Original Article

Changes in Response Properties of Periodontal Mechanoreceptors After Experimental Orthodontic Tooth Movement in Rats

Hokuto Nakanishi, DDS[a]; Yoichiro Seki, DDS, PhD[b]; Tomoko Kohno, DDS, PhD[b]; Takeshi Muramoto, DDS, PhD[c]; Kazuo Toda, DDS, PhD[d]; Kunimichi Soma, DDS, PhD[e]

Abstract: Using an in vitro preparation, we investigated chronological changes in response properties of periodontal mechanoreceptors (PMRs) in the rat right mandibular first molar (M1) after experimental orthodontic tooth movement. Orthodontic force was applied to M1 for 14 days by activating 24.5 mN superelastic titanium-nickel alloy closed coil springs anchored to the mandibular incisors. Experiments were performed on days 3, 7, 10, and 14 during application of orthodontic force and on days 7, 14, 21, and 28 after removal of orthodontic force. The rats without application of orthodontic force were used as control group. In each group, direct mechanical stimulation using von Frey hairs and electrical stimulation was applied to the distal root of M1. Results showed that compared with controls (1) the mechanical thresholds were significantly lower during application of orthodontic force; however, no significant difference was found after removal of force application and (2) conduction velocities were significantly lower from day 7 during application of orthodontic force to day 14 after removal of orthodontic force; however, no significant difference was found on days 21 and 28 after removal of orthodontic force. These results suggest that the PMRs, although having some of their response properties altered during orthodontic force application, were able to recover and adapt to the newly acquired intraoral condition after removal of the orthodontic force. (*Angle Orthod* 2004;74:93–99.)

Key Words: In vitro; Periodontal mechanoreceptor; Tooth movement; Response property; Rat

INTRODUCTION

The periodontal mechanoreceptors (PMRs) are regarded as sensory receptors playing an important role in the stomatognathic system, transmitting afferent information from the tooth to the central nervous system. The inputs from PMRs can control jaw movements and influence a variety of reflex systems in the orofacial region.[1–5] The object of clinical orthodontic treatment is to obtain both an esthetic appeal and a functional occlusion.[6] Thus, the physiological properties of PMRs should be able to adapt to new tooth position after removal of orthodontic force.

The arrangement of collagen fibers of the periodontal ligament is changed during experimental orthodontic tooth movement,[7–9] and after experimental orthodontic tooth movement, the arrangement of collagen fibers recovered to the state similar to that before the experimental orthodontic tooth movement.[10,11]

On the other hand, there have been very few reports on the physiological properties of PMRs during[12,13] or after[14] experimental orthodontic tooth movement. Therefore, this study was conducted to reveal the chronological changes in response properties of PMRs after experimental tooth movement, using in vitro preparation.[15,16]

MATERIALS AND METHODS

Animals

Fifty-three 7-week-old female Wistar albino rats were used with a mean initial body weight of 173.7 ± 0.9 g

[a] Graduate Student, Orthodontic Science, Department of Orofacial Development and Function, Division of Oral Health Sciences, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan.

[b] Clinical Fellow, Orthodontic Science, Department of Orofacial Development and Function, Division of Oral Health Sciences, Tokyo Medical and Dental University, Tokyo, Japan.

[c] Teaching Fellow, Orthodontic Science, Department of Orofacial Development and Function, Division of Oral Health Sciences, Tokyo Medical and Dental University, Tokyo, Japan.

[d] Professor, Integrative Sensory Physiology, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan.

[e] Professor, Orthodontic Science, Department of Orofacial Development and Function, Division of Oral Health Sciences, Tokyo Medical and Dental University, Tokyo, Japan.

Corresponding author: Hokuto Nakanishi, DDS, Orthodontic Science, Department of Orofacial Development and Function, Division of Oral Health Sciences, Graduate School, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, 113-8549 Tokyo, Japan (e-mail: hokuto.orts@tmd.ac.jp).

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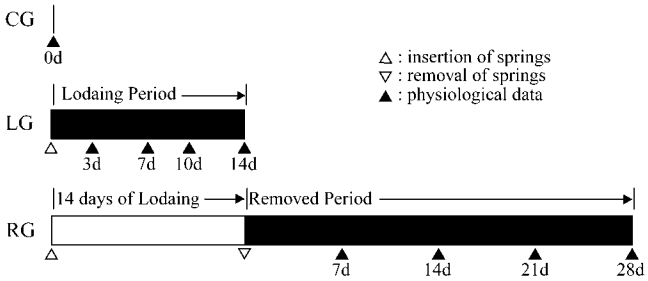

FIGURE 1. Experimental protocol. The rats without application of orthodontic force served as the CG. In the LG, orthodontic force was applied for 3, 7, 10, and 14 days. In the RG, springs were removed after 14 days of insertion and were fed for 7, 14, 21, and 28 days after removal.

(mean ± SEM; n = 53). The rats were randomly divided into one control group (CG) and two experimental groups, that is, a loading orthodontic force group (LG) and a removed orthodontic force group (RG). Furthermore, the LG was divided into 3d- (n = 6), 7d- (n = 6), 10d- (n = 7), and 14d-LG (n = 5) according to days of force application. The RG was divided into 7d- (n = 5), 14d- (n = 6), 21d- (n = 5), and 28d- RG according to days after force removal (n = 7) (Figure 1). Rats (7-week-old) without application of orthodontic force were used as the CG (n = 6). The response properties of PMRs are stable in 7- to 13-week-old rats.[17] All animals were fed ad libitum with powder diet (Rodent Diet CE-2; Japan Clea Inc, Shizuoka, Japan) and had free access to drinking water. All procedures were performed under the guidelines of the Tokyo Medical and Dental University for Animal Research.

Application of orthodontic force

The application of orthodontic force was based on a modified technique described elsewhere.[18–20] The rats were anesthetized with ketamine hydrochloride (40 mg/kg, i.p.; Veterinary Ketalar50®, Sankyo Co, Ltd, Tokyo, Japan) containing 20% xylazine hydrochloride (3.5 mg/kg, i.p.; Celactal® 2% injections, Bayer-Japan Co, Ltd, Tokyo, Japan), after initial inhalant anesthesia with diethyl ether (Wako Pure Chemical Industries, Osaka, Japan).

One end of a closed coil spring (lumen: 0.9 mm) fabricated from superelastic titanium-nickel alloy wire (diameter: 0.093 mm; Furukawa Electric Co, Ltd, Tokyo, Japan) connected with a clamp was inserted into the cervical region of the right mandibular first molar (M1) and fixed with light curing composite resin (Clearfil® Photo SC, Kuraray, Okayama, Japan). To confirm the fixation of the spring, the mandibular incisors were notched on the labial surface of the cervical region using a round steel bur (Meisinger ST1HP 008, Hager and Meing GmbH, Dusseldolf, Germany) with a dental drill, and the incisors were ligated with ligature wire (diameter: 0.25 mm; Tomy International, Tokyo, Japan) to the other end of the spring. The spring was further fixed with adhesive materials and composite resin. With this model, a mesially directed continuous force of approximately 24.5 mN[13] was subjected to M1. After 14 days of application of orthodontic force, the springs of the RG were removed under inhalant anesthesia with diethyl ether.

Histological observation

Mechanical stimulation was limited to the distal apical one-third of the distal root of M1 (Figure 2a through c), where PMRs abundantly exist.[21,22] We observed the histology of the periodontal tissue of rats under the same conditions of the CG, 14d-LG, and 7d-RG. The rats were sacrificed under ether anesthesia, and the right half of the mandible of each rat was removed en bloc. The specimens were fixed with 4% paraformaldehyde in 0.1 M cacodylic acid buffer (pH 7.3) for three days (4°C). When the fixation was completed, the appliances of the 14d-LG were removed. The specimens were decalcified in 10% ethylenediaminetetraacetic acid solution at 4°C for 35 days and embedded in paraffin by a conventional method. Sections (5 μm thick) sagittal to the long axis of the distal root of M1 were stained with hematoxylin and eosin and examined under light microscope (MICROPHOT-FXA, Nikon, Tokyo, Japan).

In vitro jaw-nerve preparation and experimental setup

Under deep anesthesia using thiamylal sodium (60 mg/kg, i.p.; Isozol®, Yoshitomi Pharmacy, Osaka, Japan), an in vitro jaw-nerve preparation was made as previously reported.[15,16] To apply direct mechanical stimulation to PMRs, three mandibular molars on the right side were extracted from the mandible. The chamber consisted of two pools (test and oil pools) separated by a thin plastic plate with a small hole drilled in its center. The inferior alveolar nerve trunk was passed through the hole and fixed in the oil pool by the cotton thread. The nerve trunk in the oil pool was slightly pulled to furnish enough tension for easy insertion of the recording electrode. The contents of the two pools were separated by filling the hole surrounding the nerve trunk with vaseline. The oil pool was filled with liquid paraffin to avoid dehydration of the nerve trunk. The mandible was placed on the rubber bed in the test pool and was fixed to the rubber bed with dental cement (GC Fuji I®, GC Corporation, Tokyo, Japan). The test pool was perfused by a modified Krebs-Henseleit solution (107.4 mM NaCl, 3.4 mM KCl, 1.5 mM $CaCl_2$, 0.7 mM $MgSO_4$, 2.2 mM NaH_2PO_4, 26.2 mM $NaHCO_3$, 9.6 mM sodium gluconate, 5.5 mM glucose, 7.6 mM sucrose) saturated with a O_2–CO_2 (95:5%) gas mixture.

Stimulation and recording

For mechanical stimulation, eight intensities (2.61, 4.74, 7.35, 8.39, 9.41, 10.62, 13.79, and 15.84 mN) of calibrated

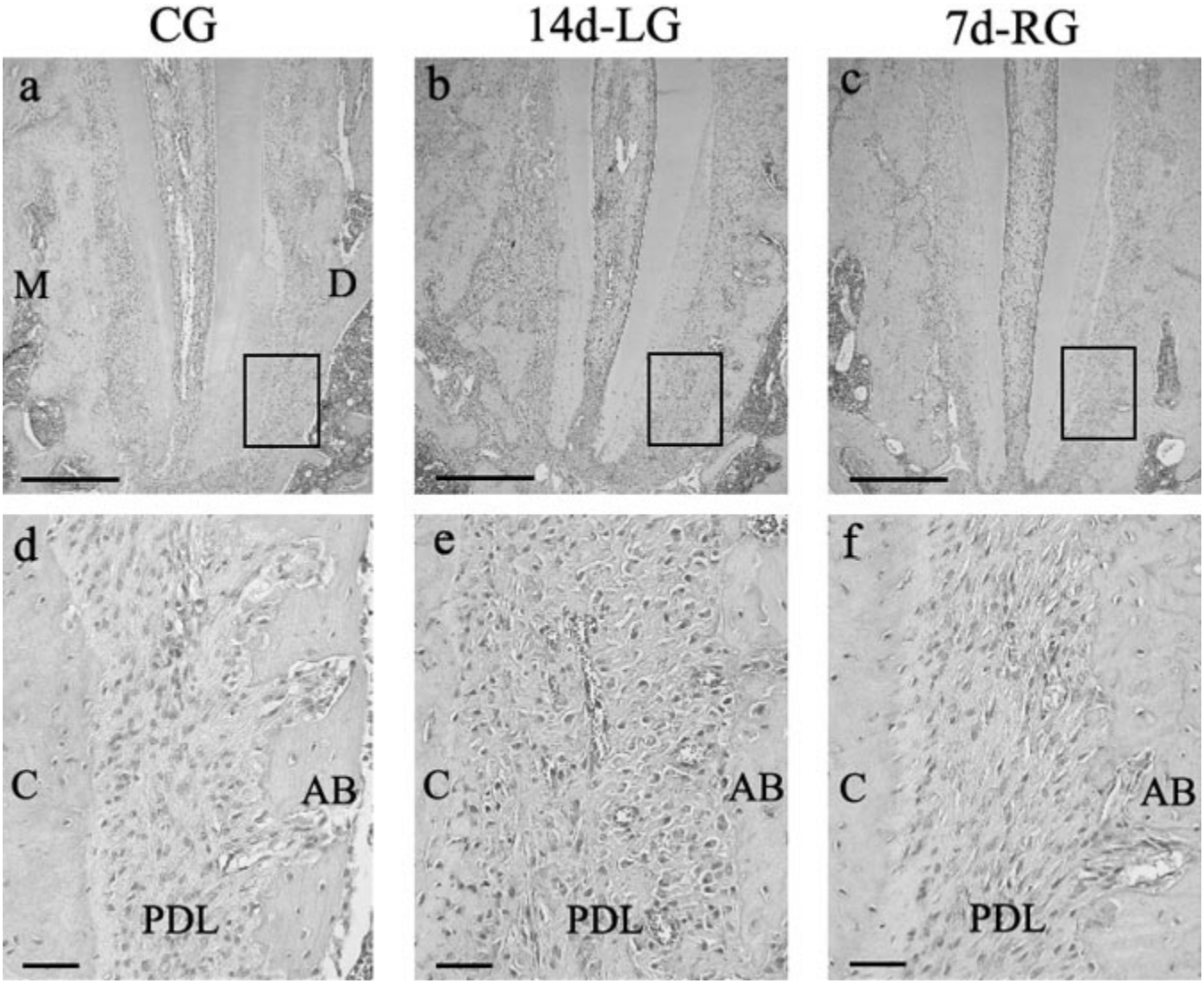

FIGURE 2. Histological findings. (a)–(c) The periodontal tissues of the distal root of M1. The enclosed region indicates the observation area, which was the apical one-third of the distal root of distal side (bars = 500 μm). (d)–(f) High-magnification view of each enclosed region (bars = 50 μm). (a) and (d) CG, the periodontal collagen fibers ran wavy between cementum over alveolar bone. (b) and (e) 14d-LG, the periodontal collagen fibers showed irregular arrangement, and the thickness of the periodontal ligament tended to increase. (c) and (f) 7d-RG, the periodontal collagen fibers showed similar arrangement, and the thickness of the periodontal ligament were almost equal to the CG. M indicates mesial side; D, distal side; AB, alveolar bone; PDL, periodontal ligament; and C, cementum.

plastic von Frey hairs (tip diameter: 0.2 mm) were manually applied (duration: 5–10 seconds) to the PMRs remaining in the stimulating site. Using the microneurographic method,[23] single-unit activities were recorded from the nerve trunk with a tungsten microelectrode (Type: 25-10-1, FHC, Brunswick USA) inserted into the nerve trunk.

The signal was fed into a high-impedance, low-noise amplifier (DAM80-E, World Precision Instruments, Sarasota, Fla). The signal was digitized and displayed on the monitor with a data analyzing software (Spike2® for Windows® Version 4.02, Cambridge Electronic Design, Cambridge, UK), using a signal processing interface (CED 1401 plus, Cambridge Electronic Design, Cambridge, UK) and a DOS-V computer.

The units were divided into two types according to their response pattern: those that discharged continuously for more than five seconds while mechanical stimulation was applied were classified as a slowly adapting (SA) type, and the other units were classified as a rapidly adapting (RA) type.

Conduction velocity of the innervating fibers

To estimate the conduction velocity of the recorded single afferent fiber, electrical stimulation was applied to the tooth sockets of the distal root of M1 by a bipolar concentric tungsten electrode (tip diameter: 0.4 mm, tip distance: 1 mm; IMB-9004, Inter Medical, Nagoya, Japan) and the

TABLE 1. Distribution of Subtypes of Units

Type	CG 0d	LG 3d	LG 7d	LG 10d	LG 14d	RG 7d	RG 14d	RG 21d	RG 28d	Total
RA-on	7	9	3	5	9	9	5	8	10	67
RA-off	5	6	11	9	4	13	10	14	10	82
RA-on/off	14	8	9	11	5	8	13	10	17	95
SA	1	—	1	—	—	—	—	1	1	4
Total	27	23	24	25	20	30	28	33	38	248

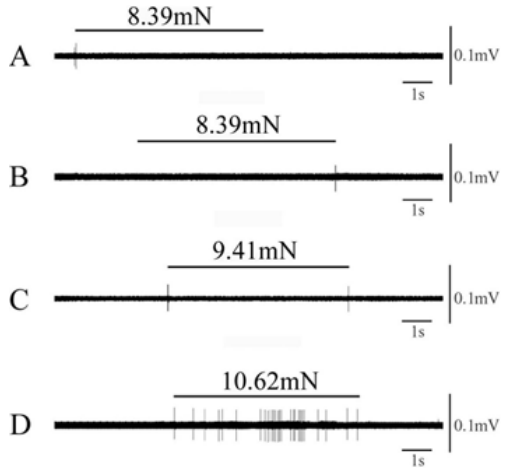

FIGURE 3. Typical examples of responses. Horizontal bar above each trace indicates the duration of the mechanical stimulation applied by von Frey stimuli. (A) RA-on type; (B) RA-off type; (C) RA-on/off type; (D) SA type.

action potential was displayed on an oscilloscope (CS-6030, KENWOOD, Kanagawa, Japan). The conduction velocity was calculated from both the conduction time and the distance between the stimulating and recording electrodes and was corrected to the value at 37°C, using the Q_{10} correlation reported by Paintal.[24] Fibers with conduction velocity of less than 2.0 m/s were regarded as unmyelinated (C), those between 2.0 and 10.0 m/s as thin myelinated (Aδ), and those over 10.0 m/s as large myelinated (Aβ) fibers.[25] In this study, only mechanoreceptors innervated by Aβ fibers were investigated further.

Statistical analysis

For statistical analysis, the data-analyzing software (StatView® for Windows®, Version 5.0, SAS Institute, Cary, NC, USA) was used. All data are expressed as mean ± SEM. The statistical differences between the CG and each experimental group were evaluated by the Mann-Whitney's *U*-test. A probability of less than .05 was considered significant.

RESULTS

Histological finding

In the CG, the periodontal collagen fibers ran wavy between cementum and alveolar bone. In the 14d-LG, the periodontal collagen fibers were irregularly arranged, and the thickness of the periodontal ligament tended to increase compared with the CG. However, in the 7d-RG, the periodontal collagen fibers showed almost the same arrangement, and the thickness of the periodontal ligament was almost equal to the CG. Excessive root resorption or inflammatory reactions were not detectable in any group (Figure 2).

Unit type

Responses to the mechanical stimulation were recorded from a total of 248 units. The units were classified into 244 RA and four SA units (Table 1). The RA units were classified into three subtypes: those that discharged at the beginning of continuous mechanical stimulation were classified as RA-on, those that discharged at the end as RA-off, and those that discharged at both the beginning and the end as RA-on/off type. The typical examples of each type are shown in Figure 3.

Mechanical threshold

Chronological changes were found in the threshold to mechanical stimulation during and after experimental orthodontic tooth movement. Table 2 shows the mean values of mechanical thresholds for each group. Figure 4 shows the chronological changes of mechanical thresholds. Groups 3d-, 7d-, 10d-, and 14d-LG had significantly lower threshold values compared with the CG. In contrast, there was no significant difference in the 7d-, 14d-, 21d-, and 28d-RG.

Conduction velocity of innervating fibers

The conduction velocity of 248 mechanoreceptive units was distributed between 10.20 and 21.15 m/s. Table 2

TABLE 2. Mean Values of the Mechanical Threshold and the Conduction Velocity[a]

	CG 0d	LG 3d	LG 7d	LG 10d	LG 14d
Mechanical threshold (mN)	9.23 ± 0.13	8.23 ± 0.21*	8.54 ± 0.21*	8.72 ± 0.12*	8.81 ± 0.14*
Conduction velocity (m/s)	17.01 ± 0.30	17.54 ± 0.26	15.43 ± 0.28*	14.15 ± 0.28*	13.89 ± 0.55*
	n = 27	n = 23	n = 24	n = 25	n = 20

[a] All data are given as mean ± SEM (*$P < .05$).

shows the mean values of the conduction velocities for each group. Figure 5 shows the chronological changes in conduction velocity. Compared with the CG, significantly lower conduction velocities were found in the 7d-, 10d-, and 14d-LG and in the 7d- and 14d-RG. No significant difference was found in the 21d- and 28d-RG.

DISCUSSION

Histological finding

The periodontal ligament incessantly receives the occlusal forces, and active tissue remodeling constantly occurs.[26] The periodontal ligament was abundantly supplied by two kinds of receptors, Ruffini-like endings and free nerve endings,[21] which have their structures changed in response to external stimuli like orthodontic force or occlusal trauma.[27,28] PMRs also have a high potential for neural plasticity.[27,28] The active remodeling of the periodontal ligament and the structural alteration of PMRs are positively related to each other.[27,29] Our histological findings suggest that structural alterations of PMRs took place. Moreover, because typical figurations to traumatic tissue,[30,31] excessive infiltration of inflammatory cells, or wide range of root resorption were not observed, occlusal trauma has no influence under these conditions. Thus, the orthodontic force was applied without triggering degenerative alterations.

Unit type

Our result showed that the RA type was the most frequently found unit type (Table 1); this result agrees with previous reports under conditions of normal occlusion.[16] In contrast, the previous study in which PMRs were stimulated indirectly has reported on the different proportion of RA and SA types.[32,33] Cash and Linden[34] hypothesized that the adaptation property depended on the stimulating method. Receptors being stimulated over a wide area such as a tooth would respond as SA; on the contrary, those being stimulated directly with the tip of fine von Frey hairs would respond as RA.[34] Thus, the discrepancy between our results and those of the authors regarding unit type proportion may be related to different methodology.

Mechanical threshold and conduction velocity

As shown in Table 2 and Figure 4, the thresholds in the LG were significantly lower compared with the CG, as previously reported.[13] In contrast, the thresholds in the RG showed no significant difference compared with the CG. One possible explanation is that the structural alteration of PMRs by orthodontic force could have been responsible for the changes in the mechanical thresholds. Histological study using growth-associated protein-43 (GAP-43), which is involved in neural development and regeneration, has shown that the Schwann cells, which are thought to perceive distortion of periodontal collagen fibers,[35] exhibited GAP-43-like immunoreactivity during experimental orthodontic tooth movement.[27] Therefore, the chronological changes of the mechanical threshold seem to be a functional alteration in accordance with the structural alteration of PMRs by the orthodontic force.

Along with mechanical thresholds, chronological changes were also found in the conduction velocity of the innervating fibers (see Figure 5; and Table 2). During experimental orthodontic tooth movement not only Schwann cells but also axon terminals show GAP-43–like immunoreactivity.[27] Thus, the afferent fibers innervating the PMRs also suffered changes after the morphological or functional alterations of PMRs resulting in lower conduction velocities.

This study revealed that different recovery periods were

TABLE 2. Extended

RG			
7d	14d	21d	28d
9.58 ± 0.17	9.21 ± 0.12	9.21 ± 0.10	9.37 ± 0.07
15.67 ± 0.21*	16.22 ± 0.26*	16.94 ± 0.28	17.58 ± 0.24
n = 30	n = 28	n = 33	n = 38

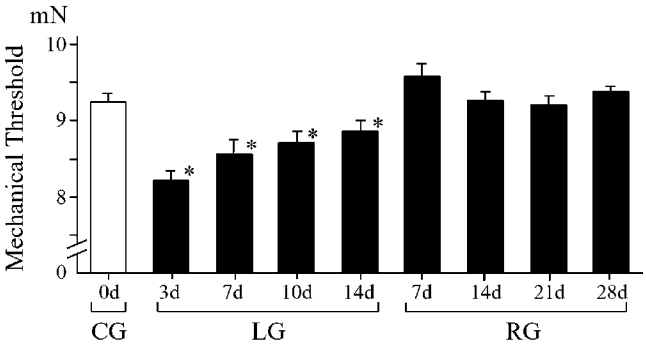

FIGURE 4. Chronological changes of mechanical thresholds. Compared with the CG, the mechanical thresholds were significantly lower in the 3d-, 7d-, 10d-, and 14d-LG. No significant difference was found in the 7d-, 14d-, 21d-, and 28d-RG. Vertical bars indicate SEM. * $P < .05$.

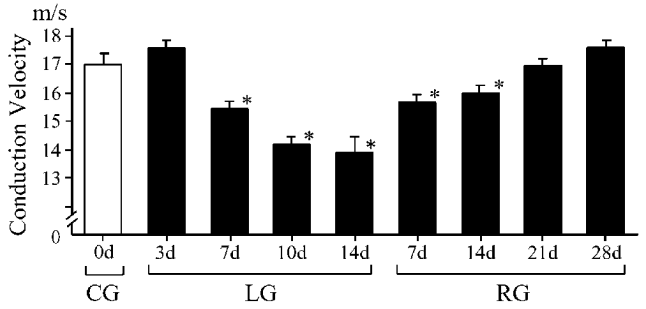

FIGURE 5. Chronological changes in conduction velocity. Compared with the CG, the conduction velocities were significantly lower in the 7d-, 10d-, and 14d-LG and in the 7d- and 14d-RG. No significant difference was found in the 21d- and 28d-RG. Vertical bars indicate SEM. * $P < .05$.

required for PMRs (mechanical threshold) and their innervating fibers (conduction velocity). These discrepancies may be due to the time lag between the morphological or functional alterations of PMRs and the characteristic alteration of afferent fibers. This idea can also be backed up by other results showing various changes in the localization pattern of GAP-43-like immunoreactivity between Schwann cells and axon terminals during experimental orthodontic tooth movement.[27]

After seven days of force removal, the periodontal collagen fiber arrangement became similar to the CG, and mechanical threshold also showed no significant difference to the CG. This result suggests that the adaptation of PMRs, due to their high potential for neural plasticity,[27,28] took place early within seven days along with the alteration of the periodontal environment. Because the recovery of the conduction velocity took a longer time, the adaptation of the afferent nerve fibers may require a longer period than that of PMRs. However, afferent nerve fibers also adapted to the new periodontal environment.

Functional adaptation after removal of orthodontic force

The present results showed that the response properties of PMRs adapted to the newly acquired tooth position. Thus, the alteration observed during experimental orthodontic tooth movement is considered recoverable. However, there still is a controversy between the present result and other reports showing an alteration of morphological or physiological characteristic of PMRs during experimental orthodontic tooth movement, without any recovery after a long period.[14,36] This discrepancy may be due to different experimental conditions, where the opposed tooth of observation site were extracted and relatively strong magnitude of orthodontic force was applied (2000 mN).[37] The mechanical stimuli from the occlusal force is considered an important factor to maintain distribution and morphology of periodontal Ruffini-like endings,[17,27,29,38] and occlusal hypofunction may affect the response properties of PMRs.[39] Furthermore, the stronger the magnitude of orthodontic force, the larger the site of pathological degeneration of periodontal tissue.[20,40] The root surface of M1 has an area that is about 20 times smaller than that of a human first molar.[37] The orthodontic force of 24.5 mN, used in this study, was almost equal to the clinical low orthodontic force of 490 mN. Unlike the other studies, we used a low orthodontic force of 24.5 mN together with the occlusal force. Therefore, a faster recovery of the response properties of PMRs results.

CONCLUSIONS

When a low orthodontic force is used, during orthodontic treatment, functional alterations may take place temporarily while the orthodontic forces are applied to the teeth; however, adaptation to the newly acquired intraoral condition can be achieved after removal of orthodontic force.

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