ORIGINAL ARTICLE

Diurnal variation in tooth movement in response to orthodontic force in rats

Kaoru Igarashi, DDS, PhD,[a] Kotaro Miyoshi, DDS,[b] Hisashi Shinoda, DDS, PhD,[c] Shuichi Saeki, DDS, PhD,[a] and Hideo Mitani, DDS, MS, PhD[d]
Sendai, Japan

The purpose of this study was to determine whether there is any difference in orthodontic tooth movement when the orthodontic force is applied at different times of the day. Twenty-four rats were divided into three experimental groups based on the time of day that maxillary expansive force was applied; i.e., the force was applied continuously throughout the entire experimental period of 21 days in animals in the whole-day group; animals in the dark-period group and the light-period group received force only during the dark period (19:00-07:00) or the light period (07:00-19:00), respectively. Maxillary expansion in the light-period group was about 2 times greater than that in the dark-period group on day 21. There was no significant difference in expansion between the light-period group and the whole-day group. An experiment that used chronologic labeling with NTA-Pb revealed that there was greater formation of new bone on the tension side in the light-period group than in the dark-period group. There was no significant difference in the width of the palate among the three groups. These results indicate that there is a diurnal variation in tooth movement in response to orthodontic external force and that the application of force during the animal's rest period may be more effective than that while it is active. (Am J Orthod Dentofacial Orthop 1998;114:8-14)

Various studies concerning diurnal rhythmic phenomena have been performed in the field of bone physiology. For example, diurnal rhythms have been found in various parameters related to bone formation and bone resorption, including plasma calcium and phosphate,[1-5] calcium-regulating hormones[1,5-7] osteocalcin,[8,9] serum markers of type I collagen synthesis and degradation,[10] and urinary excretion of bone collagen cross-links.[5,11] Diurnal variations have also been found in various local events that influence bone turnover, such as proliferation and matrix-synthetic activities of osteogenic cells,[12-16] osteoclast-bone surface contact,[17] and enzyme activities related to bone formation and bone resorption.[18,19] All of these studies strongly support the concept that diurnal rhythmicity is an essential feature of bone physiology. Considering such diurnal fluctuations in bone physiology, biological responses to the external force exerted by various orthodontic and orthopedic appliances, which inevitably include mechanically induced bone modeling in the alveolar bone, sutures, or condyles, may vary depending on the time of day such forces are applied. The purpose of the present study was to investigate whether there is any difference in orthodontic tooth movement in rats when the orthodontic force is applied at different times of the day (daytime or nighttime).

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From the Tohoku University School of Dentistry.
[a]Instructor, Department of Orthodontics.
[b]Graduate student, Department of Orthodontics.
[c]Professor and Chairman, Department of Pharmacology.
[d]Dean, Professor, and Chairman, Department of Orthodontics.
Reprint requests to: Kaoru Igarashi, Department of Orthodontics, Tohoku University School of Dentistry, Seiryo-machi 4-1, Aoba-ku, Sendai, 980-8575, Japan. E-mail: igarashi@orthod.dent.tohoku.ac.jp

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Material and methods

A total of 29 male 6-week-old Wistar rats were adapted to a 12/12 hour light/dark illumination program (with light from 07:00 to 19:00) for 2 weeks. Five animals served as a control group without force application. The rest of the animals were divided into three experimental groups (eight rats for each group) based on the time of day the force was applied, i.e., force was applied continuously throughout the entire experimental period of 21 days in animals in the whole-day group; animals in the dark-period group and the light-period group received force only during the dark period (19:00-07:00) or the light period (07:00-19:00), respectively. A uniform standardized expansive spring was set in each animal's mouth between the right and left upper first molars.[20] The expansive force was applied for 21 days. For the animals in the dark-period group and the light-period group, the appliances were set or removed at 7:00 and 19:00 every

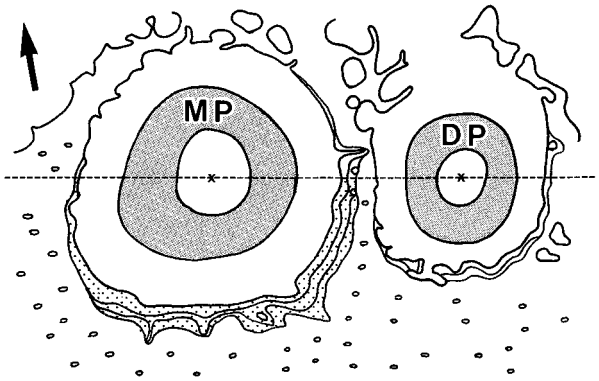

Fig. 1. Evaluation of new bone formation. Mesiopalatal root area was divided into a pressure side and a tension side based on a line drawn between the center of the mesiopalatal root (MP) and the center of the distopalatal root (DP). New bone formation was evaluated by measuring the area between the first and fourth lead-labeled lines on the tension side *(dotted area)* by image analysis. *Arrow* indicates direction of tooth movement.

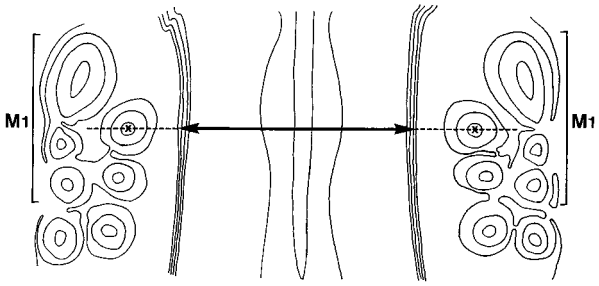

Fig. 2. Determination of the width of the palate (see text).

day under light ether anesthesia. During the experimental period, the weights of all of the animals were monitored as appropriate and the amount of food consumed by the animals in each group was measured every day. The animals were treated ethically in compliance with the regulations of Tohoku University School of Dentistry.

Maxillary expansion was evaluated by measuring the distance between the right and left crests of the mesiopalatal cusps of the upper first molars. The method has been described previously.[20] Briefly, a precise stone model of the upper jaw was made by taking an impression with silicone materials under light ether anesthesia. Measurements were made on the occlusal view of the model under ×10 magnification with a profile projector.

The animals in the three experimental groups were given 4 mg Pb/Kg of nitrilotriacetato lead (NTA-Pb) by intraperitoneal injection four times at 7-day intervals (on days 0, 7, 14, and 21) to provide chronologic bone labeling,[21,22] and were sacrificed 6 hours after the last injection. After the expansive force was applied for 21 days, the animals were sacrificed under pentobarbital anesthesia (40 mg/Kg), and the maxillary bones, including the molars, were dissected. The specimens were fixed in 4% paraformaldehyde solution for 3 days, and then decalcified in 10% ethylenediamine tetraacetic acid (EDTA) solution with 4% Na_2S for 30 days at room temperature. The addition of Na_2S to EDTA solution turns Pb that has been deposited in the tissue to less-soluble PbS, which stays in situ in the demineralized matrix. The specimens were then dehydrated in a graded series of ethanol and embedded in paraffin. Periodontal tissues of the mesiopalatal root of the upper first molar were examined with a light microscope in serial cross-sections of the molars at a bifurcation level. Sections (7 μm thick) were first gold-plated with 0.1% $HAuCl_4$ to make lead-labeled lines visible, and then treated with 5% $Na_2S_2O_3$ to tone the lines. They were then stained with hematoxylin and eosin. The mesiopalatal root area was divided into a pressure side and a tension side based on a line drawn between the center of the mesiopalatal root and the center of distopalatal root. The formation of new bone was evaluated by measuring the area between the first and fourth lead-labeled lines on the tension side (Fig. 1). The areas of new bone formation on the right and left sides in each section were added together. Values for five sections, which were selected at three section intervals, were averaged for each animal. The width of the palate was evaluated by measuring the distance between the first lead-labeled lines on the palatal periosteal side in the right and left alveolar bone along a line drawn between the centers of the right and left mesiopalatal roots at 150 μm from the furcation of the root in the direction of the apex (Fig. 2).

The data were subjected to one or two way analyses of variance (ANOVA) with pairwise comparison by Scheffe's F test; $p < 0.05$ was considered a significant difference.

RESULTS

A total of three animals were disqualified for the study because of the failure of the spring. At the end of the experiment, there were five animals in the control group, eight in the whole-day group, six in the light-period group, and seven in the dark-period group.

The animals in the control group consumed more food (89.0% of the daily food intake) during the dark period than during the light period. As shown in Fig. 3, the animals in the three experimental groups also consumed food principally during the dark period (92.4%, 91.8%, and 90.1% of the daily food intake in the whole-day group, light-period group, and dark-period group, respectively). There was no difference in either food intake or weight gain between the light-period group and the dark-period group (Figs. 3 and 4). Because of daily

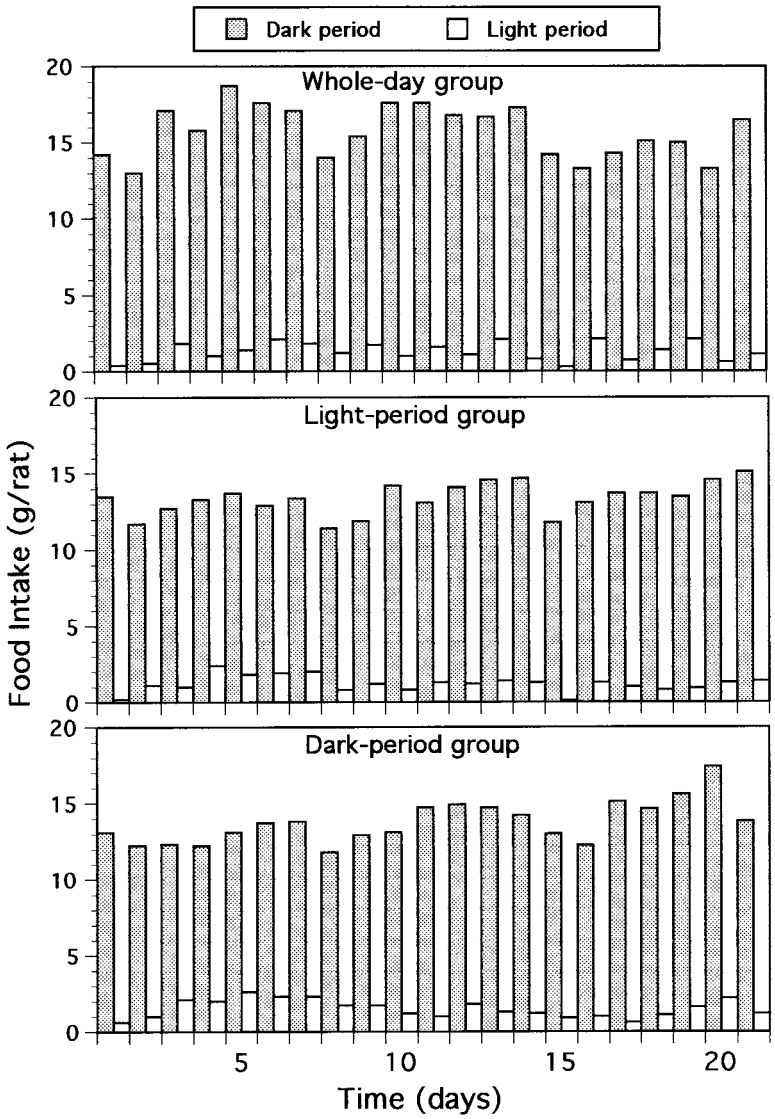

Fig. 3. Food intake patterns of rats in the three experimental groups during the experimental period. Each column represents the mean (n = 6 to 8).

anesthesia, the food intake and weight gain in these two groups were less than those in the control group and the whole-day group.

Maxillary expansion in the light-period group was significantly greater than that in the dark-period group on days 14 and 21 (Fig. 5). Mean maxillary expansion on day 21 was 1.94 ± 0.07 mm in the whole-day group, 1.77 ± 0.08 mm in the light-period group, and 0.95 ± 0.04 mm in the dark-period group. The light-period group showed 1.9-fold greater expansion than the dark-period group. There was no significant difference between the light-period group and the whole-day group. Spontaneous expansion in the control group during the experimental period of 21 days was 0.15 ± 0.03 mm.

As shown in Fig. 6, extensive new bone formation occurred on the tension side in the whole-day group and the light-period group. On the other hand, bone resorptive lacunae, rather than new bone formation, were observed in the dark-period group. Slight new bone formation occurred on the periphery of the measured bone surface. The amount of new bone formed in the light-period group was about 8-fold greater than that in the dark-period group (Fig. 7). There was no significant

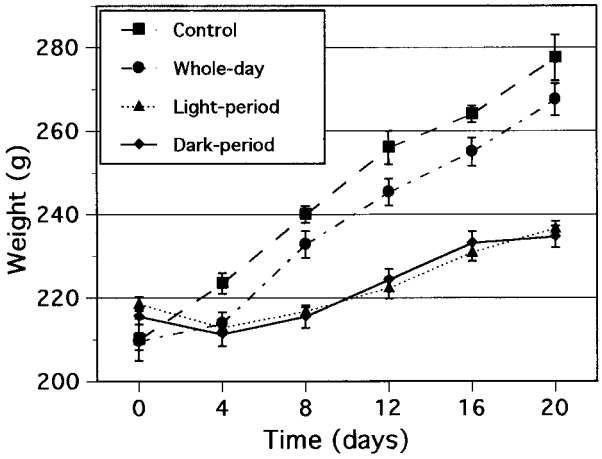

Fig. 4. Mean weight gain of rats in the control group and three experimental groups during the experimental period. Each point represents the mean ± SEM (n = 5 to 8).

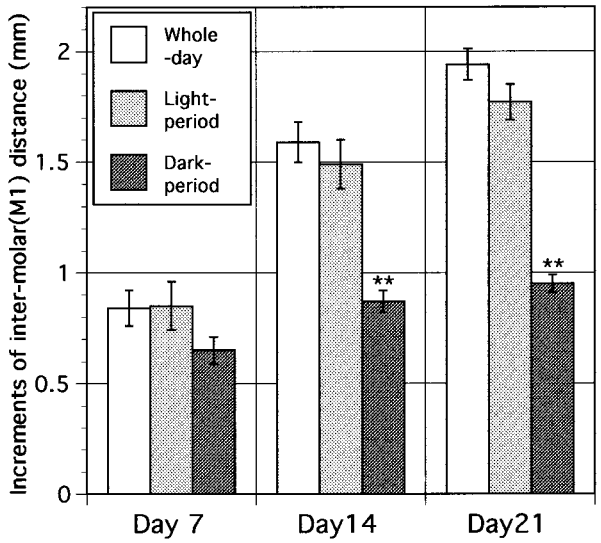

Fig. 5. Mean maxillary expansion in rats in the three experimental groups on days 7, 14, and 21. Each column represents the mean ± SEM (n = 6 to 8). The difference among the groups was significant ($p < 0.01$ for treatments and $p < 0.01$ for time by two-way ANOVA). **$p < 0.01$ vs the light-period group by Scheffe's F test.

difference between the light-period group and the whole-day group.

There was no significant difference in the width of the palate among the three experimental groups (Fig. 8).

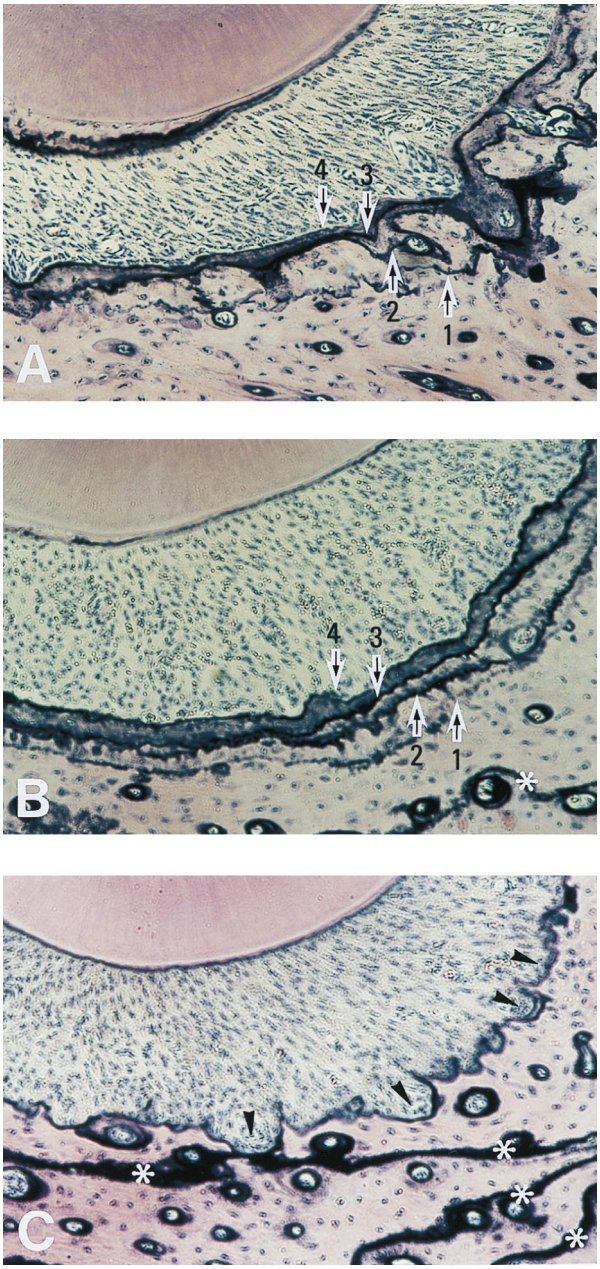

Fig. 6. Photomicrographs of tension side of mesio-palatal root of upper first molar in rats in three experimental groups on day 21. (Original magnification ×100.) Extensive new bone formation occurred on the tension side in the whole-day group **(A)** and the light-period group **(B)**. NTA-Pb was injected 4 times at 7-day intervals. *Arrows 1, 2, 3, and 4* indicate lead-labeled lines on days 0, 7, 14, and 21, respectively. On the other hand, bone resorptive lacunae *(arrowheads)*, rather than new bone formation, were observed in the dark-period group **(C)**. Slight new bone formation occurred on the periphery of the measured bone surface. *Lead-labeled lines on palatal periosteal side.

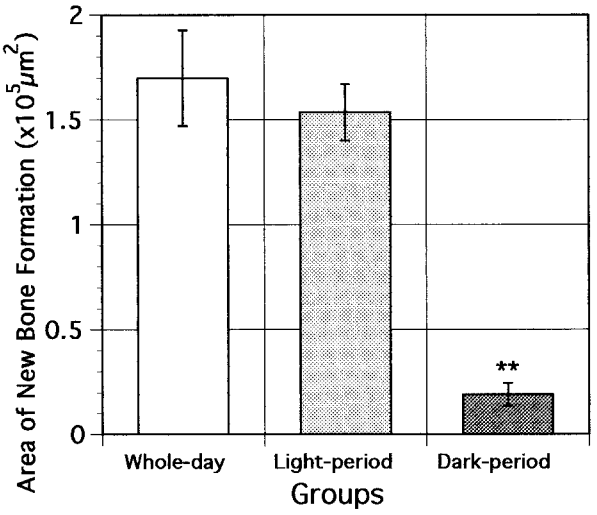

Fig. 7. Amount of new bone formation on the tension side of the mesiopalatal root of the upper first molars in rats in the study. Each column represents the mean ± SEM (n = 6 or 7). The difference among the three groups was significant ($p < 0.01$ by one-way ANOVA). $^{**}p < 0.01$ vs the light-period group by Scheffe's F test.

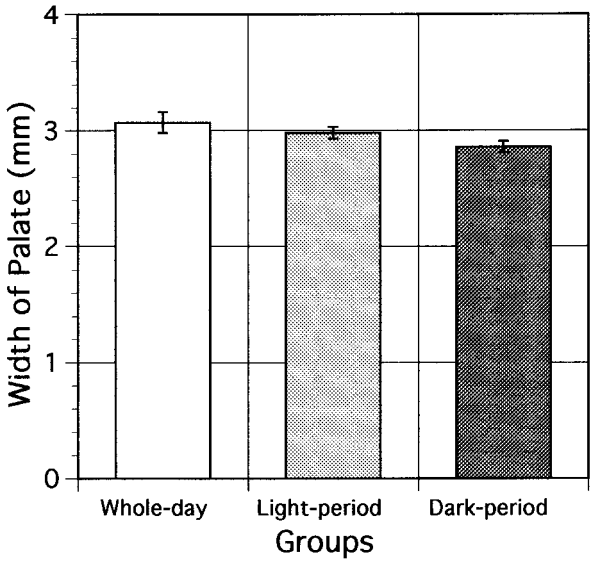

Fig. 8. Mean width of the palate in rats in the three experimental groups on day 21. Each column represents the mean ± SEM (n = 5 to 7).

DISCUSSION

The present data clearly showed for the first time that the response to orthodontic force varies depending on the time of day the force is applied. More tooth movement was achieved in rats that received orthodontic force during the light period than in rats that received force during the dark period. The maxillary expansion in the light-period group was nearly twice that in the dark-period group. Although these values may also partly involve spontaneous expansion from growth and reflect orthopedic effects such as the deformation of alveolar bone and possible expansion of the midpalatal suture, the former was only 0.15 mm. There was no significant difference in the width of the palate among the three experimental groups, however, a marked difference in new bone formation on the tension side of the mesiopalatal root was observed between the light-period group and the dark-period group. These results indicate that the differences in maxillary expansion may have been mainly due to a difference in orthodontic effects rather than to a difference in orthopedic effects.

It has been shown that the events related to matrix production by osteoblasts occur around midday in rats.[12] In growing rats, the proliferation of chondrocytes and collagen-synthetic activities of osteoblasts in the rat mandibular condyle fluctuate with a peak in the middle of the light period and a nadir in the middle of the dark period.[13,14] Under physiologic conditions, maximal cell proliferation in the periodontal ligament occurs during the daytime.[15,16] Like these other events that contribute to bone formation, bone resorption has been considered to be active during daytime when the animal is resting.[12] Shinoda and Stern[23] have shown that the release of calcium from bone is most active during the middle to late light period in rats. A morphologic study revealed that osteoclast-bone surface contact area is more extensive during the light period than in the dark period.[17] Thus, in rats, both bone formation and bone resorption are most active in the environmental light period and are minimal in the environmental dark period. In the present study, the amount of new bone formed on the tension side in the light-period group was greater than that in the dark-period group. This indicates that a greater bone-formative response can be obtained when orthodontic force is applied during the light period, the active period of bone formation, rather than during the dark period. Therefore, the magnitude of the response may depend on the underlying physiologic activity of bone formation, which varies with the time of day. However, bone resorptive lacunae, rather than new bone formation, were observed on the tension side in the dark-period group. This can be explained as follows: in the dark-period group,

teeth received orthodontic force during the period of minimal bone metabolism. When the spring was removed, relapse of the teeth may have occurred, i.e., the teeth would have moved to their original positions during the period of maximum activity. This may have resulted in minimal tooth movement with minimal bone formation and traces of bone resorption on the tension side.

Another possible explanation for this variation is that the susceptibilities of so-called mechanosensors in the target tissues could vary with the time of day. At present, however, the precise mechanisms are not known.

The causes of skeletal diurnal rhythms have not yet been clarified. Several endogenous and exogenous factors have been suggested to be involved. In rats, rhythms of cell proliferation in cartilage and bone parallel the serum corticosterone level, while the rhythm of matrix synthesis in bone is parathyroid-dependent.[12] It has been shown that diurnal rhythms in calcium metabolism in both rats and human beings are regulated by undefined serum factor(s).[5,23] Therefore, it is reasonable to assume that the observed variation in tooth movement is also caused by these hormonal rhythms.

Another possible cause may be a biomechanical rhythm, i.e., the diurnal variation in masticatory function. In the present study, the animals consumed 10 times more food during the dark period than during the light period, indicating that masticatory activities increased at night. A 24-hour electromyogram study has revealed that masseter muscle activity in rats increased during the dark period (65% of the whole-day activity).[24] Therefore, it is conceivable that increased masticatory activity during the dark period might interfere with orthodontic force, and thus may have inhibited tooth movement in the dark-period group.

An interesting finding in this study is that there was no significant difference in tooth movement between the light-period group and the whole-day group. This means that the intermittent application of force for 12 hours (07:00 to 19:00) can produce tooth movement comparable to that produced by continuous force in rats. Previous studies both in animals[25] and human beings[18] have shown that the application of intermittent or short-term forces for less than 24 hours are as effective as, or even more effective than continuous force. However, this might be true only when the force is applied at certain times of the day. Our results indicate that diurnal variation should be considered an important factor that may affect tooth movement.

CONCLUSIONS

In summary, the present results demonstrated a diurnal variation in tooth movement in response to orthodontic force in rats. More tooth movement was achieved in rats that received orthodontic force during the light period than in rats that received the force during the dark period. Because rats are known to be nocturnal, these results indicate that the application of force during the animal's rest period may be more effective than that while it is active. If this is applicable to diurnal human beings, more tooth movement would be expected at night than during the day. Indeed, Stutzmann and Petrovic[18] and Petrovic et al.[19] have shown that the rate of alveolar bone turn-over in human beings was higher in the nighttime than in the daytime. Further studies are necessary to verify whether or not a diurnal variation in orthodontic tooth movement exists in human beings, and to clarify the cellular mechanisms that produce this variation.

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