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Experimental occlusal interference induces long-term masticatory muscle hyperalgesia in rats

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ABSTRACT

Temporomandibular joint or related masticatory muscle pain represents the most common chronic orofacial pain condition. Patients frequently report this kind of pain after dental alterations in occlusion. However, lack of understanding of the mechanisms of occlusion-related temporomandibular joint and muscle pain prevents treating this problem successfully. To explore the relationship between improper occlusion (occlusal interference) and masticatory muscle pain, we created an occlusal interference animal model by directly bonding a crown to a maxillary molar to raise the masticating surface of the tooth in rats. We raised the occlusal surface to three different heights (0.2, 0.4, and 0.6 mm), and for one month we quantitatively measured mechanical nociceptive thresholds of the temporal and masseter muscles on both sides. Results showed a stimulus–response relationship between the height of occlusal interference and muscle hyperalgesia. Removal of the crown 6 days after occlusal interference showed that the removal at this time could not terminate the 1 month duration of mechanical hyperalgesia in the masticatory muscles. Lastly, we systemically administered NMDA antagonist MK801 (0.2, 0.1, and 0.05 mg/kg) to the treated rats and found that MK801 dose dependently attenuated the occlusal interference-induced hyperalgesia. These findings suggest that occlusal interference is directly related to masticatory muscle pain, and that central sensitization mechanisms are involved in the maintenance of the occlusal interference-induced mechanical hyperalgesia.

1. Introduction

Temporomandibular disorders (TMDs) and related masticatory muscle pain represent the most common chronic orofacial pain condition. Numerous studies have addressed the multifactorial etiology of these TMDs. One contributor has been suggested to be improper occlusion [15,25,37]. However, several other studies and reviews have not found strong support for an occlusal etiology of TMD, at least not as a unique or dominant factor [12,15,32,37,38,53]. This may be at least partly because TMD has potential contributions from a variety of anatomical, physiological, and psychological factors. Clinically masticatory muscle pain is frequently reported after changes in occlusion [19,24,25], yet relatively little is known about the relationship between occlusal alteration and masticatory muscle pain, or the mechanisms of the initiation and perpetuation of this type of pain. In this report, we examine the potential role of occlusion in causing nociceptive muscle behavior that may be more important than is generally accepted in the etiology of masticatory muscle pain and TMD [26,39].

Occlusion is an important variable in the overall treatment scheme for clinical restorative and prosthodontic success. Occlusion is defined as "the static relationship between the incising or masticating surfaces of the maxillary or mandibular teeth or tooth analogues" [1]. This relationship should be as balanced and stress free as possible. Occlusal interference is defined as "a tooth contact that inhibits the remaining occluding surfaces from achieving stable and harmonious contacts" [1], and may produce pathologic changes in the stomatognathic system [6,37]. In order to understand the harmful effects of occlusal interference on oromandibular function, several animal models have been developed. An early model consisted of tooth filling with amalgam, but the height of occlusal alteration could not be quantified. Later, the method of bonding restorations or pins into pulp cavities was used, which made occlusal alteration semi-quantified, but pulpectomy was required. Pulpectomy is a very specialized form of nerve injury [20], which may result in both structural and functional changes in the brainstem where the central terminals are found [17,21,29]. Recently, with the development of new bonding materials, direct

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bonding of an interference apparatus onto the tooth surface became possible [9,16,28,34,45]. With this method, pulpectomy was avoided. Most of these later investigations indicated that experimental occlusal interference in animals could result in mandibular condyle bone remodeling [16] and pathological changes in masticatory muscles, dental pulp and periodontium [2,7,27,28,34,45]. However, few studies were designed to test for occlusion-related nociceptive mechanisms [9].

To explore the relationship between improper occlusion (occlusal interference) and masticatory muscle nociceptive behavior, we created an occlusal interference animal model using crowns with a thickness of 0.2, 0.4, or 0.6 mm, which were directly bonded to rat maxillary molars. We found that nociceptive threshold decreases in masticatory muscle were directly related to the height of occlusal interference, and that removal of the crown after 6 days did not reduce the hyperalgesia observed for at least 1 month after the induced occlusal interference. We also found that the *N*-methyl-D-aspartate (NMDA) antagonist MK801 dose dependently reduced the hyperalgesia caused by the occlusal interference.

2. Materials and methods

2.1. Subjects

The experimental protocol was reviewed and approved by our Institutional Animal Care and Use Committee. Sixty-four male Sprague–Dawley rats weighing from 220 to 250 g were used (Vital River Laboratory Animal Technology Co. Ltd., Beijing). Rats were housed under a 12-h light/dark cycle with food and water available *ad libitum*.

2.2. Animal model of occlusal interference

Under ether anesthesia, impressions of the rats' maxillary dentitions were made with irreversible hydrocolloid (Heraeus-kulzer, Germany) poured into stone casts. Crowns/bands for the maxillary first molars on the right side were made from nickle-chromium by: trimming the master cast – waxing up – casting – fitting on the cast – grinding to a specific thickness – polishing. The modified cast *crown (active occlusal interference)* was designed to cover the occlusal, buccal, lingual, and medial surfaces (Fig. 1), while the *band (sham occlusal interference)* was fabricated to cover the buccal, lingual and medial surfaces without changing the occlusal surface (Fig. 1). Rats were anesthetized by i.p. injection of pentobarbital sodium (40 mg/kg) and the crowns/bands were bonded to the right-side maxillary first molars with dental resin cement (Panavia F, Kuraray, Japan).

2.3. Measurement of nociceptive threshold to mechanical pressure of the masticatory muscles

Animals were habituated prior to commencement of testing. Testing was similar to that described by Ren [40]. The rat was habituated to standing on its hind paws and against the tester's gloved hand. The "Paw Pressure" mode of the electronic von-Frey anesthesiometer (IITC Life Science, CA, USA) was used. The unit was supplied with a rigid plastic tip, capable of transmitting pressure onto the masticatory muscles. We designed a round cap (diameter 3 mm) fixed to the plastic tip in order to diminish the effective skin stimulation, but still produce muscle pressure elicited by the rigid plastic tip of the von-Frey anesthesiometer (Fig. 2). Two orofacial areas were tested, one was the temporal muscle region at the central point of the line between the orbit and the tragus. The other was the masseter muscle belly region, at a site 10 mm inferior to the temporal muscle testing point. At these locations obvious muscular contraction could be palpated during mastication. When testing, force was applied with the probe oriented perpendicular to the sagittal plane. The force in grams needed to elicit head withdrawal indicative of nociceptive response was recorded five times for each animal at 1-min intervals. The average of these five values was used as the withdrawal threshold. For this study we defined mechanical hyperalgesia as a statistically significant decreased withdrawal threshold following treatment compared to pre-treatment baseline.

For all data reported here, the observer was blinded to the occlusal, inflammation or sham treatments.

2.4. Experimental design

2.4.1. Nociceptive behavior measurement following CFA injection

In order to test our modified anesthesiometer, we used the well-established injection of CFA into muscles in rats [3,4]. Under ether anesthesia, unilateral muscle inflammation in rats ($n = 4$) was produced by Complete Freund's adjuvant (CFA, 0.05 mL, 0.025 mg *Mycobacterium tuberculosis*, Sigma) suspended in saline (1:1) injection into the masseter muscle with a 30-gauge needle. Saline injection into the masseter served as a control ($n = 4$). Measurement of nociceptive threshold to mechanical pressure on the masseter was performed: pre-injection, and 4 h, 1, 3, 5 and 7 days after CFA/saline injection.

2.4.2. Nociceptive behavior measurement following experimentally occlusal interference

Crowns with thickness of 0.2, 0.4, or 0.6 mm, represented three severities of occlusal interference. Rats were randomly assigned to one of five groups, five rats in each group: (1) sham-application

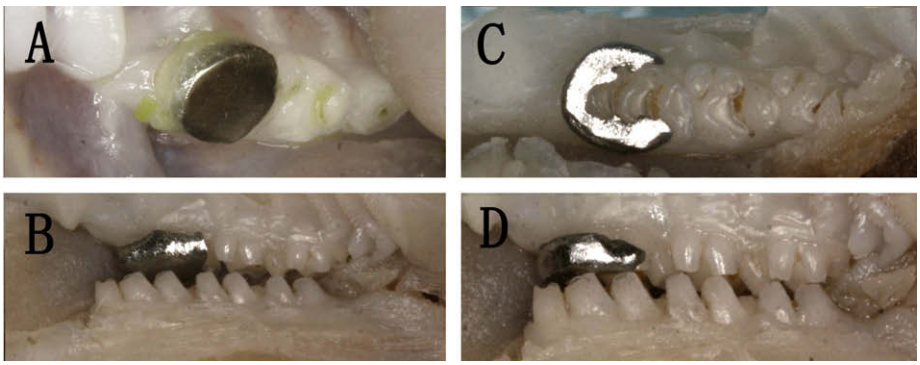

Fig. 1. Photographs of a crown on the right side of the maxillary first molar (A) and the alteration of occlusion in the rat's mouth (B). A band which did not cover the occlusal surfaces (C), did not interfere with occlusion (D).

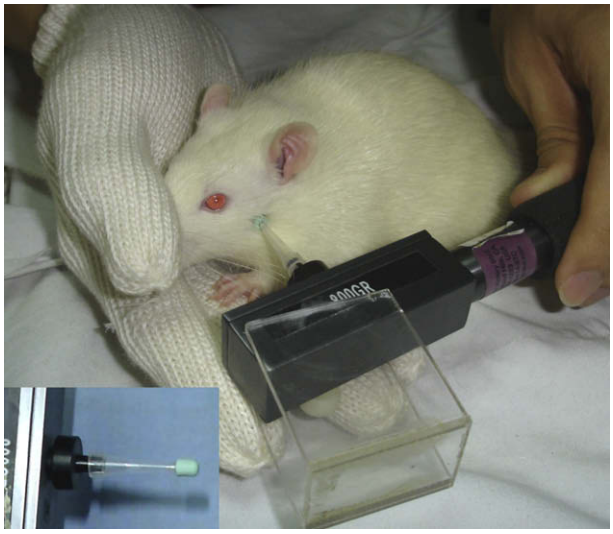

Fig. 2. Photograph illustrating the method of assessing mechanical nociceptive thresholds in a rat. The experimenter's hand provided a comfortable "nest" for the rat. The modified electronic von-Frey anesthesiometer is probing the masseter. The inset shows the rigid plastic tip covered by a rounded cap.

control – these rats were anesthetized and mouths were forced open for about 5 min (same duration as other groups) but restorations were not applied; (2) under anesthesia sham-occlusal interference control – bands were bonded to the right maxillary first molars which did not interfere with occlusion; (3) under anesthesia occlusal interference group with a 0.2 mm thick crown; (4) under anesthesia occlusal interference with a 0.4 mm thick crown; (5) under anesthesia occlusal interference with a 0.6 mm thick crown.

Behavioral testing was performed at 10:00–12:00 a.m. everyday for 6 days before and 28 days after (total 34 days) the corresponding dental applications (occlusal interference, sham-occlusal interference and sham-application). On pre-application days 4, 5, and 6, rats were placed in assessment environments 30 min/day to familiarize them to the testing surroundings. On pre-application days 1, 2, 3, and post-application days 1, 3, 5, 7, 10, 14, 21 and 28, nociceptive thresholds were determined from stimulation of temporal and masseter muscles on both sides of the head. The average of the pre-application nociceptive threshold was calculated as the baseline.

2.4.3. Nociceptive behavior measurement following removal of occlusal interference appliance

Rats were randomly divided into three groups, five rats in each group. Group (1) had occlusal interference. These rats were bonded with 0.4 mm thickness of crown that was described above. Group (2) had occlusal interference appliance removed on day 6 after wearing 0.4 mm thick crowns. Their crowns were removed under anesthesia by i.p. injection of pentobarbital sodium (40 mg/kg). Group (3) rats were sham-application controls.

Nociceptive behavior was tested at the same time points as Section 2.4.2.

2.4.4. Nociceptive behavior measurement following systemic MK801 treatment

All rats in these experiments were treated with a 0.4 mm occlusal interference appliance, and nociceptive behavior thresholds were measured on day 1, day 3, and day 5. On day 6, drug vehicle (saline, $n = 4$) or the selective NMDA antagonist MK801 0.2 mg/kg ($n = 4$), 0.1 mg/kg ($n = 4$), and 0.05 mg/kg ($n = 4$) was injected intraperitoneally in the same volume [52], and nociceptive thresholds were re-evaluated 1 h after the injections.

2.5. Statistical analysis

All data were reported as the means ± SEM for each treatment group of animals. Time course measures for mechanical nociceptive thresholds between CFA- and saline-injected group, and among different occlusal treated groups were compared by repeated measures ANOVA followed by a LSD post hoc test first. Then multiple covariance analyses (multivariate test of the general linear model in SPSS) were used for comparison among different groups in a pairwise fashion at each time point. The difference between the bilateral sides at each time point in each group was compared using paired *t*-tests. The comparison of behavioral changes following MK801 treatment was performed by one-way ANOVA followed by a Bonferroni post hoc test. Calculations were carried out by means of SPSS12.0. A probability level <0.05 was chosen for all hypothesis testing.

3. Results

3.1. Mechanical hyperalgesia was induced by ipsilateral muscular injection of CFA into the masseter muscle

Animals with a unilateral masseter CFA injection showed marked signs of edema and skin redness on the injected side. These inflammatory indicators persisted for 2–3 days, and subsided 5 days following injection. The head withdrawal thresholds to mechanical pressure on the ipsilateral side were significantly reduced at 4 h, day 1, day 3, and day 5 following CFA injection, and were back to the pre-injection baseline on day 7 (Fig. 3). No reduction of the head withdrawal threshold was found on the contralateral side following CFA injection, nor on either side following saline injection.

3.2. Experimental occlusal interference induced a long-term masticatory muscle hyperalgesia

Repeated testing showed no significant change in head withdrawal thresholds during the four weeks tested in the sham-application control rats compared to baseline (Fig. 4). Sham-occlusal interference controls showed a slight, transient reduction of head withdrawal threshold during day 3 to day 7 both on the ipsi- and the contralateral side, but these reductions were not significantly different from the sham-application controls (Repeated Measures ANOVA followed by LSD post hoc test, ipsilateral: $P = 0.205$; contralateral: $P = 0.147$).

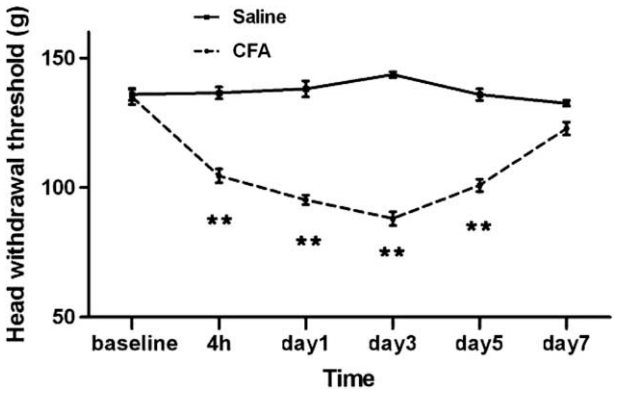

Fig. 3. The time course of head withdrawal thresholds following injection of CFA and saline into the masseter muscle. The head withdrawal thresholds at 4 h, 1day, 3 days and 5 days following injection of CFA were significantly decreased, compared to those of the saline-injected group. $^{**}P < 0.01$.

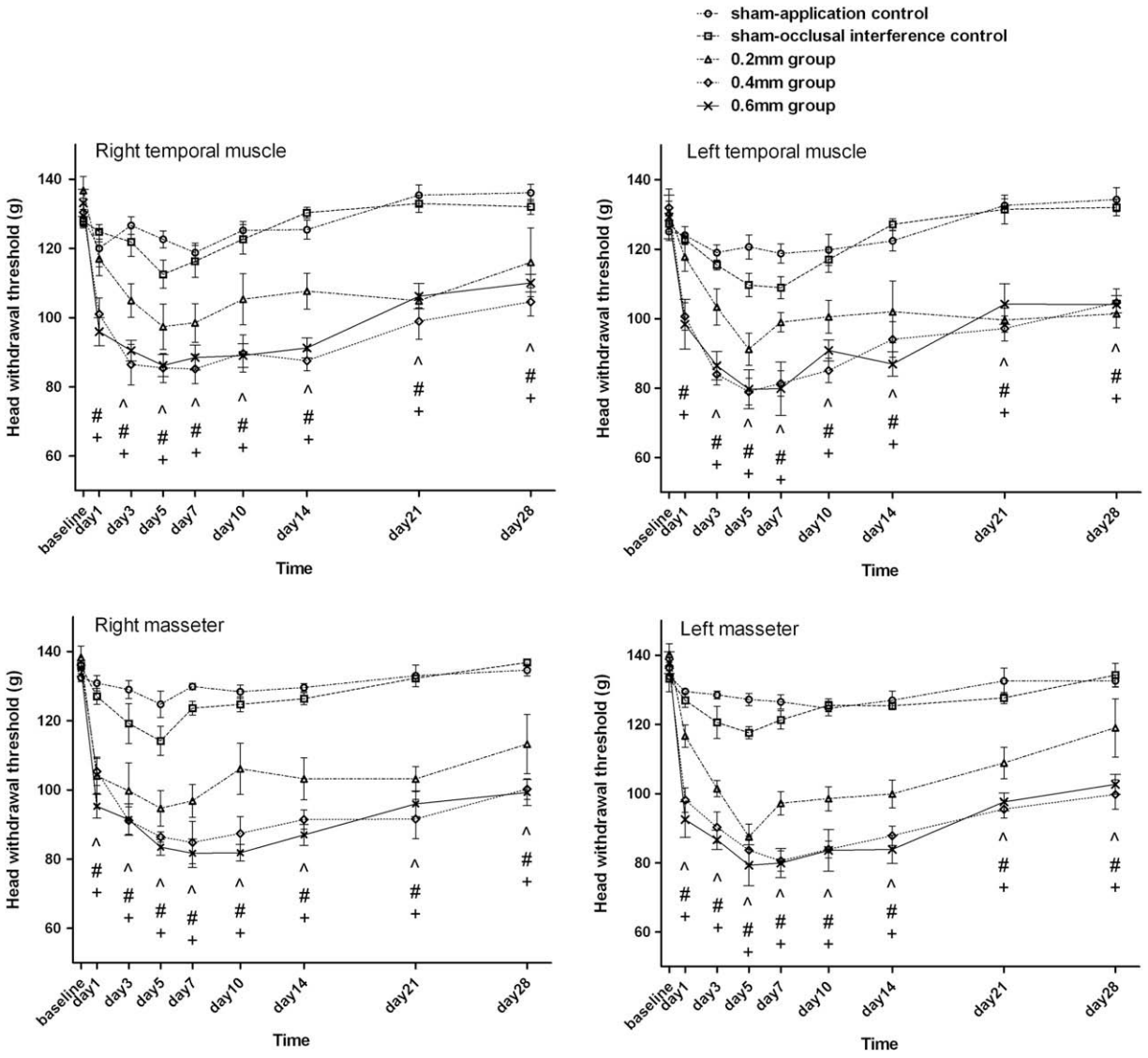

Fig. 4. The time course of the head withdrawal thresholds caused by stimulating masseter and temporal muscles on both sides following experimental occlusal interference. All three heights of the occlusal alterations resulted in reduction of the head withdrawal thresholds by stimulation of muscles on both sides. The mechanical nociceptive threshold started to decrease on day 1 after occlusal interference application, peaked on days 5–7, and lasted until the end of the experiment. $^{\wedge}P < 0.05$, a significant difference between the 0.2 mm group and the sham-application control, $^{\#}P < 0.05$, a significant difference between the 0.4 mm group and the sham-application control, $^{+}P < 0.05$, a significant difference between the 0.6 mm group and the sham-application control.

Mechanical hyperalgesia (decreased head withdrawal threshold to mechanical pressure) was induced in both temporal muscles and masseter muscles on both sides of the head following occlusal interference (Fig. 4). The occlusal interference-induced mechanical hyperalgesia persisted for 4 weeks, starting on day 1, peaking from day 5 to day 7, and lasting until the end of experiment (Fig. 4). The occlusal interference groups with 0.4 and 0.6 mm crowns showed greater mechanical hyperalgesia than the group with 0.2 mm crowns, but no significant difference was present between the group with 0.4 mm crown and 0.6 mm crown. All rats with occlusal interference showed bilateral hyperalgesia. The contralateral side tended to be greater than the ipsilateral side, but no significant differences were detected (paired *t*-test, $P > 0.05$).

3.3. Removal of occlusal interference appliance did not block the mechanical hyperalgesia

The animals with 0.4 mm occlusal alteration demonstrated mechanical hyperalgesia after wearing crowns. The crowns were removed on day 6, and the hyperalgesia was significantly reduced at days 10, 14 and 21, compared to that of 0.4 mm occlusal interference group (Fig. 5), but still exhibited significantly decreased head withdrawal thresholds on both sides until the last day of the experiment (Fig. 5). Similar results were found in both masseter and temporal muscles (Fig. 5).

3.4. MK801 reversed the experimental occlusal interference-induced mechanical hyperalgesia

All animals with 0.4 mm occlusal interference appliances showed mechanical hyperalgesia. On day 6, i.p. saline injection did not change the masseter nociceptive thresholds. However, i.p. injection of 0.2 mg/kg MK801 completely reversed the occlusal interference-induced mechanical hyperalgesia. MK801 (0.1 mg/kg) partially reversed the mechanical hyperalgesia, but 0.05 mg/kg MK801 showed no effect on nociceptive thresholds (Fig. 6). These results indicated that MK801 dose dependently attenuated the occlusal interference-induced hyperalgesia. No behavioral side effects were observed during the experiment after i.p. administration of the three doses of MK801.

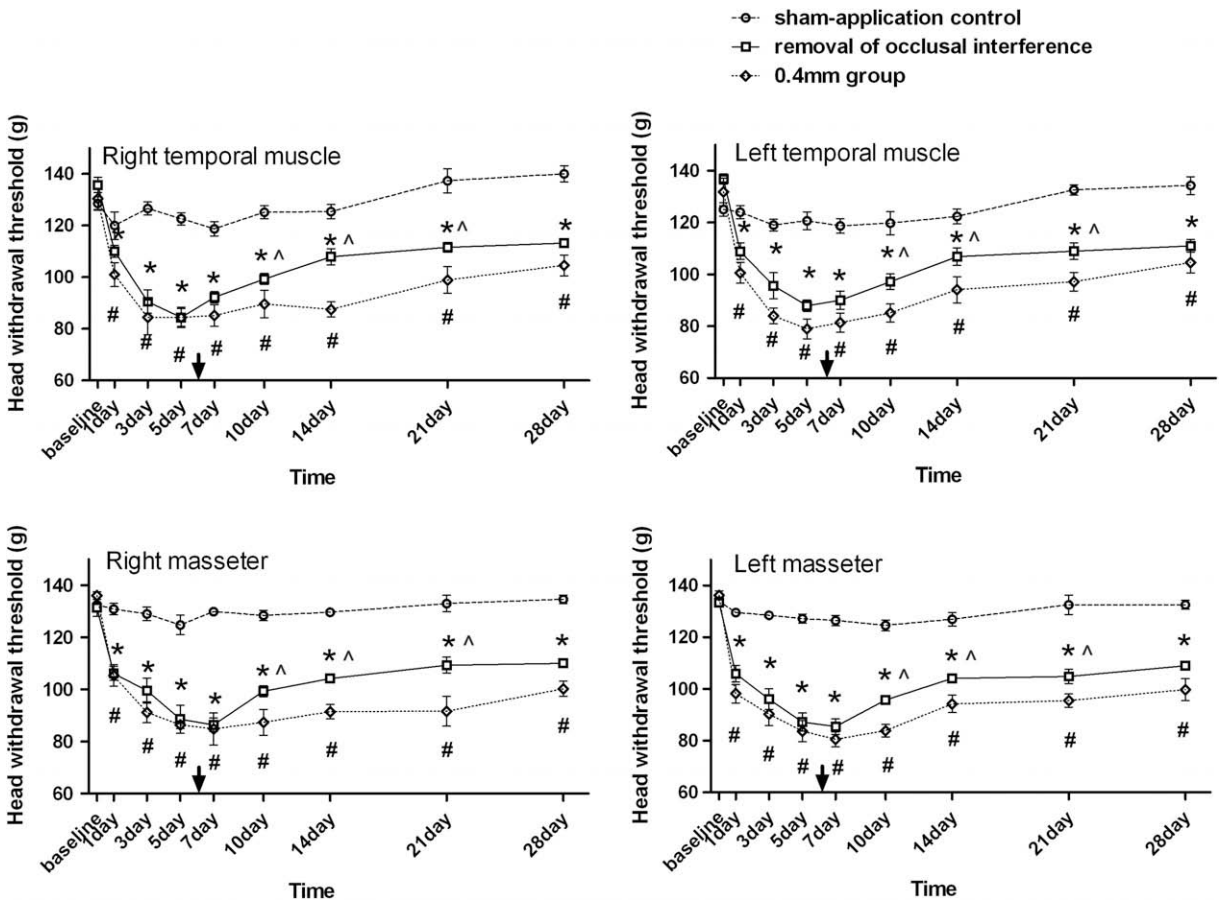

Fig. 5. The time course of the head withdrawal thresholds following the removal of occlusal interference. The crown was removed on day 6 after the experimental occlusal interference application. Animals in the group with the crown removed showed muscle hyperalgesia that was reduced at days 10, 14, and 21 (compared to animals in group in which the crown was not removed), but still continued demonstrating significantly decreased mechanical nociceptive thresholds until day 28. $^{\wedge}P < 0.05$, a significant difference between the removal of occlusal interference group and the 0.4 mm group, $^{*}P < 0.05$, a significant difference between the removal of occlusal interference group and the sham-application control group, $^{\#}P < 0.05$, a significant difference between the 0.4 mm group and the sham-application control.

4. Discussion

4.1. A modification for the tip of von Frey filament

The method for evaluation of mechanical nociceptive threshold in the orofacial region of rats was first described by Ren et al. [40], which has been successfully used in many studies [3,4,18,22,48,51]. While this procedure has many variables, the repeatability across laboratories has been extensively documented. Recently, rigid von Frey filaments (1 mm diameter) coupled with a force transducer have been used to test mechanical nociceptive behavioral responses evoked by stimulation of the masseter muscles [3,4]. However, using von Frey hairs or a sharp tip stimulator may elicit responses more from cutaneous rather than muscle nociceptors. Takahashi et al. [50] determined that mechanical nociceptive thresholds measured with larger ($\geqslant$1.6 mm) probes reflect the nociceptive threshold of deep tissues, possibly muscle, while smaller diameter probes reflect nociceptive thresholds from skin. Therefore, we developed a modified tip for the von Frey filament. The rigid tip of an electronic anesthesiometer was covered by an elastic round cap (diameter 3 mm), capable of transmitting pressure onto the masticatory muscles, avoiding skin stimulation that would evoke cutaneous pain as is elicited by sharp von Frey filaments. CFA injection into TMJ or muscle is a well-accepted inflammatory pain model. We used the modified electronic anesthesiometer to measure the mechanical nociceptive thresholds of the masticatory muscles in naive rats and CFA-inflamed rats. The results were comparable to that of others' [3,4], indicating that the rounded cap probe with a diameter of 3 mm was adequate to induce nociceptive behaviors that were modified by CFA injection.

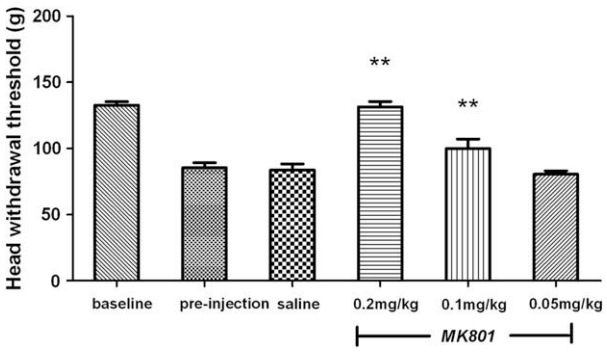

Fig. 6. A comparison of head withdrawal threshold of the right masseter before and after the i.p. injection of MK801 or saline on day 6 following experimental occlusal interference application. $^{**}P < 0.01$, compared to pre-injection data.

4.2. Major findings

In the present study, we designed an occlusal interference animal model by putting a crown on the first maxillary molar. This interference produced long-term (at least one month) bilateral masticatory muscle mechanical hyperalgesia. The application of the appliance itself did not affect the mechanical hyperalgesia, because wearing non-occlusal modifying bands instead of crowns did

not change the muscle nociceptive thresholds. The hyperalgesia was significantly associated with the height of the occlusal alteration; animals with 0.4 and 0.6 mm crowns showed more decreased nociceptive thresholds than those with 0.2 mm crowns, demonstrating a cause and effect relationship between occlusal interferences and muscle pain. There was no significant difference between the groups with 0.6 and 0.4 mm crowns, probably because of a ceiling effect – i.e. the 0.4 mm effect already produced maximal hyperalgesia. The stimulus–response effects we report here suggest that occlusal interference could be an important factor causing chronic masticatory muscle pain.

4.3. Mechanisms by which occlusal interference leads to chronic pain

Clinical trials have found that iatrogenic occlusal interference is a frequent occurrence and may be a direct cause of masticatory muscle pain [47]. Facial pain was reported by volunteers within 3 days after production of a rigid unilateral intercuspid occlusal highspot, and the symptoms remained for at least 6 days [30]. Unfortunately, to our knowledge there has been no investigation of the mechanisms of occlusal interference-related chronic pain using animal models.

Peripherally, fatiguing exercise results in changes in the muscle tissue that includes increases in lactate, ATP, phosphate, creatinine kinase, decreases in pH, and infiltration of neutrophils [5,13,14,35]. Changes in these substances could directly activate nociceptors and increase the release of cytokines and prostaglandins [31,36,46]. Histopathological studies on masticatory muscle following occlusal interference have been reported by other researchers demonstrating extended connective tissue, exudation of inflammatory cells, and hypertrophied muscle fibers [2,7,34]. Although we did not perform histological analyses here, it is possible that muscle fatigue or injury after occlusal interference initiated the occlusal interference-induced mechanical hyperalgesia. However, a peripheral mechanism alone is insufficient to explain all of the features of chronic pain observed here. Occlusal interference-induced mechanical hyperalgesia could not be terminated by removal of occlusal interference appliance (peripheral stimulus). The hyperalgesia persisted and still could be detected until the last day of experiment, three weeks after the removal of the crown. Three weeks would seem to be a prolonged period for muscle recovery, if only peripheral factors are involved. In the first experiment of our present study, tissue injury induced by intramuscular injection of CFA produced mechanical hyperalgesia lasting for only one week.

During the past decades, researches have provided new insights into the difference between the mechanism of acute pain and chronic pain. Acute pain is mainly due to damage or inflammation of peripheral tissues (peripheral sensitization), while chronic pain is considered to be an augmentation of CNS responsiveness (central sensitization). Central NMDA receptors appear to play a major role in central sensitization caused by deep tissue injury or inflammation [8,41,43,44]. MK801, a NMDA receptor antagonist, when given systemically, is able to antagonize NMDA receptor activation in the CNS, and thereby inhibit central sensitization [33,42,52]. Trigeminal central sensitization can also be caused by noxious stimulation of jaw muscles, TMJ and dental tissues, and this sensitization can also be reversed by NMDA antagonists [8,10,54]. Our present finding that systemic injection of MK801 dose dependently attenuates the occlusal interference-induced hyperalgesia, combined with the prolonged time course of pain behavior following occlusal interference suggests that central pain mechanisms in addition to peripheral tissue injury are involved in the occlusal interference-induced pain mechanisms.

4.4. Masticatory muscle pain models and occlusal alteration models

Masticatory muscle pain models have been produced by excessive jaw movements [11,23,49] and by intramuscular injection of algesic agents [3,4]. Hyper-function induced by excessive jaw movements is difficult to produce and may lead to localized hypoxia causing immediate ischemic muscle pain, which usually lasts for only a few minutes [49]. Intramuscular injection of algesic agents do induce masticatory muscle pain, but do not match dental clinical conditions, although the subjective descriptions of the pain produced do resemble those of clinically encountered pain [47]. An animal model for doing research on occlusion-related pain obviously would be more realistic if occlusion alterations could be used to induce the pain. Several methods of occlusal alteration have been extensively used for decades [2,7,16,27,28,34,45], but few of these have been used in pain research [9]. Moreover, most of these protocols had obvious defects. For example, occlusal alteration could not be evaluated quantitatively, or the occlusion modification was too large (0.5–1 mm or more) to mimic the conditions that exist in clinic [2,7,16,27,28,34,45]. Recently, raising the occlusal surface by bonding orthodontic square wire was used. This method could be quantified and easily applied [9,45], but in all these previous models, it was hard to create a sham occlusal interference group to exclude the effect of the appliance itself. In our work, we applied clinical restorative procedures. We put a crown on the maxillary first molar, to establish a model of occlusal interference in rats. The height of the crown could be adjusted and the relationship to occlusal interference could be determined. A band applied with the same restorative procedure could be applied as the sham occlusal interference. The crown had stronger retention than an orthodontic square wire bonded to dental surface, and could be kept in place for the entire 1 month period of experiment. We weighed those animals with occlusal interference for 2 weeks. Compared to sham and non-application controls, we did not find significant weight loss after adding a crown, indicating that the procedure for producing the animal model did not influence the animals' general health.

4.5. Comparison with clinical findings in human

Masticatory muscle pain could be quickly induced in our occlusal interference animal model, and maintained for at least one month, which mimics the clinical condition of occlusion-related pain. Features of occlusion-related chronic masticatory muscle pain in patients include tenderness to palpation of masticatory muscles, complaint of discomfort or pain bilaterally in the orofacial region, and psychogenic phenomena that develops when the pain becomes chronic. In our animal model, the long-term hyperalgesia was induced bilaterally, both in masseter muscles and in temporal muscles. Ideally, a balanced occlusion is in harmony with the TMJ and the masticatory muscles, providing for optimal orthopedic stability. A unilateral occlusal interference may interrupt this balance and change the mandibular intercuspal position, occlusal vertical dimension, and chewing styles bilaterally. Due to this imbalance, masticatory muscles on the contralateral side may also be injured during functional movements. It is not unusual for patients with longstanding pain to become depressed, anxious, and show other psychological problems. The animals with the occlusal interference appliance also appeared to be anxious following the occlusal treatment (data not shown).

Our data strongly indicate that occlusal interference can directly cause long-term masticatory muscle pain in a laboratory animal model. Whether this mechanism accounts for some cases of TMD in humans will need further investigation.

4.6. Summary

We report here an occlusal interference animal model produced by directly bonding a crown to a maxillary molar from male rats. Our results indicate that the experimental occlusal interference produced long-term masticatory muscle hyperalgesia, and that

central sensitization may play an important role in the maintenance of the occlusal interference-induced muscle hyperalgesia. The animal model described here mimics clinical masticatory muscle pain and provides a method to further investigate mechanisms of occlusion-related muscle hyperalgesia, and to explore possible pain management strategies.

Conflicts of interest

The authors declare no conflicts of interest.

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