Original Article

Functional changes in the temporomandibular joint mechanoreceptors associated with experimentally induced condylar resorption in rats

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ABSTRACT

Objectives: To evaluate the influence of experimentally induced progressive condylar resorption (PCR) on temporomandibular joint (TMJ) mechanoreception.

Materials and Methods: Twenty 13-week-old male albino Wistar rats were divided equally into control and PCR groups. A compressive force was loaded on the left TMJ of PCR group rats to induce condylar resorption. Single-unit activities of TMJ mechanoreceptors were also induced through passive jaw movement. Recording was performed for the left Gasserian ganglion at 3 days and 1 week after the establishment of PCR group. The effects of PCR on TMJ units were assessed by measuring the firing threshold, maximum instantaneous firing frequency, and average firing frequency.

Results: Compared with the control group, there were no significant differences in the firing threshold of the PCR group after 3 days. The thresholds were significantly higher 1 week after compressive force loading on the condyle. The maximum instantaneous firing frequencies and the average firing frequencies showed no significant differences after 3 days. However, these were significantly lower 1 week after compressive force loading.

Conclusions: The findings suggest that compressive force loading on the condyle may influence the function of TMJ mechanoreceptors. (*Angle Orthod.* 2020;90:831–836.)

KEY WORDS: Functional changes; Temporomandibular joint; Condylar resorption

INTRODUCTION

Progressive condylar resorption (PCR) is a clinical complication that is sometimes identified in orthodontic patients. Patients with PCR usually show progressive resorption with a flattened and malformed condylar shape.[1] Most PCR patients suffer from a decline in posterior facial height, retrognathism, and progressive anterior open bite with a clockwise mandible rotation.[2] Although there were studies on the exceptional deteriorative change in occlusion, the etiology and pathogenesis of this condition remain poorly understood.[3] Currently, only a few studies have investigated the physiological changes associated with PCR.

Sensory inputs from low-threshold orofacial proprioceptors, such as the temporomandibular joint (TMJ) mechanoreceptors, are one of the important afferents in the regulation of occlusion.[4,5] These mechanoreceptors, which exist in the lateral and posterior regions of the TMJ capsule, are activated in pace with condylar movements.[4] PCR may alter the properties of TMJ mechanoreceptors, consequently affecting the physiologic regulation of the jaw position.

Conventionally, establishment of animal models for PCR studies was limited to scraping of the articular surface and anterior disc displacement, and involved surgical invasion of the TMJ capsule.[6,7] It was necessary to create an animal model for PCR without using these methods to avoid the unfavorable and or unexpected side effects of surgery. In a study by Zhao and colleagues, the animal's mandible was pulled mechanically and deviated posteriorly on one side, which induced condyle resorption in both the ipsilateral and contralateral TMJs.[8] In their study, the contralateral TMJ showed resorption without using direct force. Therefore, they created an animal model for bilateral

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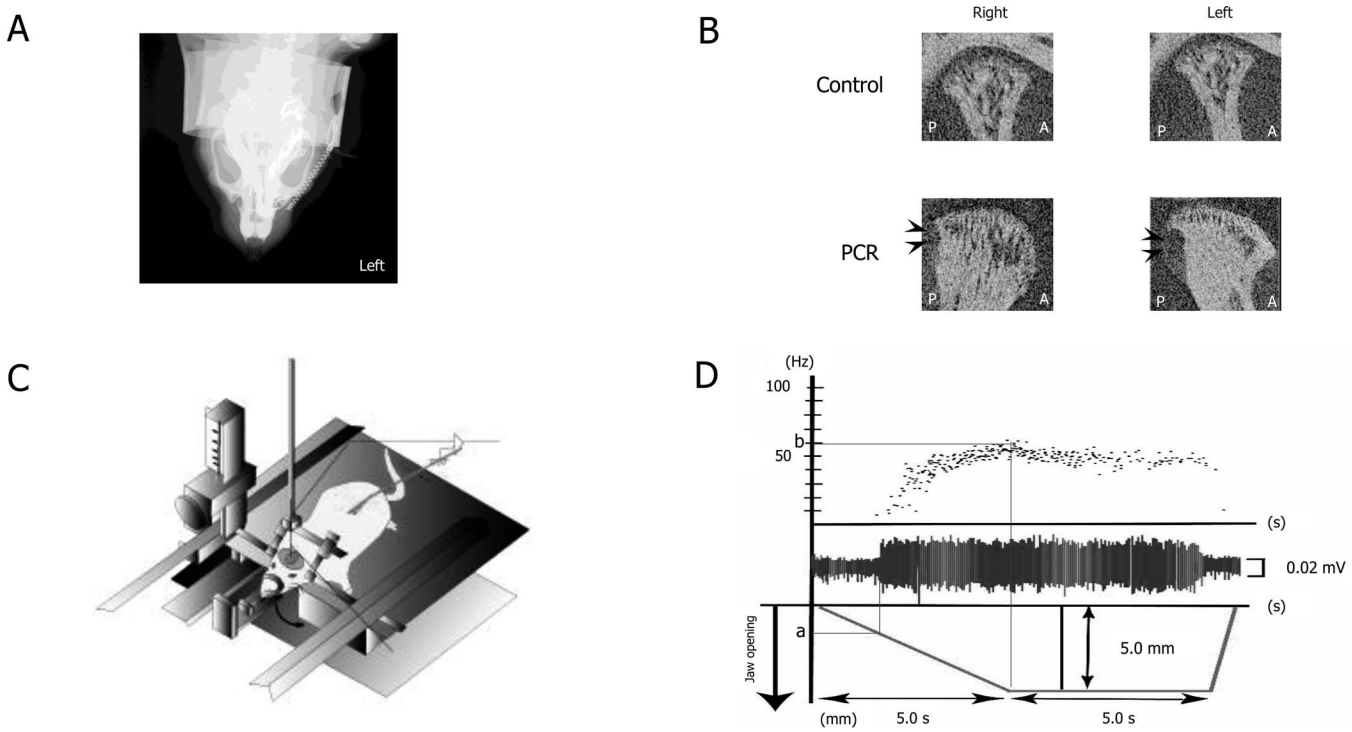

Figure 1. Experimental design. (A) X-ray film (vertical view) of an animal from the PCR group placed in a traction appliance. (B) Micro-CT images of the condyles in the control (*upper*) and PCR (*lower*) groups. A lesion (*arrowheads*) was found in the posterior region of the condyles bilaterally in the PCR animal. (C) Schematic drawing of the experimental setup. The animal's head was fixed to a stereotaxic frame. A small aperture approximately 3.0 mm wide was prepared in the skull and monopolar tungsten microelectrodes were inserted into the left trigeminal Gasserian ganglion. A thread was attached to the mandible and ramp-and-hold jaw movement was achieved using an automatic pulling machine. (D) Simultaneous recording of the firing frequency of the recorded unit (*top trace*), single-unit activity (*middle trace*), and open-close jaw movement (*bottom trace*). (*a*) The firing threshold was considered as the magnitude of jaw-opening observed at the first spike. (*b*) The maximum instantaneous firing frequency was considered as the minimum firing interval between the two consecutive spikes. Average firing frequency was the mean of the instantaneous firing frequency between 5-6 seconds during open jaw movement A indicates anterior; P, posterior.

PCR without invading the TMJ capsule. In the current study, by utilizing and applying the concept of a non-invasive PCR animal model, the effects of an experimentally induced PCR on the functional characteristics of TMJ mechanoreceptors in rats were investigated. It was hypothesized that the TMJ mechanoreceptors would show no physiological changes in experimentally induced PCR.

MATERIALS AND METHODS

The experimental procedures were approved by the Animal Welfare Committee (#0100204, #0110997, #0110337A) and performed in accordance with the Animal Care Standards of Tokyo Medical and Dental University. Twenty male Wistar albino rats (13 weeks old) were equally divided into a control group and a PCR group.

Animal Preparation

Animals in the PCR group were lightly anesthetized intraperitoneally with 60 mg/kg thiamylal sodium (Isozol, Yoshitomi Pharmaceutical Co. Ltd., Osaka, Japan). Compressive force (ca. 25gF) was loaded using a coil-spring traction appliance (Figure 1A) for 24 hours per day for a week on the condyle of the PCR group's left TMJ. Resorption of the TMJ on both sides was confirmed using micro-CT 1 week after starting traction (Figure 1B). The animals were returned to their cages and allowed to recover from anesthesia. The body weight of rats in the control and PCR groups was monitored throughout the experimental period.

Stimulation and Recording

Electrophysiological recordings of the control and PCR groups were performed five times each on day 3 and 1 week after loading. The animals were anesthetized again via intraperitoneal injection of 80 mg/kg thiamylal sodium. The amount of anesthesia injected was monitored by checking the pupil size, flexion and corneal reflexes, and heart rate. Additional 5.0 mg/kg thiamylal sodium was administered when a firm pinch applied to the tail resulted in increased respiratory and heart rates. The animal was placed in models SN-2

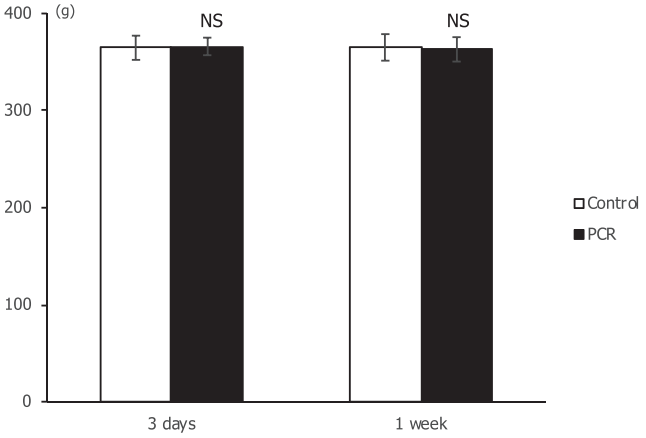

Figure 2. Comparisons of body weight in the control and PCR groups during the experimental period. There was no significant difference between the two groups after 3 days and 1 week. NS indicates not significant.

and SM-15M stereotaxic apparatus (Narishige Scientific Instrument Lab, Tokyo, Japan) in a prone position (Figure 1C). For the indirect stimulation of TMJ mechanoreceptors during passive jaw movement, one end of a cotton thread was fixed to the mandibular symphysis while the other end was attached to an automatic pulling machine.[9–11] Jaw-opening movement was constantly directed straight downward. The maximum jaw-opening distance was set to 5.0 mm (ramp duration of 5.0 seconds and hold duration of 5.0 seconds) from the rest position (Figure 1D). Passive jaw movement was attempted three times per recording session. Stimulation was performed from the position in which the lower jaw was loosened by anesthesia.

The sensory unit activities were recorded from the Gasserian ganglion. To introduce the recording electrode, the scalp was incised at the midline and two small apertures about 1.0 mm wide were prepared symmetrically in the skull using a stereotaxic microengine. Monopolar tungsten microelectrodes 250 μm in diameter with an 8.0° tapered tip and a 5.0 MΩ AC impedance (A-M Systems, LLC, Carlsborg, WA, USA) were used to record the single-unit activities of the TMJ mechanoreceptors. The recording electrode was inserted into the left Gasserian ganglion with reference to the stereotaxic coordinates[12] as previously reported.[9–11] Electrical stimulation of the auriculotemporal nerve evoked responses with a latency of 0.125 ± 0.01 ms (mean ± standard deviation [SD]) in the nucleus of the left Gasserian ganglion. The conduction distance from the stimulation site to the recording electrode in the Gasserian ganglion was estimated to be 5.0 mm. Therefore, the mean conduction velocity recorded in the afferents was 40.5 ± 3.5 m/s, indicating that these were probably large myelinated (eg, Aß fibers) fibers.[13] Spike signals were recorded and amplified using a DAM-80 differential amplifier with 10003 gain and 300 Hz and 3.0 kHz for low- and high-pass filters, respectively (WPI, Sarasota, FL, USA). All data were captured using a CED 1401 interface and analyzed using Spike2 software Version 4.02a for Windows (Cambridge Electronic Design Limited, Cambridge, UK).

Histologic Identification of the Electrode Position

After each recording session, the electrode position was marked by passing a 50-mA negative current for 10 seconds. At the end of the experiment, the rats were euthanized via intraperitoneal injection of 120 mg/kg thiamylal sodium. The brains were removed and embedded in paraffin, cut into 5-mm-thick sections, and stained with cresyl violet. The position of the electrode tip was confirmed histologically based on the electrolytic marks and signs of electrode penetration in the horizontal section.[14] Electrolytic marking was performed for each unit recorded in 20 rats.

Data Analysis

Prior to the study, power analysis was performed using the free software EZR[15] to consider the sample size. In power analysis, the required number of samples was more than 3, a condition fulfilled by this study.

The effects of experimentally induced PCR on TMJ units were assessed by measuring the firing threshold, maximum instantaneous firing frequency, and average firing frequency. The firing threshold indicates the level of jaw opening when the first spike response was observed (Figure 1D). The maximum instantaneous firing frequency was the minimum firing interval between the two consecutive spikes. The average firing frequency was the mean instantaneous firing frequency from 5 seconds (maximum jaw-opening) to 6 seconds. Significance of the mean difference between the control and PCR groups was evaluated using Mann-Whitney *U*-test ($P < .05$). The software StatView version 5.0 for Windows (SAS Institute, Cary, NC, USA) was used for the statistical analysis. The method of statistical analysis employed in the study was the same as in previous studies.[9–11]

RESULTS

There was no significant difference in the body weight of the control and PCR groups throughout the experiment (Figure 2). In both groups, firing activities were recorded from 20 TMJ mechanoreceptor units. Results of the TMJ units recorded from the Gasserian

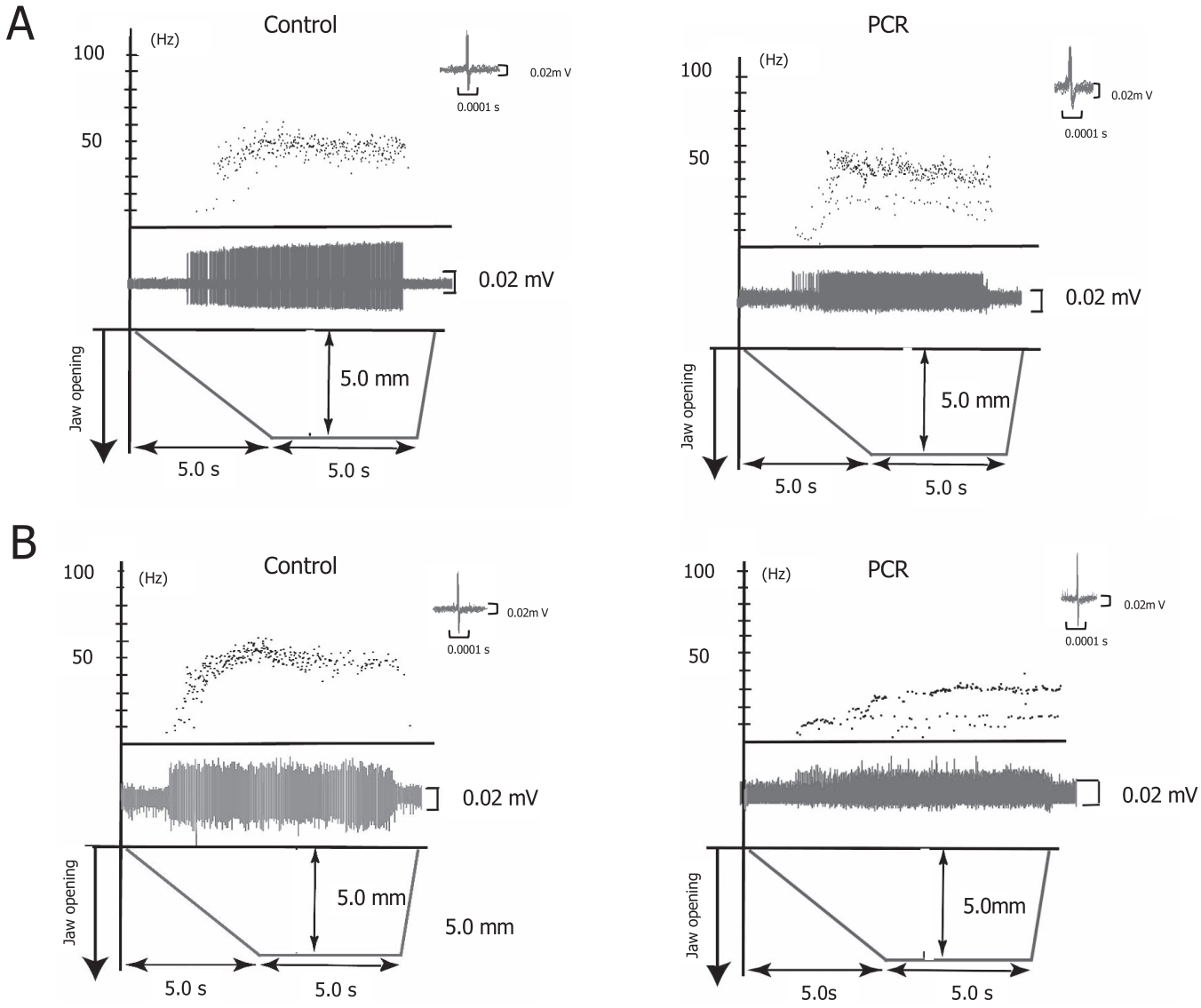

Figure 3. Examples of responses recorded from the control and PCR groups after (A) 3 days and (B) 1 week. Insets denote the single-unit activities in the corresponding records.

ganglion of each group after 3 days and 1 week are shown in Figure 3.

Firing Threshold

After 3 days, there was no significant difference between the control (1.59 ± 0.08 mm) and PCR (1.59 ± 0.08 mm) groups (Figure 4A). On the other hand, it was significantly higher in the PCR group (1.92 ± 0.06 mm) compared with the control group (1.58 ± 0.05 mm) 1 week after the compressive force loading on the condyle.

Maximum Instantaneous Firing Frequency

After 3 days, there was no significant difference between the control (57.04 ± 2.03 Hz) and PCR (55.45 ± 3.97 Hz) groups, while it was significantly lower in the PCR group (36.52 ± 1.35 Hz) compared with the control group (56.76 ± 1.64 Hz) 1 week after the compressive force loading on the condyle (Figure 4B).

Average Firing Frequency

After 3 days, there was no significant difference between the control (51.69 ± 3.42 Hz) and PCR (47.93 ± 2.67 Hz) groups (Figure 4C). By contrast, it was significantly lower in the PCR group (24.51 ± 1.35 Hz) compared with the control group (56.76 ± 1.64 Hz) 1 week after the compressive force loading on the condyle.

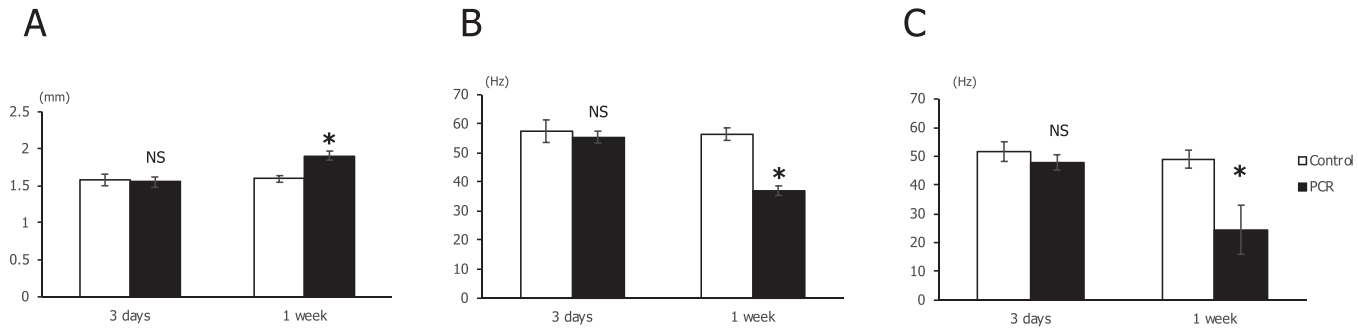

Figure 4. Comparison of the functional properties of TMJ mechanoreceptor afferents. (A) The firing threshold after 3 days and 1 week. (B) The maximum instantaneous firing frequency after 3 days and 1 week. (C) The average firing threshold after 3 days and 1 week. NS indicates not significant. * $P < .05$.

DISCUSSION

In the literature, there was a report of a rodent model that used mechanical loading in the hypofunctional TMJ[16] and a low-dose mono-iodoacetate[17] to induce condyle derangement. Mechanical loading in the hypofunction TMJ induced the PCR-like lesion in the posterosuperior region of the condyle. Caution is needed in interpreting the findings because hypofunction of the TMJ has been known to affect TMJ mechanoreceptors.[10] In the animal model using low-dose monoiodoacetate, possible involvement in the peripheral nerves cannot be separated. The use of drugs may affect peripheral nerves. In the present study, an animal model with mechanically induced PCR was used. The model was developed non-invasively and did not have systemic side effects in the animal. Since TMJ mechanoreceptors are more densely packed in the posterolateral areas than other areas of the capsule, a model was adopted that applied the load in the posterolateral direction. Successful application was confirmed by micro-CT images showing histological changes had occurred in the designated region.

The recording electrode was inserted into the left Gasserian ganglion with reference to the stereotaxic coordinates previously reported to record the single-unit activities of TMJ mechanoreceptors.[9–11] The Gasserian ganglion contains the cell bodies of trigeminal sensory neurons from periodontal mechanoreceptors. Identification of TMJ-afferent was complemented by electrical stimulation of the auriculotemporal nerve. The auriculotemporal nerve was exposed by removing the skin around the head. The posterior parts of the temporal and masseter muscles were detached to expose the TMJ region. Thus, the mandibular condyle and the TMJ capsule became visible and accessible to electrical stimulation.[18] Conduction velocity and conduction delay were estimated using the distance between the stimulating and recording electrodes. The criterion of conduction velocity adopted the classification of a previous study.[13] Additionally, the teeth were not stimulated in this study, thus the trigeminal sensory neurons from periodontal mechanoreceptors were not involved in the recordings. Therefore, the probability that the recording of the sensory units was performed exclusively from the TMJ mechanoreceptors was high.

PCR causes a decrease in condyle size resulting in clockwise mandible rotation, an increase in overjet and negative overbite, and a decline in posterior facial height.[2] These morphological changes are often clinically difficult to treat and to anticipate good prognosis.[2] In this study, the maximum instantaneous firing frequency associated with mandibular condylar resorption showed a significant decline after 1 week. It has often been suggested that the inputs from TMJ mechanoreceptors are involved in the physiologic regulatory mechanism of occlusal vertical dimension and thus play a role in regulating the mandibular position.[11] The false information that the level of jaw-opening is “smaller” than the actual amount may be transmitted to the brain, hence motor commands from the brain may cause the jaw to open wider by deactivating the jaw-closing neuromuscular system. A similar phenomenon was previously reported in the regulatory central mechanism of oral respiration where jaw opening is essentially required.[19] If this were the case in PCR, functional derangement of TMJ mechanoreception would further worsen open bite.

In a previous study, mandibular lateral shift was experimentally induced in rats to investigate its effect on the functional characteristics of TMJ mechanoreceptors. Results showed a decrease in the firing threshold, while the maximum firing frequency increased in the PCR group.[9] The previous animal model differs from the current PCR model in at least three points. First, it was a functional lateral shift model, meaning the condyle was laterally guided only when the mandible was functional, mainly during mastication. Second, the force applied to the TMJ

was not measured; however, in the current study, a constant force of 25 gF was applied. Third, it was not confirmed whether histological changes in the condyle occurred in the previous model, while micro-CT imaging in the present study confirmed that lesion was observed in the posterior part of the condyle. The differences between the previous and current PCR animal models suggest that TMJ mechanoreception varies depending on the circumstances of bone resorption or apposition on the condyle.

It is generally understood that the rat TMJ has a different morphology compared with that in humans.[20] Thus, interpretation of the findings in this study should be made with caution even if it is applicable to humans; overloading can cause systematic and physiological changes in the TMJ which are not desirable. Additionally, this study lacks a long-term prognosis; therefore, further studies are necessary to elucidate the exact mechanism of PCR. In the future, it would be beneficial to determine whether TMJ mechanoreceptors would adapt to or keep degenerating when PCR is occurring.

CONCLUSIONS

- Compressive force loading can experimentally induce PCR in the rat condyle. PCR is associated with the changes in the characteristics of TMJ mechanoreceptors that play a role in regulating mandibular position. Therefore, dysfunction of TMJ mechanoreceptors may be one of the etiological factors of anterior open bite, which is associated with PCR.
- In association with PCR, a significant decline in detection of the amount of jaw opening would enhance further jaw opening by deactivating the jaw-closing muscle activity, resulting in aggravation of open bite.

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