Article

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Investigation of the Effects of Occlusal Reconstruction on Neural Function and Pain Alleviation in Rats

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Background: Tooth wear (TW) typically requires occlusal reconstruction as an effective treatment. Progression of TW into a pathological state may cause various symptoms, including temporomandibular joint (TMJ) pain, occlusal disorders, and aesthetic concerns. Previous studies mainly focused on oral and facial tissues and electromyographic activity of masticatory muscles rather than the nervous system. This study aims to investigate the effects of bite reconstruction following TW on the peripheral and central nervous systems (CNS).

Methods: The bilateral maxillary molars of 60 Sprague-Dawley rats were examined, and a subgroup of 20 rats was selected to establish an occlusal reconstruction model. Subsequently, four time points were designated for general observation and measurement of pressure pain threshold (PPT). Immunofluorescence was employed to evaluate the expression of substance P (SP), calcitonin gene-related peptide (CGRP), and phosphorylated extracellular signal-regulated kinase (p-ERK) in trigeminal ganglion and medulla oblongata tissues. quantitative real-time polymerase chain reaction (qPCR) was performed to measure expression of *tachykinin precursor 1* (*Tac1*), *calcitonin-related polypeptide alpha* (*CALCA*), *tumor necrosis factor-alpha* (*TNF-*α), *interleukin-1 beta* (*IL-1*β), and *brain-derived neurotrophic factor* (*BDNF*). Additionally, Western blot (WB) was conducted to analyze expression of mitogen-activated protein kinase p38 (p38), phosphorylated p38 (p-p38), extracellular signal-regulated kinase (ERK), and p-ERK proteins.

Results: No bleeding or ulceration was observed in any rat group following each operation. Additionally, no significant tooth loosening or loss occurred, and facial symmetry during chewing and stable body weight were maintained in all groups. In the occlusal reconstruction group, PPT values significantly decreased on the third day post-modeling ($p < 0.001$) but gradually returned to baseline levels by day 28. SP levels significantly increased over time following occlusal reconstruction ($p < 0.001$) and returned to normal levels by day 28. The expression of p-ERK increased significantly 3 days after reconstruction ($p < 0.001$); although the number of positive cells decreased at day 14, it remained significantly higher than the control group ($p < 0.01$) before returning to baseline levels by day 28. Similarly, the expression of *Tac1*, *CALCA*, *TNF-*α, *IL-1*β, and *BDNF* genes exhibited a consistent downward trend on days 3, 7, and 14 post-reconstruction ($p < 0.01$). WB analysis revealed that expression of p-p38 and p-ERK proteins peaked on day 3 in the occlusal reconstruction group ($p < 0.001$) and gradually decreased thereafter.

Conclusions: Occlusal reconstruction may alleviate pain perception and nerve injury through activation of the p38 mitogen-activated protein kinase (MAPK)/ERK signaling pathway and regulation of factors such as SP, CGRP, and BDNF.

Keywords: tooth wear; temporomandibular joint pain; occlusal reconstruction; pain regulation; neuroregulation; p38 MAPK/ERK signaling pathway

Introduction

Tooth wear (TW) is a physiological phenomenon occurring throughout the human lifespan, and the stomatognathic system undergoes adaptive changes following TW. These changes include continuous tooth eruption, remodeling of the temporomandibular joint (TMJ), and shortening of the dental arch due to tooth displacement [1]. When TW exceeds the rate of tooth eruption and causes patient discomfort or aesthetic concerns, it is considered excessive wear and is defined as pathological TW [2]. Notably, TW is a common clinical problem encountered by dentists, with a reported prevalence ranging from 64.7% to nearly 100% [3]. Functionally, TW can lead to TMJ pain, occlusal disorders, masticatory muscle pain, prosthetic complications, and tooth cracks, which are the primary reasons patients with TW seek dental care [4]. In addition, long-term occlusal disorders may impair chewing efficiency, potentially leading to digestive problems, such as indigestion, and contributing to the development of temporomandibular disorders (TMD) [5].

Occlusal vertical dimension (OVD) is defined as the distance between the upper and lower jaws when the teeth are in maximum intercuspation [6]. TW can lead to a reduction in OVD, abnormal mandibular movements, clicking and crepitus in the TMJ region [7], as well as aesthetic and phonetic disorders [8]. Occlusal reconstruction has been shown to be an effective treatment for severe TW. In our preliminary study, 20 patients with severe TW and an OVD loss greater than 5 mm underwent occlusal reconstruction [9]. The results showed that 98% of patients reported satisfaction, while 68% experienced transient clinical symptoms, including masseter muscle discomfort and TMJ discomfort.

A previous study on occlusal reconstruction has mainly focused on changes in orofacial tissues and electromyographic activity of the masticatory muscles [10], whereas the neural response has been insufficiently investigated. In addition, TMD has been shown to be significantly associated with headache and migraine. Patients with TMD frequently report headache symptoms, particularly those with chronic daily or episodic headaches, in whom TMD symptoms are more prevalent [11]. Moreover, the incidence of migraine is higher in patients with TMD, which may be related to psychological factors and other comorbidities [12]. Another study also reported that tooth loss is associated with cognitive impairment [13].

Therefore, a rat model of occlusal reconstruction was established to investigate the interaction between the p38 mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) signaling pathway and proinflammatory mediators, and to explore the effects of occlusal reconstruction on neurological function and pain relief mechanisms in rats.

Materials and Methods

Animals and Experimental Protocol

A total of 60 eight-week-old male Sprague-Dawley rats, weighing 200–300 g, were provided by the experimental animal center of Changzhi Medical College. Rats were housed individually in cages at 25 °C, with 40%–60% humidity and a 12-hour light/dark cycle, and given free access to food and water. They were randomly assigned to three groups (n = 20 per group): (1) Occlusal reconstruction group (Group T), in which rats' occlusal surfaces were ground (0.3 mm per operation, with a total of 0.6 mm reduction) followed by occlusal reconstruction; (2) Operation simulation group (Group S), in which procedures mimicked those of Group T without actual grinding; and (3) Control group (Group C), in which no procedures were performed. Each group was further subdivided into four subgroups based on sacrifice day (day 3, 7, 14, and 28).

Establishment of Animal Model

Rats were anesthetized using intraperitoneal injection of sodium pentobarbital (40 mg/kg). Under a surgical microscope (ASOM-4, Chengdu Keaoda Optoelectronic Technology Co., Ltd., Chengdu, China), the occlusal surfaces of maxillary posterior teeth were reduced using a 0.3 mm ball drill operated at low speed (35,000 r/min). The reduction was performed twice, 0.3 mm each, at five-day intervals, achieving a total reduction of approximately 0.6 mm to simulate severe TW [14]. After occlusal reduction, rats were housed in individual cages at 22 °C under natural day-night cycles, with free access to food and water for 45 days, to adapt to the simulated severe TW condition.

After the adaptation period, occlusal splints were constructed as follows:

Maxillary posterior teeth impressions were taken using silicone rubber (2507092, Shanghai Huge Medical Devices Co., Ltd, Shanghai, China), and plaster casts were prepared from these impressions. Methyl methacrylate was placed into the impressions and attached to the worn plaster model. After polymerization, splints were removed, trimmed, and polished. The thickness of each occlusal splint was adjusted to 0.6 mm using a vernier caliper.

Rats' maxillary posterior teeth were dried using cotton balls and an air gun. An acid etchant was then applied to the prepared teeth and evenly dispersed using an air gun. Occlusal splints were bonded using resin adhesive (40417A, Single Bond Universal, 3M Company, Sao Paulo, MN, USA), positioned appropriately, and cured by light for 60 seconds. Afterward, passive occlusal movements were conducted, occlusal adjustments were made, excess resin was removed, and rats were returned to their cages. Rats in Group S underwent anesthesia and simulated surgical procedures without actual tooth grinding or bonding, while Group C received no intervention.

General Observations and Pressure Pain Threshold (PPT) Detection

Changes in body weight were recorded at the experiment start, the first and second tooth grinding operations, occlusal reconstruction, and days 3, 7, 14, and 28 post-reconstruction, to assess growth and nutrient intake. The PPT of rats was measured at the same intervals. Before testing, rats were allowed to adapt to the environment. Tests were performed with the rats' hind paws upright and front paws resting on the experimenter's hands. An electronic von Frey pain meter (Alemo 2390-5, IITC Life Sciences Inc., Woodland Hills, CA, USA) was applied to the masseter muscle area bilaterally (1 cm below and behind the midpoint between the tragus and eyeball), gradually increasing pressure. When the rat shook its head, the reading was recorded as the tenderness threshold. Each test was repeated three times, and the average pain sensitivity value was calculated.

Table 1. Primers sequences.

Primer	Forward primer (5′→3′)	Reverse primer (5′→3′)	Size (bp)
Tac1	GTCCGACCGCAAAATCCAAC	AGCATCCCGTTTGCCCATTA	239
CALCA	GCTGCCCAGATCAAGAGTCA	TCCCTGAGCAGGAACCTCAG	115
TNF-α	GGCTTTCGGAACTCACTGGA	GGGAACAGTCTGGGAAGCTC	164
IL-1β	AGCTTCAGGAAGGCAGTGTC	TCAGACAGCACGAGGCATTT	239
BDNF	AGGGAAATCTCCTGAGCCGA	CTGCGCCCTAGCACAAAAAG	152
GAPDH	TCTCTGCTCCTCCCTGTTCT	GTTCACACCGACCTTCACCA	91

Tac1, Tachykinin precursor 1; *CALCA*, calcitonin-related polypeptide alpha; *TNF-α*, tumor necrosis factor-alpha; *IL-1β*, interleukin-1 beta; *BDNF*, brain-derived neurotrophic factor; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase.

Immunofluorescence Staining

Rats were anesthetized using intraperitoneal injection of sodium pentobarbital (40 mg/kg). Cardiac perfusion was performed on days 3, 7, 14, and 28 after occlusal reconstruction. Rats were initially perfused with 0.9% saline until blood drainage was nearly complete, followed by fixation with 4% paraformaldehyde. Complete rigidity of the rats was observed after perfusion. The bilateral trigeminal ganglia and medulla oblongata tissues were removed and embedded in paraffin. After dewaxing and dehydration, the sections for substance P (SP), calcitonin gene-related peptide (CGRP), and phosphorylated extracellular signal-regulated kinase (p-ERK) analysis were immersed in citric acid buffer (ZLI-9065; Beijing Zhongshan Jinqiao Biotechnology Co., Ltd., Beijing, China) and blocked with bovine serum albumin (GC305010, Servicebio, Wuhan, China) for 1 h at room temperature. Sections were subsequently incubated overnight at 4 °C with primary antibodies: goat polyclonal anti-CGRP (1:200; ab36001, Abcam PIc, Cambridge, UK), mouse monoclonal anti-SP (1:200; ab14184, Abcam, UK), and rabbit polyclonal anti-p-ERK (1:100; bs-3016R, YaJi Biological, Shanghai, China). Afterwards, sections were incubated for 2 h at room temperature with secondary antibodies: FITC-labeled anti-goat IgG (1:100, GB23404, Servicebio, Wuhan, China), anti-mouse IgG (1:100, GB22301, Servicebio, Wuhan, China), and anti-rabbit IgG (1:100, GB22303, Servicebio, Wuhan, China). Stained sections were examined under a fluorescence microscope (VS 200 and OlyVIA; Olympus, Tokyo, Japan).

Real-Time Polymerase Chain Reaction

Total RNA was isolated separately from bilateral trigeminal ganglia and medulla oblongata tissues at 3, 7, 14, 28 days after occlusal reconstruction. RNA samples were reverse-transcribed into cDNA using a Reverse Transcription Kit (2690S, TaKaRa, Japan). Primer sequences are listed in Table 1. Subsequently, quantitative real-time PCR (qPCR) was performed under the following conditions: initial denaturation at 95 °C for 30 sec, followed by 45 cycles of 95 °C for 5 sec, 55 °C annealing for 30 sec, and 72 °C extension for 30 sec. The comparative CT method ($2^{-\Delta\Delta Ct}$ method) was employed to evaluate mRNA expression levels of *tachykinin precursor 1* (*Tac1*), *calcitonin-related polypeptide alpha* (*CALCA*), *tumor necrosis factor-alpha* (*TNF-α*), *interleukin-1 beta* (*IL-1β*), and *brain-derived neurotrophic factor* (*BDNF*).

Western Blot (WB)

Rats were euthanized by perfusion fixation at 3, 7, 14, and 28 days after occlusal reconstruction. The brainstem and trigeminal spinal nucleus-related areas of the medulla oblongata were quickly removed and homogenized on ice for 1 min using a tissue grinder. An appropriate amount of tissue was weighed, centrifuged, and the supernatant was discarded. Lysis buffer was then added, and the supernatant was homogenized again on ice. Extracted proteins were quantified using a bicinchoninic acid assay solution (BCA) protein assay kit (C500053-0050, Sangon, Shanghai, China). Total protein (20 µg) was separated by electrophoresis and transferred onto a polyvinylidene difluoride membrane (PVDF) membrane using a Bio-Rad blotting system (1704150, Bio-Rad Laboratories, Hercules, CA, USA). The membrane was incubated in blocking solution and subsequently treated overnight at 4 °C with primary antibodies: rabbit anti-p38 (8690P, Cell Signaling Technology, Inc., Danvers, MA, USA), p-p38 (4511L, Cell Signaling, USA), rabbit anti-ERK (4695P, Cell Signaling, USA), and p-ERK polyclonal antibody (9101L, Cell Signaling, USA), diluted in tris-buffered saline with tween-20 (TBST) solution. After washing, the membrane was incubated at room temperature for 1 h with horseradish peroxidase-labeled anti-rabbit IgG (1:500, SC-2004, Santa Cruz Biotechnology, Inc., Dallas, TX, USA) diluted in TBST. Bands were visualized using chemiluminescence, and images were analyzed quantitatively using Olympus Image Pro-Plus software (7.0, Media Cybernetics, Silver Spring, MD, USA).

Statistical Analysis

All data were presented as mean ± standard error. Two-way analysis of variance (ANOVA) with Tukey's test and the *t*-test for comparisons between two means were performed. Statistical significance was defined as $p < 0.05$.

Results

Weight Changes in Rats

The general condition of rats was systematically observed following each operation. Specifically, no bleeding or ulcers occurred postoperatively. Additionally, no significant tooth loosening or loss was observed. Facial symmetry during chewing was maintained without obvious midline deviation or unilateral mastication. Daily behaviors remained normal, without abnormal activities such as restlessness or face scratching. Body weight measurements revealed no significant differences among groups T, S, and C at each time point before and after modeling ($p > 0.05$) (Fig. 1).

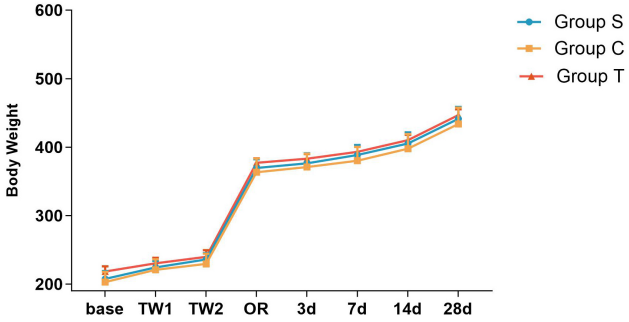

Fig. 1. Body weight changes of SD rats. Body weights recorded at baseline (before experimental procedures), first tooth grinding (TW1), second tooth grinding (TW2), and days 3, 7, 14, and 28 after occlusal reconstruction. n = 5. SD, Sprague-Dawley; TW, Tooth wear.

Pain Sensitivity Threshold of Masseter Muscle in Rats

According to PPT results, baseline values showed no significant differences among groups prior to experimental procedures ($p > 0.05$). After modeling, PPT values in Group T rats at days 3, 7, and 14 were significantly lower compared with Group C and Group S rats ($p < 0.001$). By day 28, PPT values in Group T were no longer significantly different from those in Group C and Group S ($p > 0.05$) (Fig. 2).

Compared with baseline values, PPT values in Group S and Group C remained stable at all subsequent time points without significant changes. In contrast, PPT values in Group T showed a significant decreasing trend, reaching a minimum on day 3 after modeling. Although these values slightly rebounded over the following 14 days, they remained significantly lower than baseline values, returning to baseline by day 28.

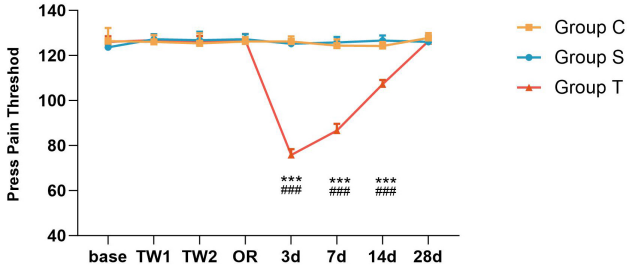

Fig. 2. Changes in PPT values of rats. PPT values recorded before experimental operations served as baseline values. Measurements after modeling were recorded at days 3, 7, 14, and 28 (n = 5). $^{###}p < 0.001$ versus Group C; $***p < 0.001$ versus Group S. PPT, pressure pain threshold.

Immunofluorescence Staining of SP and p-ERK in the Peripheral Nervous System

Immunofluorescence analysis showed that SP and p-ERK were predominantly localized in the cytoplasm of neuronal cells, with positive staining in green and neuronal nuclei counterstained in blue. Compared with Groups C and S, the number of SP-positive neurons in Group T was significantly increased at days 3, 7, and 14 following occlusal reconstruction ($p < 0.001$). The number of positive neurons gradually declined over time and returned to levels comparable to the control group by day 28 ($p > 0.05$) (Fig. 3). Similarly, p-ERK levels significantly increased on day 3 post-reconstruction ($p < 0.001$), decreased by day 21 but remained significantly higher than those in the control group ($p < 0.01$), and returned to baseline by day 28 ($p > 0.05$) (Fig. 4). These findings suggest that occlusal reconstruction regulates the expression of SP and p-ERK, potentially contributing to therapeutic effects, alleviating pain, and facilitating tissue repair.

qPCR Analysis of Tac1, CALCA, TNF-α, IL-1β, and BDNF mRNA Expression Levels

According to qPCR results, no significant differences in *Tac1*, *CALCA*, *TNF-α*, *IL-1β*, and *BDNF* mRNA expression levels were observed between Group S and Group C ($p > 0.05$). Compared with Groups C and S, these mRNA levels were significantly upregulated in Group T at days 3, 7, and 14 after occlusal reconstruction ($p < 0.01$, $p < 0.001$). Expression peaked on day 3 and progressively declined thereafter. By day 28, expression returned to baseline levels, with no significant differences compared to Group C ($p > 0.05$) (Fig. 5). Thus, as occlusal relationships gradually normalized after reconstruction, expression of factors associated with pain conduction, neuroregulation, and inflammatory responses progressively decreased. These changes may contribute to pain relief, periodontal tissue repair, and alveolar bone reconstruction.

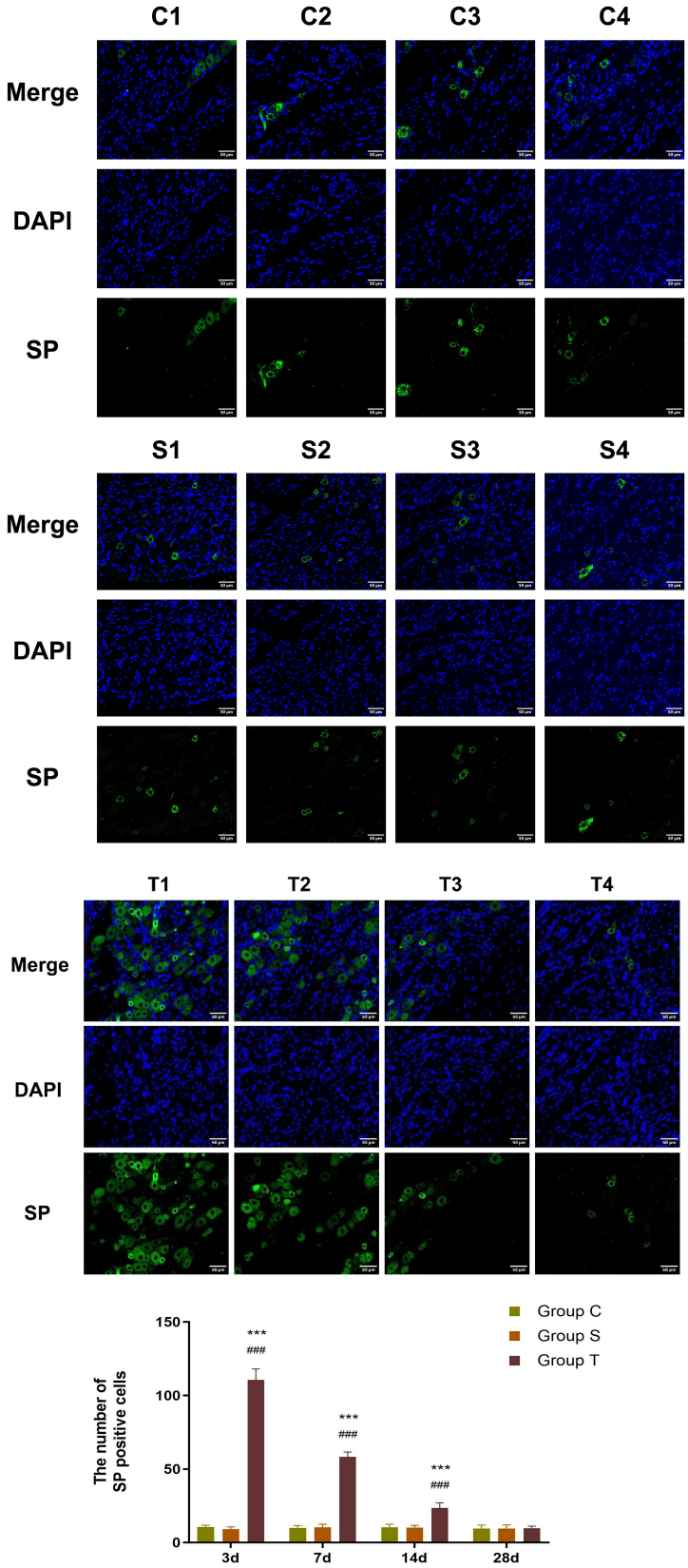

Fig. 3. SP immunofluorescence staining of trigeminal ganglion tissues in rats after occlusal reconstruction. $^{###}p < 0.001$ versus Group C; $^{***}p < 0.001$ versus Group S. C1, day 3 of Group C; C2, day 7 of Group C; C3, day 14 of Group C; C4, day 28 of Group C; S1, day 3 of Group S; S2, day 7 of Group S; S3, day 14 of Group S; S4, day 28 of Group S; T1, day 3 of Group T; T2, day 7 of Group T; T3, day 14 of Group T; T4, day 28 of Group T. n = 5. DAPI, 4′,6-Diamidino-2-phenylindole; SP, Substance P.

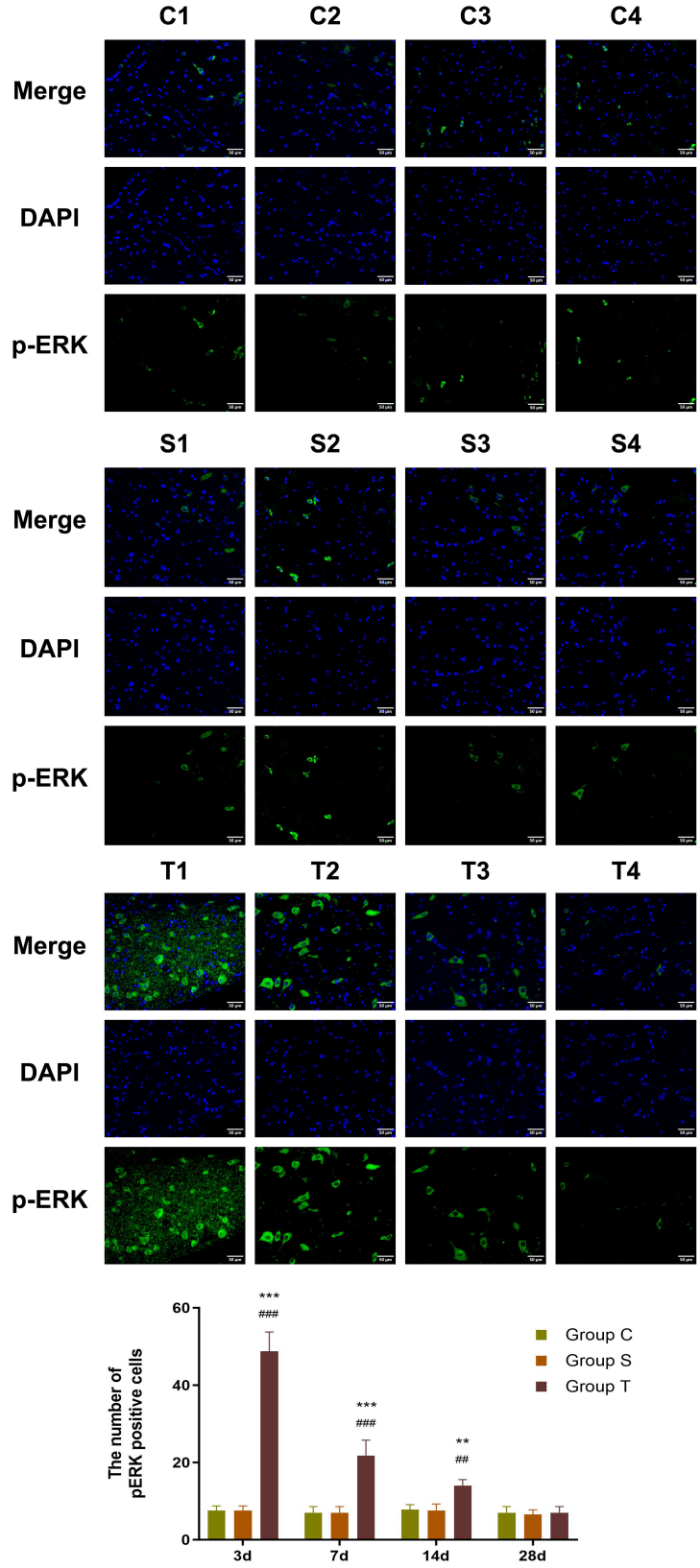

Fig. 4. p-ERK immunofluorescence staining of medulla oblongata tissues in rats after occlusal reconstruction. $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ versus Group C; $^{**}p < 0.01$, $^{***}p < 0.001$ versus Group S. C1, day 3 of Group C; C2, day 7 of Group C; C3, day 14 of Group C; C4, day 28 of Group C; S1, day 3 of Group S; S2, day 7 of Group S; S3, day 14 of Group S; S4, day 28 of Group S; T1, day 3 of Group T; T2, day 7 of Group T; T3, day 14 of Group T; T4, day 28 of Group T. n = 5. p-ERK, phosphorylated extracellular signal-regulated kinase.

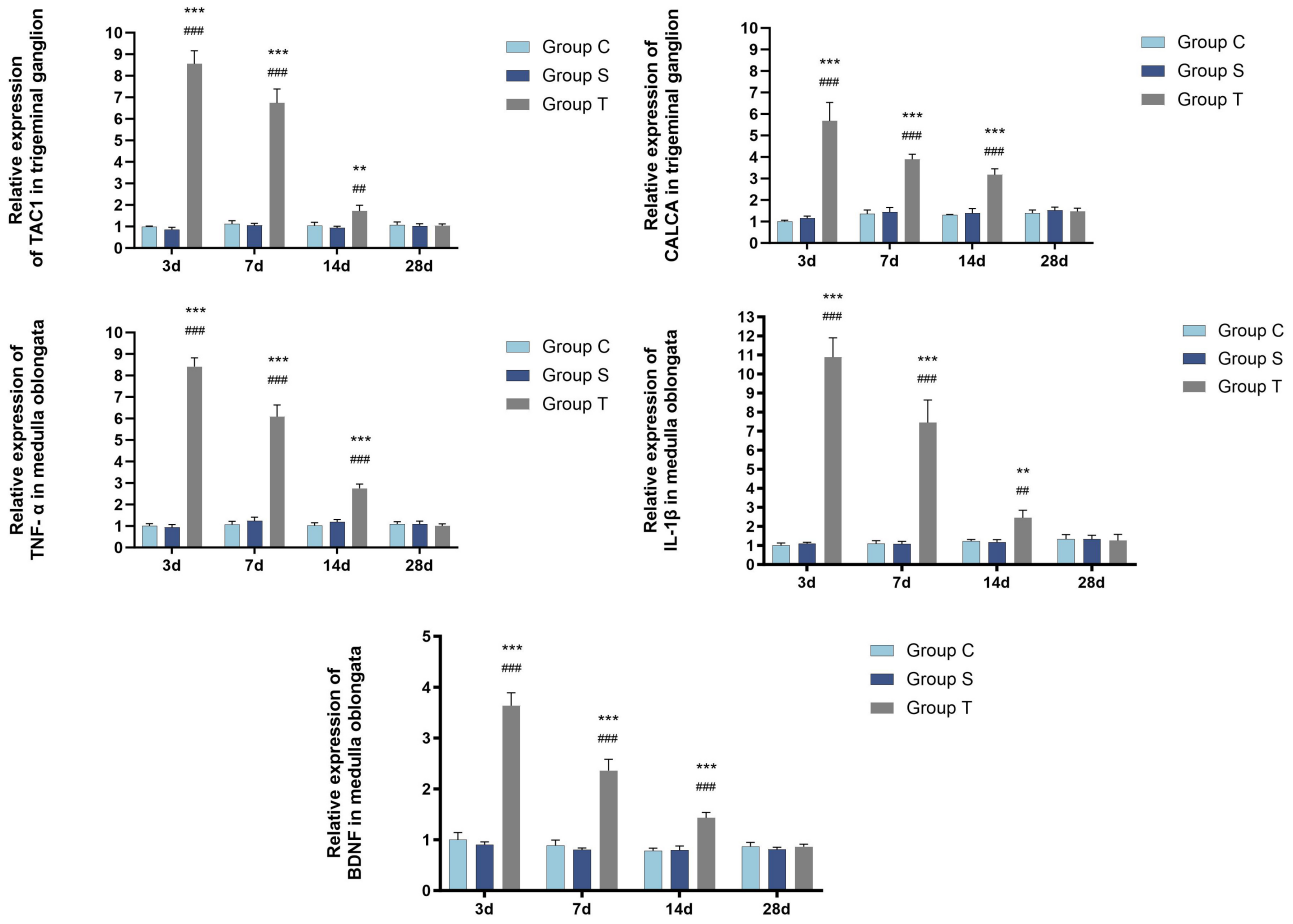

Fig. 5. qPCR analysis of Tac1, CALCA, TNF-α, IL-1β, and BDNF mRNA levels. n = 5. $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ versus Group C; $^{**}p < 0.01$, $^{***}p < 0.001$ versus Group S. qPCR, Quantitative real-time PCR; Tac1, Tachykinin precursor 1; CALCA, calcitonin-related polypeptide alpha; TNF-α, tumor necrosis factor-alpha; IL-1β, interleukin-1 beta; BDNF, brain-derived neurotrophic factor.

WB Analysis of p38, p-p38, ERK, and p-ERK Proteins in the Brainstem and Medulla Oblongata

WB results showed no significant differences in p-p38 and p-ERK protein expression between Group S and Group C ($p > 0.05$). Compared with Groups C and S, p-p38 and p-ERK proteins were significantly upregulated in Group T at days 3, 7, and 14 after occlusal reconstruction ($p < 0.001$). Protein expression peaked on day 3 and gradually decreased thereafter. By day 28, protein expression returned to baseline levels, with no significant differences compared to Group C ($p > 0.05$) (Fig. 6). These results suggest that TW activates the p38 MAPK/ERK signaling pathway, and the gradual reduction of p-p38 and p-ERK expression following occlusal reconstruction may alleviate pain and reduce tissue damage.

Discussion

Currently, animal experiments are commonly used in research on stomatognathic system-related diseases. For instance, well-established animal models exist for occlusal interference, unilateral mastication, and occlusal trauma. Rats are ideal experimental animals because their anatomical structure and TMJ movements closely resemble those of humans. Moreover, rats are frequently used as research subjects for stomatognathic system diseases [15]. The use of rats as experimental subjects also offers advantages, such as low cost and ease of handling, and rats were therefore chosen as the model animals for this study [16,17]. Functionally, occlusal splint fixation was effective in all experimental groups. Nutritional intake and growth in rats are closely related to changes in body weight; thus, the effects of occlusal reconstruction were evaluated by monitoring body weight variations [18]. Additionally, no statistically significant differences in body weight changes were observed among the three groups, indicating that this method did not affect the nutritional intake or growth of rats.

In contrast to inflammatory stimulation models involving the injection of formalin or complete Freund's adjuvant (CFA) into the face, rats in the occlusal reconstruction model showed no symptoms of acute orofacial pain and no active face-scratching behavior [19]. However, a decreas-

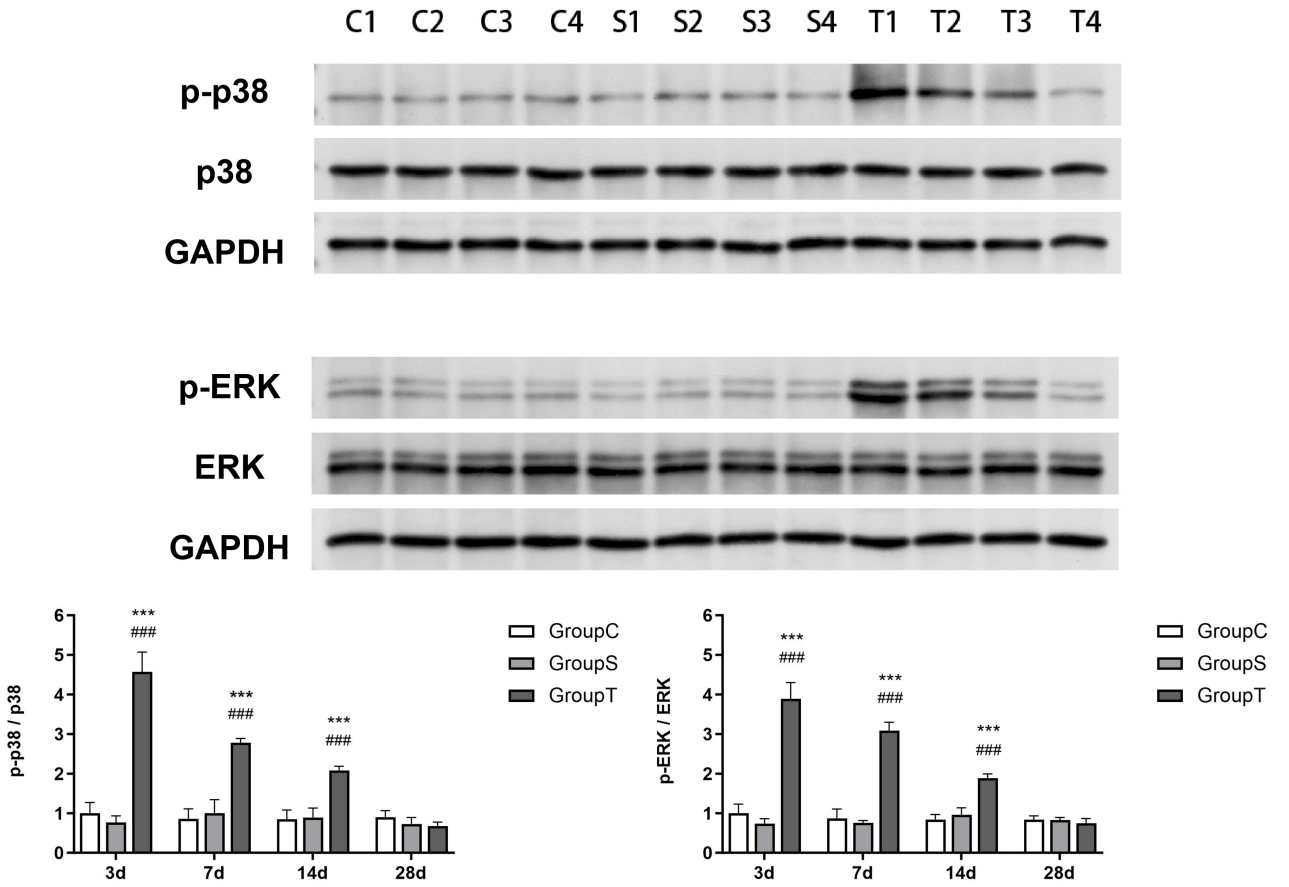

Fig. 6. WB analysis of p38, p-p38, ERK, and p-ERK protein expression. $^{###}p < 0.001$ versus Group C; $***p < 0.001$ versus Group S. Molecular weights: p38, 41 kDa; p-p38, 43 kDa; ERK1/2, 44/42 kDa; p-ERK1/2, 44/42 kDa; GAPDH, 36 kDa. C1, day 3 of Group C; C2, day 7 of Group C; C3, day 14 of Group C; C4, day 28 of Group C; S1, day 3 of Group S; S2, day 7 of Group S; S3, day 14 of Group S; S4, day 28 of Group S; T1, day 3 of Group T; T2, day 7 of Group T; T3, day 14 of Group T; T4, day 28 of Group T. n = 5. WB, Western blot; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

ing trend in the mechanical tenderness threshold of bilateral masseter muscles was observed, lasting for nearly one month. Rats in Group S showed no changes in masseter muscle PPT, indicating that the experimental manipulation itself did not affect the results. This further supported the conclusion that occlusal reconstruction directly contributed to changes in masseter muscle PPT in Group T rats. PPT values in Group T significantly decreased, reaching a minimum three days after modeling, suggesting increased masseter muscle sensitivity due to increased occlusal vertical dimension. Subsequently, muscle pain sensitivity returned to baseline levels over time. Overall, these results indicate that occlusal reconstruction altered mechanical tenderness sensitivity of rat masseter muscles, consistent with clinical observations in patients who experience transient pain during chewing following occlusal reconstruction.

SP is a highly conserved neuropeptide belonging to the tachykinin family, widely expressed across the animal kingdom. SP mediates pain signal transmission through activation of the neurokinin-1 receptor (NK1R), primarily conveying signals from primary afferent fibers to secondary neurons located in the spinal cord and brainstem [20]. Functionally, the SP/NK1 system contributes to thermal hyperalgesia associated with inflammation or nerve injury and is involved in maintaining persistent facial pain [21]. CGRP is another neuropeptide widely distributed throughout the nervous system, acting as a neuromodulator of glutamate signaling and contributing to central sensitization via both presynaptic and postsynaptic mechanisms. CGRP and SP act synergistically to modulate pain signal transmission in neural circuits underlying pain perception. Studies have shown that the release of CGRP and SP within the trigeminal nervous system is closely associated with pain transmission, particularly in migraines and other facial pain disorders [22]. Investigation into the expression and localization of CGRP and SP in neurons may provide deeper insights into mechanisms underlying pain transmission pathway alterations during occlusal reconstruction [23]. Expression of CGRP within the trigeminal ganglion plays an essential role in modulating pain transmission. Additionally, CGRP upregulation is strongly correlated with heightened pain perception during facial pain induced by tooth move-

ment [24]. In this study, immunofluorescence staining was employed to analyze SP and CGRP expression changes in the trigeminal ganglion after occlusal reconstruction. Fluorescence intensity of SP and CGRP peaked on day 3 post-reconstruction and subsequently returned to baseline levels by day 28. Pressure pain sensitivity in peripheral masticatory muscles, induced by occlusal reconstruction, was closely correlated with SP and CGRP expression dynamics in the trigeminal ganglion. Immunofluorescence staining successfully localized these neurotransmitters within trigeminal ganglion tissues. Changes in SP- and CGRP-positive cells suggested peripheral neurotransmitters significantly modulate chronic maxillofacial pain. Moreover, qPCR analysis of *Tac1* and *CALCA* genes (encoding SP and CGRP, respectively) revealed expression trends consistent with observed protein-level changes.

TW can disrupt occlusal relationships, subjecting periodontal tissues to abnormal occlusal forces. This process imposes excessive mechanical stimulation on nerve endings, causing nerve fiber damage and triggering inflammatory responses in periodontal tissues [25]. BDNF plays a critical role in transmitting and modulating pain signals [26], primarily exerting its effects by activating tropomyosin receptor kinase B (TrkB) receptors in peripheral and central nervous systems (CNS). Functionally, BDNF participates in the development and persistence of inflammatory and neuropathic pain, modulating pain perception through regulation of synaptic plasticity and neuronal excitability [27]. BDNF is markedly upregulated after peripheral nerve injury, predominantly in the early post-injury phase [28]. BDNF expression progressively normalizes during occlusal reconstruction and facilitates regeneration and functional recovery of damaged nerves by promoting axonal growth, branching, and synaptic formation. TNF-α and IL-1β are key pro-inflammatory cytokines that significantly contribute to inflammation during TW and related oral diseases. They modulate local inflammatory responses and affect systemic health through various mechanisms [29]. Additionally, substantial amounts of these cytokines are released during TW, triggering neurogenic inflammation. Elevated levels of IL-1β and IL-6 in gingival crevicular fluid and serum in patients with periodontal disease indicate their critical roles in periodontal disease pathogenesis [30]. Expression levels of TNF-α and IL-1β peaked 3 days after occlusal reconstruction and subsequently decreased over time. Overall, these findings suggest that occlusal reconstruction effectively alleviates inflammation resulting from TW.

The p38 protein is an important member of the MAPK family, which can be activated by upstream mitogen-activated protein kinase kinase 3 (MKK3)/MKK6 kinases. It plays crucial roles in environmental stress responses, inflammatory processes, and pain-related signaling pathways. ERK and p38, both belonging to the MAPK signaling family, have strong associations with pain modulation. Specifically, p38 participates in pain regulation, particularly neuropathic and inflammatory pain. The level of p-p38 reflects pain intensity, and activated p-p38 is predominantly localized in microglia. One study reported that phosphorylated p38 expression in spinal cord microglia increased following spinal cord ligation in rats, reaching a peak on the first and third days post-ligation, and subsequently decreased after 3 weeks [31]. Lee established a trigeminal neuralgia model by inserting an implant into the alveolar bone of rats to injure the inferior alveolar nerve. The study found that p-p38 expression peaked on day three after surgery and remained elevated until day seven. Additionally, p38 protein kinase inhibitors effectively reduced mechanical hyperalgesia and p-p38 protein expression levels [32]. These findings indicate that reducing p-p38 protein expression can alleviate pain intensity. However, it has no direct effect on peripheral tissue damage and inflammation, suggesting that pain intensity is primarily influenced by central pain sensitization following pain signal transmission to the CNS. When the oral and maxillofacial regions are subjected to noxious stimuli, damaged cells release neurotransmitters that diffuse locally and are transported to the CNS, leading to central sensitization, lowered sensitization thresholds, and physiological changes such as ectopic discharges. WB and immunofluorescence staining results revealed that, compared with Group C and Group S, the levels of p-p38 and p-ERK proteins were significantly elevated at 3 d, 7 d, and 14 d after occlusal reconstruction, returning to normal levels at 28 d post-occlusal interference. Overall, the p38 MAPK/ERK signaling pathway plays a crucial role in modulating pain responses in this model.

Conclusions

In conclusion, the present study demonstrated that occlusal reconstruction can alter mechanical pain sensitivity in the masseter muscle and affect neuronal excitation levels in rats. The underlying mechanism may involve modulation of the p38 MAPK/ERK signaling pathway and regulation of the expression of pain-related neurotransmitters (e.g., SP and CGRP), thereby attenuating the transmission of nociceptive signals. Additionally, occlusal reconstruction appears to alleviate inflammatory responses. Nevertheless, due to the complexity of the regulatory mechanisms associated with occlusal reconstruction and related pain responses, a more systematic and thorough analysis is necessary in future studies. Such efforts would facilitate the development of effective therapeutic strategies for relevant disorders.

Availability of Data and Materials

The datasets generated and/or analyzed during the current study are not publicly available due to the need for follow-up research but are available from the corresponding author on reasonable request.

Author Contributions

RR, JC and WL designed the research study; RR, JC, WL, AA and MM performed the research; JC and WL collected and analyzed the data. AA and MM have been involved in drafting the manuscript. All authors contributed significantly to the important editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

The USM Institutional Animal Care and Use Committee (USM IACUC) approved this study (Approval No.: USM/IACUC/2023/(139)(1256)).

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Conflict of Interest

The authors declare no conflict of interest.

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