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Inflammatory pain memory facilitates occlusal interference-induced masticatory muscle hyperalgesia in rats

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Abstract

Background: Patients with an orofacial pain history appear to be more susceptible to occlusal interference pain in dental practice for unknown reasons. Pain memory has a critical function in subsequent pain perception. This study aims to explore whether orofacial pain memory could affect the masticatory muscle pain perception for occlusal interference.

Methods: Cross-injection of 2% carrageenan into bilateral masseters in male rats was carried out to establish the inflammatory pain memory model. The effects of pain memory on masseter muscle nociception were tested by applying crowns with heights beyond the occlusal plane by 0.2 or 0.4 mm onto a maxillary molar 2 weeks after inflammation in the right masseter. The 0.4-mm crowns were removed on day 2 or day 4 after application to further confirm the effects of pain memory. Moreover, memory impairment was established using ibotenic acid (IBO) infusion into the bilateral hippocampus, followed by behaviour tests, including the Morris water maze test and the locomotor activity test. The relationship between pain memory and occlusal interference-induced masseter muscle pain perception was subsequently re-examined. The head withdrawal thresholds of masseters on both sides were measured to reflect the perception.

Results: Inflammatory pain memory aggravated the 0.2-mm crown-induced mechanical hyperalgesia of the masseters, but not in the 0.4-mm crown group. However, the recovery of the 0.4-mm crown-induced mechanical hyperalgesia was postponed. The effects of pain memory were reversed in rats with impaired mnemonic function of the hippocampus.

Conclusions: Inflammatory pain memory facilitated occlusal interference-induced masseter muscle pain.

1. Introduction

Patients with an orofacial pain history appear to be more susceptible to occlusal interference pain in dental practice. Pain is an aversive experience and refractory in clinical practice. Previous pain experience can lead to the development of pain memory and influence subsequent perception (Albanese et al., 2007). The definition of memory is the retention of information that modifies future behavioural or neuronal responses. Most phantom limb pain patients have experienced pain in the limb prior to amputation (Giummarra et al., 2011; Nikolajsen et al., 2013). Pain memories resulting from long-lasting preamputation pain are powerful elicitors of

What's already known about this topic?
- Pain memory has a critical function in subsequent pain perception.
- Patients with orofacial pain history appear to be more susceptible to occlusal interference pain in dental practice.

What does this study add?
- Inflammatory pain memory could facilitate occlusal interference-induced masticatory muscle pain. The facilitatory effects could be reversed when the hippocampal memory function is impaired.
- A reasonable explanation is provided for the sensitivity to occlusal interference of patients with orofacial pain history.

phantom limb pain (de Roos et al., 2010). What people remember about previous painful events, such as paediatric pain (Noel et al., 2012a), visceral pain (Gramsch et al., 2014), injections (Noel et al., 2012b) and surgery (Costantini et al., 2011; Burgoyne et al., 2012), influences future pain perception. In animals, pain memory is presented and measured through changes in performance or behaviour after prior exposure rather than through recalling past pain in words. Rats with a history of inflammatory pain present an increase in the second phase of formalin-induced pain behaviour (Li et al., 2012a).

The hippocampus has a pivotal role in mnemonic function (Kennedy and Shapiro, 2004; Li et al., 2012b). This function has been widely studied in several clinical reports that involved memory deficiency in patients after bilateral excision or hippocampal lesions (Scoville and Milner, 1957; Zola-Morgan et al., 1986). Previous studies have indicated that damage in the hippocampus is sufficient to produce memory impairment in human or animal models (Zola-Morgan et al., 1986, 1992; Winocur et al., 2013). To conduct studies on behavioural manifestations or molecular mechanisms, hippocampal lesions are typically induced by chemicals, which is a common method used in addition to the more recent transgenic animals (Gimbel et al., 2010; Sondergaard et al., 2012).

Clinically, the reaction to occlusal interference may vary in patients with different medical histories. Subjects without temporomandibular disorder (TMD) history have been reported to adapt fairly well to experimental occlusal interference, whereas those with TMD history (especially with an orofacial pain history) showed significant increase in clinical signs (Le Bell et al., 2002, 2006). Orofacial pain is a frequent reaction to occlusal interference. Therefore, the question arises as to whether a preceding pain experience can exacerbate occlusal interference-induced masticatory muscle hyperalgesia.

We have previously developed an animal model of occlusal interference with crowns higher than the occlusal plane after bonding onto the right maxillary first molar, which produced long-term masticatory muscle hyperalgesia (Cao et al., 2009, 2013). In the present study, we used this occlusal interference model in addition to an inflammatory pain memory model developed by another group (Kissin et al., 2006) to show that the preceding inflammation could generate pain memory and subsequently facilitate occlusal interference-induced masticatory muscle hyperalgesia. Moreover, this facilitatory effect was reversed when the hippocampal memory function was damaged.

2. Materials and methods

2.1 Animals and occlusal interference model

The experimental protocol was approved by the Animal Care and Use Committee of Peking University (Beijing, China), and was consistent with the Ethical Guidelines of the International Association for the Study of Pain.

The methods used to produce occlusal interference have been described previously in detail (Cao et al., 2009, 2013). Although orofacial pain is more frequent in women than in men (Carlsson, 1999), the roles of estrogens in pain remain controversial (Craft, 2007). To exclude the complicated effects of female hormones, only male Sprague–Dawley (SD) rats (180–200 g; Academy of Military Medical Sciences, Beijing, China) were used. All rats were housed under controlled temperature (22 ± 1 °C) conditions with a 12 h/12 h light/dark cycle; food and water were available *ad libitum*. Crowns with heights beyond the occlusal plane by 0.2 or 0.4 mm were bonded onto the right maxillary first molar using adhesive bonding material (Panavia F; Kuraray Co., Osaka, Japan) for the occlusal interference groups.

2.2 Measurement of head withdrawal threshold

The head withdrawal threshold was measured with a modified electronic von-Frey anesthesiometer (Bioseb, FL, USA) as previously reported (Cao et al., 2009, 2013). We chose the masseter muscle areas as

the test region. The force was recorded automatically until the head was withdrawn. The head withdrawal threshold was calculated as the mean ± standard deviation (SD), based on five measurements per point. For all data reported in this study, the observer was blinded to the treatments used.

2.3 Experiment design

2.3.1 Inflammatory pain memory model

We established the inflammatory pain memory model by two cross-over injections of 2% carrageenan (CA) into the bilateral masseter muscles by a 2-week interval based on a previous pain memory model induced in the hindpaws (Kissin et al., 2006). Two groups with six rats per group were injected with 100 μL of 2% CA or 0.9% saline into the right masseter on day 0. On day 15, all rats were injected with 100 μL of 2% CA into the left masseter. The naïve group received no injection. The head withdrawal threshold was measured by testing the bilateral masseters before injection and at 5 h, 12 h, 1 days, 3 days, 5 days, 7 days and 14 days after the first and second injections. The experimental procedure is illustrated in Fig. 1A.

2.3.2 Effects of inflammatory pain memory on occlusal interference-induced masseter muscle hyperalgesia

Rats were randomly divided into six groups, namely, A, B, C, D, E and F, with six rats in each group. On day 0, groups B, C and D were injected with 100 μL of 2% CA into the right masseter, whereas groups E and F were injected with 100 μL of 0.9% saline into the right masseter. On day 15, groups C and E received 0.2-mm crowns for occlusal interference, whereas groups D and F received 0.4-mm crowns for occlusal interference. For Group B, the mouth was only kept open for 3 min. The group A comprised naïve controls. The head withdrawal threshold was measured by testing the bilateral masseters before injection and at 5 h, 12 h, 1 days, 3 days, 5 days, 7 days, 14 days, 16 days, 18 days, 20 days, 22 days, 25 days, 29 days, 36 days and 43 days after the injection. The experimental procedure is illustrated in Fig. 2A.

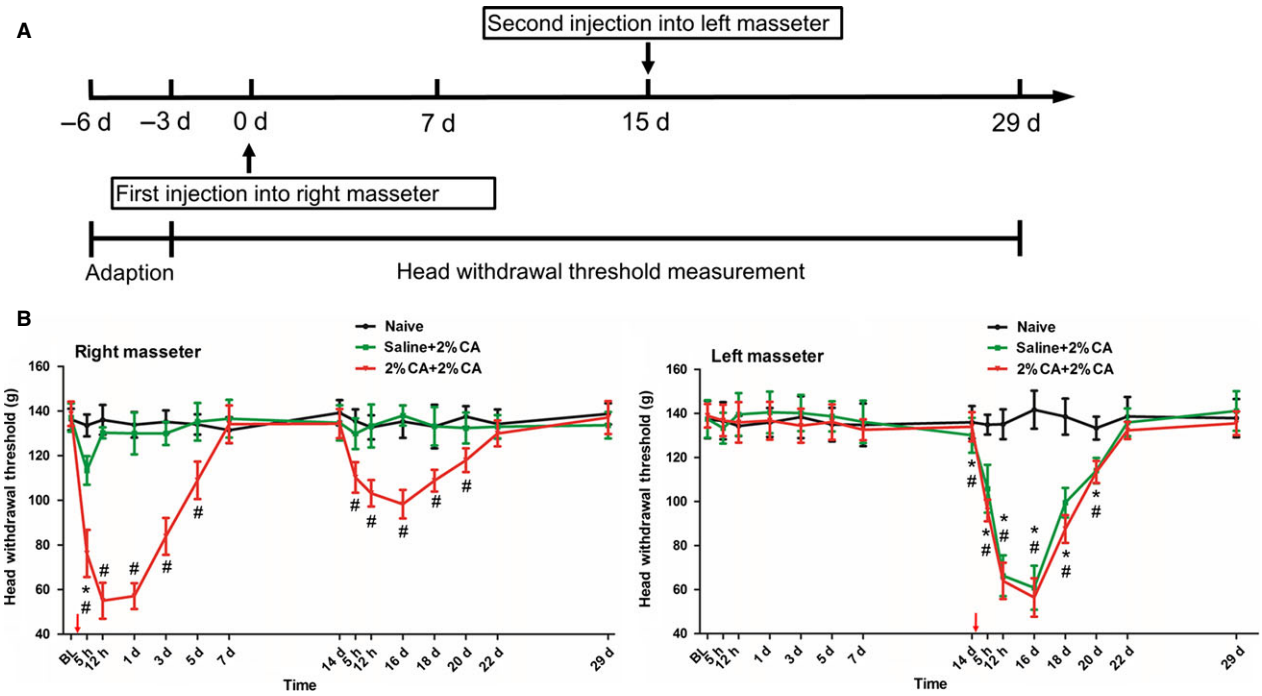

Figure 1 The inflammatory pain memory model was prepared by cross-injections of 2% carrageenan into masseter muscles. (A) Schematic graph of time course. (B) The time course of the head withdrawal threshold on both sides following cross-injections of 2% carrageenan. *$p < 0.05$, comparison between saline + 2% CA group and naïve control; #$p < 0.05$, comparison between 2% CA + 2% CA group and naïve control; $n = 6$. Red arrowhead: injection into masseter muscle.

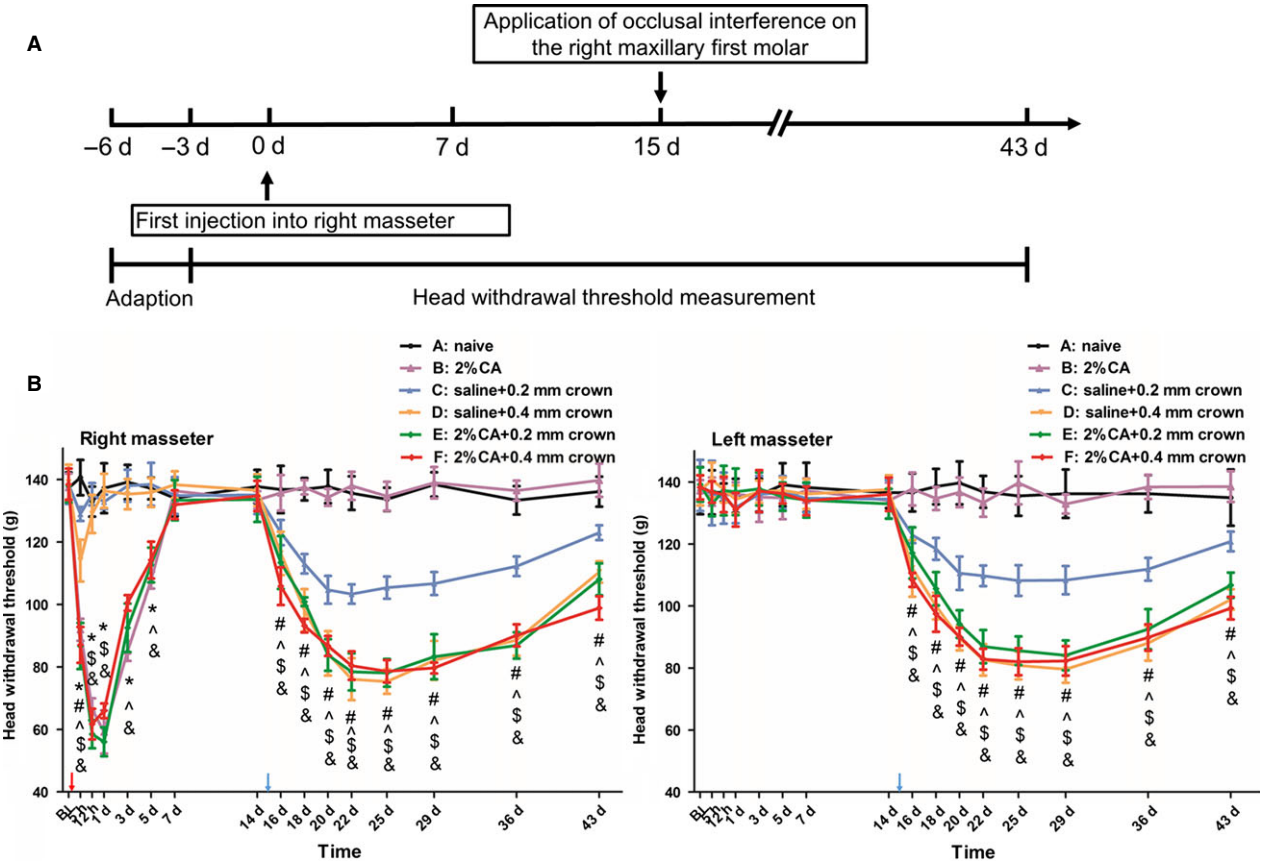

Figure 2 Inflammatory pain memory exacerbated occlusal interference-induced mechanical hyperalgesia of masseter muscles. (A) Schematic graph of time course. (B) The time course of the head withdrawal threshold on both sides following inflammation and different degrees of occlusal interference. $^{*}p < 0.05$, comparison between groups A and B; $^{\#}p < 0.05$, comparison between groups A and C; $^{\wedge}p < 0.05$, comparison between groups A and D; $^{\$}p < 0.05$, comparison between groups A and E; $^{\&}p < 0.05$, comparison between groups A and F; $n = 6$. Red arrowhead: injection into masseter muscle; blue arrowhead: application of occlusal interference.

2.3.3 Effects of inflammatory pain memory on masseter muscle hyperalgesia following removal of occlusal interference

The rats were randomly divided into five groups, namely, A, B, C, D and E, with six rats in each group. Group A were the naïve controls. On day 0, groups B and C were injected with 100 μL of 0.9% saline into the right masseter, whereas groups D and E were injected with 100 μL of 2% CA into the right masseter. On day 15, the rats of groups B, C, D and E received 0.4-mm crowns for occlusal interference. The occlusal interference appliance was removed on day 17 (2 days after wearing the crowns) for groups B and D, and on day 19 (4 days after wearing the crowns) for groups C and E. The head withdrawal threshold was measured by testing the bilateral masseters before injection and at 5 h, 12 h, 1 days, 3 days, 5 days, 7 days, 14 days, 16 days, 18 days, 20 days, 22 days, 25 days, 29 days, 36 days and 43 days after the injection. The experimental procedure is illustrated in Fig. 3A.

2.3.4 Effects of inflammation pain memory on occlusal interference-induced masticatory muscle hyperalgesia after memory impairment

2.3.4.1 Induction of memory impairment

The model of memory impairment was established by injection of ibotenic acid (IBO) into the bilateral hippocampus as in previous studies (Heo et al., 2009; Babaei et al., 2012). Rats were anesthetized and positioned in a stereotaxic instrument. Two guide cannulas were bilaterally inserted into the hippocampal region according to the stereotaxic atlas (Paxinos and Watson, 1997) and our previous report (Wu et al., 2010). The rats were allowed to recover from surgery for 7 days. IBO (1 μg/μL; Sigma, St. Louis, MO, USA) or vehicle (PBS) injections were

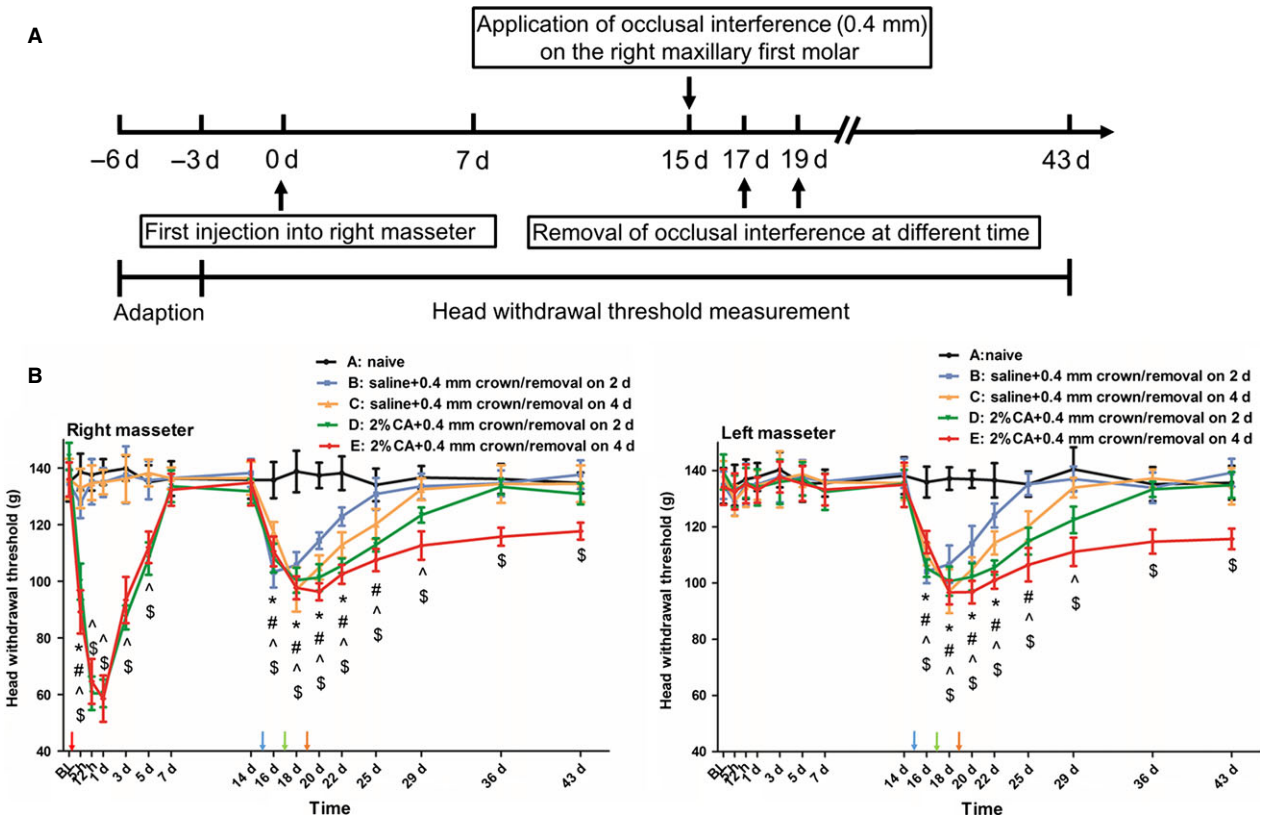

Figure 3 Inflammatory pain memory prolonged or blocked the recovery of mechanical hyperalgesia when the occlusal interference of 0.4 mm was removed. (A) Schematic graph of time course. (B) The time course of the head withdrawal threshold on both sides following inflammation and 0.4-mm occlusal interference, which was removed at different times. *$p < 0.05$, comparison between groups A and B; $^{\#}p < 0.05$, comparison between groups A and C; $^{\wedge}p < 0.05$, comparison between groups A and D; $^{\$}p < 0.05$, comparison between groups A and E; $n = 6$. Red arrowhead: injection into masseter muscle; blue arrowhead: application of occlusal interference; green arrowhead: removal of crowns on day 2 after occlusal interference; orange arrowhead: removal of crowns on day 4 after occlusal interference.

performed with a micromanipulator mounted with two 10-μL Hamilton microsyringes. Bilateral infusions of 5 μL of IBO or the vehicle were simultaneously conducted over 5 min. The dose of IBO was based on a previous report (Babaei et al., 2012). The injection cannulas were left in place for an additional 3-min period to prevent back diffusion of the solution. After 2 weeks, the subsequent behaviour tests were conducted.

2.3.4.2 Morris water maze task

The Morris water maze test was conducted and modified according to a previous report (Morris et al., 1982). A circular pool with black-painted inner surface (160 cm in diameter, 50 cm in height) and a black escape platform (10 cm in diameter, 30 cm in height) were used. The acquisition phase was carried out for five consecutive days with warm water (22 ± 1 °C) filled to 1.0 cm above the platform. The time latency to reach the platform was recorded. The probe trial was conducted on the sixth day, wherein the platform was removed. Rats were given a single 60-s period to find the target. The time spent in the target quadrant and the numbers of target annulus crossovers were measured. The visible platform test was conducted on the seventh day. The platform was then returned to the previous position 1.0 cm below the water. All experimental sessions were video recorded.

2.3.4.3 Locomotor activity test

All rats were subjected to a locomotor activity test after the Morris water maze test. Locomotor activity was measured by an automated video tracking system (DigBehv-LG, Shanghai, China) with four activity chambers (49 cm × 49 cm × 59 cm) situated in sound-attenuating cabinets (Liu et al., 2012; Li et al., 2014a). Horizontal locomotor activity was recorded by a monochrome video camera mounted at the top of each chamber. The video data were analysed by

the DigBehv analysis software. The test was recorded for 60 min for each animal.

2.3.4.4 Head withdrawal threshold assessment

Group A was composed of naïve controls. Rats with IBO infusion were divided randomly into groups B and C, whereas rats with vehicle infusion were divided randomly into groups D and E. Rats that failed to present memory impairment in the IBO group were excluded. On day 0, groups B and D were injected with 100 μL of 2% CA into the right masseter, whereas groups C and E were injected with 100 μL of 0.9% saline. Two weeks after the injection, i.e. on day 15, the rats of groups B, C, D and E received 0.2-mm occlusal interference. The head withdrawal threshold was measured by testing the bilateral masseters before injection and at 5 h, 12 h, 1 days, 3 days, 5 days, 7 days, 14 days, 16 days, 18 days, 20 days and 22 days after the injection.

2.4 Statistical analysis

Statistical analyses were performed with SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA). All data were expressed as mean ± SD. Time course measures for the head withdrawal threshold among groups and the time course for escape latency in the Morris water maze test were compared by repeated measures ANOVA, followed by a Bonferroni *post hoc* test. Subsequently, multiple covariance analyses were used for pair-wise comparisons among different groups at each time point followed by a Bonferroni *post hoc* test. Differences between the bilateral sides at each time point in each group were compared by a paired *t*-test. Differences between groups in the Morris water maze test and the locomotor activity test were examined by one-way ANOVA followed by an LSD *post hoc* test. A value of $p < 0.05$ was considered to indicate statistical significance.

3. Results

3.1 Inflammatory pain memory generated by two cross-injections of CA into masseter muscles

We first assessed whether inflammatory pain memory could be generated after injection of 2% CA into the masseter. No significant change in the head withdrawal threshold was noted on either side during the whole test in the naïve group as compared with the baseline. The head withdrawal threshold by testing the right masseter (injected side) markedly decreased from 5 h (the time started to measure) until day 5 after the first injection of 2% CA ($p < 0.01$); this value had recovered to the baseline on day 7 ($p = 0.659$). However, the head withdrawal threshold was not changed as evidenced by testing the left masseter after the first 2% CA injection. A slight, transient reduction in the head withdrawal threshold at 12 h was observed by testing the right masseter following the first injection of saline ($p < 0.01$). No significant change in the head withdrawal threshold was observed later by testing the bilateral masseters until day 14 (Fig. 1B).

The second injection of 2% CA or saline into the left masseter was performed on day 15 after the first injection. The change in head withdrawal threshold by testing the left masseter was similar to that by testing the right masseter after the first injection of 2% CA or saline into the right masseter, i.e. decreased from 5 h until day 5 post-second injection (day 15 and 5 h until day 20 post-first injection; $p < 0.01$), and recovered to baseline on day 7 post-second injection (day 22 post-first injection; $p = 0.532$). However, the head withdrawal threshold decreased again in the group that received the first injection of 2% CA, which had already recovered to baseline; the observed decrease was less than the previous value (Fig. 1B), whereas the head withdrawal threshold by testing the right masseter in the group that received the first injection of saline showed no change.

3.2 Inflammatory pain memory exacerbated occlusal interference-induced masseter muscle mechanical hyperalgesia

To determine whether pain memory could affect the masseter muscle hyperalgesia caused by occlusal interference, we bonded crowns of 0.2 or 0.4 mm (representing two severities of occlusal interference) onto the right maxillary first molar on day 15 after the injection of CA into the right masseter when no hyperalgesia was observed after 1 week. As shown in Fig. 2B, the head withdrawal threshold tested on both the right and left masseters of the groups with injection of saline decreased sharply and bilaterally after occlusal interference (0.2 or 0.4 mm) as compared with the baseline ($p < 0.05$), but the 0.4-mm crowns induced greater mechanical hyperalgesia than the 0.2-mm crowns ($p < 0.01$). These results were similar to our previous report (Cao et al., 2009). For the groups injected with 2% CA, the 0.2-mm crowns induced the head withdrawal

thresholds, as tested on both the right and left masseters, which were significantly lower than that of the saline group with 0.2-mm crowns ($p < 0.01$) and comparable to that of the saline group with 0.4-mm crowns. These results suggest that the previous inflammatory pain exacerbated the occlusal interference-induced masseter hyperalgesia. However, no significant differences in the head withdrawal thresholds appeared among the saline group with 0.4-mm crowns and the 2% CA groups with 0.2- or 0.4-mm crowns ($p > 0.05$). No significant differences in the head withdrawal thresholds were found between the right masseters and the contralateral sides after the occlusal interference ($p > 0.05$).

3.3 Inflammatory pain memory delayed or blocked recovery of mechanical hyperalgesia when occlusal interference was removed

We designed an additional experiment to examine whether the previous inflammatory pain would affect the recovery from 0.4-mm crown-induced hyperalgesia after the crown was removed (Fig. 3A). Our preliminary experiment suggested that masseter muscle hyperalgesia could recover back to normal, if the occlusal interference (0.4-mm crown) was removed within 6 days (Li et al., 2014b). Consequently, we removed the crowns at two time points (days 2 and 4 post-bonding). As shown in Fig. 3B, the head withdrawal threshold of the saline group with crowns removed on day 2 post-bonding had recovered to the baseline level on day 10 post-bonding or day 25 post-injection, whereas the head withdrawal threshold of the saline group with crowns removed on day 4 post-bonding had returned back to baseline on day 14 post-bonding or on day 29 post-injection. However, the head withdrawal threshold of the CA group with crowns removed on day 2 post-bonding had recovered to baseline on day 21 post-bonding or day 36 post-injection, which was 11 days later than the saline group with crowns removed on day 2 post-bonding. Moreover, the head withdrawal threshold in the CA group with crowns removed on day 4 post-bonding continued to decrease until day 43 at the end of the experimental period ($p < 0.05$; Fig. 3B). No significant differences in head withdrawal threshold were found between the masseters of both sides after occlusal interference ($p > 0.05$).

Based on the above results, the preliminary conclusion is that inflammatory pain memory facilitated occlusal interference-induced masseter muscle pain. We raised another hypothesis as to whether this facilitatory effect would vanish if the memory function was destroyed. To test this, we carried out the following experiments (Fig. 4A).

3.4 Memory deficit was successfully established by injecting IBO into hippocampus

In the Morris water maze task, the IBO-injected rats moved more irregularly and took significantly more time to locate the platform in the hidden platform trial (Supporting Information Fig. S1A). The IBO group showed a longer escape latency than the naïve and vehicle rats. During the subsequent probe trial, the swimming tracks of the naïve and vehicle groups were concentrated in the target quadrant, whereas those of the IBO group were scattered (Supporting Information Fig. S1B), suggesting that the naïve and vehicle rats spent more time in the target quadrant than the IBO group (Supporting Information Fig. S1C). Moreover, the frequency of swimming across the target annulus in both the naïve and vehicle groups was higher than that in the IBO group by almost four times (Supporting Information Fig. S1D). Statistical analysis showed no significant differences in the escape latency for the visible platform trial (Supporting Information Fig. S1E).

3.5 Memory deficit did not affect locomotor activity

Four indices of movement (total distance, total movement, movement time and movement velocity) over a 60 min-period were measured to determine whether IBO injection into the hippocampus affected the spontaneous locomotor activity in rats. No statistically significant differences were noted in any of the above-mentioned indices (Supporting Information Fig. S2A–D). These results indicated that IBO injection had no effect on locomotion in rats.

3.6 Memory impairment reversed the facilitatory effect of inflammatory pain memory on occlusal interference-induced hyperalgesia

To confirm whether the destruction of memory reversed the facilitatory effect, we repeated the injection of 2% CA into the right masseter and the application of 0.2-mm occlusal interference in the IBO rats as previously described (two rats were excluded because of failure in memory impairment). After the 2% CA injection, the head withdrawal threshold significantly decreased, as verified by testing the right masseter (injection side) in the memory impairment group; however, this decrease was less than that of

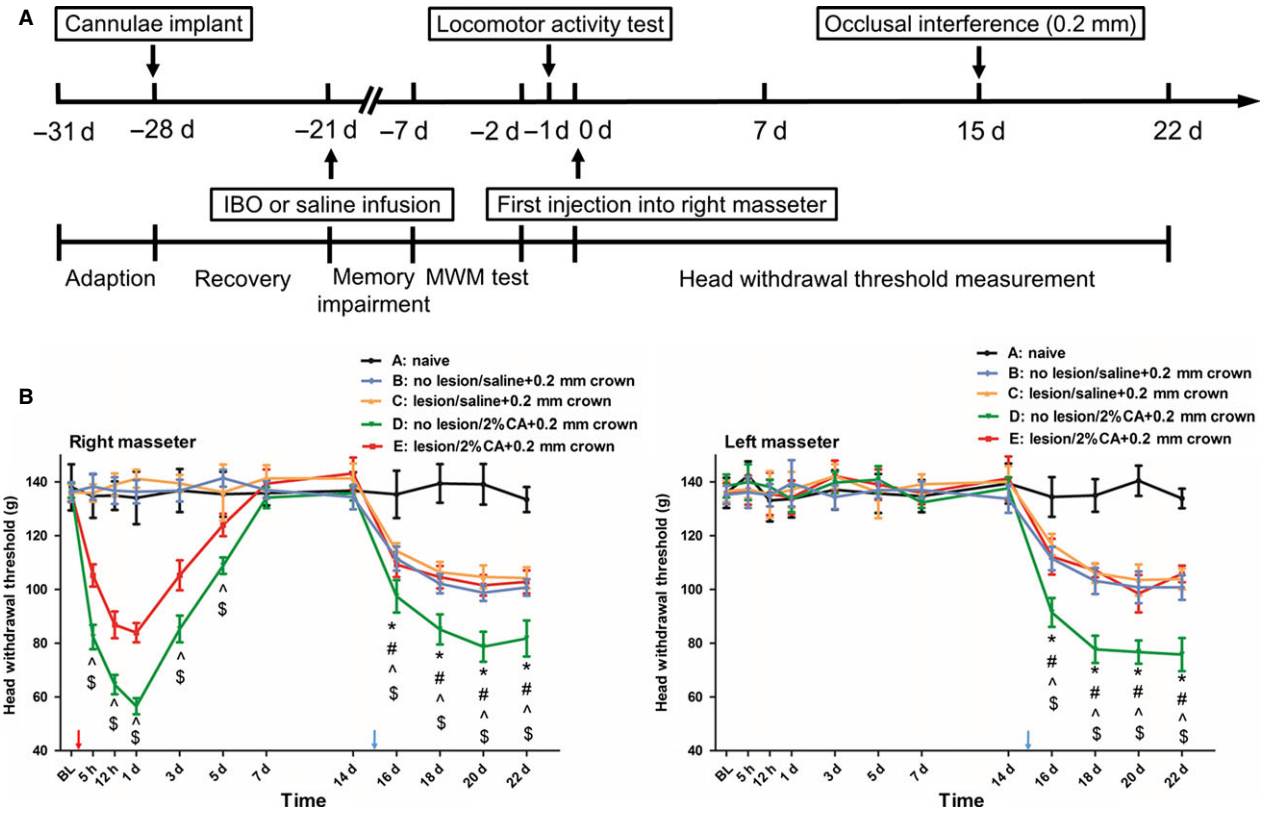

Figure 4 Memory impairment reversed the facilitatory effect of inflammatory pain memory on masseter muscle hyperalgesia induced by occlusal interference. (A) Schematic graph of experiment design. (B) The time course of the head withdrawal thresholds on both sides following inflammation and occlusal interference of 0.2 mm under conditions of memory impairment or not. $^{*}p < 0.05$, comparison between groups A and B ($n = 7$); $^{\#}p < 0.05$, comparison between groups A and C ($n = 7$); $^{\wedge}p < 0.05$, comparison between groups A and D ($n = 6$); $^{\$}p < 0.05$, comparison between groups A and E ($n = 6$). Lesion: impairment of hippocampus memory function; no lesion: no impairment of hippocampus memory function; red arrowhead: injection into masseter muscle; blue arrowhead: application of occlusal interference.

the group without memory impairment ($p < 0.05$). In addition, the decrease was comparable to that of head withdrawal threshold in the no memory impairment group with 0.6% CA injection (Supporting Information Methods and Results in S1; Supporting Information Fig. S3). The head withdrawal threshold as measured by testing the left masseter showed no significant change from that of the right masseter in the memory impairment group after saline injection (Fig. 4B). When 0.2-mm occlusal interference was applied, the head withdrawal threshold of the no memory impairment groups with 2% CA or saline injection demonstrated a consistent trend, as previously observed. For the memory impairment groups, the 0.2-mm occlusal interference bilaterally induced a head withdrawal threshold that was lower than that of the naïve group, but higher than that of the no memory impairment group with 2% CA injection ($p < 0.05$). These results suggested that memory impairment could abolish the facilitation effect of previous pain on occlusal interference-induced hyperalgesia. Although a higher concentration of CA was injected, the stronger facilitatory effect on occlusal interference-induced hyperalgesia was produced. After applying 0.2-mm occlusal interference, the head withdrawal threshold in the no memory impairment group with 0.6% CA injection decreased to a value lower than that of the memory impairment group with 2% CA injection ($p < 0.05$), thereby suggesting that the 0.6% CA injection-induced inflammatory pain could still facilitate the 0.2-mm occlusal interference-induced masseter muscle pain (Supporting Information Fig. S3). No significant difference in the head withdrawal threshold was detected between the left and right side after occlusal interference ($p > 0.05$).

4. Discussion

In the present study, we provided several lines of evidence to show the facilitatory effects of pain memory on occlusal interference-induced masseter

muscle pain. To the best of our knowledge, our study is the first to associate pain memory with orofacial pain in an animal model.

Pain memory can be an important regulator of the perception of occlusal interference-induced masseter muscle pain. Pain memory has a key function in the later life of an individual (Chen et al., 2000; Liu et al., 2012; Noel et al., 2012a). However, its role in occlusal interference-induced masseter muscle pain has not yet been defined. Consequently, we examined the head withdrawal threshold of both masseters after the cross-induction of muscle inflammation. We observed that pain memory was formed after the first induction of inflammation in the right masseter because the recovered head withdrawal threshold of the right masseter decreased upon the second induction of inflammation in the left masseter. This model was consistent with previous observations after cross-injection of 2% CA into the hind paws (Kissin et al., 2006). We further showed that this carrageenan-induced inflammatory pain memory facilitated the occlusal interference-induced masseter muscle hyperalgesia. Our results were consistent with previous studies, which showed that the later perception of pain is associated with the previous inflammatory stimulation in a model of repeated inflammatory pain in the hind paws (Kayser et al., 1998; Li et al., 2012a). Our results were also in good agreement with results of the clinical trials, which showed that children who displayed great distress at lumbar punctures experienced aggravated distress during subsequent lumbar punctures (Chen et al., 2000); their direct experience of pain intensity and their adult behaviour during venipuncture were related to their memories of past procedures (Noel et al., 2010). Unlike the previous studies which gave the same stimulus, we used two different stimuli, i.e. inflammatory stimulus followed by occlusal interference. Our results also showed that the facilitatory effect of pain memory could be exerted by different types of stimuli, even though the previous painful stimulus was removed. Therefore, in dental practice, more attention should be given to the patients with previous orofacial pain history during treatment especially when occlusal adjustment is involved.

The facilitatory function of pain memory in occlusal interference-induced masseter muscle pain was also reflected into the postponed recovery of hyperalgesia. We observed that a previous injection of carrageenan into the right masseter did not further enhance the 0.4-mm crown-induced hyperalgesia. We considered this phenomenon a ceiling effect, i.e. the 0.4-mm occlusal interference had already produced the maximal degree of hyperalgesia, and the facilitatory effect of pain memory was overlapped. Therefore, we explored whether previous injection of carrageenan could have any effect on recovery of occlusal interference-induced hyperalgesia. The results showed that the carrageenan injection postponed the recovery of 0.4-mm occlusal interference-induced mechanical hyperalgesia, another profile of the facilitatory effect of pain memory. Our results were consistent with previous clinical observations, which showed that phantom limb pain is established and persists after acute traumatic amputation or chronic disease-caused amputation (Montoya et al., 1998; Schley et al., 2007).

The hippocampus has an important role in pain memory; its involvement in memory formation is well known. Hippocampal memory impairment was confirmed by the Morris water maze and locomotor activity after the infusion of IBO acid into the bilateral hippocampus; similarly, the injection of 2% CA into the right masseter in rats with hippocampal lesions lessened the decrease in the head withdrawal threshold, i.e. less hyperalgesia. These results were consistent with previous observations in the patients showing amnesia and reduced pain after extensive bilateral hippocampectomy (Scoville and Milner, 1957; Gol and Faibish, 1967). Moreover, the facilitation effect of a previous inflammatory pain experience on the occlusion interference-induced mechanical hyperalgesia of masseter muscles was also abrogated or reversed in the memory-impaired rats. We excluded the possibility that this abrogation was caused by the decreased hyperalgesia of 2% CA injection in the memory impairment rats because the facilitation effect of previous pain memory was still observed in the nonmemory-impaired rats with 0.6% CA injection, which produced similar hyperalgesia to that of 2% CA injection in the memory-impaired rats. Therefore, the memory function of the hippocampus is involved in the previous inflammatory pain's facilitation of the occlusal interference-induced hyperalgesia. These data also provided a new profile for the memory function of the hippocampus involved in the pain experience.

However, the underlying mechanism of the memory function of the hippocampus, which is involved in the exacerbating effect of a preceding pain experience on the occlusal interference-induced masseter muscle hyperalgesia remains unknown. A possible explanation is that the long-term potentiation (LTP) in the hippocampus might be involved. LTP is expressed as a persistent increase in the size of the synaptic component of the evoked response (Bliss

and Collingridge, 1993). Since LTP was first observed in the hippocampus (Bliss and Gardner-Medwin, 1973; Bliss and Lomo, 1973), it has been found in all excitatory pathways of the structure (Morris et al., 1990; Doyere and Laroche, 1992; Bliss and Collingridge, 1993). LTP could be a prominent property of hippocampal neurons, although it has also been found in several other brain regions. Nevertheless, LTP is universally considered a fundamental neuronal model of learning and memory formation (Bliss and Collingridge, 1993). Although the hippocampus is generally not considered an important structure related to pain, its involvement in pain has been evidenced in humans (Scoville and Milner, 1957) and animals (Gol et al., 1963; McKenna and Melzack, 1992; Soleimannejad et al., 2006, 2007). Extensive hippocampectomy results in reduction in pain in the patients with intractable pain; similar results were also observed in animals (Gol et al., 1963; McKenna and Melzack, 1992; Soleimannejad et al., 2007). Therefore, we speculated that the hippocampus might function as an 'amplifier' in the pain pathway, as facilitated by the LTP of synaptic transmission. To explain the role of the hippocampus concretely in the present study, we speculated the following reflex arc of head withdrawal: a preceding nociceptive stimulus, such as inflammation in the masseter, first activates the peripheral nociceptors; signals are then sent from these nociceptors to the trigeminal ganglion (first-order neuron) to be relayed by the spinal trigeminal nucleus (second-order neuron) and the thalamus (third-order neuron); these signals somehow reach the hippocampus to be amplified and also stored as the so-called pain memory through LTP, and finally interpreted by the cortex as pain; the application of a second nociceptive stimulus, such as occlusal interference triggers a prolonged increase in the excitability and synaptic efficacy of hippocampal neurons to make the animals much more sensitive to the stimuli, as shown by the lower head withdrawal threshold in the present study.

To some extent, this hypothesis might explain the memory function of the hippocampus for facilitating pain perception in the present study. The possible reflex arc of head withdrawal is summarized in Supporting Information Fig. S4. However, much detail remains to be elucidated, including whether the hippocampus receives impulses from the thalamus before it sends them to an unidentified part of the cortex, among others.

Although orofacial pain is more frequent in women than in men (Carlsson, 1999), the present study solely explored the effects of pain memory in male rats. This could be one defect of our study, which ideally should have been performed also in female rats, or in both female and male rats. However, male rats alone were used because of the following concerns. First, the effect of pain memory was not easy to evaluate, as demonstrated by the complex design of the experiments in the present study. Second, the roles of estrogens in pain remain controversial (Craft, 2007), which would complicate the effects of pain memory to be evaluated. Third, serum estradiol levels are difficult to control through the menstrual cycle in rats or via estradiol replacement; more experience with this kind of study is still needed. Therefore, a future similar study on female rats is necessary to fully evaluate the effects of a preceding pain experience on occlusal interference.

In our study, the injection of 2% CA into the right masseter could only produce ipsilateral hyperalgesia. Our results are consistent with previous studies, which also showed that a lower concentration of CA does not induce hyperalgesia in the contralateral side (Kissin et al., 2006; Yokoyama et al., 2007). Therefore, the subsequent decrease in the head withdrawal threshold of the left masseter after the occlusal interference was caused by pain memory, rather than the long-term contralateral effect of 2% CA injection into the right masseter.

In conclusion, our study demonstrated that inflammatory pain memory facilitated occlusal interference-induced masseter muscle pain. These results may help explain why patients with orofacial pain history are susceptible to occlusal interference and lead to the development of a novel strategy to deal with this clinical issue.

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Author contributions

T.T.D., X.X.X., Y.C. and C.R.L. carried out the animal and laboratory experiments. T.T.D., X.X.X. and Y.C. contributed to the data analysis. T.T.D. wrote a draft of the manuscript. Q.F.X. and Y.H.G. contributed to the concept and design of the study, the coordination of all experiments and critical review of the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. IBO injection into the hippocampus impaired the memory function of SD rats in the MWM. Escape latency in the hidden platform test during a 60-s session was measured over 6 days.

Figure S2. Memory deficit did not affect locomotor activity in the open field test.

Figure S3. The time course of the head withdrawal threshold on both sides following different degrees of inflammation and 0.2-mm occlusal interference.

Figure S4. Schematic diagram of head withdrawal reflex arc.

Methods S1: We observed that 2% CA injection induced lower hyperalgesia when hippocampus was impaired, so we considered to figure out whether this lower hyperalgesia contributed to the later abolishment of facilitatory effect. We used a series of concentrations of CA to find out the same level of inflammatory pain perception with that induced by 2% CA injection in memory impairment rats and observe the changes of facilitatory effect. Rats were divided into five groups, named A, B, C, D and E, with six rats in each group. Group A were naïve controls. On day 0, groups B, C, D and E were injected with 100-μL CA into the right masseter, with concentration of 0.3%, 0.6%, 0.9% and 1%, respectively. On day 15, the rats of groups B, C, D and E received 0.2-mm crowns for occlusal interference. Group F was memory impairment rats with 2% - CA injection. The head withdrawal threshold was measured by testing the bilateral masseters before injection, and 5 h, 12 h, 1 day, 3 days, 5 days, 7 days, 14 days, 16 days, 18 days, 20 days and 22 days after the injection.
Results S1: The head withdrawal threshold of naïve group showed no significant changes. The head withdrawal threshold by testing the right masseter (injected side) was decreased from 5 h (the time started to measure) till day 5 after the first injection of CA with different concentrations ($p < 0.01$), and recovered to the baseline on day 7 ($p > 0.05$). The level of hyperalgesia revealed concentration-related effects, i.e. the head withdrawal thresholds decreased more lowly as the concentration of CA increased gradually. We found that 0.6% CA-induced hyperalgesia was comparable to that induced by 2% CA injection in memory impairment rats. However, we found that 0.2-mm occlusal interference induced the head withdrawal threshold tested on both right and left masseters significantly to be lower in nonmemory impairment group with 0.6% CA injection than memory impairment group with 2% CA injection ($p < 0.05$). These results suggested that 0.6% CA-induced inflammatory hyperalgesia still could facilitate 0.2-mm occlusal interference-induced masseter muscle hyperalgesia, suggesting that the abolishment of facilitatory effect in memory impairment group with 2% CA injection was mainly due to pain memory impairment rather than lower inflammatory hyperalgesia.