Original Article

Alterations of the Rat Temporomandibular Joint in Functional Posterior Displacement of the Mandible

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Abstract: Functional malocclusion that induces posterior condylar displacement may affect the remodeling processes of the temporomandibular joint structures. We tested the hypothesis that intermittent posterior condylar displacement due to functional malocclusion traumatizes condylar cartilage and joint innervated nerve fibers. Thirty-nine eight-week-old Wistar rats were used. To induce functional posterior condylar displacement, guiding appliances were attached to maxillary incisors of 24 rats for four, seven, and 14 days. Fifteen normal rats served as controls. Sections were stained with hematoxylin and eosin or processed for immunohistochemistry of protein gene product 9.5 and growth-associated protein-43 (GAP-43). Functional posterior condylar displacement led to a diminution in proliferative cells, reduction in cartilage width, and re-expression of GAP-43–immunoreactive nerve fibers. These results indicate that intermittent posterior condylar displacement due to functional malocclusion causes dysfunctional remodeling of condylar cartilage and nerve injury. (*Angle Orthod* 2004;74:677–683.)

Key Words: Condylar cartilage; Functional malocclusion; GAP-43; Intermittent mastication force; Posterior condylar displacement

INTRODUCTION

Functional malocclusion (occlusal interference) is considered to be one of the factors likely to cause temporomandibular joint disorders (TMD). TMD has been defined as ''a collective term embracing a number of clinical problems that involve the masticatory muscles, the temporomandibular joint (TMJ) and associated structures, or both''.[1] In the occluded condition, functional malocclusion can displace the condyle from the position that it normally occupies in the center of the glenoid fossa. In clinical studies, there are many controversial reports about occlusion and TMD. Various authors have reported that there are no scientifically established risk factors between malocclusion and TMD.[2–4] Others have found a high correlation between TMD and posterior condylar displacement resulting from malocclusion,[5–7] especially severe deep overbite in both Class I and Class II malocclusions. In patients with posterior displacement of the condyle, the incidence of clicking (81%) and pain (65%) is high.[8] However, the underlying changes resulting from functional posterior displacement of the condyle are still unknown.

Displacement of the condyle may result in abnormal loading of the tissues in and around the joint. Like other articular cartilage of the body, the condylar cartilage is mainly a load-bearing structure for induced biomechanical stresses. The thickness of articular cartilage has also been suspected to be subjected to functional adaptation.[9] If nonphysiological stress adversely affects the mechanical function of the TMJ, remodeling of the TMJ will be dysfunctional and cause histological alterations and a decrement in condylar head volume.[10]

Investigations of condylar cartilage under many conditions in animals have been reported over the years. Retrusion of the mandible in adult and older rats shows less metabolic activity and cartilage formation than in younger ones.[11] Posterior displacement of the mandible with an unstable occlusion (maxillary and mandibular teeth were not in contact) resulted in a reduction of the number of cartilaginous cells in the rabbit TMJ.[12] Experimentally induced posterior condylar displacement with a continuous force demonstrated a decrease in the proliferation of chondro-

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cytes and the amount of extracellular matrix.[13] However, only a few studies about the effects of intermittent force on the TMJ in vivo during mastication have been reported. Furthermore, the posteriorly displaced condyle may cause inflammation, which can spread to the nerve fibers passing through the narrow posterior joint space.[5] The above occurrences may predispose the joints to TMD.

Therefore, because posterior condylar displacement is likely to generate nonphysiological stress on the TMJ, this study poses the question whether intermittent posterior condylar displacement due to functional malocclusion traumatizes the condylar cartilage and the innervated nerve fibers. With the rat as the experimental model, we examined the condyles and retrodiscal tissues for histological parameters as well as immunohistochemical changes after intermittent posterior condylar displacement due to functional malocclusion.

MATERIALS AND METHODS

Animal and tissue preparations

The Institutional Animal Care and Use Committee of the Tokyo Medical and Dental University approved the animal protocols. The experiment was properly carried out under the control of the University's Guidelines for Animal Experimentation.

Thirty-nine eight-week-old male Wistar rats weighing between 255 and 265 g were divided into an experimental group of 24 rats and a control group of 15 rats. The experimental group and the control group were each randomly divided into three subgroups.

All the experimental rats were anesthetized by an intraperitoneal injection with a 4:1 mixture of ketamine hydrochloride and 20% xylazine hydrochloride (0.1 mL/100 g body weight). To induce posterior displacement of condyles in the occluded condition, we modified an earlier reported guiding appliance.[12] The modified guiding appliances were constructed from band material (0.180 × 0.005 inch, Rocky Mountain Orthodontics, Denver, Colo) sized 8 × 14 × 4 mm and were attached to the maxillary incisors with composite resin (Clearfil SC composite resin, Kuraray, Japan) for four, seven, and 14 days (Figure 1b). After attachment of the appliances, all experimental rats could function with the mandible normally. All animals were fed standard rat chow (CE-2, Japan Clea Inc, Shizuoka, Japan) and water ad libitum in a 12-hour light-dark environment at a constant temperature of 23°C. The rats were weighed three times a week.

Each experimental subgroup, together with its control, was deeply anesthetized with diethyl ether and intraperitoneal injection, and then sacrificed by transcardiac perfusion with saline followed by ice-cold 4% paraformaldehyde with 0.2% picric acid in 0.1 M phosphate buffer, pH 7.4. Lateral radiographs of the rat skulls were made to confirm molar relationship by using a Sofron SRO-M50 (Sofron Ltd, Tokyo, Japan).

Undecalcified ground section preparation

We used an undecalcified ground section technique to confirm the relationship between the TMJ and surrounding structures seven and 14 days postattachment of the appliances, together with the controls. The skulls were fixed in 10% buffered formalin for three days, dehydrated in serial concentrations of ethanol, cleared with monomer, and embedded in polyester resin (Rigolac, Showa High Polymer Co Ltd, Tokyo, Japan). Sections of 70-μm thickness were cut with a diamond band saw (BS-3000, EXAKT, Norderstedt, Germany) and ground with a grinding-sliding machine (Microgrinding MG-4000, EXAKT).

Decalified tissue section preparation

Both TMJs were dissected and immersed en bloc in the same fixative for an additional 12 hours at 4°C. After fixation, the tissue specimens were decalcified in 10% ethylenediamine tetraacetic acid–2Na solution, pH 7.4, for 4–5 weeks. The decalcified tissues were then placed in 20% sucrose overnight and embedded in OCT compound (Tissue Tek, Sakura, Japan). Frozen sections, 20 μm in thickness, were cut sagittally using a cryostat (Leica CM3000, Nussloch, Germany). We investigated morphological changes of the condylar cartilage using hematoxylin and eosin (H&E) staining on the left-side TMJ and of the joint innervated nerve fibers by immunohistochemistry of protein gene product 9.5 (PGP 9.5), which is a general marker for neural elements,[14,15] and growth-associated protein-43 (GAP-43), a marker for nerve injury because it is an axonal membrane protein involved in neuronal development and repair,[16–19] on the right-side TMJ.

Quantitative evaluation of condylar cartilage widths

Twenty H&E-stained sections of each left-side TMJ were selected and photographed with a digital camera (Nikon DXm1200, Kanagawa, Japan). A vertical line was drawn through the middle of the condyle in an anteroposterior direction. A horizontal line, perpendicular to the vertical line, was drawn through the most prominent posterior point of the condylar head. The intersection of these lines was marked. We measured the widths of condylar cartilage from the lowest border of the hypertrophic layer to the outer border of the fibrous layer along with 30°, 45°, and 60° angulations to verify the cartilage width at the posterior region by using the Image-Pro Plus image analysis software (V 4.0, Media Cybernetics, Silver Spring, Md) (Figure 3a).

Statistical analysis

The condylar cartilage widths of the left-side TMJs were represented as mean ± standard error of the mean. Statis-

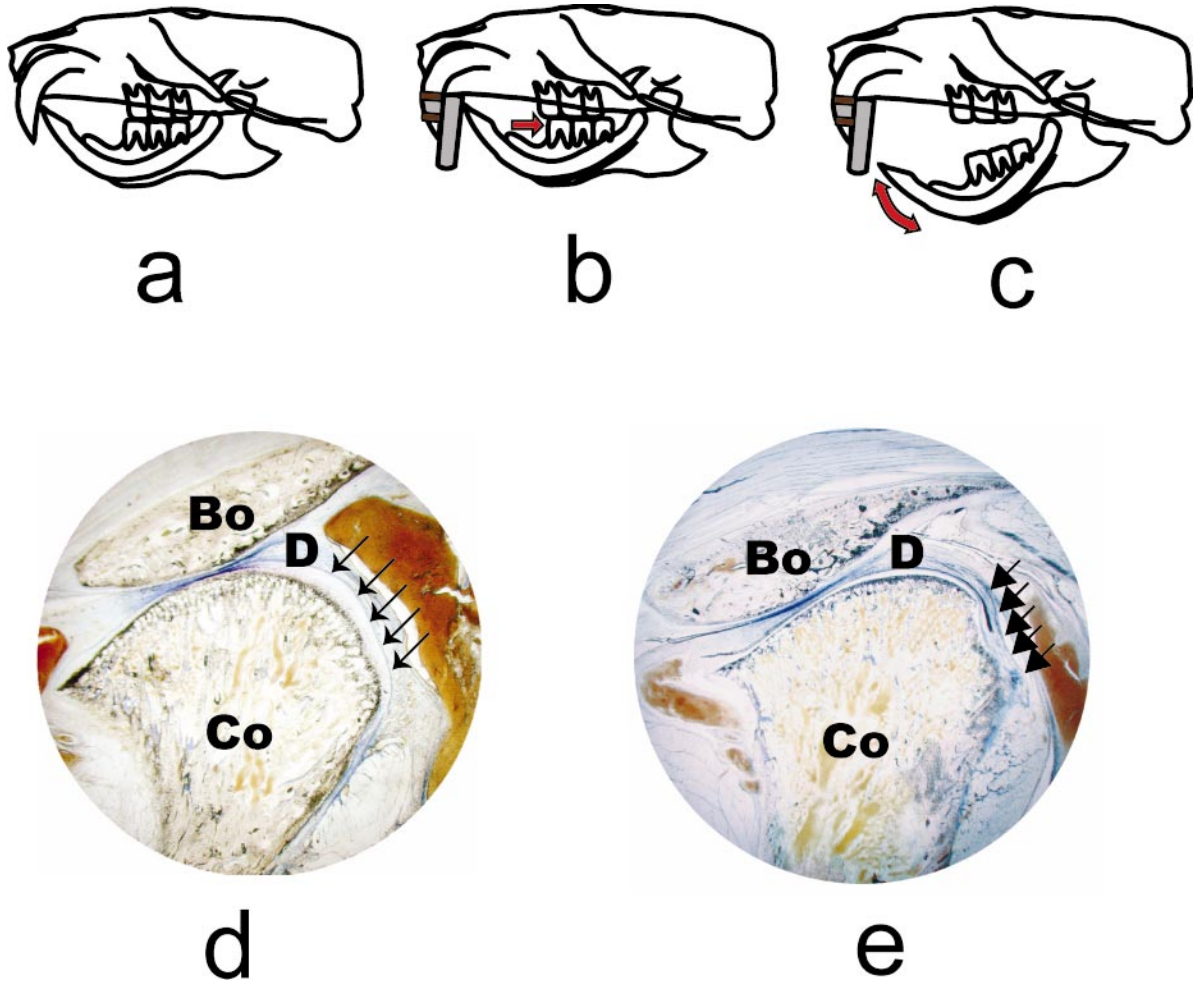

FIGURE 1. Position of the mandible and condyle after appliance seating. (a) Tracing of lateral radiograph of rat skull in control rat at centric occlusion. (b) In experimental rat at centric occlusion. (c) In experimental rat during jaw opening. (d) Undecalcified ground section of TMJ in control rat showing the normal position of condyle and convex shape of condyle at posterior region, ←. (e) In experimental rat, after seven days postattachment of appliance showing a posterior displacement of condyle and flattening of the condyle at the posterior region, ←. Co, condyle; D, articular disc; and Bo, bone.

tically significant differences between control and experimental subgroups were assessed by Mann-Whitney *U*-test using SPSS statistical software (V 11.0 for Windows, SPSS Inc, Chicago, Ill).

Immunohistochemistry of PGP 9.5 and GAP-43

The prepared sections of the right-side TMJs were rinsed with 0.3% Triton X-100 in phosphate buffer and then treated with methanol containing 0.3% H_2O_2, followed by preincubation with 2% normal goat serum. The primary antibody was rabbit antisera to PGP 9.5 (1:10,000; Ultraclone, Cambridge, UK) or GAP-43 (1:1000; Novus Biologicals Inc, Littleton, Co). The secondary antibody was biotinylated goat anti-rabbit immunoglobin G (IgG) (1:200; Vector Laboratories, Burlingame, Calif), and the diaminobenzodine method was used to observe final staining. Negative controls were obtained by replacing the primary antibodies with nonimmune rabbit serum or by omitting the antirabbit IgG. Specimens of rat brain served as positive control.

RESULTS

Radiographic findings

Tracings of the lateral radiographs of the controls showed that the mandibular first molars were mesial to the maxillary first molars in the occluded condition. In contrast, the mandibular first molars in the experimental rats after postattachment of appliances were in a distal relationship to the maxillary first molars. No incisal attrition of the mandibular incisors was observed in the experimental rats even after 14 days postattachment of the appliance (Figure 1a–c).

Undecalcified ground section findings

Undecalcified ground sections of the experimental rats after seven and 14 days postattachment showed that the

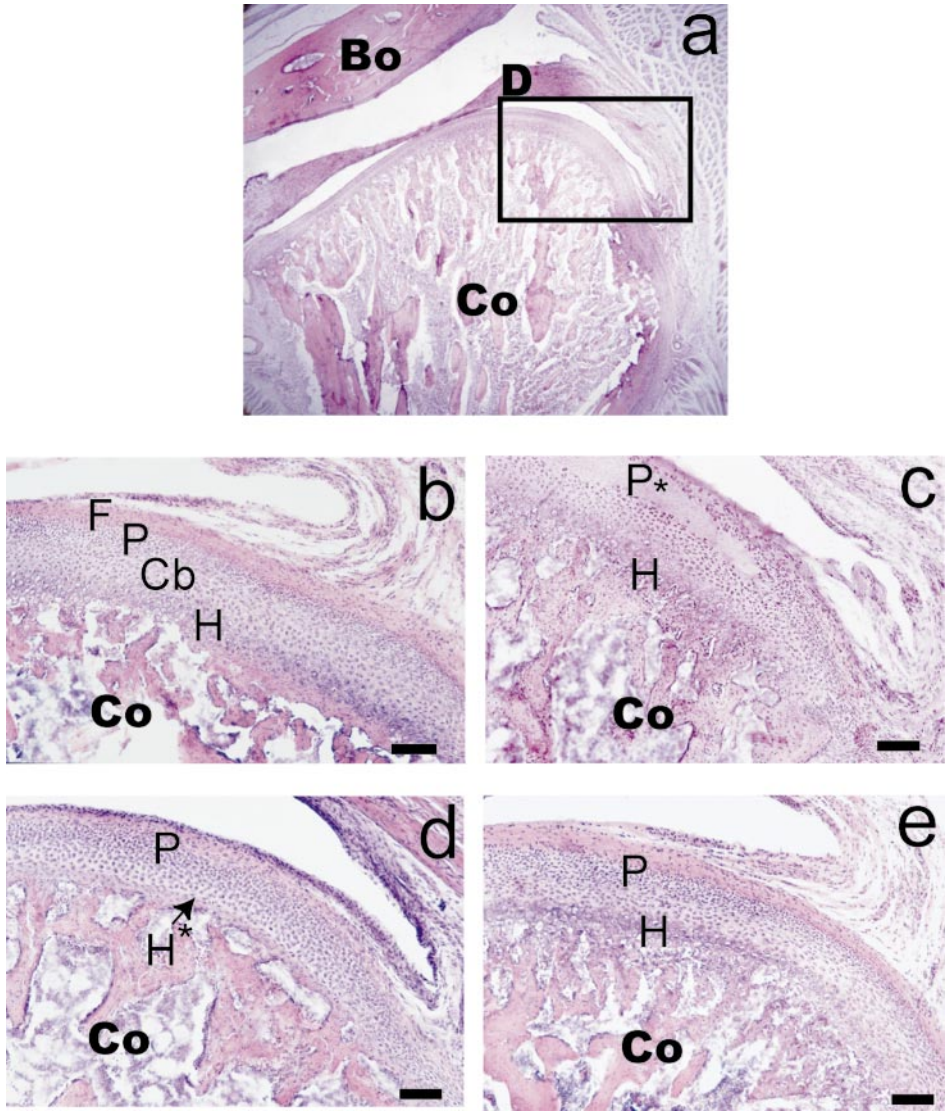

FIGURE 2. Photomicrographs of mandibular condyle sections stained with hematoxylin and eosin. (a) Observation area. (b) Control rat. (c) Experimental rat four days postattachment of appliance. (d) Experimental rat seven days postattachment of appliance. (e) Experimental rat 14 days postattachment of appliance. Co, condyle; D, articular disc; Bo, bone; F, fibrous layer; P, proliferative layer; Cb, chondroblast layer; H, hypertrophic layer; P*, disappearance of cells in proliferative layer; and H*, disappearance of hypertrophic layer. Bar = 100 μm.

condyles in the occluded condition were displaced posteriorly. The posterior regions of the condyles were flattened compared with the controls (Figure 1d,e).

Histological examination of condylar cartilage

In normal rat condyles, the layers of cartilage are regionalized to a fibrous layer, a proliferative layer, a chondroblastic layer, and a hypertrophic layer (Figure 2b). There was no significant difference among these subgroups of the controls. All control rats were pooled. However, in the experimental rats, after four days postattachment of the appliance, cells in the proliferative layers at the posterior regions of the condyles were obviously diminished and also exhibited irregular cellular distributions, whereas the hypertrophic layers were the same as those in the controls (Figure 2c). Seven days postattachment, the calcified chondrocytes in the hypertrophic layers had almost totally vanished (Figure 2d). After day 14, cells in the proliferative layers had recovered nearly to the controls, and the hypertrophic layers also tended to increase compared with day 7 (Figure 2e).

Quantitative analysis

Condylar cartilage widths at 30°, 45°, and 60° measurements showed no significant differences. We calculated the width of cartilage by using all the measurements. The cartilage width significantly decreased on day 7 (0.173 ± 0.011 mm; $P < .001$) and day 14 (0.236 ± 0.003 mm; P

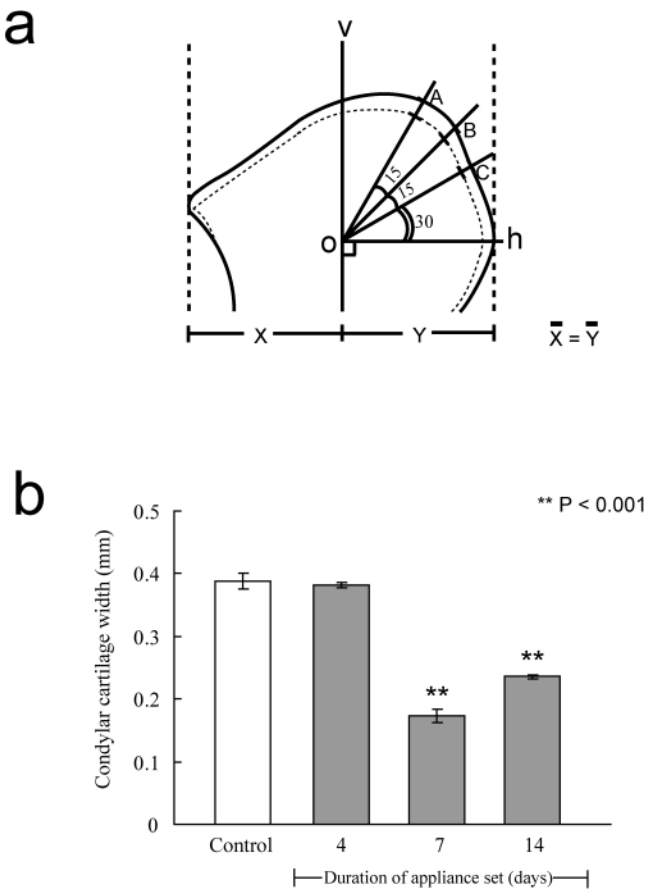

FIGURE 3. (a) Measurement of condylar cartilage widths at posterior region. The vertical line (ov) was drawn through the middle of the condyle in anteroposterior direction. The horizontal line (oh), which was perpendicular to the vertical line, was drawn through the most posteriorly prominent point of the condyle. The condylar cartilage widths were measured at A, B, and C (60°, 45°, and 30°, respectively). (b) Quantitative analysis of posterior condylar cartilage width in control and experimental rats. The data represent mean ± standard error of the mean. ** indicates $P < .001$ vs control.

$< .001$) compared with the controls (0.388 ± 0.013 mm) (Figure 3b).

Immunohistochemical changes of nerve fibers

In sections of all groups, PGP 9.5–immunoreactive (IR) nerve fibers were observed in peripheral portions of the discs, posterior disc attachments, joint capsules, and synovial membranes. However, no nerve fibers were observed in the central portions of the discs (Figure 4b). In the control rats, GAP-43–IR nerve fibers were only found surrounding blood vessels (Figure 4c), whereas in the experimental rats after seven days postattachment, GAP-43–IR nerve fibers were showing not only around blood vessels but also at the posterior disc attachments and synovial membranes (Figure 4e). These findings were true on day 14 also (Figure 4f).

DISCUSSION

In this study, we used rats as an experimental model because the rat TMJ is widely accepted for these studies.[11,20–25] The modified guiding appliance in this study induced functional malocclusion that displaced the mandible posteriorly in the occluded condition, which caused intermittent posterior displacement of the condyle. This experimental model established a disto-occlusion in the rat without changing the vertical dimension because no attrition of the incisal edges of the mandibular incisors was observed throughout the experimental period. The rat was able to occlude the posterior teeth during mastication and also able to open and close the mandible normally.

Physiological stress to the TMJ, if optimal, is of great importance for the development of TMJ structures during adolescence and condylar remodeling in the adult.[26,27] In contrast, nonphysiological stress is the stress that harms tissue structures. In this experimental model, however, nonphysiological stress probably occurred at the posterior region of the condyle and disc because of the anatomy of the TMJ for there is a limitation of movement in the posterior direction. Furthermore, in humans there is the postglenoid process; therefore, nonphysiological stress due to mechanical obstruction to the posterior condylar displacement in humans must be greater. We assume that there would be more condylar resorption at the posterior region of the human TMJ and resorption of the anterior surface of the postglenoid spine may be found, as in the study of the adult monkey TMJ (*Macaca mulatta*).[28]

The sequence of growth of the condylar cartilage begins with mesenchymal cells in the proliferative layers and differentiates into chondroblasts and elaborates an extracellular matrix followed by hypertrophy of the chondrocytes. The matrix calcifies circumferentially to become calcified chondrocytes in a hypertrophic layer. The calcified chondrocytes around the lowest level of the hypertrophic layer is resorbed next by chondroclasts in the zone of erosion.[21,22]

This study demonstrated that dysfunctional remodeling of condylar cartilage was substantially influenced by nonphysiological stress. We found a diminution of cells in the proliferative layers at the posterior regions of the condyles on day 4. This data showed that when chondroprogenitor cells for the growth cartilage were destroyed, the calcified matrices in the hypertrophic layers could not be produced and disappeared during the experimental period (day 7). Thus, the average cartilage width significantly decreased as shown on days 7 and 14. On day 14, there was some adaptation of cartilaginous cells. Although the development was slow and the change was abrupt, a significant decrease in cartilage width was observed. However, we suppose that if there were still an intermittent posterior condylar displacement, the nonphysiological stress would still occur.

The nonphysiological stress not only occurs on the surface of the condyle but also is distributed on the articular

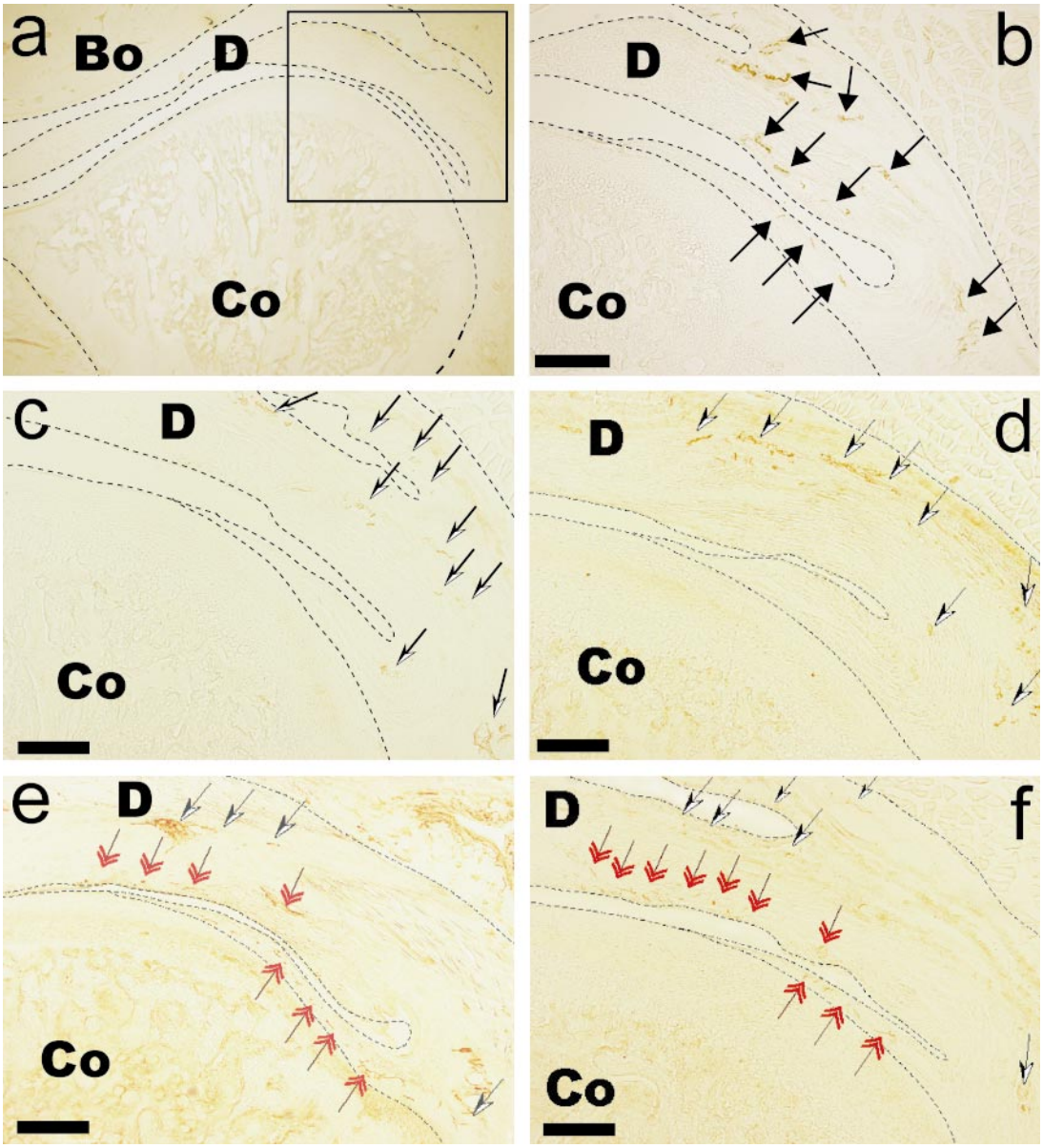

FIGURE 4. Innervation and regeneration of nerve fibers. (a) Observation area. (b) Overall innervation at posterior region of TMJ as shown by PGP 9.5-immunoreactivity in the control rat. (c) GAP-43–immunoreactive nerve fibers existed only around blood vessels in control rat. (d) Four days postattachment of appliance, the expression pattern of GAP-43 was similar to that of the control. (e) Seven days postattachment of appliance, at lower posterior disc attachment and synovial membrane exhibited the expression of GAP-43. (f) On day 14, as in (e). →, PGP 9.5–immunoreactive nerve fibers; ↠, GAP-43–immunoreactive nerve fibers around blood vessels; ⇛, regeneration of GAP-43–immunoreactive nerve fibers at lower posterior attachment of disc and synovial membrane. Co, condyle; D, articular disc; and Bo, bone. Bar = 200μm.

disc in the anterior disc displacement position.[29] PGP 9.5–IR nerve fibers that were observed in the posterior attachments of the discs in the control rats indicated that there was a distribution of nerve fibers, whereas in the same observation area, GAP-43–IR nerve fibers were not observed in the posterior disc attachments, except around the blood vessels. This agrees with reports that the GAP-43–IR nerve fibers did not exist after growth had finished or if there was no injury to the nerve fibers[30] but only existed throughout sympathetic nerve fibers.[31]

In the experimental rats, nonphysiological stress to the posterior attachments of the discs caused injury to the innervated nerve fibers because we observed re-expression of GAP-43–IR nerve fibers at the posterior disc attachments on days 7 and 14. Many studies in various animals have reported that expression of GAP-43 has an initial delay due to axon elongation after the injury,[32,33] and the expression is not fully activated until axon elongation is well underway.[16] The delay of expression for GAP-43 suggested that alterations in both condylar cartilage and innervated nerve

fibers might occur at almost the same period of time (day 4).

CONCLUSIONS

Our study reveals that the functional posterior displacement of the mandible induces dysfunctional remodeling of condylar cartilage and causes injury to the innervated nerve fibers. However, further studies for long-term effects are necessary to clarify whether inflammation or pain is also involved in the TMJ under this condition.

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