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Original Article

Changes in response properties of periodontal mechanoreceptors during tooth movement in rats

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Using an *in vitro* model, we investigated the chronological effects of orthodontic force on the response properties of periodontal mechanoreceptors (PMRs) in the rat mandibular first molar (M1). Experimental tooth movement was obtained by attaching a super-elastic titanium-nickel (Ti-Ni) alloy closed coil spring from the mandibular incisors to the right M1. On 1, 2, 3, 4, 7 and 14 days after the appliances were set, three right mandibular molars were extracted and direct stimulation with von Frey hairs was applied to the PMRs remaining in the tooth sockets of right M1. Single unit discharges were recorded from the inferior alveolar nerve. Following results were obtained; (1) in the 1-, 2- and 3-day groups, the mechanical thresholds were significantly lower than those in the control group. In the 4-day group, the mechanical threshold was significantly higher than that of the 3-day group. (2) In the 3-, 4-, 7- and 14-day groups, the conduction velocities of Aβ units were lower than those in the control group. These results imply that orthodontic force applied to M1 induced functional changes in the PMRs within a few days, suggesting that the PMR seems to respond to orthodontic force at early stage of tooth movement.

Key words: *in vitro*, periodontal mechanoreceptor, rat, response property, tooth movement.

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Introduction

In clinical orthodontic treatment, orthodontic appliances are generally adjusted or activated about once per month. Within several days after the appliance is adjusted and sustained force is applied to the teeth, some patients complain of senses of incongruity of teeth[1]. It has been reported that such unusual sensations are diminished after one or two weeks[1,2]. It has been an important matter of concern to examine the effect of orthodontic force toward the periodontal tissue, especially, the periodontal ligament during tooth movement.

Many researchers have investigated the physiological properties of the periodontal mechanoreceptors (PMRs) in various animals[3-5] and in man[6] by several physiological approaches. From microscopic studies in rats, it has been demonstrated that two types of mechanoreceptors, identified as free nerve endings and Ruffini-like endings, richly exist in the periodontal ligament. Toda *et al.*[7] developed an *in vitro* jaw-nerve preparation enabled precise study of the response properties of the rat PMRs. Using the preparation, the

response properties of PMRs to mechanical stimuli in the normal occlusal conditions have been investigated[8].

Recently, many kinds of orthodontic appliances that are made from Ti-Ni alloy have often been used in orthodontic treatment. On the other hand, quite a heavy force has been used in experimental tooth movement in rats. In addition, few quantitative studies have been carried out by physiological approaches in teeth which orthodontic force was applied.

The purpose of the present study was to examine the chronological changes in response properties of PMRs of rat teeth to which was applied a continuous orthodontic force.

Materials and Methods

Animals

Forty 12-week-old female Wistar albino rats were used. The weight of the rats was 213.6 ± 16.1 g (mean ± SEM). The rats were acclimatized in plastic cages with a standard 14-hour light and 10-hour dark cycle, and fed a diet of laboratory food and water *ad libitum*. Animals were divided into one control (Cont) and six experimental groups by day, Cont (n = 8) and 1- (n = 5), 2- (n = 5), 3- (n = 5), 4- (n = 6), 7- (n = 6) and 14-day (n = 5) groups. The present study was approved by the Animal Care and Use Committee, and the experiment was properly carried out under the Guidelines for Animal Experimentation in Tokyo Medical and Dental University.

Application of orthodontic force

The appliance for moving the tooth used in the present study was based on a modified technique described by Steigman and Michaeli[9]. The orthodontic appliance consisted of a closed coil spring (lumen: 0.9 mm) fabricated from the super-elastic Ti-Ni alloy wire (diameter: 0.093 mm; Furukawa Electric, Tokyo, Japan). In advance, the magnitude of the force was verified using a load cell under the constant temperature of 37 °C as previous reserchers described[10,11]. Fig. 1A shows the relationship between load and displacement of super-elastic Ti-Ni alloy closed coil spring used in the present study. The range remarked by a bold line implies the spring genetated force of 2-2.5 gf during experimental tooth movement. After the appliance was set, the right mandibular M1 was subjected to a mesially directed continuous force of 2.5 gf approximately. In appliance insertion, rats were anesthetized by injection of ketamine hydrochloride (40 mg/kg, i.p.;

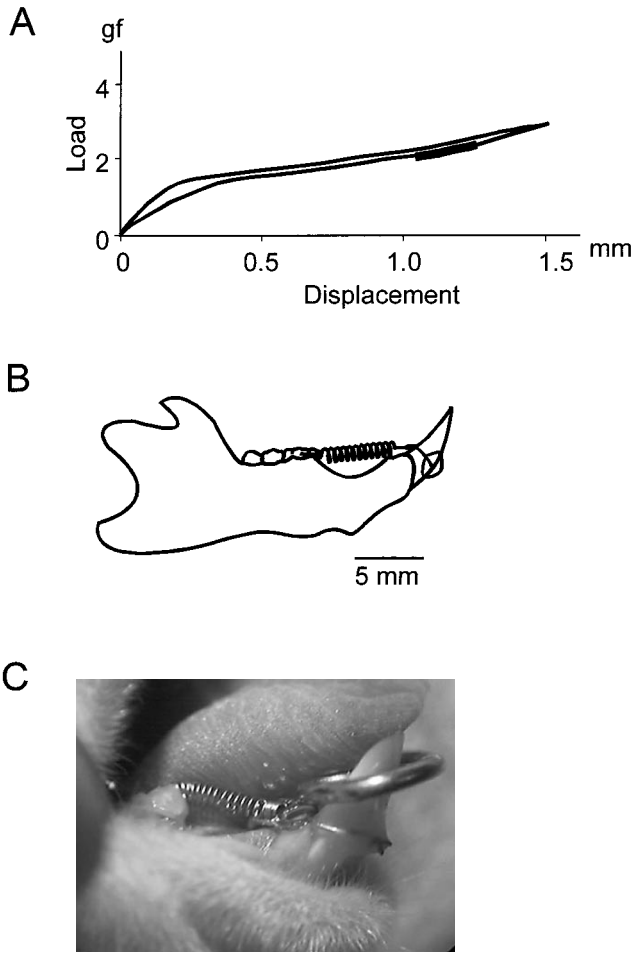

Fig. 1. (A) A load-displacement curve of super-elastic Ti-Ni closed coil spring. The remarked range with bold line indicates the range of load and displacement used in the present study. (B) A schematic drawing of the orthodontic appliances. (C) A photograph of the appliance practically applied in rat oral cavity.

Veterinary Ketalar 50®, Sankyo, Tokyo, Japan) containing 20% xylazine hydrochloride (3.5 mg/kg, i.p.; Celactal® 2% injection, Bayer-Japan, Tokyo, Japan) as a muscle relaxant, after inhalant anesthesia with diethyl ether (Wako Pure Chemical Industries, Osaka, Japan). Incisors were notched on the mesial surface using a round bur with dental drill to ensure maximum retention of the spring. The spring was ligated with ligature wire (diameter: 0.25 mm; Tomy International, Tokyo, Japan) between the right mandibular M1 and two mandibular incisors. The springs were further fixed with adhesive materials, consisting of a resin adhesive system (Clearfil® Liner bond IIΣ, Kuraray, Okayama, Japan) and a light curing composite resin (Clearfil® Photo SC, Kuraray, Okayama, Japan). The spring was activated upon insertion and was not reac-

tivated during the experimental period. The rats of Cont group were subject to the same treatment, but springs were not activated throughout the experimental period (1 day). Fig. 1B shows a schematic drawing of the experimental appliances for moving M1 and Fig. 1C shows a photograph of the appliances that were practically applied in oral cavity.

Measuring the magnitude of tooth movement

After experimental tooth movement, the impressions of mandibular teeth were taken and then we made plaster models of mandibular molars. Magnitude of tooth movement was measured using a non-contact digital microscopic gauge (resolution: 10 μm; MS-214, Fusoh, Tokyo, Japan). The distance between the mesio-occlusal pits of M1 and the second molar (M2) was mesured both on the appliance side (right) and non-appliance side (left). The magnitude of tooth movement was calculated as following numeral expression:

$$d_M = d_R - d_L$$

where d_M is the magnitude of tooth movement, d_R is the distance of appliance side and d_L is that of non-appliance side. The mean magnitudes of tooth movement in each group were calculated.

Preparation and experimental set up

On 1, 2, 3, 4, 7 and 14 days post-appliance insertion, the rats of each group were deeply anesthetized with thiamylal sodium (60 mg/kg, i.p.; Isozol®, Yoshitomi Pharmacy, Osaka, Japan), and then an *in vitro* jaw-nerve preparation was made as previously reported[7]. Three right mandibular molars were extracted using a pair of forceps with round chisels (F-15, Natsume, Tokyo, Japan). Using a binocular microscope (×20; Nikon-46207, Nikon, Tokyo, Japan), we cheked if periodontal tissue was remaining in the tooth sockets of M1[12]. The mandible was placed on a plastic chamber and the lateral side was fixed to the bottom with a soft-type dental wax (Utility wax®, GC, Tokyo, Japan).

The chamber design is shown in Fig. 2. 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 placed in the oil pool. The contents of two pools were separated by filling the space in the hole surrounding the nerve trunk with vaseline.

The oil pool was filled with liquid paraffin not to dry the nerve trunk. 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 O_2/CO_2 (95:5) gas mixture. The pre-warmed fluid was driven by a peristaltic pump, and heated by a Peltier-type heater attached to the microtube near the test pool to maintain the temperature at 31 °C using a feedback controller. The temperature of the solution was measured with a thermometer (BAT-12, Bailey Instruments, Saddlebrok, USA). The nerve trunk in the oil pool was slightly pulled to give enough tension to enable the recording electrode to be inserted easily.

Stimulating and recording procedures

For mechanical stimulation, seven intensities (2.9, 7.8, 9.3, 10.8, 11.8, 13.7 and 15.0 mN) of calibrated plastic von Frey hairs (tip diameter: 0.2 mm) were applied. The PMRs remaining in the tooth sockets of M1 were stimulated manually (duration: 5-10 seconds). Using the microneurographic method[13], single unit activities were recorded from the nerve trunk with a tungsten microelectrode (#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, USA) and displayed on a storage-oscilloscope (N-5113, Sony-Tektronix, Beaverton, USA). Using signal-processing interface (CED 1401 plus, Cambridge Electronic Design, Cambridge, UK) and a personal computer, the signal was digitized and displayed on a CRT monitor with the data analyzing software (Spike2® for Windows® Ver. 2.19, Cambridge Electronic Design, Cambridge, UK).

In the present study, we divided the units into two types according to their response pattern: those discharged continuously for more than five seconds while mechanical stimulation was applied were classified as slowly adapting (SA) type and the other units were all classified rapidly adapting (RA) type. Furthermore, RA units were classified into three subtypes, that is, those discharged at the begining of continuous mechanical stimulation were classified into RA-on, those discharged at the end into RA-off and those discharged at both the beginning and the end into RA-on-off type.

Conduction velocity

To estimate the conduction velocity of the recorded single afferent fiber, bipolar concentric tungsten electrodes (tip diameter: 0.4 mm, tip distance: 1 mm, IMB-9004, Inter Medical, Nagoya, Japan) were used to apply electrical stimulation to the tooth sockets of M1.

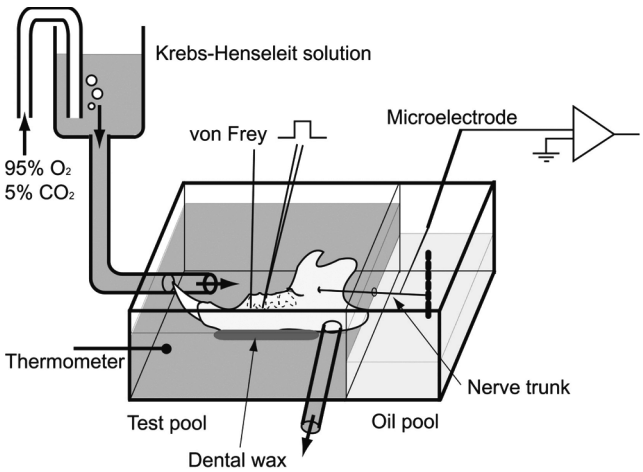

Fig. 2. A schematic drawing of the experimental apparatus. The chamber consisted of test and oil pools.

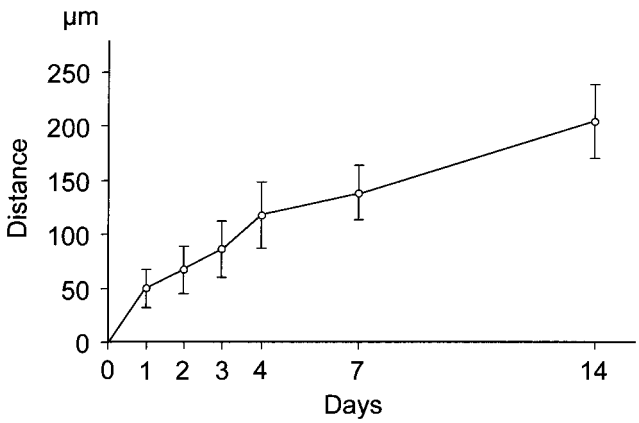

Fig. 3. The relationship between magnitude of tooth movement and time course. Vertical bars indicate SEM. The mean magnitude of tooth movement increased gradually.

The conduction velocity was calculated from both the distance between the stimulating and recording electrodes and the conduction time, and was corrected to the value at 37 °C using the Q_{10} correlation reported by Paintal[14]. Fibers with conduction velocity less than 2.0 m/s were regarded as unmyelinated (C), those between 2.0 and 10.0 m/s were regarded as thin myelinated (Aδ) and those over 10.0 m/s as large myelinated (Aβ) fibers[15]. C fibers were not examined because nocicepters were selectively excluded in the present study.

Statistical analysis

For statistical analysis, the data analizing software (StatView® for Windows® Ver. 5.0, SAS Institute, Cary, USA) was used. All data expressed as mean ± SEM. Differences among each group were compared using analysis of variance (ANOVA) followed by Fisher's PLSD post hoc test and were considered statistically significant at $P < 0.05$.

Results

Magnitude of tooth movement

The mean magnitudes of tooth movement in each experimental group were as follows, 1-day: 51 ± 17, 2-day: 68 ± 21, 3-day 87 ± 25, 4-day: 118 ± 31, 7-day: 138 ± 26 and 14-day: 205 ± 34 μm. Fig. 3 shows the relationship between the magnitude of tooth movement and time course. The magnitude of tooth movement gradually increased.

Unit type

Responses to mechanical stimuli were recorded from a total of 182 single units (Cont: n = 65 and 1-: n = 15, 2-: n = 12, 3-: n = 18, 4-: n = 17, 7-: n = 28 and 14-day: n = 27). The units were classified into 179 RA and 3 SA type units. Table 1 shows the distribution of the recorded single unit activities. The RA type units were classified into 21 on, 11 off and 147 on-off type units. Majority of the RA type units responded with on-off pattern (147 units). Typical samples of responses are shown in Fig. 4 (A: RA, B: SA).

Mechanical threshold

The mean thresholds of the mechanical stimuli were calculated in each group. The changes in threshold with time after application of orthodontic force were observed. Table 2 shows the mean values of the mechanical threshold in each group. Fig. 5A shows the relationship between the mechanical threshold and time course. Compared with the Cont group, significant lower thresholds were found in the 1-, 2- and 3-day groups. Significant difference was found between 3- and 4-day groups.

Conduction velocity

The conduction velocity of 182 mechanoreceptive units was distributed between 4.9 and 19.8 m/s. 159 units were identified as Aβ and 23 units were as Aδ units. Table 2 shows the mean values of the conduction velocities in each group. Aβ units were predominant in each group. Fig. 5B shows the relationship between the conduction velocities of Aβ units and time course. In regard to the Aβ units, compared with the Cont group,

significant lower conduction velocities were found in the 3-, 4-, 7- and 14-day group. Significant difference was found between 2- and 3-day groups.

Discussion

Propriety of the experimental tooth movement model

There are few quantitative studies on physiological properties of PMRs in teeth to which sustained orthodontic forces are applied. Loescher *et al.*[16] was only reported. In the study, elastic modules were used for moving cat canines, which initial force was 2 N. The value is rather strong for cat canines. The method developed by Waldo *et al.*[17], which makes teeth moved by the placement of an elastic wedge between two teeth, has been frequently used for many experiments in small mammals. The intensity of the initial force that this orthodontic appliance generates was calculated approximately 80-200 gf[18]. This value is equivalent to 1.6-4 kgf for man because the surface of roots of man's first molar has an area of about 20 times larger than that of rat molar[19].

In the present study, on the other hand, we used closed coil springs that continuously generated the approximate force of 2.5 gf. The intensity of the force was estimated approximately 50-60 gf for man by the calculating method that Sato *et al.*[19] described. Super-elastic Ti-Ni alloy wire has many properties as follows, that is, shape memory, super-elasticity and excellent springback[10]. These properties contributed to the present study because changing intraoral environment such as changing temperature and/or spring deformation affected less the load of the spring. It was reported that the magnitude of the pressure which hardly affects the blood flow in the periodontal ligament was less than 80 gf per square centimeters[20]. Kirino *et al.*[21] reported that the value was equivalent to 10 gf for rat

Table 1. Adaptation properties of units.

Type		Cont	Post-treatment group						Total
			1-day	2-day	3-day	4-day	7-day	14-day	
RA	on	16	-	-	1	-	2	2	21
RA	off	4	2	1	2	1	1	-	11
RA	on-off	45	13	10	13	16	25	25	147
SA		-	-	1	2	-	-	-	3
Total		65	15	12	18	17	28	27	182

Number of units.

Table 2. Mean values of mechanical thresholds and conduction velocities.

	Cont	Post-treatment group					
		1-day	2-day	3-day	4-day	7-day	14-day
Mechanical Threshold (mN)	12.9 ± 1.0	10.3 ± 0.7	10.1 ± 1.1	9.4 ± 0.7	13.4 ± 0.6	13.6 ± 0.4	13.6 ± 0.5
Conduction Velocity (m/s)							
Aβ units	13.8 ± 1.5 (n = 59)	14.4 ± 0.7 (n = 15)	14.1 ± 1.5 (n = 11)	11.5 ± 0.6 (n = 11)	11.9 ± 0.4 (n = 14)	11.1 ± 1.0 (n = 25)	10.4 ± 0.5 (n = 24)
Aδ units	6.5 ± 1.9 (n = 6)	- -	9.6 (n = 1)	8.6 ± 0.9 (n = 7)	9.5 ± 0.3 (n = 3)	8.8 ± 0.5 (n = 3)	8.8 ± 0.9 (n = 3)

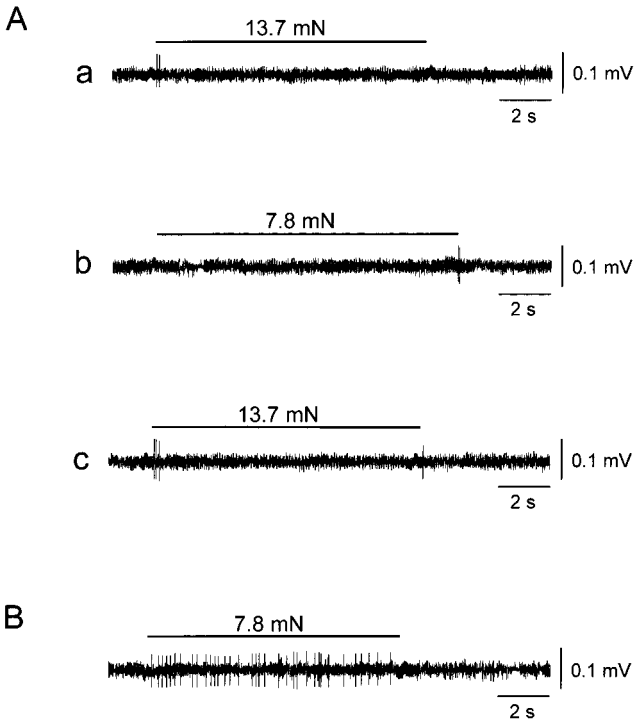

Fig. 4. Typical samples of responses when mechanical stimuli were applied to the PMRs. Horizontal bars above each trace indicate the duration of mechanical stimulation. (A) a: RA-on, b: RA-off and c: RA-on-off type. (B) SA type.

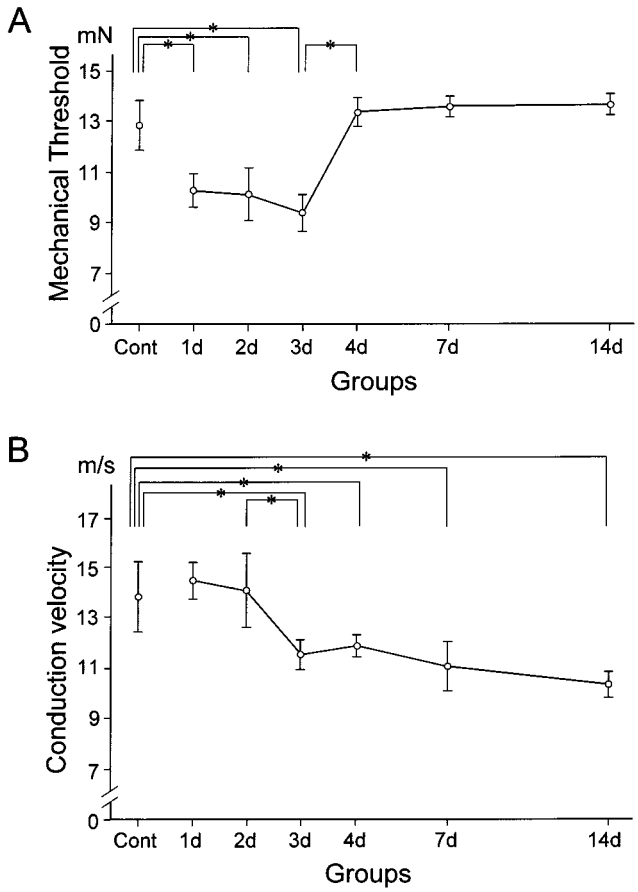

Fig. 5. (A) The relationship between mechanical threshold and time course. (B) The relationship between conduction velocity of A β units and time course. Vertical bars indicate SEM. * represents P<0.05.

M1. Therefore, we considered that the spring that we used in the present study was suitable for experimental tooth movement in rats and that the force level was equal to the clinical level for man.

Unit type

In the present study, only three units were SA type, and the rest of all was RA type. In the control group, all of the units were RA type, as Ishii reported elsewhere[8]. The adaptation properties of PMRs are influenced by the direction or intensity of mechanical stimuli applied to the receptor[22]. On the other hand, Appenteng *et al.*[23] and Linden and Millar[24] suggested that there is only one type of the PMR in cats and that its response property depends on the receptor's spatial position. Therefore, it is considered that SA type units found in the present study may have density changes of PMRs following the deformation of periodontal ligament caused by tooth movement. We observed that most of the RA type units responded with an on-off type. Our findings agree with a previous study by Ishii[8].

Mechanical threshold

We observed that in the 1-, 2-, and 3-day groups showed lower mechanical threshold compared with the control group. In an attempt to explain the alterations in mechanoreceptive properties during the application of orthodontic force, several possibilities were considered in previous studies. It is within the several days of orthodontic treatment that patients may feel discomfort of teeth. Histological studies have shown that the number of axons in the periodontal ligament which contain CGRP (calcitonin gene-related peptide) increased rapidly within 3-5 days of application of orthodontic force[25]. A recent study showed that NFP (neurofilament protein) within nerve fibers increased after experimental tooth movement in the rat molar[26]. Loescher *et al.*[16] reported that three factors were possible explanation for the alterations in receptor characteristics as follows: (1) the change in tooth mobility, (2) disruption of the collagen fiber matrix within the liga-

ment, (3) direct injury to the nerve fibers or nerve terminals themselves. Therefore, combining above possibilities, not only the deformation of PMRs but also factors involved in the development or regeneration of nerve fibers may have influenced the threshold of PMRs.

Conduction velocity

Nerve fibers innervating the PMRs were classified according to the conduction velocities of the units recorded in the present study. In regard to the Aβ units, the results showed that in the 3-, 4-, 7-, and 14-day groups had lower conduction velocities than those in the control group. One possible explanation may be due to that PMRs innervated by large-diameter nerves were easy to change their physiological properties after experimental tooth movement. These findings indicate that the PMR seems to respond to orthodontic force at early stage of tooth movement.

Acknowledgements

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