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Original Article

Melatonin induces a state-dependent analgesic effect, but not after chronic administration in rats

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ABSTRACT

Introduction: Temporomandibular disorders are the primary source of nonodontogenic orofacial pain, affecting the patients' quality of life. Pharmacological interventions, including opioids, nonsteroidal anti-inflammatories, steroids, and immunosuppressant drugs, have shown adverse effects, limiting their use. This study aimed to investigate the impact of acute and repeated administration of morphine and/or melatonin on pain thresholds and physiological markers modulated by chronic pain and treatments in a temporomandibular joint arthritis (TMJA) model. **Methods:** Fifty-nine male Wistar rats (≈250 g) were randomly assigned to two groups, receiving an intra-temporomandibular joint injection of Complete Freund's Adjuvant (CFA, 1 mg/ml) or saline. After 14 days, treatment with melatonin (50 mg/kg/i.p.) and/or morphine (5 mg/kg/s.c.) was carried out for 8 days. The facial von Frey test was employed to assess mechanical allodynia on experimental days 0 (baseline), 15, and 22. At the end of the protocol, rats were euthanized, and structures (cerebral cortex, pons, and spinal cord) were dissected to determine the levels of BDNF (brain-derived neurotrophic factor), IL-10 (interleukin-10), and TNF-α (tumor necrosis factor alpha). **Results:** The TMJA group presented facial mechanical allodynia at D15, but not at D22. Melatonin and morphine induced analgesia at D15. The TMJA model increased spinal cord IL-10 and pontine BDNF levels, whereas melatonin decreased pontine BDNF levels and joint inflammation. **Conclusion:** Morphine and melatonin exhibited acute analgesic effects, yet melatonin failed to maintain the analgesic effect after multiple doses in the TMJA groups.

Keywords: *Melatonin; Morphine; Complete Freund's Adjuvant; Chronic Pain; Mechanical Allodynia.*

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INTRODUCTION

Temporomandibular disorders (TMDs) are the main cause of nonodontogenic orofacial pain, significantly impairing the patients' quality of life and functionality. These disorders affect the temporomandibular joints (TMJ), which are highly complex and frequently used. TMDs commonly involve pain in the masticatory muscles and the joint and include conditions such as internal derangement, arthritis, trauma, and pain-dysfunction syndrome[1,2]. TMDs are typically induced by trauma, strain, and autoimmune or rheumatic diseases, which result in inflammation.

Managing TMDs is challenging due to persistent pain and multiple comorbidities. Its treatment aims to alleviate TMJ pain, restore masticatory function, and instruct patients to minimize stressful situations. However, the inefficacy and adverse effects of opioids, non-steroidal anti-inflammatory drugs (NSAIDs), steroids, and immunosuppressants, existing pharmacological agents used to manage this pain condition, limit their use[3]. This underscores the need for safer and more effective alternative therapies.

In this context, centrally or peripherally administered melatonin or its analogs produce long-term and dose-dependent antinociceptive effects in inflammatory, neuropathic, and acute pain models[4]. In the orofacial region, melatonin has demonstrated promising effects for dental disorders, including periodontal disease and dental implant osseointegration, likely due to its

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anti-inflammatory and osteoconductive properties[5]. Therefore, it is also reasonable to investigate the role of melatonin for temporomandibular joint arthritis (TMJA). Supporting its therapeutic application, we have previously demonstrated that melatonin exerts antinociceptive effects by reducing inflammatory pain[6-8]. In addition, melatonin has been associated with antidepressant, anxiolytic, locomotor-regulating, neuroprotective, anti-inflammatory, antioxidant, and pain-modulating properties. It also promotes sleep and contributes to the regulation of blood pressure, retinal and vascular physiology, seasonal reproduction, ovarian physiology, osteoblast differentiation, and tumor suppression[9].

A study by our research group showed that short-term melatonin administration induces high and low BDNF levels in the spinal cord and prefrontal cortex, respectively, in rats with acute inflammation induced by Complete Freund's Adjuvant (CFA), potentially related to the pain-modulating and neuroprotective effects of this neurotrophin[8]. In another study, we demonstrated specific tissue profiles of neuroimmunomodulators (BDNF, NGF, IL-6, and IL-10) associated with the effects of melatonin in the brainstem in rats submitted to the TMJA model induced by intra-articular administration of CFA[6]. These results suggest that melatonin may act as a potential therapeutic agent for TMDs, since preclinical studies indicate that CFA-induced TMJA increases NGF, IL-10, IL-6, and BDNF levels in the trigeminal ganglia and brainstem following inflammatory pain in male rats[6,10]. In female rats, TMJA reduces brainstem TNF-α levels without affecting BDNF or TNF-α levels in the hippocampus[11].

Furthermore, a recent study indicated that intra-articular morphine injections may provide prolonged relief from TMJ pain; however, larger, rigorously designed trials are necessary to standardize protocols and confirm their long-term efficacy[12]. Subcutaneous morphine reduces thermal sensitivity with sex-dependent and short-lasting effects[13]. However, tolerance to morphine and hyperalgesia commonly occur during extended use; with the cessation of its administration, pain sensitivity may escalate[14].

Considering the relevance of this topic and the limited research focusing on melatonin and morphine as treatments for TMJ disorders, this study aimed to evaluate the effects of repeated administration of melatonin and morphine, either alone or in combination, on nociceptive, neurotrophic, and neuroinflammatory parameters in a rat model of TMJA.

MATERIAL AND METHODS

Animals

A total of 59 adult male Wistar rats, provided by the Animal Breeding and Experimentation Center (CREAL/UFRGS), were used in this study. The rats were approximately 65 days old (200-250 g) at the beginning of the experiment. Animals were housed in the Animal Experimentation Unit (UEA) of the Hospital de Clínicas de Porto Alegre (HCPA), in a controlled environment with a 12-hour light-dark cycle (lights on at 7:00 a.m.), ambient temperature (22°C ± 2°C), and relative air humidity (40–60%), with free access to water and rodent chow (Nuvilab CR1®, Quimtia, PR, Brazil). The cages were environmentally enriched with pieces of wood, ropes, cardboard rolls, and shredded paper. All experiments and procedures were approved by the Institutional Ethics Committee on the Use of Animals (CEUA/HCPA protocol #2020.0689), in compliance with Brazilian guidelines for the use of animals in research (Law #11,794), and the ARRIVE guidelines[15]. The sample size was determined based on the study by Scarabelot and colleagues[6].

Drugs and chemicals

Complete Freund's adjuvant (CFA, an inactivated *Mycobacterium tuberculosis* compound) was purchased from Sigma–Aldrich® (St. Louis, MO, USA). Saline (S) solution (0.9% NaCl) was used as a diluent and was administered intra-articularly to the SHAM group. Morphine (M) was diluted in 0.9% saline and was administered subcutaneously (s.c.) at a dose of 5 mg/kg[16]. Melatonin (Me) was diluted in 40% propylene glycol, combined with saline solution, and administered at a dose of 50 mg/kg. The solution was preheated (54ºC) to maximize solubility before intraperitoneal (i.p.) injection[6]. All compounds were administered at an established dose within the mg/kg limits and in accordance with animal safety standards.

Experimental design

Upon arrival at the housing facility (UEA), the rats underwent a 14-day acclimatization period (Figure 1).

First phase

On experimental day 0 (D0), animals were randomly allocated according to body weight and baseline facial mechanical nociceptive threshold into two main groups:

1. **SHAM** (n = 29), which received an intra-articular injection of 0.9% saline into the TMJ;
2. **TMJA** (n = 30), which received CFA (1 mg/mL) injected into the right TMJ to induce TMJA.

The establishment of the TMJA model was confirmed 15 days later (D15) using the electronic von Frey (VF) test.

Second phase

Beginning on experimental D15, animals received daily treatment with either morphine, melatonin, or their combination for 8 consecutive days. Rats were further subdivided into eight experimental groups according to the interventions administered. The group nomenclature was defined as follows: the first letter represented the initial condition, S (SHAM) or C (CFA-induced TMJA), whereas the subsequent abbreviations indicated treatment allocation: S (saline; morphine vehicle), Me (melatonin), M (morphine), and V (melatonin vehicle). Accordingly, the experimental groups were SSV, SMV, SSMe, SMMe, CSV, CSMe, CMV, and CMMe.

The treatment effects were assessed on D15, after the first dose of drug administration, and on D22, following repeated administration. All investigators, except the principal investigator, were blinded to group allocation throughout the experimental procedures and data collection.

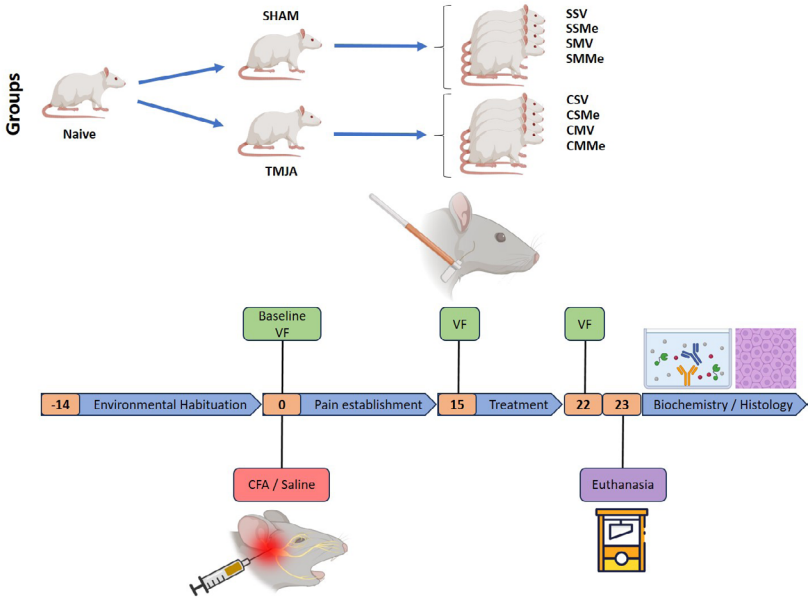

Figure 1: Experimental timeline. CFA = Complete Freund's Adjuvant; VF = von Frey test. SSV: SHAM + Saline + Vehicle; SMV: SHAM + Morphine + Vehicle; SSMe: SHAM + Saline + Melatonin; SMMe: SHAM + Morphine + Melatonin; CSV: CFA + Saline + Vehicle; CMV: CFA + Morphine + Vehicle; CSMe: CFA + Saline + Melatonin; CMMe: CFA + Morphine + Melatonin. Made via BioRender® and Microsoft PowerPoint®.

Temporomandibular joint arthritis (TMJA) model induction

Chronic inflammatory orofacial pain and mechanical allodynia were induced by the intra-articular administration of 50 µl of CFA (*Mycobacterium tuberculosis*, 1 mg/mL, Sigma–Aldrich, St. Louis, MO, USA) into the right TMJ (TMJA group)[17]. SHAM animals received an injection of 50 µl of 0.9% sterile saline solution into the right TMJ.

Electronic von Frey assay

The facial mechanical nociceptive threshold of the TMJ region was assessed via an electronic VF analgesiometer (Insight, São Paulo, Brazil). The experimental protocol was performed according to Kroeff and colleagues[11].

Euthanasia, tissue collection, and sampling

The animals were euthanized via guillotine one day following the conclusion of the behavioral tests (D23). The right TMJ was subsequently dissected and processed for histological examination. The central nervous system was surgically exposed, and the spinal cord, pons, and cerebral cortex were removed and preserved (-80°C) for posterior biochemical analysis. All samples were homogenized using a protease inhibitor cocktail (P8340, Sigma–Aldrich®), centrifuged, and the supernatant was equally aliquoted for subsequent analysis.

Protein and biochemical marker quantification

Tumor necrosis factor alpha (TNF-α), interleukin-10 (IL-10), and brain-derived neurotrophic factor (BDNF) levels were quantified in the tissue homogenates via commercial ELISA-specific kits for rats (DY248, DY522, and DY510, respectively; R&D Systems, Minneapolis, MN, USA), following the manufacturer's instructions. The total protein content of the homogenates was quantified using the Bradford colorimetric method[18], employing bovine serum albumin at multiple concentrations to

produce a standard curve. The results are expressed as pg/mg (corrected by protein quantification).

Histology and histological scoring

Twenty-three days after the CFA injection, the TMJ and adjacent tissue were excised and subsequently fixed in 10% buffered formalin for a period of 7 days. Afterward, the samples were decalcified with a 5% nitric acid solution. The samples were then subjected to dehydration via a series of alcohols (70–100%) for 6 hours, followed by embedding in paraffin and sectioning at 3 µm slices. Slides containing the tissues adjacent to the TMJ were prepared and stained with hematoxylin and eosin (HE). A qualitative assessment of the degree of inflammation in the region was conducted via light microscopy by an observer who was blind to the treatment. Scoring was based on the methodology established by Laste and colleagues[19]. The percentage of infiltrating mononuclear cells was scored as follows: 0 = absent; 1 = mild (1–10%); 2 = moderate (11–50%); and 3 = severe (51–100%).

Statistical analysis

The Shapiro–Wilk test and histogram analysis were used to verify the data distribution. To evaluate the establishment of the pain model, behavior and biomarkers were analyzed via an independent-samples *t*-test. To assess treatment effects, behavioral and biomarker data were analyzed via one-way ANOVA followed by Duncan's *post hoc* test. The Spearman correlation coefficient was used to assess associations between behavioral and biochemical variables. All statistical analyses were conducted using SPSS® (Statistical Package for the Social Sciences), version 18.0. Data are expressed as the mean ± standard error of the mean (SEM), and statistically significant differences were considered when $p < 0.05$.

RESULTS

Nociceptive behavior

No significant differences in the mechanical nociceptive threshold were observed between the groups at baseline ($t_{(57)} = -0.403$, $p = 0.689$; Fig. 2a, n = 29-30/group). Fifteen days post-injection, rats injected with CFA exhibited mechanical allodynia compared with those injected with saline, confirming the efficacy of the pain model ($t_{(57)} = 2.017$, $p = 0.048$; Fig. 2b, n = 29-30/group). A similar effect was observed when comparing only the SSV and CSV groups at D15 ($t_{(12)} = 5.672$, $p < 0.001$; Fig. 2c, n = 5-10/group); however, by D22, no significant differences were observed between the SSV and CSV groups ($t_{(12)} = -0.008$, $p = 0.994$; Fig. 2d), demonstrating reversal of the pain model. In this way, animals that received the CFA injection and those receiving saline were analyzed independently for treatment effects.

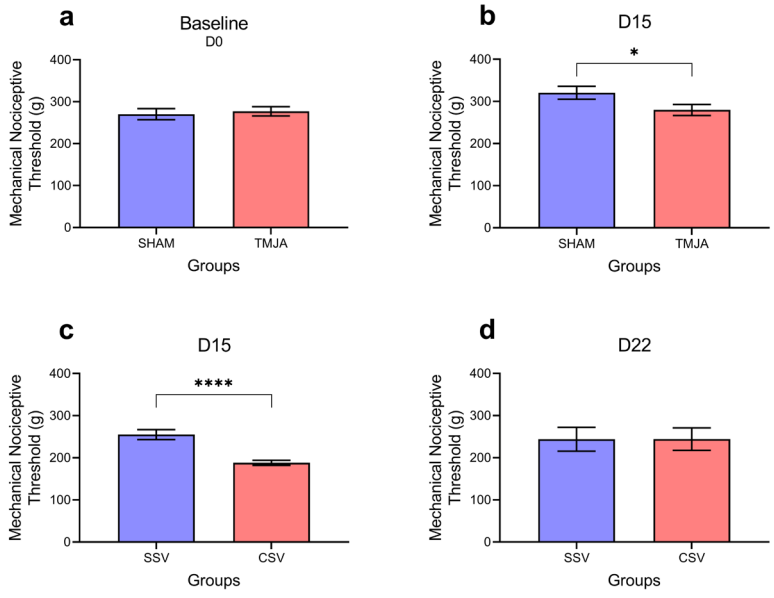

Figure 2: Mechanical nociceptive threshold assessed via the facial VF test. The data are expressed as the means ± SEMs. **Panel A.** SHAM and TMJA groups at baseline (D0; n = 29 to 30/group). **Panel B.** Effect of intra-TMJ CFA injection on D15 (all animals; n = 29 to 30/group). **Panel C.** Effect of intra-TMJ CFA injection on D15. **Panel D.** Effect of intra-TMJ CFA injection on D22 (Saline/Vehicle-treated animals only; n = 5 to 10/group). SHAM: Animals injected with saline intra-TMJ. TMJA: Animals injected with CFA intra-TMJ; SSV: SHAM + saline + vehicle; CSV: TMJA + saline + vehicle; * $p < 0.05$; **** $p < 0.001$. Made via GraphPad Prism 9.0®.

After the initial dose of treatments, the one-way ANOVA demonstrated significant differences between the SHAM groups ($F_{(3,25)}$ = 7.807, p = 0.001; n = 29). Paired comparisons between these groups revealed that both groups of animals receiving morphine (SMV and SMMe) presented analgesia (D15) (Duncan, p < 0.05; Fig. 3a), with no effects of melatonin (SSMe) (Duncan, p > 0.05; Fig. 3a). With respect to the TMJA groups, the one-way ANOVA also revealed differences between groups ($F_{(3)}$ = 46.873, p < 0.001; n = 30). Comparisons between the TMJA groups revealed that both melatonin and morphine induced an analgesic effect after the initial dose of treatment (D15); however, in the morphine groups (CMV and CMMe), this effect was significantly greater than that observed in the melatonin group (CSMe) (Duncan, p < 0.05; Fig. 3b).

The analysis of all SHAM groups revealed no differences at D22 (one-way ANOVA, $F_{(3)}$ = 1.752, p = 0.182; Fig. 3c). With respect to the TMJA groups, the one-way ANOVA revealed differences between groups at D22 ($F_{(3)}$ = 3.220, p < 0.039). Comparisons between the TMJA groups revealed a morphine analgesic effect at D22 (Duncan, p < 0.05; Fig. 3d), which was not observed when melatonin was present (Duncan, p > 0.05).

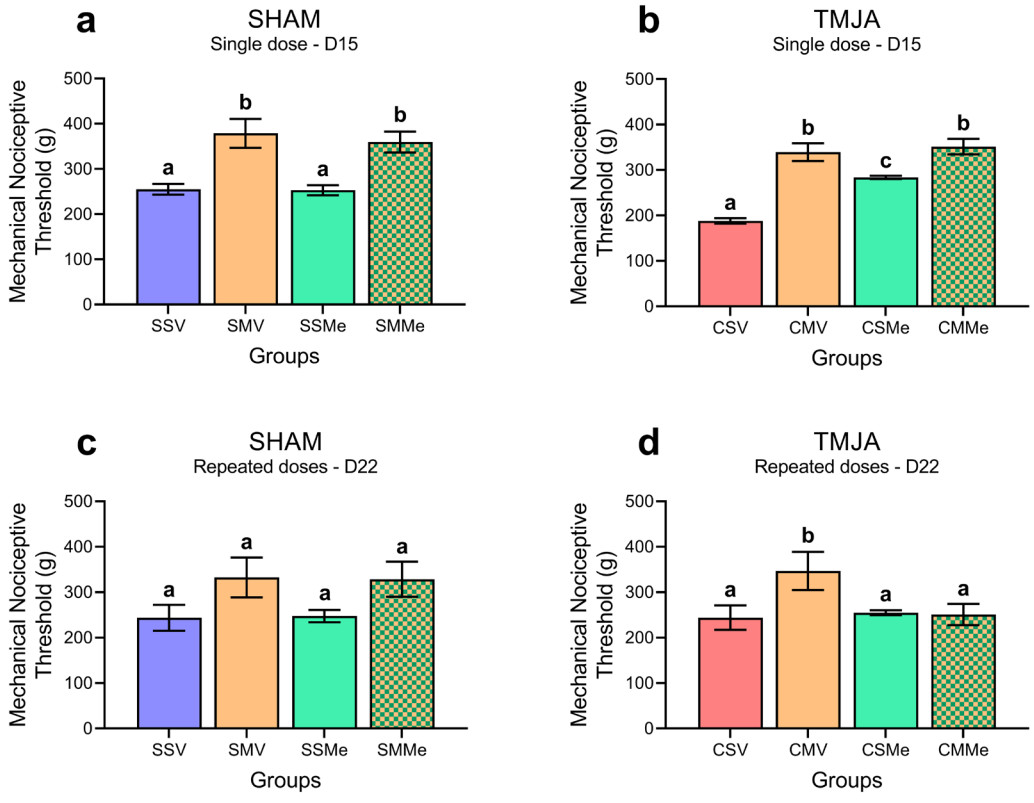

Figure 3: Mechanical nociceptive threshold assessed by the facial VF test. The data are expressed as the means ± SEMs. **Panel A.** SHAM groups after a single dose of treatment (D15). **Panel B.** TMJA groups after a single dose of treatment (D15). **Panel C.** SHAM groups after repeated doses of treatment (D22). **Panel D.** TMJA groups after repeated doses of treatment (D22). SSV: SHAM + Saline + Vehicle; SMV: SHAM + Morphine + Vehicle; SSMe: SHAM + Saline + Melatonin; SMMe: SHAM + Morphine + Melatonin; CSV: CFA + Saline + Vehicle; CMV: CFA + Morphine + Vehicle; CSMe: CFA + Saline + Melatonin; CMMe: CFA + Morphine + Melatonin. $^{a, b, c}$ - Different letters indicate significant differences between groups. Made via GraphPad Prism 9.0®.

Central biochemical markers

With respect to the neurochemical variables, the analysis was first conducted between the SSV and CSV groups (pain model effect). TMJA (CFA injection) increased the spinal cord levels of IL-10 ($t_{(12)}$ = -2.743, p = 0.018; Fig. 4a) and BDNF levels in the pons ($t_{(12)}$ = -5.523, p < 0.001; Fig. 4b). For the remaining central structures and markers assessed, no significant differences were observed between these two groups (Supplementary Table 1).

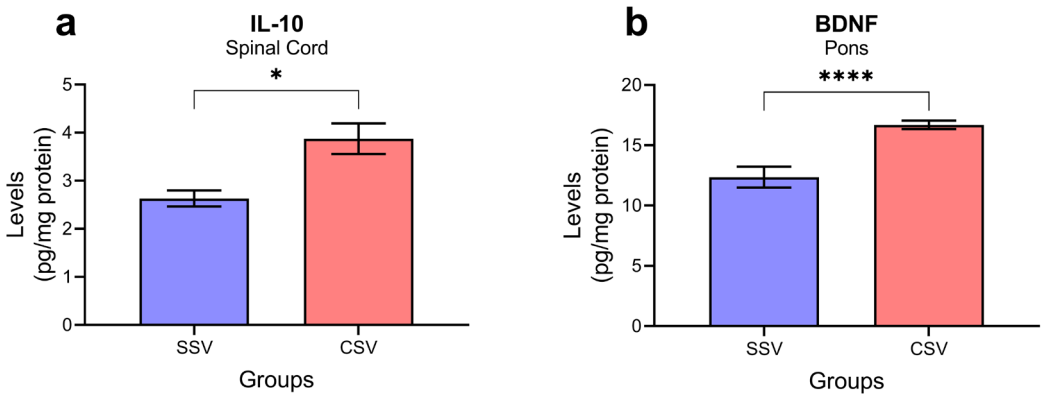

Figure 4: Effects of the pain model on the central levels of IL-10 and BDNF. The data are expressed as the means ± SEM (pg/mg protein). **Panel A.** Effect of TMJA on the levels of IL-10 in the spinal cord. **Panel B.** Effect of TMJA on the levels of BDNF in the spinal cord. SSV: SHAM + Saline + Vehicle; CSV: TMJA + Saline + Vehicle. * $p < 0.05$. Made via GraphPad Prism 9.0®.

As with the VF data, the analysis between those animals receiving saline and those receiving CFA was conducted independently. For the SHAM animals, the one-way ANOVA revealed significant differences in the levels of IL-10 in the spinal cord between the groups ($F_{(3,25)} = 9.556$, $p < 0.001$). Paired comparisons between the SHAM groups revealed that melatonin and morphine induced an increase in the spinal cord levels of IL-10; nonetheless, the increase in IL-10 levels was significantly greater in the melatonin groups (SSMe and SMMe) than in the morphine group (SMV) (Duncan, $p < 0.05$; Table 1).

Table 1: Effects of treatment on central neurochemical marker levels in SHAM animals.

Variable (pg/mg protein)	Groups ("n")				P value
	SSV (5)	SMV (7)	SSMe (7)	SMMe (10)	
TNF-α					
Cerebral Cortex	5.93 ± 1.25	6.42 ± 1.06	6.80 ± 0.44	7.89 ± 1.16	0.590
Pons	42.21 ± 3.29	47.85 ± 2.75	36.46 ± 5.65	39.57 ± 1.39	0.136
Spinal Cord	2.64 ± 0.46	2.27 ± 0.38	4.12 ± 0.43	2.85 ± 0.52	0.070
IL-10					
Cerebral Cortex	1.34 ± 0.28	1.39 ± 0.26	1.28 ± 0.19	1.34 ± 0.35	0.809
Pons	8.40 ± 0.17	7.14 ± 0.51	6.78 ± 0.75	7.96 ± 0.28	0.126
Spinal Cord	2.63 ± 0.17 [a]	3.30 ± 0.12 [b]	3.84 ± 0.26 [c]	3.80 ± 0.10 [c]	**< 0.001**
BDNF					
Cerebral Cortex	2.68 ± 0.48	2.81 ± 0.31	2.50 ± 0.19	3.23 ± 0.47	0.580
Pons	12.36 ± 0.87	12.67 ± 0.83	11.83 ± 0.88	11.91 ± 0.56	0.844
Spinal Cord	6.55 ± 0.72	7.00 ± 0.67	8.02 ± 0.36	7.55 ± 0.26	0.226

Caption - Data are expressed as mean ± SEM (pg/mg protein). BDNF: Brain-Derived Neurotrophic Factor; IL-10: Interleukin 10; TNF-α = Tumor Necrosis Factor alpha; SSV: SHAM + Saline + Vehicle; SMV: SHAM + Morphine + Vehicle; SSMe: SHAM + Saline + Melatonin; SMMe: SHAM + Morphine + Melatonin. [a, b, c] - Different letters indicate significant differences between groups.

For the TMJA animals, the one-way ANOVA revealed differences in pons BDNF levels among the groups ($F_{(3,26)} = 7.273$, $p = 0.001$). Paired comparisons between the TMJA groups indicated that the increase induced by the pain model was reversed by treatments with melatonin or morphine, whether administered independently or in combination (Duncan, $p < 0.05$; Table 2).

Table 2: Effects of treatment on central neurochemical marker levels in TMJA animals.

Variable (pg/mg protein)	Groups ("n")				P-value
	CSV (8-9)	CMV (5-6)	CSMe (9)	CMMe (5-6)	
TNF-α					
Cerebral Cortex	6.86 ± 1.16	8.26 ± 1.68	7.11 ± 1.00	4.78 ± 0.45	0.339
Pons	53.16 ± 5.88	46.15 ± 9.24	54.42 ± 6.08	38.38 ± 6.12	0.358
Spinal Cord	3.36 ± 0.61	3.19 ± 0.56	3.02 ± 0.37	3.87 ± 0.78	0.772
IL-10					
Cerebral Cortex	1.57 ± 0.32	1.58 ± 0.48	1.65 ± 0.27	1.37 ± 0.23	0.950
Pons	8.83 ± 0.56	7.61 ± 1.22	8.05 ± 0.47	7.81 ± 0.55	0.597
Spinal Cord	3.87 ± 0.32	3.84 ± 0.47	3.68 ± 0.07	3.94 ± 0.56	0.953
BDNF					
Cerebral Cortex	3.12 ± 0.47	3.00 ± 0.63	2.93 ± 0.39	2.46 ± 0.40	0.805
Pons	16.69 ± 0.34 [a]	12.61 ± 1.68 [b]	12.72 ± 0.94 [b]	10.88 ± 0.66 [b]	**0.001**
Spinal Cord	7.71 ± 0.35	7.70 ± 0.65	7.46 ± 0.32	7.72 ± 0.50	0.961

Caption - Data are expressed as mean ± SEM (pg/mg protein). BDNF: Brain-Derived Neurotrophic Factor; IL-10: Interleukin 10; TNF-α = Tumor Necrosis Factor alpha; CSV: CFA + Saline + Vehicle; CMV: CFA + Morphine + Vehicle; CSMe: CFA + Saline + Melatonin; CMMe: CFA + Morphine + Melatonin. [a, b] - Different letters indicate differences between groups.

Associations between behavioral and biochemical variables

The Spearman correlation coefficient indicated several significant associations between behavioral and biochemical variables, which were predominantly positive in nature. The nociceptive threshold at D15 was negatively correlated with BDNF levels in the pons. Positive associations were observed between the nociceptive threshold at D22 and the cortical levels of TNF-α, IL-10, and BDNF (Table 3).

The biochemical variables also exhibited significant correlations among structures and markers. The levels of TNF-α in each analyzed central structure were positively correlated with the levels of IL-10 and BDNF within the same structure. One exception to the correlation between distinct structures was observed in TNF-α levels in the pons and BDNF levels in the spinal cord. Like those of TNF-α, the levels of IL-10 in all three central structures were positively correlated with the levels of BDNF within the same structure. Additionally, IL-10 levels in the pons correlated negatively with cortical BDNF levels (Table 3).

Table 3: Most relevant correlations using the Spearman coefficient.

Correlated Variables			Spearman	
			R^2	P
VF D15	X	BDNF PS	- 0.362	0.005
VF D22	X	TNF-α Ctx	0.386	0.003
VF D22	X	IL-10 Ctx	0.419	0.001
VF D22	X	BDNF Ctx	0.394	0.002
TNF-α Ctx	X	IL-10 Ctx	0.820	< 0.001
TNF-α Ctx	X	BDNF Ctx	0.835	< 0.001
TNF-α SC	X	IL-10 SC	0.452	< 0.001
TNF-α SC	X	BDNF SC	0.649	< 0.001
TNF-α PS	X	IL-10 PS	0.540	< 0.001
TNF-α PS	X	BDNF PS	0.539	< 0.001
TNF-α PS	X	BDNF SC	0.330	0.011
IL-10 Ctx	X	BDNF Ctx	0.860	< 0.001
IL-10 SC	X	BDNF SC	0.635	< 0.001
IL-10 PS	X	BDNF Ctx	- 0.270	0.039
IL-10 PS	X	BDNF PS	0.488	< 0.001

Caption - Data from the Spearman correlation test showing significant associations between several pairs of variables. BDNF = Brain-Derived Neurotrophic Factor; Ctx = cerebral cortex; IL-10 = interleukin 10; PS = pons; SC = spinal cord; TNF-α = tumor necrosis factor alpha; VF D15 = nociceptive threshold before treatment; VF D22 = nociceptive threshold after the end of treatment. n = 59.

TMJ inflammatory score

According to the representative visual assessment of the histological slides (Fig. 5), changes in the TMJ resulting from the TMJA model and/or melatonin treatment were observed in the structures embedded in paraffin blocks, sectioned, and stained with HE. Figures 5a and 5b (SSV group, 100x and 200x magnification, respectively) demonstrate a preserved, normally stained morphological structure devoid of significant inflammatory characteristics (score = 0). In slices from TMJA animals (CSV, Fig. 5c), a distinct morphological profile is apparent, characterized by significant CFA accumulation in the tissue, a pronounced inflammatory response (high eosinophilia, score 3), and extensive infiltration of adjacent mononuclear immune cells (likely lymphocytes and monocytes). Despite a lower number of immune cells, the TMJ structures from animals administered morphine (CMV, Fig. 5e) or a combination of morphine and melatonin (CMMe, Fig. 5f) exhibited the inflammatory profile and scoring outlined for the TMJA group (Fig. 5c). Slices from animals infiltrated with CFA and treated solely with melatonin (CSMe, Fig. 5d) exhibited a modest reduction in eosinophilia and morphological changes (inflammatory profile, score = 2).

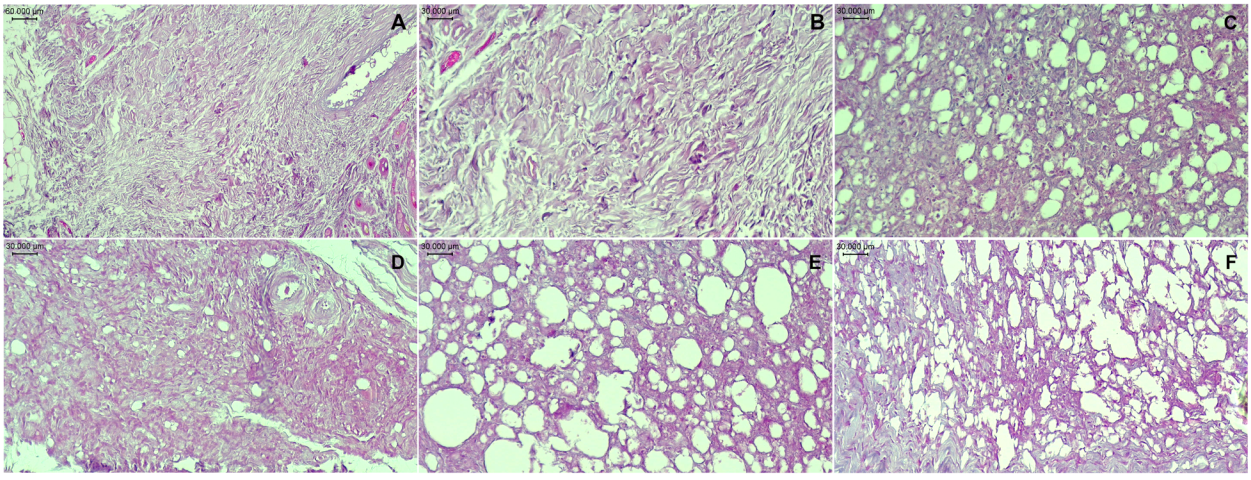

Figure 5: Inflammatory score. Hematoxylin and eosin (HE) staining. **A - B:** SHAM - SSV group - tissue integrity (100x, score 0 - 200x, score 0 - discrete presence of mononuclear cells). **C:** TMJA - CSV group (200x, score 3 - accumulation of CFA and intense inflammatory reaction). **D:** TMJA + Saline + Melatonin (200x, score 2 - moderate presence of mononuclear cells). **E:** TMJA + Morphine + Vehicle (200x, score 3 - exacerbated mononuclear cell infiltrate). **F:** TMJA + Melatonin + Morphine (200x, score 3 - exacerbated mononuclear cell infiltrate).

DISCUSSION

This study demonstrates that CFA infiltration into the TMJ is an effective TMJA model in rats, characterized by mechanical allodynia (D15), neuroinflammation, and a morphological profile with CFA accumulation and increased inflammatory responses (D22). Furthermore, acute treatment with morphine and melatonin (D15) increased the facial mechanical nociceptive threshold, reversing mechanical allodynia induced by the pain model. However, mechanical allodynia resolved spontaneously by D22. In this way, the findings of this study should be interpreted considering the temporal dynamics of the CFA-induced inflammatory model in the TMJ.

Our previous investigations revealed an acute analgesic effect of melatonin on chronic inflammatory pain[6,7], supporting the findings of the present study. Furthermore, a subsequent investigation demonstrated that systemic administration of melatonin significantly reduced the visceral motor response to colorectal distension in rats with trinitrobenzenesulfonic acid-induced colonic inflammation, while having no effect on nonsensitized rats[20], corroborating our findings. Numerous studies on inflammatory and neuropathic pain models substantiate the antiallodynic action of melatonin[21,22]. Multiple orofacial pain conditions have pointed to the relevance of melatonin treatment. A preclinical investigation demonstrated improved oxidative stress and insulin resistance in rats with apical periodontitis on a high-fat diet[23]. Rats with apical periodontitis exhibit low insulin sensitivity and impaired insulin signaling, which could be rescued by melatonin[24]. In vivo studies have proved that melatonin is able to decrease osteoclastic activity and reduce hyperglycemia-induced oxidative stress and alveolar bone loss in diabetic rats with periodontitis[25,26]. In a ligature-induced periodontitis model in rats, treatment with melatonin seemed to alleviate gingival inflammation due to the inhibition of inflammatory cytokines[27].

Furthermore, systemic administration of melatonin was shown to inhibit alveolar bone destruction by reducing RANKL expression and inflammatory cell infiltration, while increasing osteoblastic activity in a rat model of osteoporosis and periodontitis[28].

Melatonin reduces derivatives of arachidonic acid, diacylglycerol, and intracellular calcium levels[29], and the synthesis of nitric oxide synthase and cyclooxygenase-2[30]. In addition, it inhibits nitric oxide production and polymorphonuclear recruitment at the inflammation site, while activating nuclear factor kappa B[30], modulating excitatory synaptic transmission via NMDA and/or 5-HT receptors[22]. Furthermore, a prior study demonstrated that melatonin possesses immunomodulatory and anti-inflammatory properties[31], potentially offering protective benefits against pain-related inflammation, as evidenced in the current study. Also, the trigeminal tract, trigeminal nucleus, thalamus, and spinal cord dorsal horn possess melatonin membrane receptors (MT1Rs and MT2Rs) that are implicated in nociceptive transmission[32]. These receptors are likely necessary for the melatonin-mediated antinociception in nociceptive, neuropathic, and inflammatory pain models[31].

Interestingly, in the current study, melatonin had modest efficacy in diminishing eosinophilia and morphological changes in the TMJ of rats; however, this effect was not observed when melatonin was administered in association with morphine. In a CFA injection model, melatonin had a mild peripheral anti-inflammatory impact on the footpad, diminishing inflammatory cells in the subcutaneous tissues of the ankle joints[7]. The melatonin's anti-inflammatory properties may include direct interactions with binding sites on lymphocytes and macrophages[33]. It enhances cell proliferation and cytokine synthesis in activated lymphocytes and mononuclear cells through NF-kB[34].

Prior research indicates that CFA inflammatory models of the TMJ may exhibit temporary sensitization, followed by a gradual recovery of nociceptive thresholds. The spontaneous resolution observed here may be related to both the CFA concentration and the rat strain used. Higher CFA doses (300 µg to 20 mg/mL) are associated with more persistent arthritic pain, whereas lower concentrations (0.5–1.0 mg/mL) may lead to partial or complete remission of nociceptive behavior over time[35]. Additionally, strain-related differences have been reported; rats form the Sprague-Dawley strain show more sustained pain responses than from the Wistar strain[6,10]. Consistent with our findings, previous studies from our group using Wistar rats and 1 mg/mL CFA into the TMJ demonstrated mechanical hypersensitivity up to approximately days 20[36] and 21, followed by spontaneous recovery in later stages (D25)[17]. In another study using female rats submitted to the same pain model, the CFA groups exhibited mechanical hyperalgesia until day 21 after CFA injection[11]. The natural resolution of the model may involve a reduction in the release of pro-inflammatory mediators, restoration of tissue homeostasis, and a decrease in peripheral and central nociceptive excitability[37]. Therefore, the spontaneous recovery of the TMJA control group limits definitive conclusions regarding the maintenance of the long-term analgesic effects or any loss of therapeutic efficacy in the later phases of the experiment.

Although mechanical allodynia was not observed by D22, neuroinflammation (D23) was evident in rats with TMJA, as evidenced by elevated spinal cord IL-10 and pontine BDNF levels, a neurotrophin associated with the development and maintenance of pain. Given the analgesic effect of morphine in the TMJA group and the reliance of a single pain assessment method, it is unreasonable to rule out pain in these animals. Interestingly, the lower nociceptive threshold at D15 was negatively associated with an increase in BDNF levels in the pons at D22 and positively associated with cortical TNF-α, IL-10, and BDNF levels. Punctate stimulation, like the von Frey test, activates all cortical areas involved in non-nociceptive and allodynic responses[38], and identical physical stimuli can activate the cortex differently in allodynic and non-allodynic regions[39]. Nonpainful stimulation activates the contralateral primary, posterior parietal, insular, and bilateral secondary somatosensory cortex[40]. This may result from the anti-inflammatory and anti-nociceptive properties of IL-10 and the neuroprotective benefits of BDNF, which counteract the pro-inflammatory effects of TNF-α and maintain central nervous system homeostasis.

Melatonin and/or morphine prevented the TMJA model's BDNF increase in the pons. Previous investigations from our research group revealed a significant increase in BDNF levels in the brainstem of Sprague–Dawley rats submitted to the TMJA model[6,10]. Interestingly, acute melatonin administration in inflammatory models (facial and plantar) decreased brainstem BDNF levels[6,7], supporting the pontine findings. BDNF can sensitize distinct nociceptive pathways and play a complex role in central sensitization and neuroinflammation[41], which may explain the increase in its levels induced by TMJA. In addition, the BDNF-TrkB receptor cascade in the rostral ventromedial medulla circuit contributes to chronic pain following inflammation[42], and negatively regulates the $K^{(+)}$-$Cl^{(-)}$ cotransporter (KCC2), reducing GABA inhibition and promoting pain signaling[43]. Thus, at least partially, the recognized analgesic properties of melatonin and morphine may be associated with their capacity to reduce central levels of BDNF[7,19].

The phenomenon of morphine tolerance is well established in the literature[44]. In the current study, this effect was observed only in the SHAM group,

since repeated morphine administration in the TMJA animals provided analgesia in rats at D22, despite the absence of mechanical allodynia, indicating a dual effect of morphine dependent on the animal's pain state. Moreover, variations in disease severity may account for a significant confounding effect when assessing tolerance in the management of chronic pain disorders[45]. It may be suggested that morphine reestablishes homeostasis by inhibiting the adenylate cyclase activity, activating potassium channels, and reducing calcium conductance, while interacting with G protein-coupled opioid receptors[46]. On the other hand, the phenomenon of melatonin tolerance is not completely established or comprehended throughout the scientific community. Melatonin desensitizes natural and recombinant MT1Rs and MT2Rs following prolonged exposure. These data support the TMJA group's lack of melatonin effect at D22, which could be the result of desensitization, a critical factor influencing hormone efficacy owing to daily exposure. This hypothesis is further supported by a study indicating that prolonged exposure of cell lines to melatonin desensitizes receptors and reduces the receptor potency[47]. However, the causes of melatonin receptor desensitization, which makes them resistant to agonists, remain unknown.

While the present study identified elevated central levels of IL-10 in TMJA, another study showed no changes in its peripheral levels in the same model[17]. This may be inherently associated with a more advanced (resolutive) phase of the inflammatory process[48]. Notably, IL-10 levels in all three assessed central structures exhibited a positive correlation with BDNF levels within the corresponding region. Nonetheless, cortical IL-10 levels are negatively correlated with BDNF levels in the pons. Interestingly, the pons is a primary source of specific branches of the trigeminal nerve that innervate the region of the TMJ affected by inflammatory insult[49]. Melatonin increased IL-10 levels solely in SHAM animals, indicating a fundamental immunomodulatory effect independent of the inflammatory state. The lack of further IL-10 modulation in TMJA animals may indicate the activation of endogenous counter-regulatory mechanisms during inflammation or a potential ceiling effect. The higher IL-10 levels found in SHAM rats, which do not exhibit hypernociceptive behavior, may indicate that cytokine modulation alone may not directly result in active analgesia[50,51].

CFA injections into the TMJ of rodents cause soft tissue alterations, including inflammatory cell infiltration, early pannus formation, and increased IL-1β and TNF-α levels[52]. This hypothesis predicts elevated TNF-α levels in the brainstem, as the TMJ region affected by inflammation is innervated by trigeminal nerve branches originating in the brainstem, particularly in the pons[49]; however, this study showed no changes in central TNF-α levels induced by TMJA. A strong association was found between TNF-α, IL-10, and BDNF levels within the corresponding structure, and between pontine TNF-α levels and spinal cord BDNF levels. These findings emphasize the role of these central biomarkers in the development, maintenance, and resolution of the TMJA model. Our recent study revealed an increase in IL-1β levels in the ipsilateral trigeminal ganglion (TG) compared to those in the contralateral side at 27 days after the TMJA model induction[17]. However, in the present investigation, central IL-1β levels were assessed 23 days following pain model induction. These data indicate that temporal factors and tissue type are essential for neuroinflammatory profiles. A study by our group employing the same TMJA model demonstrated that CFA significantly reduces TNF-α levels in the brainstem of ovariectomized rats[11]. Therefore, differences in sex and the employed surgical, pharmacological, and hormonal interventions must be considered.

Some limitations of this study are as follows: 1. The exclusion of female rats was intentional to mitigate the confounding influence of hormonal fluctuations on the nociceptive threshold, particularly in adult rats, which might affect the results. Nevertheless, including females would require numerous animals. Our recent investigation has prioritized female rats in animal models of diseases that affect predominantly or exclusively females[11]. 2. We employed a single nociceptive assessment. Another recent study employing a similar model used a facial thermal hyperalgesia test (OPAD)[17]; however, this test demands long-term exposure of animals to the apparatus, limiting the investigation of the temporal effects of pharmacological treatments. 3. The lack of serum biochemical markers of inflammation; however, the histological changes corresponded to the drug's effects *in situ*, rendering them less susceptible to bias.

Lastly, both melatonin and morphine relieve orofacial pain. Only the TMJA group exhibited melatonin analgesia, but both showed morphine analgesia. By D22, only morphine administered to the TMJA group yielded analgesia, despite the absence of mechanical allodynia, yet it was ineffective in the SHAM group. Melatonin did not prevent morphine tolerance in the SHAM group, nor did it sustain the analgesic efficacy in TMJA animals following repeated doses. Due to its association with reduced central BDNF levels, melatonin may affect many pathways. Furthermore, melatonin exhibited stronger anti-inflammatory benefits, evidenced by increased IL-10 levels. Our findings underscore the potential of melatonin to act as an analgesic and anti-inflammatory agent in chronic orofacial inflammatory pain.

REFERENCES

1. Ingawalé S, Goswami T. Temporomandibular joint: disorders, treatments, and biomechanics. Ann Biomed Eng. 2009;37(5):976-96.

2. Detamore MS, Athanasiou KA. Structure and function of the temporomandibular joint disc: implications for tissue engineering. J Oral Maxillofac Surg. 2003; 61(4):494-506.

3. Peimani A, Sardary F. Effect of low-level laser on healing of temporomandibular joint osteoarthritis in rats. J Dent (Tehran). 2014;11(3):319-27

4. Yu C-X, Zhu C-B, Xu S-F, Cao X-D, Wu G-C. The analgesic effects of peripheral and central administration of melatonin in rats. Eur J Pharmacol. 2000;403(1-2):49-53.

5. Permuy M, López-Peña M, González-Cantalapiedra A, Muñoz F. Melatonin: A Review of Its Potential Functions and Effects on Dental Diseases. Int J Mol Sci. 2017;18(4):865.

6. Scarabelot VL, Medeiros LF, Oliveira C, Adachi LNS, Macedo IC, Cioato SG, et al. Melatonin Alters the Mechanical and Thermal Hyperalgesia Induced by Orofacial Pain Model in Rats. Inflammation. 2016;39(5):1649-59.

7. Laste G, Macedo IC, Rozisky JR, Silva FR, Caumo W, Torres IL. Melatonin administration reduces inflammatory pain in rats. J Pain Res. 2012;5:359-62.

8. Laste G, Rozisky JR, Caumo W, Torres IL. Short- but not long-term melatonin administration reduces central levels of brain-derived neurotrophic factor in rats with inflammatory pain. Neuroimmunomodulation. 2015;22(6):358-64.

9. Ahmad SB, Ali A, Bilal M, Rashid SM, Wani AB, Bhat RR, et al. Melatonin and Health: Insights of Melatonin Action, Biological Functions, and Associated Disorders. Cell Mol Neurobiol. 2023;43(6):2437-58.

10. Scarabelot VL, Oliveira C, Medeiros LF, Macedo IC, Cioato SG, Adachi LNS, et al. Transcranial direct-current stimulation reduces nociceptive behaviour in an orofacial pain model. J Oral Rehabil. 2019;46(1):40-50.

11. Kroeff GPH, Castro JM, Braga HB, Dal Bosco T, Oliveira TC, Morais ITS, et al. Hormone replacement therapy did not alleviate temporomandibular joint inflammatory pain in ovariectomized rats. Odontology. 2025;113(1):232-44.

12. Agrawal G, Askar G, Kaur T, Kamdar D, Kumar SS, Khan J. The efficacy of intra-articular morphine injections in arthrogenous temporomandibular joint pain: a systematic review of randomized controlled trials. Oral Surg Oral Med Oral Pathol Oral Radiol. 2025;141(1):23-35.

13. Armendariz A, Nazarian A. Morphine antinociception on thermal sensitivity and place conditioning in male and female rats treated with intraplantar complete Freund's adjuvant. Behav Brain Res. 2018;343:21-7.

14. Bahrami N, Goudarzi M, Hosseinzadeh A, Sabbagh S, Reiter RJ, Mehrzadi S. Evaluating the protective effects of melatonin on di(2-ethylhexyl) phthalate-induced testicular injury in adult mice. Biomed Pharmacother. 2018;108:515-23.

15. 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.

16. Silva Torres IL, Cucco SN, Bassani M, Duarte MS, Silveira PP, Vasconcellos AP, et al. Long-lasting delayed hyperalgesia after chronic restraint stress in rats-effect of morphine administration. Neurosci Res. 2003;45(3):277-83.

17. Vicenzi CB, Stein DJ, Castro JM, Silveira BL, Melo AS, Callai EMM, et al. Decreased anxiety-like behavior and trigeminal ganglion BDNF levels persist in rats with temporomandibular joint arthritis even after resolution of the nociceptive process. Neurosci Lett. 2025;851:138166.

18. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248-54.

19. Laste G, Vidor L, Macedo IC, Rozisky JR, Medeiros L, Souza A, et al. Melatonin treatment entrains the rest-activity circadian rhythm in rats with chronic inflammation. Chronobiol Int. 2013;30(9):1077-88.

20. Mickle A, Sood M, Zhang Z, Shahmohammadi G, Sengupta JN, Miranda A. Antinociceptive effects of melatonin in a rat model of post-inflammatory visceral hyperalgesia: a centrally mediated process. Pain. 2010;149(3):555-64.

21. Ambriz-Tututi M, Granados-Soto V. Oral and spinal melatonin reduces tactile allodynia in rats via activation of MT2 and opioid receptors. Pain. 2007;132(3):273-80.

22. Arreola-Espino R, Urquiza-Marín H, Ambriz-Tututi M, Araiza-Saldaña CI, Caram-Salas NL, Rocha-González HI, et al. Melatonin reduces formalin-induced nociception and tactile allodynia in diabetic rats. Eur J Pharmacol. 2007;577(1-3):203-10.

23. Santos RM, Tsosura TVS, Belardi BE, Chaves-Neto AH, Chiba FY, Mattera MSLC, et al. Melatonin decreases plasma TNF-α and improves nonenzymatic antioxidant defence and insulin sensitivity in rats with apical periodontitis fed a high-fat diet. Int Endod J. 2023;56(2):164-78.

24. Tavares BS, Tsosura TVS, Mattera MSLC, Santelli JO, Belardi BE, Chiba FY, et al. Effects of melatonin on insulin signaling and inflammatory pathways of rats with apical periodontitis. Int Endod J. 2021;54:926-40.

25. Balci Yuce H, Karatas O, Aydemir Turkal H, Pirim Gorgun E, Ocakli S, Benli I, et al. The Effect of Melatonin on Bone Loss, Diabetic Control, and Apoptosis in Rats With Diabetes With Ligature-Induced Periodontitis. J. Periodontol. 2016;87(4):e35-43.

26. Kose O, Arabaci T, Kara A, Yemenoglu H, Kermen E, Kizildag A, et al. Effects of Melatonin on Oxidative Stress Index and Alveolar Bone Loss in Diabetic Rats With Periodontitis. J. Periodontol. 2016;87:e82-90.

27. Kara A, Akman S, Ozkanlar S, Tozoglu U, Kalkan Y, Canakci CF, et al. Immune modulatory and antioxidant effects of melatonin in experimental periodontitis in rats. Free Radic Biol Med. 2013;55:21-6.

28. Doğan SSA, Toker H, Göze ÖF. Melatonin Decreases Alveolar Bone Loss in Rats with Experimental Periodontitis and Osteoporosis: A Morphometric and Histopathologic Study. Biomedicines. 2024;12(3):684.

29. Chen D, Zhang T, Lee TH. Cellular Mechanisms of Melatonin: Insight from Neurodegenerative Diseases. Biomolecules. 2020;10(8):1158.

30. Zhao M, Qiu D, Miao X, Yang W, Li S, Cheng S, et al. Melatonin Delays Arthritis Inflammation and Reduces Cartilage Matrix Degradation through the SIRT1-Mediated NF-κB/Nrf2/TGF-β/BMPs Pathway. Int J Mol Sci. 2024;25(11):6202.

31. Xie S, Fan W, He H, Huang F. Role of Melatonin in the Regulation of Pain. J Pain Res. 2020;13:331-43.

32. Liu J, Clough SJ, Hutchinson AJ, Adamah-Biassi EB, Popovska-Gorevski M, Dubocovich ML. MT1 and MT2 Melatonin Receptors: A Therapeutic Perspective. Annu Rev Pharmacol Toxicol. 2016;56:361-83.

33. Ahmad SB, Ali A, Bilal M, Rashid SM, Wani AB, Bhat RR, et al. Melatonin and Health: Insights of Melatonin Action, Biological Functions, and Associated Disorders. Cell Mol Neurobiol. 2023;43(6):2437-58.

34. Szczepanik M. Melatonin and its influence on the immune system. J Physiol Pharmacol. 2007;58(Suppl 6):115-24.

35. Noh A'SM, Chuan TD, Khir NAM, Zin AAM, Ghazali AK, Long I, et al. Effects of different doses of complete Freund's adjuvant on nociceptive behaviour and inflammatory parameters in polyarthritic rat model mimicking rheumatoid arthritis. PLoS One. 2021;16(12):e0260423.

36. Marini LL, Stein DJ, Fiuza KJ, Braga HB, Farias VEF, Dal Bosco T, et al. The analgesic effect of very low-dose naltrexone (VLDN) was not enhanced by repetitive transcranial magnetic stimulation (rTMS) in rats with temporomandibular joint arthritis. Odontology. 2026;114(2):652-63.

37. Yong-C B, Kyoung-A W, Nak-H L, Min-K L, Min-K P, Gwi-Y Y, et al. A Blockade of the Central MAPK Pathway Attenuates Referred Pain in Rats with Complete Freund's Adjuvant - Induced Inflammation of the Temporomandibular Joint. Int J Oral Biol. 2010;35(3):83-9.

38. Hans GH, Vandervliet E, Deseure K, Parizel PM. Cerebral activation during von Frey filament stimulation in subjects with endothelin-1-induced mechanical hyperalgesia: a functional MRI study. Biomed Res Int. 2013;610727.

39. Geha PY, Baliki MN, Wang X, Harden RN, Paice JA, Apkarian AV. Brain dynamics for perception of tactile allodynia (touch-induced pain) in postherpetic neuralgia. Pain. 2008;138(3):641-56.

40. Maihöfner C, Handwerker HO, Birklein F. Functional imaging of allodynia in complex regional pain syndrome. Neurology. 2006;66(5):711-17.

41. Xiong H-Y, Hendrix J, Schabrun S, Wyns A, Van Campenhout J, Nijs J, et al. The Role of the Brain-Derived Neurotrophic Factor in Chronic Pain: Links to Central Sensitization and Neuroinflammation. Biomolecules. 2024;14(1):71.

42. Mazzitelli M, Kiritoshi T, Presto P, Hurtado Z, Antenucci N, Ji G, et al. BDNF Signaling and Pain Modulation. Cells. 2025;14(7):476.

43. Hamze M, Brier C, Buhler E, Zhang J, Medina I, Porcher C. Regulation of Neuronal Chloride Homeostasis by Pro- and Mature Brain-Derived Neurotrophic Factor (BDNF) via KCC2 Cation-Chloride Cotransporters in Rat Cortical Neurons. Int J Mol Sci. 2024;25(11):6253.

44. Zhou J, Ma R, Jin Y, Fang J, Du J, Shao X, et al. Molecular mechanisms of opioid tolerance: From opioid receptors to inflammatory mediators (Review). Exp Ther Med. 2021;22(3):1004.

45. Petersen KL, Meadoff T, Press S, Peters MM, LeComte MD, Rowbotham MC. Changes in morphine analgesia and side effects during daily subcutaneous administration in healthy volunteers. Pain. 2008;137(2):395-404.

46. Listos J, Łupina M, Talarek S, Mazur A, Orzelska-Górka J, Kotlińska J. The Mechanisms Involved in Morphine Addiction: An Overview. Int J Mol Sci. 2019;20(17):4302.

47. Witt-Enderby PA, Bennett J, Jarzynka MJ, Firestine S, Melan MA. Melatonin receptors and their regulation: biochemical and structural mechanisms. Life Sci. 2003;72(20):2183-198.

48. Iyer SS, Cheng G. Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease. Crit Rev Immunol. 2012;32(1):23-63.

49. Perry SK, Emrick JJ. Trigeminal somatosensation in the temporomandibular joint and associated disorders. Front Pain Res (Lausanne). 2024;5:1374929.

50. Huang N-C, Wong C-S. Melatonin as an Integrative Adjunct in Multimodal Analgesia: Linking Circadian Regulation, Anti-Inflammatory Modulation, and Opioid-Sparing Mechanisms. Int. J. Mol. Sci. 2026;27(4):2046.

51. Cho JH, Bhutani S, Kim CH, Irwin MR. Anti-inflammatory effects of melatonin: A systematic review and meta-analysis of clinical trials. Brain Behav Immun. 2021;93:245-53.

52. Morel M, Ruscitto A, Pylawka S, Reeve G, Embree MC. Extracellular matrix turnover and inflammation in chemically-induced TMJ arthritis mouse models. PLoS One. 2019;14(10):e0223244.

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