Mechanical and Thermal Hypersensitivities Associated with Orthodontic Tooth Movement: A Behavioral Rat Model for Orthodontic Tooth Movement–Induced Pain

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Aim: To test whether orofacial mechanical and thermal hypersensitivities occur in rats during orthodontic tooth movement (OTM). **Methods:** Sprague-Dawley rats (140 to 160 g) were divided into an experimental (E) group (n =7), with an active orthodontic spring placed in the right side of their mouth, and a sham (S) group (n = 7), with an inactive orthodontic spring. Mechanical sensitivity was tested preoperatively (1 day before attaching the orthodontic spring) and postoperatively (1 hour, 3 hours, 6 hours, days 1 to 7, day 14, day 21, and day 28 after orthodontic spring attachment) on the cheek, upper lip, and maxillary incisor labial gingiva bilaterally by recording the threshold for a head withdrawal response evoked by von Frey filaments. Thermal sensitivity was also tested preoperatively and postoperatively on the cheek bilaterally by applying a noxious thermal stimulus and measuring head withdrawal response duration, response score, and response percentile rate. Statistical analyses involved a mixed-model repeated-measures analysis of variance (MMRM ANOVA). **Results:** The mechanical and thermal sensitivities at all bilateral sites were significantly increased ($P < .01$) in the E group in the early postoperative period (1 to 5 days), with peaks reached on day 1, and then returned to and remained at preoperative levels until postoperative day 28. However, there was no significant change from the preoperative levels in mechanical and thermal sensitivities for the S group for all the tested sites. **Conclusion:** This rat OTM-induced pain model correlates with the time course of OTM-induced pain in humans and suggests that OTM-induced mechanical and thermal hypersensitivities may be useful measures of OTM-induced pain. *J Oral Facial Pain Headache 2015;29:60–69. doi: 10.11607/ofph.1336*

Key words: *behavioral changes, malocclusion, orofacial, orthodontic appliances, sensitization*

Orthodontic treatment is a common clinical procedure, and most orthodontic patients experience pain that has been suggested to affect treatment outcomes and compliance.[1–8] A majority of the patients undergoing orthodontic treatment first experience pain approximately 4 hours after orthodontic appliance placement, and pain reaches its peak levels at 24 hours, usually lasts for 2 to 3 days, and then gradually dissipates completely by 5 to 6 days.[9–17] An immediate and delayed painful response after orthodontic force application has also been reported in the initial few days of OTM.[12,18,19] The immediate pain has been attributed to compression of the periodontal ligament (PDL) and the resulting input from activated nociceptors, and the delayed pain to a hyperalgesic state. However, the etiology and pathophysiology of OTM-induced pain are still largely unknown, and no animal model has been developed to simulate clinical OTM-induced pain in humans.

A number of animal models have been developed to study various types of pain-related behaviors and mechanisms.[20–23] In the orofacial region, the infraorbital nerve ligation model[24,25] and the inferior alveolar nerve injury model[26–28] have been particularly used (for review, see Dubner et al[29] and Iwata et al[30]). Other behavioral models have been developed to study temporomandibular joint (TMJ), masticatory muscle,

and pulp pain mechanisms by characterizing the nociceptive behavioral responses induced by the injection of algesic chemicals into these tissues or by using other approaches to disrupt these tissues[31–50] (for review, see Khan and Hargreaves[51] and Sessle[52]).

The majority of behavioral studies using orofacial pain models in animals have typically assessed pain behavior in terms of evoked withdrawal responses and hypersensitivity. These include behavioral hyperalgesia and allodynia.[24,25,30,53] Quantification of behavioral responses to cutaneous hyperalgesia in the orofacial region in animals has relied upon mechanical testing[24,25,54–56] and thermal testing procedures.[57–60] Since there is a need for animal models of clinical OTM-induced pain in humans, and since OTM induces sensorimotor cortical neuroplasticity that conceivably could be a result of OTM-induced pain,[61] the aim of this study was to test whether orofacial mechanical and thermal hypersensitivities occur in rats during OTM. Some findings of this study have been published briefly in an abstract.[62]

Materials and Methods

Animal Preparation

Experiments were performed on 14 young adult male Sprague-Dawley rats (140 to 160 g) that were 6 weeks old and housed in cages (27 cm × 45 cm × 20 cm) in a temperature- (21°C ± 1°C) and humidity- (50% ± 5%) controlled environment under a 12-hour light/dark cycle (lights on at 07:00 am) and that received water and a mashed diet (Rodent diet #2018M, Harlan Teklad) ad libitum. The rats were acclimatized to the environment for 1 week before the initiation of the study. All experimental procedures were approved by the University of Toronto Animal Care Committee, in accordance with the Canadian Council on Animal Care Guidelines and the regulations of the Ontario Animals for Research Act (RSO 1990), and the guidelines of the International Association for the Study of Pain. All experimental procedures were carried out by one investigator (to ensure consistency in the experimental procedures), who was blinded to the animal groups; the data analyses were performed by another experimenter who was also blinded to the animal groups.

Study Groups and Orthodontic Springs

The rats were separated into two groups, an experimental (E) group (n = 7) that received a nickel-titanium (Ni-Ti) closed-coil orthodontic spring (coil diameter 0.22 mm, eyelet diameter 0.56 mm, force on activation 10 cN, GAC) activated to induce OTM, and a sham (S) group (n = 7) that received the orthodontic spring but in an inactive state. The orthodontic springs were attached between the maxillary right molars and both the maxillary incisors under general anesthesia (inhalation isoflurane 5% induction, 2%~2.5% maintenance). The extension of the facemask that was used for general anesthesia was limited to the snout of the rat and permitted free access to the oral cavity to place the orthodontic spring.

Stimulation Procedures and Behavioral Recordings

Rats were placed in a cylindrical restrainer (10 cm diameter, 20 cm length) with an open posterior end and a small opening in the anterior end through which the rats could place their snouts. This restrainer permitted the investigator access to the face of the rat, but covered the eyes so that the rat could not visualize the approach of the investigator to perform mechanical and thermal stimulation. Further, the restrainer was large enough to allow the rat to rotate to the posterior end to escape the stimulus. Multiple grooves cut into the side of the restrainer posterior to the initial 1 cm from the anterior end permitted the investigator to visualize the rat after the stimulus induced a withdrawal response of the head into the restrainer. Before the stimulation session, rats were adapted to the restrainer for 15 minutes daily, and then the series of mechanical and thermal stimulations were initiated. Stimulations were applied perpendicular to the sagittal plane of the head when the rat was neither moving nor freezing. A stimulus was applied only when the rat resumed this position and at least 30 seconds after the preceding stimulation. Three consecutive days before the placement of the orthodontic spring, the rats were habituated to the restrainer. One day prior to the placement of the orthodontic spring, the preoperative values for the mechanical and thermal tests were obtained. Rats were then tested for mechanical and thermal sensitivities at 3 hours, 6 hours, days 1 to 7, 14, 21, and 28 after the placement of the orthodontic spring. Testing was conducted at times between 0700 and 1500 hours of the day. Rats were tested in the test room with a constant background noise (50 dB) that was used to decrease any interference of sudden auditory stimulation.

For mechanical stimulation, von Frey filaments (Pressure Aesthesiometer, Stoelting Co) of varying diameters were used; the force required to bend each filament was 1 g, 1.4 g, 2 g, 4 g, 6 g, 8 g, 10 g, or 16 g, in an ascending series. During one session, the complete series of von Frey hair intensities was presented in an ascending series until a response was obtained. Each testing site was stimulated with each filament five consecutive times in an ascending order starting with 1 g. When three out of five stimulations for a filament resulted in a head withdrawal response (see below), the force level of that filament

was considered the threshold mechanical response level. Once the threshold response was obtained, the threshold value was confirmed by dropping down to one size smaller in the series of von Frey filament stimulation intensities and observing if the withdrawal response could no longer be evoked by that stimulus intensity.[20,24,25,63,64] The sites were tested in a random order with at least 1 minute time lag between the different test sites. Further, a time lag of 30 seconds was maintained between each filament contact at each testing site. Mechanical stimuli were applied to the cheek, upper lip, and maxillary incisor labial gingiva. Radiographic images and anatomical landmarks of the head of the rat were used to select the stimulation sites. The site of the cheek was the midpoint vertically between the second and the third row of whiskers and horizontally the most posterior extent of the vibrissal pad where it meets the furry skin of the cheek. This point was midway on the cheek between the maxillary incisor and the maxillary molar teeth, and although being on the hairy vibrissal pad, was covered with only a small amount of hair and thereby provided an unobstructed area for mechanical testing. Secondly, the anterior opening of the restrainer permitted this area of the rat's face to be easily accessible for testing. For the upper lip, the testing was done at a spot midway along the length and the width of the upper lip. In testing for the maxillary incisor labial gingiva, caution was taken to prevent making any contact with the upper lip. These various areas were stimulated on both sides of the face, ie, ipsilateral and contralateral to the side where the orthodontic spring was attached.

For thermal testing, a beam of noxious radiant heat was aimed at the cheek. The stimulation was limited to the ipslilateral and the contralateral cheek site only, and the site was similar to the one used for mechanical testing on the cheek. The light source was made from the lamp housing of a fiber optic microscope illuminator (Lype Laser). The radiant heat stimulus was a focused beam of light from a modified microscope illuminator, the aperture of which was 10 cm from the stimulation site. Thermal testing was done at a strength of 22 A and duration of 200 ms to generate a noxious radiant heat.[65] Guideline marks on the laboratory table helped maintain a constant distance between the skin and the heat source. Head withdrawal latency along with the response duration was measured by using a stopwatch to give the total response duration (TRD). It was determined five times on each side of the face with 2-minute intervals between each stimulus; any response that occurred in at least three out of five thermal stimuli was considered a positive response. Since the values for head withdrawal latency and response duration could not be separated because the response was very rapid, the TRD value was measured and used in the analysis. A thermal stimulation cutoff of 6 seconds was used to prevent tissue damage.

Behavioral Scoring

For mechanical testing, the behavioral response threshold was noted for each of the testing sites when there was a head withdrawal response, ie, the rat pulled its head briskly backward when the stimulus was applied.[25,64] Further, to compare if the change in mechanical response threshold affected one of the tested sites more than the other in the E group, the change in postoperative response threshold from the preoperative response threshold was compared amongst the different tested sites on analogous days. For thermal testing, in addition to measuring the TRD (see above), each response was scored[57–59,66,67] by grading the response from 0 to 3 (0 = no response, 1 = slight twitch [approximately 0.5 seconds], 2 = distinct movement away from the stimulus/brief stroke of the face by the rat's paw [range of 0.7 to 1.2 seconds], 3 = very strong movement/turn around). Also, the frequency of a positive response during the thermal testing procedure was calculated as the response percentile rate.

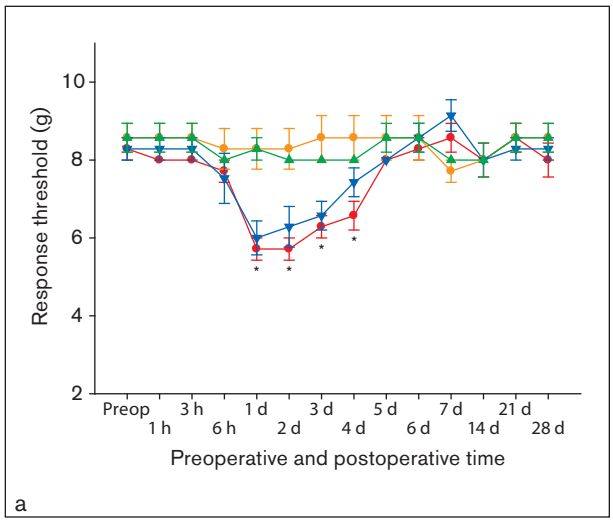
Statistical Analyses

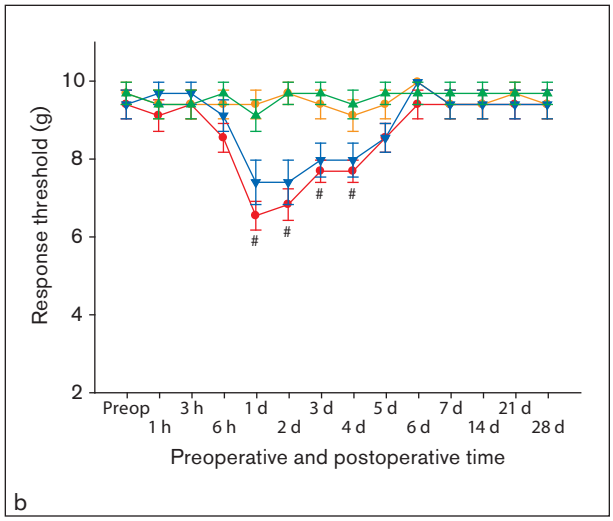
Statistical differences between groups at different time points were determined by using multivariate (mixed-model repeated-measures [MMRM] analysis of variance [ANOVA]) analyses, followed by post-hoc Sidak-adjusted pairwise comparisons as appropriate. A probability level of $P < .05$ was considered statistically significant. Data were analyzed by a statistician using the SAS statistical software program (version 9.3). All values are expressed as mean ± SEM.

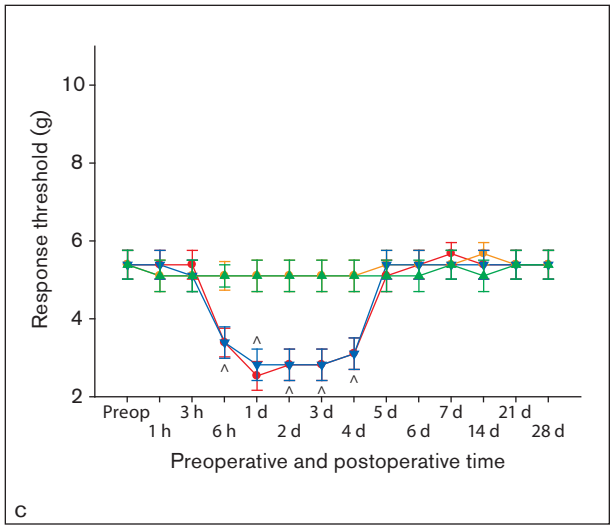
Results

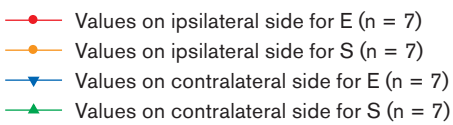
The condition of the rats was monitored daily during the experiment and no abnormal behavior or orthodontic spring-related complications were apparent. Before the orthodontic spring was placed, the E- and S-group animals had a similar daily gain in body weight. However, after the placement of the orthodontic spring, the E group had a small but significant loss of weight for day 1 as compared to the S group ($P < .001$), but thereafter gained weight again at a similar rate to that of the S group. Significant changes in mechanical and thermal sensitivities following OTM were documented during the testing period, which extended from 1 day before (preoperative) and 28 days after (postoperative) the placement of the orthodontic spring on the maxillary molars and the maxillary incisors.

Figs 1a to 1c Response threshold evoked by bilateral mechanical stimulation at the cheek, upper lip, and maxillary incisor gingival sites. **(a)** At the cheek site, post-hoc analysis revealed a significant decrease in the response thresholds in the E group on each of the postoperative days 1 to 4 compared to the preoperative value ($P < .0001$) and compared to analogous days in the S group (*$P < .0001$). **(b)** A similar trend of decrease in postoperative response threshold was observed at the upper lip site in the E group on each of the postoperative days 1 to 4 compared to the preoperative value ($P < .0001$) and compared to analogous days in the S group (#$P < .01$). **(c)** At the maxillary incisor gingival site, a significant decrease in the response thresholds in the E group was revealed on 6 hours and each of the days 1 to 4 compared to the preoperative value ($P < .0001$), and compared to the analogous time period in the S group (^$P < .01$).





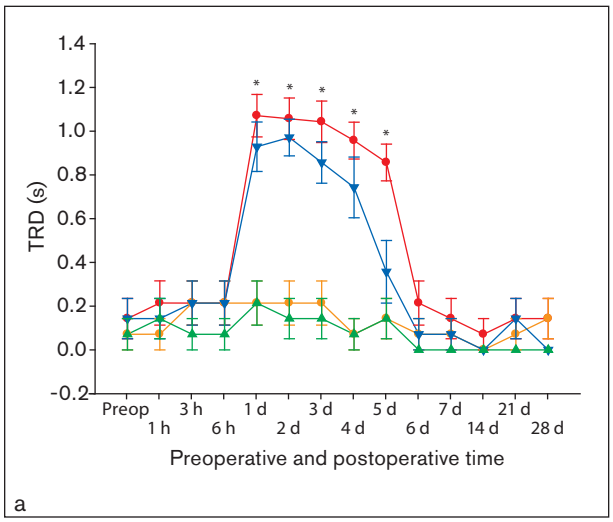


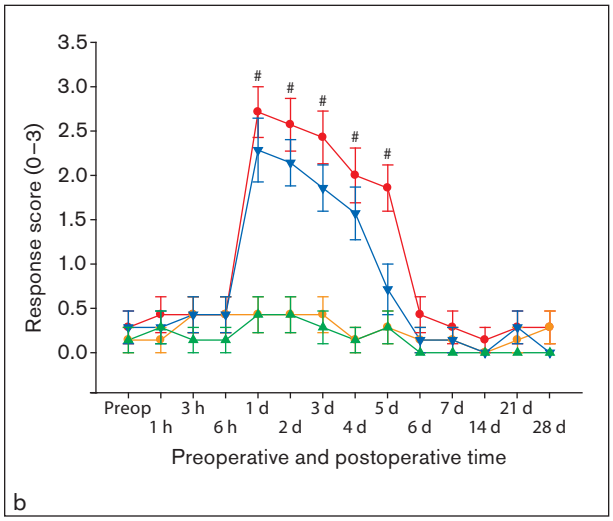


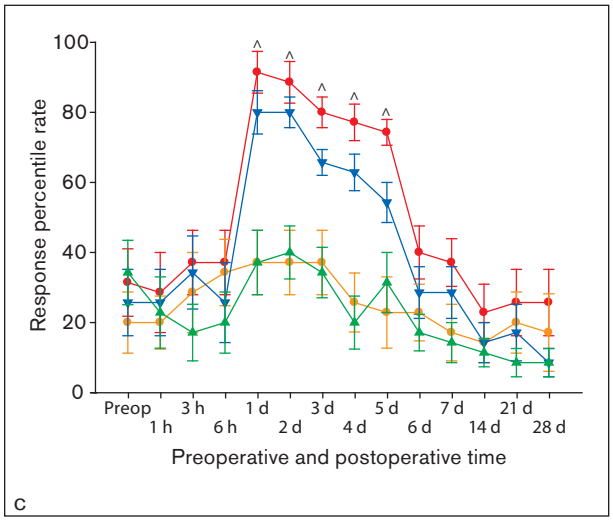
Response Threshold Related to Mechanical Stimulation

In the S group, the postoperative response threshold at any of the tested sites (cheek, upper lip, and maxillary incisor gingiva) did not differ significantly from the preoperative response threshold value at that same site ($P > .05$). However, MMRM ANOVA revealed significant differences in treatment ($P = .036$). In the E group, a significant difference was revealed in time ($P < .0001$) and also in treatment × time interaction effects ($P < .0001$). There was no significant difference between the mean values of the ipsilateral and the contralateral sides of the tested sites ($P = .69$), indicating that the contralateral response threshold decrease reached statistical significance at the same time as the ipsilateral side (at postoperative day 1 for all sites, except at 6 h for the maxillary incisor gingival site). The significant change was reflected in a decrease in response threshold that reached its peak on postoperative day 1, but none of the significant changes in response thresholds to mechanical stimulation of the orofacial areas lasted longer than postoperative day 4 (Fig 1).

At the cheek site, post-hoc analysis revealed a significant decrease in the response thresholds in the E group on each of the postoperative days 1 to 4 compared to the preoperative value ($P < .0001$) and compared to analogous days in the S group ($P < .0001$) (Fig 1a). A significant decrease in postoperative response threshold was also observed at the upper lip site in the E group on each of the postoperative days 1 to 4 compared to the preoperative value ($P < .0001$) and compared to analogous days in the S group ($P < .01$) (Fig 1b). At the maxillary incisor gingival site, a significant decrease in the response thresholds in the E group was revealed at 6 hours and each of the postoperative days 1 to 4 compared to the preoperative value ($P < .0001$) and compared







Values on ipsilateral side for E (n = 7)
Values on ipsilateral side for S (n = 7)
Values on contralateral side for E (n = 7)
Values on contralateral side for S (n = 7)

Figs 2a to 2c Responses evoked by noxious thermal stimulation on the cheek site tested, as reflected by the total response duration (TRD), response score, and response percentile rate. **(a)** There was a significant increase in the TRD in the E group on each of the postoperative days 1 to 5 compared to the preoperative value ($P < .0001$) and compared to the analogous days in the S group (*$P < .0001$). **(b)** There was a significant increase in the response score in the E group on each of the postoperative days 1 to 5 compared to the preoperative value ($P < .0001$) and compared to the analogous days in the S group (#$P < .0001$). **(c)** There was a significant increase in the response percentile rate in the E group on each of the postoperative days 1 to 5 compared to the preoperative value ($P < .0001$) and compared to the analogous days in the S group (^$P < .001$).

to the analogous time period in the S group ($P < .01$) (Fig 1c). The postoperative response threshold at each of these sites returned to the preoperative levels by day 5, and thereafter remained at those levels until day 28. Amongst the three tested sites, the lowest response thresholds were detected at the maxillary incisor gingival site (Fig 1). Further comparison of the early postoperative decrease in mechanical response threshold to the preoperative value between the three sites for the E group on the analogous days revealed that there was no significant difference in the level of decrease in response thresholds compared to their preoperative value at all the sites.

Responses Related to Thermal Stimulation

The responses evoked by noxious thermal stimulation applied bilaterally to the cheek site were measured in terms of three parameters described below. In the S group, the postoperative value at the tested site did not differ significantly from the preoperative test value for that site, but multivariate analysis revealed significant differences in treatment ($P < .05$). In the E group, a significant difference was also revealed in time ($P < .0001$), and also in treatment × time interaction effects ($P < .0001$). There was no significant difference in any of the measured thermal response parameters between the sides of the tested sites ($P > .05$), indicating that the contralateral thermal response values reached statistical significance around the same time as those for the ipsilateral side (at day 1), but none of the significant changes to thermal stimulation of the cheek site lasted longer than postoperative day 5 (Fig 2).

Post-hoc analysis revealed a significant increase in TRD for each side in the E group on each of the postoperative days 1 to 5 compared to the preoperative value ($P < .0001$) and compared to that for the analogous days in the S group ($P < .0001$) (Fig 2a).

Further, a significant increase for each side in the response score (0 to 3) in the E group on each of the postoperative days 1 to 5 compared to the preoperative value ($P < .0001$) and compared to that for the analogous days in the S group ($P < .0001$) was revealed (Fig 2b). Also, a significant increase occurred for each side in the response percentile rate in the E group on each of the postoperative days 1 to 5 compared to the preoperative value ($P < .0001$) and compared to that for the analogous days in the S group ($P < .001$) (Fig 2c).

Discussion

This is the first study to document that orofacial mechanical and thermal sensitivities change in a rat OTM-induced pain model. The mechanical hypersensitivities reflected a decrease in the mechanical response thresholds, and the thermal hypersensitivities consisted of an increase in the response duration, response score, and response percentile rate. The mechanical and thermal sensitivities at all sites tested were significantly increased in the E group in the early postoperative period and then returned to preoperative levels around postoperative days 4 to 6; they were maintained at this level for the rest of the 28-day testing period. However, there was no significant change in mechanical and thermal sensitivities for the S group from the preoperative control level for all the tested sites for the entire testing period.

Both mechanical and thermal hypersensitivities occurred in contralateral as well as ipsilateral tested sites, although the active orthodontic spring was attached on the right side intraorally. This finding is in line with reports of both contralateral and ipsilateral orofacial hypersensitivities in acute and chronic orofacial pain models[24,25,30,35,37,49,50,52] that are suggestive of neuroplastic changes reflecting central sensitization in trigeminal brainstem subnucleus caudalis,[26,28,68–70] and likewise with contralateral as well as ipsilateral hyperalgesia in pain models involving spinal nerve injury.[71–73] The finding is also consistent with the projection of some trigeminal primary afferents to the contralateral subnucleus caudalis, and with projections from the subnucleus caudalis on one side to the contralateral brainstem.[74–76] Furthermore, the orthodontic spring was attached to both the maxillary incisors, and a transmedian pathway via the trigeminal ganglion may have contributed to the contralateral hypersensitivities.[77]

Peripheral and Central Mechanisms of OTM Pain

The OTM-induced mechanical and thermal hypersensitivities documented in the present study would have involved both peripheral and central neural mechanisms. OTM may result from an inflammatory process in the PDL[78–80] and dental pulp[81–84] due to compression of the PDL by the movement of the tooth.[85] The hypersensitivities apparent in the early postoperative period in the present study are consistent with a gradually developing hyperalgesic state attributed to injury-induced peripheral sensitization of nociceptive afferent endings in the PDL and associated structures.[19,78,79]

Furthermore, the peripheral sensitization of the nociceptive afferent endings can result in an augmented afferent barrage conducted along the nociceptive afferents into the central nervous system (CNS) and the production of trigeminal central sensitization that can contribute to orofacial hyperalgesia.[52,86,87] Thus, central sensitization could have been involved in the orofacial hypersensitivities in the early period of OTM. Since the present study demonstrated a return of the mechanical and thermal sensitivities to the preoperative control level by postoperative day 6 and this level was maintained for the rest of the 28-day testing period, long-term central sensitization mechanisms were unlikely present beyond the early period of OTM. Peripheral and central mechanisms may also have contributed to shortening the duration of OTM-induced pain behavior, since peripheral receptor mechanisms exist (eg, opioid, GABA) that mediate analgesic effects that are prominent in painful orofacial inflammatory conditions.[52,88–90] In addition, OTM-induced nociceptive afferent inputs have been suggested to activate the descending inhibitory systems in the periaqueductal grey and its projections to the trigeminal subnucleus caudalis at postoperative day 1 of OTM.[91–94] Therefore, these antinociceptive mechanisms may have contributed to the dissipation of the OTM-induced orofacial hypersensitivities by postoperative day 6.

OTM is also associated with an impairment of chewing and biting ability, a decrease in biting force,[95,96] a decrease in the frequency of tooth contacts during the early stages of OTM, and a decrease in pressure pain threshold for the masseter and anterior temporalis muscles.[95,97–99] Thus, occlusal changes during the early period of OTM may also have contributed through intraoral and muscle hyperalgesia to the short-term hypersensitivities documented in the present study.

Mechanical and Thermal Hypersensitivities of the Orofacial Region as an Index of OTM-Induced Pain

The time-dependent hypersensitivity changes in the present study correlated well with the early period of OTM-induced pain reported in human and rodent studies.[9–11,13–17,100] Furthermore, the mechanical and

thermal sensitivities for the S group were maintained at the preoperative control level for all the tested sites for the entire testing period. This finding addresses the concern that response parameters to repeated application of the same series of mechanical and thermal stimuli might be influenced by factors such as tissue damage, learning effect, animal fatigue, and increased animal irritability introduced by the testing procedures.[53] Hence, these observations in this rat OTM-induced pain model provide strong evidence for the validity of using mechanical and thermal hypersensitivities as a measure of OTM-induced pain.

Another model of OTM-induced pain that monitors facial expression suggests a threshold force magnitude between 20 and 40 cN may be needed to evoke orthodontic pain.[100] The difference in force magnitude used in that study and the present study to induce OTM-pain may be due to different sensitivities of the methods employed to assess pain. Also, studies in rats indicate that forces of less than 10 cN may be the ideal force magnitude for inducing an optimal rate of OTM,[101–103] and this was the force used in the present study that could induce mechanical and thermal hypersensitivities.

Strengths, Limitations, and Future Directions

Most OTM pain studies in humans and animals have used the Waldo's method[104] (1954), which consists of placing an intermaxillary elastic between the molars to induce OTM-associated pain. However, unlike the present study, these studies have not tested whether orofacial pain behavior occurs in these models. The use of Waldo's method has orthodontically related disadvantages in that the intensity of the initial force generated by the elastic has been calculated to be approximately 80 to 200 g.[102,105] This value is equivalent to 1.6 to 4 kg for humans because the roots of the human first molar have a surface area of about 20 to 50 times larger than that of the rat molar.[102,106] This heavy force applied to rat molars may not simulate the clinical condition of OTM in humans, and when this heavy a force is applied to molars in a rat, it may lead to an immediate forced eruption of the tooth and thereby cause hyperocclusion. Studies of occlusal trauma have documented hyperocclusion-based hypersensitivity.[107] Also, the elastic used in Waldo's method suffers from a rapid dissipation of force.[102] Therefore, the use of an orthodontic appliance that generates a mild constant force that better simulates clinical conditions, like the Ni-Ti orthodontic spring used in the present study, is a more appropriate means of inducing OTM for experimental pain studies.

In the present study, a stopwatch was used to record the TRD for thermal testing. Although one experimenter performed these experiments and had extensive training in operating the stopwatch, there would have been a latency for eye and button-press responses that might have affected actual TRD values; however, this would not have undermined the study conclusions about differences between groups, since the same method was used for all animals.[108] Also, the present study design was limited in its aim to study OTM-induced nociception that was related to the changes in evoked behaviors, and future studies to document spontaneous behavioral changes related to pain are indicated to clarify mechanisms relevant to spontaneous OTM-induced pain. Furthermore, future studies are warranted to test for the presence of any secondary allodynia and hyperalgesia beyond the trigeminal innervation domain,[52,53,59,87] and to delineate the impact of OTM on different neural structures of the PDL and their influence on mechanical and thermal sensitivities.

Conclusions

This project has introduced an OTM rat model that uses orthodontic force parameters that are well defined and within the physiologic limits when applied to the teeth of rats and that correlate well with the orthodontic forces applied clinically in humans. The present study has also characterized the orofacial mechanical and thermal hypersensitivities that occur in this OTM model. The mechanical and thermal hypersensitivities were apparent bilaterally and were significantly increased in the early postoperative period (days 1 to 5), with peaks reached on postoperative day 1, and then returned to preoperative levels. The hypersensitivities correlate well with the time-dependent pain reported in clinical OTM pain studies in humans. This behavioral model of allodynia/hyperalgesia related to OTM can serve as an important experimental approach to assess OTM-induced nocifensive behaviors and to study the mechanisms underlying OTM-induced pain.

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