structures (brainstem, cerebral cortex, spinal cord and hippocampus). Data expressed as mean ± SEM (pg/mg of protein) of biomarkers levels in the central nervous structures and serum of rats.

Biomarker	Structure	Control group	Fibromyalgia group	P value
BDNF (pg/mg of protein)	Brainstem	3.23±0.21	3.44±0.21	0.507
	Cerebral Cortex	0.61±0.15	0.46±0.12	0.453
	Spinal Cord	3.11±0.44	2.59±0.75	0.576
	Serum	0.60±0.01	0.45±0.02*	<0.001

Continues...

Table 1: Continuation.

Biomarker	Structure	Control group	Fibromyalgia group	P value
TNF-a (pg/mg of protein)	Brainstem	2.15±0.44	3.29±0.84	0.262
	Cerebral Cortex	1.87±0.49	0.88±0.29	0.120
	Hippocampus	2.17±1.05	0.71±0.13	0.213
	Serum	10.86±2.59	13.29±1.79	0.459

* independent Student t test.

Behavioral tests and BDNF: correlation Analysis

We have investigated the correlation between behavioral tests (mechanical and thermal threshold and immobility time) with BDNF levels (brainstem, cerebral cortex, spinal cord, and serum) in Figure 3. It was found a positive correlation between mechanical nociceptive threshold and serum BDNF levels (Spearman's correlation, P < 0.001, r = 0.841); however, there were no correlation between mechanical nociceptive threshold and brainstem BDNF levels (P = 0.474), and spinal cord BDNF levels (P = 0.201) and cerebral cortex BDNF levels (P = 0.748).

Also, a negative correlation was found between thermal nociceptive threshold and spinal cord BDNF levels (Spearman's correlation, P=0.031, r = - 0.558) and a positive correlation between serum BDNF levels was found (P = 0.034, r = 0.532); however there were no correlation between thermal nociceptive threshold and brainstem BDNF levels (P = 0.242), and cerebral cortex BDNF levels (P = 0.879).

In addition, a negative correlation was found between immobility time and serum BDNF levels (Spearman's correlation, P = 0.006, r = - 0.796); however there were no correlation between immobility time and spinal cord BDNF levels (P = 0.700), brainstem BDNF levels (P = 0.455) and cerebral cortex BDNF levels (P = 0.815).

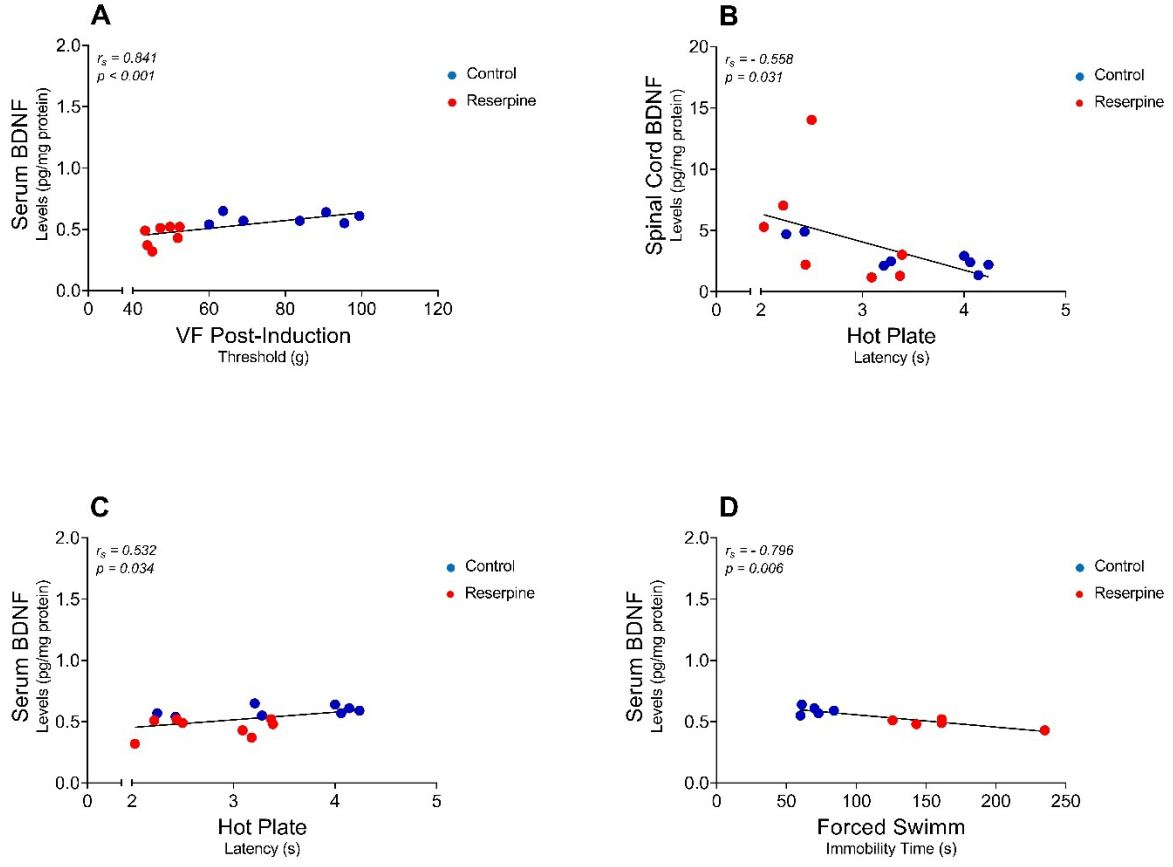


Figure 3: Correlation analysis between behavioral measurements and BDNF levels.

A: Spearman's correlation between mechanical nociceptive threshold and serum BDNF levels; B: Spearman's correlation between thermal nociceptive threshold and spinal cord BDNF levels; C: Spearman's correlation between thermal nociceptive threshold and serum BDNF levels.

DISCUSSION

The results of the current study demonstrated that rats submitted to the fibromyalgia model presented mechanical and thermal hyperalgesia, an increase in immobility time and a reduced serum BDNF levels. In addition, there was a positive correlation between mechanical and thermal nociceptive threshold and serum BDNF levels. On the other hand, there is a negative correlation between thermal nociceptive threshold and spinal cord BDNF levels. Also, there was a negative correlation between immobility time and serum BDNF levels. Altogether, these results outline that reserpine-induced fibromyalgia-like models can unleash behavioral phenotypes related to chronic pain, probably with participation of the peripheral BDNF levels.

It is important to note that on the third day of reserpine administration for fibromyalgia model induction, the rats showed an eye compression, a vocalization at the manipulation moment, an arched posture, a weight loss and a prostration behaviors that indicate the presence of pain[12]. According to the Grimace scale for rats (orbital tightening, nose/cheek flattening, ear changes and whisker change)[13], in the current study, the rats presented an indication of severe pain, and loss of weight (data not shown). Altogether, our findings showed that rats in the fibromyalgia model displayed a hyper nociceptive behavior, such as thermal and mechanical hyperalgesia, both linked to central sensitization[14,15].

Additionally, in the current study, the lower thermal threshold was characterized by presenting only ocular compression and vocalization for thermal sensitivity signaling, and not presenting the expected response which was the “tap dance” movement and the licking of the feet (data not shown)[9]. Previous study showed that monoamines (serotonin, norepinephrine, and dopamine) are reduced in the spinal cord in rats subjected to an animal model of fibromyalgia[5], which may contribute to lower thermal threshold. However, it is possible to suggest that the fibromyalgia rats present alterations in the supraspinal pain process, since the hot plate test involves complex supraspinal sensory integration[16].

A clinical diagnosis from the patient’s complaints related to several complicated spots throughout the body, such as pain, aches, palpitations, and often psychopathological changes, such as anxiety and depression[17]. Also, evidence suggests that patients diagnosed with fibromyalgia have reduced serum levels of BDNF, corroborating the worsening of the psychopathologies involved in this condition[18]. In agreement, this current study showed that the fibromyalgia model induced a reduction in serum BDNF levels of rats. It is important to note that the scientific literature showed that BDNF is an important biomarker in different types of pain, including fibromyalgia[19], also in the peripheral and central nervous structures. Moreover, it is suggested the role of cytokines in pathogenesis of fibromyalgia; an increased plasma TNF-α, IL-10 and IL-8 were observed in fibromyalgia patients; a reduced level of anti-inflammatory cytokine IL-5, but a normal level in IL-10[20]. However, we did not observe any effect on TNF-α central and peripheral levels.

We have found a negative correlation between thermal nociceptive threshold and BDNF spinal cord levels. Also, there was a positive correlation between mechanical and thermal nociceptive threshold and BDNF serum levels. Our results suggest a different role of BDNF in central and peripheral levels. Confirming our hypothesis, a previous preclinical study showed that a single intrathecal injection of BDNF was able to decrease the nociceptive threshold in naive rats, for at least a 42-day period[21]. Another study showed decreased development of allodynia and hyperalgesia in the post-injury period in BDNF-deficient subjects[22]. This effect of BDNF can be seen in both the peripheral sensory neuron and the spinal cord, where BDNF is released by microglia[22]. Corroborating these data, a clinical study showed that the concentration of plasma BDNF levels was positively associated with the heat pain threshold and numeric rating scale of pain[23]. In this way, the possible BDNF upregulation in the dorsal root ganglion (DRG) and spinal cord, which contributes to chronic pain hypersensitivity, may indicate that blocking BDNF in sensory neurons provides a new target for the development of novel drugs[24].

Furthermore, depressive-like behavior was assessed, the fibromyalgia rats presented a significant increase in immobility time, indicating the depressive-like behavior. Previous studies have used this model also to evaluate antidepressant drugs[25,26]. Thus, the depletion of monoamines resulting from the administration of reserpine may be reproducing, in addition to fibromyalgia, comorbidities attributed to this condition, such as depression. It is known that the pathophysiology of depression is complex, but there is an involvement with deficiency of monoaminergic neurotransmitters, specifically norepinephrine (NE) and serotonin (5-HT)[27]. In addition, a negative correlation was found between immobility time and BDNF serum levels. This result corroborated another study that showed a reduction in this neurotrophin induced by a fibromyalgia-like model conducted by intermittent cold stress with slight modification[28]; despite this, we did not observe change in the central BDNF levels, at least in the structures evaluated. In agreement with our data, a previous study showed a significant negative correlation between BDNF expression levels with immobility time[29].

Indeed, neurotrophic factors play a central role in the synaptic plasticity, and they may be involved in the maladaptive neuroplasticity in depression.

In conclusion, this study provides confirmation that the fibromyalgia-like model induced by reserpine effectively replicates the symptoms of fibromyalgia. Additionally, it provides evidence supporting the involvement of BDNF in the development of this pain model. Rats with fibromyalgia exhibited a reduction in peripheral levels of BDNF. Furthermore, there was a positive correlation observed between the reduced levels of BDNF and both the thermal and mechanical nociceptive threshold. On the other hand, there was a negative association between the central levels of BDNF and the thermal nociceptive threshold. The statement above emphasizes the importance of identifying biomarkers for fibromyalgia that contribute to the differentiation of diagnosis and facilitation of therapy. Additionally, this will also provide a substantial contribution to future research endeavors aimed at assessing the efficacy of both pharmaceutical and non-pharmacological therapeutic methods.

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Authors' contributions

VAS, LFM, CLO, HRM, JADS, AS and ILST have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data. VAS, LFM, DJS and ILST have been involved in drafting the manuscript or revising it critically for important intellectual content. All authors have given final approval of the version to be published.

REFERENCES

1. Ceca D, Pablos A, Elvira L, López-Hernández L, Ortega AL. Effectiveness of a self-myofascial conditioning programme on pain, depression, anxiety and sleep quality in people with Fibromyalgia. *Cuad Psicol del Deport*. 2020;20(1):147-65.
2. Poluha RL, Grossmann E. Does pregabalin improve sleep disorders in fibromyalgia? TT – A pregabalina melhora os distúrbios do sono na fibromialgia? *BrJP*. 2018;1(2).
3. Yunus MB. Fibromyalgia and overlapping disorders: the unifying concept of central sensitivity syndromes. *Semin Arthritis Rheum*. 2007;36(6):339-56.
4. Burgmer M, Pogatzki-Zahn E, Gaubitz M, Wessoleck E, Heuft G, Pfleiderer B. Altered brain activity during pain processing in fibromyalgia. *Neuroimage*. 2009;44(2):502-8.
5. Nagakura Y, Oe T, Aoki T, Matsuoka N. Biogenic amine depletion causes chronic muscular pain and tactile allodynia accompanied by depression: a putative animal model of fibromyalgia. *Pain*. 2009;146(1-2):26-33.
6. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: The arrive guidelines for reporting animal research. *PloS Biol*. 2010;8(6):e1000412.
7. Nagakura Y, Takahashi M, Noto T, Sekizawa T, Oe T, Yoshimi E, et al. Different pathophysiology underlying animal models of fibromyalgia and neuropathic pain: Comparison of reserpine-induced myalgia and chronic constriction injury rats. *Behav Brain Res*. 2012;226(1):242-9.
8. Cioato SG, Medeiros LF, Marques Filho PR, Vercelino R, de Souza A, Scarabelot VL, et al. Long-lasting effect of transcranial direct current stimulation in the reversal of hyperalgesia and cytokine alterations induced by the neuropathic pain model. *Brain Stimul*. 2016;9(2):209-17.
9. Woolfe G, Macdonald AD. The evaluation of the analgesic action of pethidine hydrochloride (Demerol*). J Pharmacol Exp Ther*. 1944;80(3):300-7.
10. Moreira SFS, Nunes EA, Kuo J, de Macedo IC, Muchale A, de Oliveira C, et al. Hypoestrogenism alters mood: Ketamine reverses depressive-like behavior induced by ovariectomy in rats. *Pharmacol Rep*. 2016;68(1):109-15.
11. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein–dye binding. *Anal Biochem*. 1976;72:248-54.
12. Neves SMP, Mancini Filho J, de Menezes EW. *Manual de cuidados e procedimentos com animais de laboratório do Biotério de Produção e Experimentação da FCF-IQ/USP*. São Paulo: FCF-IQ/USP; 2013.
13. Sotocinal SG, Sorge RE, Zaloum A, Tuttle AH, Martin LJ, Wieskopf JS, et al. The Rat Grimace Scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. *Mol Pain*. 2011;7:55.
14. Price DD, Staud R, Robinson ME, Mauderli AP, Cannon R, Vierck CJ. Enhanced temporal summation of second pain and its central modulation in fibromyalgia patients. *Pain*. 2002;99(1-2):49-59.
15. Staud R, Rodriguez ME. Mechanisms of disease: pain in fibromyalgia syndrome. *Nat Clin Pract Rheumatol*. 2006;2(2):90-8.
16. Kiso T, Moriyama A, Furutani M, Matsuda R, Funatsu Y. Effects of pregabalin and duloxetine on neurotransmitters in the dorsal horn of the spinal cord in a rat model of fibromyalgia. *Eur J Pharmacol*. 2018;827:117-24.
17. Alves RC, Nepomuceno VR, Marson PG, Bartholomeu Neto J, Silveira JM, Silveira JM, et al. Aspectos epidemiológicos e diagnóstico da

fibromialgia na região Norte do Brasil. *Res Soc Dev*. 2022;11(4): e3511427704.

18. Merino AS, Simon D. Fatores genéticos associados à fibromialgia: uma revisão narrativa. *Res Soc Dev*. 2022;11(3): e11211326421.

19. Siracusa R, Di Paola R, Cuzzocrea S, Impellizzeri D. Fibromyalgia: pathogenesis, mechanisms, diagnosis and treatment options update. *Int J Mol Sci*. 2021;22(8):3891.

20. Singh L, Kaur A, Bhatti MS, Bhatti R. Possible molecular mediators involved and mechanistic insight into fibromyalgia and associated co-morbidities. *Neurochem Res*. 2019;44(7):1517-32.

21. Constandil L, Aguilera R, Goich M, Hernández A, Alvarez P, Infante C, et al. Involvement of spinal cord BDNF in the generation and maintenance of chronic neuropathic pain in rats. *Brain Res Bull*. 2011;86(5-6):454-9.

22. Coull JAM, Beggs S, Boudreau D, Boivin D, Tsuda M, Inoue K, et al. BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature*. 2005;438(7070):1017-21.

23. Sorkpor SK, Galle K, Teixeira AL, Colpo GD, Ahn B, Jackson N, et al. The relationship between plasma BDNF and pain in older adults with knee osteoarthritis. *Biol Res Nurs*. 2021;23(4):629-36.

24. Obata K, Noguchi K. BDNF in sensory neurons and chronic pain. *Neurosci Res*. 2006;55(1):1-10.

25. Gao ZY, Yang P, Huang QJ, Xu HY. The influence of dizocilpine on the reserpine-induced behavioral and neurobiological changes in rats. *Neurosci Lett*. 2016;614:89-94.

26. Kim YR, Park BK, Seo CS, Kim NS, Lee MY. Antidepressant and anxiolytic-like effects of the stem bark extract of fraxinus rhynchophylla hance and its components in a mouse model of depressive-like disorder induced by reserpine administration. *Front Behav Neurosci*. 2021;15:650833.

27. Miri AL, Hosni AP, Gomes JC, Kerppers II, Pereira MCS. Estudo do L-triptofano na depressão ocorrida pela doença de Alzheimer em modelos experimentais. *J Phys Educ*. 2017;28(1):e-2839.

28. Lee H, Im J, Won H, Kim JY, Kim HK, Kwon JT, et al. Antinociceptive effect of Valeriana fauriei regulates BDNF signaling in an animal model of fibromyalgia. *Int J Mol Med*. 2018;41(1):485-92.

29. Sohroforouzani AM, Shakerian S, Ghanbarzadeh M, Alaei H. Effect of forced treadmill exercise on stimulation of BDNF expression, depression symptoms, tactile memory and working memory in LPS-treated rats. *Behav Brain Res*. 2022;418:113645.

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