Time-Related Changes in Periodontal Mechanoreceptors in Rat Molars after the Loss of Occlusal Stimuli*

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Summary. The effect of a loss of occlusal stimuli upon the distribution and structure of the periodontal mechanoreceptors of the rat mandibular molar was examined after extracting opposing molars.

The hypofunctional periodontal ligament narrowed significantly two weeks after tooth extraction, associated with an altered morphology of the Ruffini endings that showed typical dendritic profiles in normal controls. At four weeks and later periods after extraction, the Ruffini endings—including those without light microscopic changes—demonstrated unusual ultrastructural features such as the eccentric localization of mitochondria along the axonal membrane and loss of other cell organelles, unusual elongation of axonal microprojections, or a deep invagination of the Schwann sheath into the axoplasm. Immunoreactivity for the growth-associated protein-43 (GAP-43) in the Ruffini endings was restricted to the Schwann element in both the normal and hypofunctional periodontal ligament, but the reaction was weaker and even negligible in some cases in the latter ligament.

The present results suggest that occlusal stimuli are essential for maintaining the structural integrity of the periodontal ligament, including that of periodontal mechanoreceptors. A decreased immunoreactivity for GAP-43 in the Schwann sheaths supports the notion of a possible functional alteration in the Ruffini endings that showed no structural abnormality.

The periodontal ligament serves as a supporting and buffering apparatus for the teeth and also as a receptor for mechanical sensation. Recent morphological studies have shown that it possesses both free nerve endings and specialized endings that function as nociceptors and mechanoreceptors, respectively (BYERS, 1985; MAEDA et al., 1987; SATO et al., 1989;

BYERS and DONG, 1989). These specialized mechanoreceptors are known to be involved in the induction of various oral reflexes, which enable normal, smooth mastication (LINDEN, 1990). Recent electrophysiological studies have indicated that there is a single type of mechanoreceptor in the periodontal ligament (CASH and LINDEN, 1982; LINDEN and MILLAR, 1988; LINDEN et al., 1994) which corresponds to the Ruffini ending recognized by microscopists in the periodontal ligament in humans and various animals, including rats (MAEDA et al., 1987, 1990).

The periodontal Ruffini endings are known to have stage-specific morphological features that are related directly to the development of the tooth germ, particularly to the timing of tooth eruption and beginning of occlusion. Functional stimuli have been implicated in the final differentiation and maturation of this type of ending (NAKAKURA et al., 1993, 1995). Also in the established periodontal ligament, there seems to be a close correlation between retention of the developed Ruffini endings and occlusal function.

Although numerous papers have been available dealing with the effect of occlusal hypofunction on histological changes in periodontal tissues, few studies seem to have examined the neuroanatomical changes associated with periodontal hypofunction.

Protein gene product 9.5 (PGP 9.5) is a cytoplasmic protein universally distributed in central and peripheral neurons, and has been fully characterized previously (DORAN et al., 1983; THOMPSON et al., 1983). Growth-associated protein-43 (GAP-43), a membranous phosphoprotein present in neurons, is a useful marker of axonal growth in developing and regenerating neurons (GORDON, 1987; SKENE, 1989; TETZLAFF

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Fig. 1. Soft-X ray image of the first molar region of the lower jaws of a 21-week-old rat 16 weeks after the extraction of all opposing teeth (**R**) and that of an untreated control (**L**). Note the excessive elongation of the root of the experimental molar compared with the control. The width of the periodontal ligament is smaller in the experimental molar (**R**, arrowheads) than that of the control (**L**, arrowheads). Arrows indicate distal direction in each jaw. $\times 8$

Fig. 2. Reference points for measurement of the elongation increment of the mandibular first molar. Distance between the bottom of the distal occlusal sulcus and the upper margin of the lower incisor was measured along the longitudinal axis of the distal root.

et al., 1989). In mature neurons, the synthesis of this protein is repressed or downregulated (SKENE, 1989; CURTIS et al., 1992). However, GAP-43 immunoreactivity is exhibited in the periodontal nerve elements of adult rat molars, the axons of free endings, and Schwann cells of the mechanoreceptors, suggesting a continuous remodeling of these nerve endings associated with the dynamic reconstruction of ligament fibers (MAEDA and BYERS, 1996; KOBAYASHI et al., 1998).

The present study aims to demonstrate time-related changes in the distribution and morphology of Ruffini endings in the periodontal ligament of rat mandibular molars following extraction of the opposing teeth. Immunohistochemistry of PGP 9.5 and GAP-43 were applied respectively for structural and functional evaluations of the periodontal Ruffini endings. Histochemical analyses of tartrate-resistant acid phosphatase (TRAP) and tissue nonspecific alkaline phosphatase (ALPase) activities were also performed to reveal possible changes in cellular dynamics in the alveolar bone associated with occlusal hypofunction.

MATERIALS AND METHODS

Animals

Seventy-two 5-week-old male Wistar rats (body weight 145–155 g) were used. The animals were anesthetized by an intraperitoneal injection of chloral hydrate (400 mg/kg). The right maxillary molars (M1, M2, M3) were extracted from 36 rats. The remaining 36 animals were not subjected to extraction and served as controls. All animals were given *ad libitum* access to standard rat chow (CE-2, Japan Clea Inc, Shizuoka, Japan) and free access to drinking water. Feeding, drinking, grooming behavior, and body weight were monitored throughout the experimental period.

At 2, 4, 8 and 16 weeks after extraction, both experimental and control animals were sacrificed by transcardiac perfusion of either 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for light microscopy (5 animals per group), or a mixture of 4% paraformaldehyde and 0.025% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for electron microscopy (4 animals per group).

In all experiments, the upper and lower jaws of the fixed animals were cleaned with soft tissues, and subjected to soft X-ray radiography using a Sofron SRO-M50 (Sofron Ltd. Tokyo, Japan). The lower jaws were isolated and further fixed in the same fixative solution at 4°C for an additional 12 h. Following decalcification with Plank-Rychlo solution for 3–5

Fig. 3. ALPase (blue) and TRAP (red) reactivities in the distal root of mandibular first molars in control and experimental rats viewed in horizontal frozen sections at the apical 1/3 of the respective roots. The periodontal ligament in experimental rats is much narrower than that in the controls, and remains almost unchanged during the experimental period. Osteoblastic ALPase reactivity is localized to the mesial half of the alveolar bone surface and the osteoclastic TRAP reactivity is localized to the distal half in both control and experimental rats. *Thick open arrow* indicates the direction of physiological tooth movement. *M* mesial side, *D* distal side, *p* pulp, *d* dentin, *c* cementum, *ab* alveolar bone. $\times 38$

Fig. 4. Periodontal ligament of the distal side of a distal root of a mandibular first molar showing PGP 9.5 immunoreactions in horizontal sections. In control rats, blood vessels and nerve elements (*arrows*) are located in the alveolar half of the periodontal ligament in all cases. At 4 and 8 weeks after the extraction of opposing teeth, some nerve elements (*arrowheads*) can be found around the cementum in narrow periodontal ligament. Note the increased number of sinusoids in the experimental 16th week molar ligament (*asterisks*). C cementum, PL periodontal ligament, AB alveolar bone. $\times 140$

days at 4°C for light microscopy, or with 5% EDTA-2Na (ethylene diamine tetraacetic acid disodium) solution for 4-5 weeks at 4°C for electron microscopy, the specimens were immersed in an anti-freezing agent (30% sucrose solution) overnight at 4°C, and processed for freeze-sectioning in a cold microtome (Yamato Kohki, Saitama, Japan). Both sagittal and horizontal sections (40 μ m thick) of the lower jaws were prepared.

ALPase and TRAP double staining

Free-floating sections of the prepared lower jaws were first subjected to histochemical localization of alkaline phosphatase (ALPase) activity using an alkaline phosphatase substrate kit SK-5300 (Vector, CA, U.S.A.) for 60 min at room temperature. After thorough rinsing in 0.01 M phosphate buffered saline (PBS), the sections were further incubated for tartrate-resistant acid phosphatase (TRAP) staining using the Azo dye method (BURSTONE, 1961). Tartaric acid (L+) (50 mM) (Wako, Tokyo, Japan) was added to inhibit acid phosphatase to yield TRAP activity. Incubation was carried out for 5-10 min at room temperature. The sections were mounted with glycerin after rinsing in PBS.

Light microscopic immunohistochemical analysis of PGP9.5 and GAP-43

Nerve elements in the periodontal ligament were immunostained with the rabbit polyclonal anti-human PGP 9.5 antibody (Ultraclone, Cambridge, U. K.). The cryostat sections were processed for indirect immunostaining by the avidin-biotin-complex (ABC) method (HSU et al., 1981) using a commercially available kit (Vector). After inactivation of endogenous peroxidase with 0.3% H_2O_2 in absolute methanol for 30 min, nonspecific immunoreactivity was blocked by pre-incubation in 2% normal goat serum in PBS. The sections were first treated with the rabbit polyclonal antibody to human PGP 9.5 diluted at 1:10,000 with 2% normal goat serum overnight at 37°C, followed by two consecutive incubations with biotinylated anti-rabbit IgG (1:400) (Chemicon, CA, U. S. A.) and ABC complex, respectively. The immunoreactive sites were visualized with 0.02% 3,3-diaminobenzidine tetrahydrochloride (Dojin Chemical, Kumamoto, Japan) and 0.01% H_2O_2 in 0.05 M Tris-HCl buffer (pH 7.6). Sections were then mounted with glycerin.

For localization of GAP-43, the sections were first treated with the rabbit polyclonal anti-rat GAP-43 antibody (Chemicon) diluted at 1:10,000 with 2% normal goat serum overnight at room temperature, followed by the same procedure as used for PGP 9.5.

Fig. 5. Light micrograph of a characteristic Ruffini ending in a control periodontal ligament showing intense PGP 9.5 immunoreactivity (8-week group). Arrows indicate small granular reactions representing microprojections of the axon terminal. $\times 720$

Immunoelectron microscopy of PGP9.5 and GAP-43

Free-floating sections (40 μ m thickness) were processed for PGP 9.5 and GAP-43 immunostaining as described above, except that the pre-treatment with H_2O_2 -methanol was omitted. After being photomicrographed, the sections were demounted, freed in PBS, and subsequently fixed in 1% osmium tetroxide solution reduced with 1.5% potassium ferrocyanide for 2 h at 4°C. After osmication, the specimens were dehydrated in a graded ethanol series and flat-embedded in Epon 812 (Taab, Berkshire, U. K.). Ultrathin sections were cut with a diamond knife (Diatome, Bienne, Switzerland) and double-stained with uranyl acetate and Reynold's lead solution, to be examined under a Hitachi H-7100 transmission electron microscope (Hitachi, Tokyo, Japan) at an accelerating voltage of 75 kV.

Immunohistochemical specificity controls

To confirm the specificity of the immunoreactivity, either the primary antibody was replaced with non-immune rabbit serum, or the treatment with anti-rabbit IgG or ABC complex was omitted.

Fig. 6. Various profiles of PGP 9.5 immunoreactive Ruffini endings in the periodontal ligament of experimental rats at the 4th (**a**, **b**), 8th (**c**), and 16th (**d**) week after the extraction of opposing teeth. **a**: Spear-shaped type, **b**: drumstick type, **c**: thin funicular type (transverse view), **d**: thin funicular type (longitudinal view). a-d: $\times 720$

RESULTS

There was no remarkable difference in the pattern or amount of weight gain seen in the experimental and control rats during the postoperative period. Furthermore, no remarkable differences in drinking or grooming behavior were noticed between the two groups.

Elongation of mandibular first molars

The rate of eruption of the lower first molars differed markedly between the control and experimental animals at all time points following tooth extraction, as indicated by the soft X-ray images (Fig. 1). Sixteen weeks after extraction, the maximal elongation increment of the right mandibular first molar was 0.9 mm, less than the mean height of the crown of the first maxillary molar (1.2 mm) (Fig. 2).

Site of histological and histochemical observation

The Ruffini endings and other periodontal elements were evaluated in the distal half of the periodontal ligament of the distal root of the lower first molar because periodontal mechanoreceptors have been shown to be most abundant in the apical 1/3 of this root, particularly in the distal half when viewed in horizontal sections (authors' observation, data not depicted).

Histochemical analyses of ALPase and TRAP activity

In horizontal sections of the distal root of the lower first molar in the experimental rats, the periodontal ligament showed a dramatic decrease in thickness by 2 weeks after extraction (Fig. 3), without further changes up to the 16th week.

In the control animals, the periodontal tissues of the lower first molars displayed characteristic localization of the enzymatic reactions in which ALPase and TRAP were localized along the mesial and distal aspects, respectively, of the inner surface of the alveolar bone (Fig. 3 control group). The distinct localization of these two enzymatic reactions in the alveolar socket remained unchanged during the experimental period. In the operated rats, the periodontal tissues of the lower first molars without occlusal stimuli showed identical patterns of ALPase and TRAP activity as the control teeth (Fig. 3 experimental group).

Blood vessels and nerve elements in the periodontal ligament

Under physiological conditions, blood vessels were located mainly in the middle zone of the periodontal ligament, while some were closely associated with the alveolar bone or extended towards the root surface (Fig. 4). Sinusoidal vessels of larger caliber were mostly located close to the alveolar bone.

Immunostaining for PGP 9.5 demonstrated Ruffini endings distributed around the blood vessels and also between the vessels and alveolar bone surface. Only a small percentage of PGP 9.5 immunoreactive fibers extended toward the root surface. At higher magnifications, the periodontal Ruffini endings were shown to possess an irregular outline punctuated by numerous fine finger-like microprojections (Fig. 5). The GAP-43 immunoreactive Ruffini endings were also distributed near the blood vessels (Fig. 9a), demonstrating a typical dendritic profile with expanded terminal buttons. The GAP-43 reactive terminals generally showed a thick and irregular outline, similar to that demonstrated by anti-PGP 9.5 antibody staining. However, staining with the antibody against GAP-43 did not reveal distinct microprojections (compare Fig. 9c with Fig. 5).

Transmission electron microscopy showed the PGP 9.5 immunoreactive Ruffini endings as large swollen axon terminals with variable contours which were filled with electron-dense granular materials reactive to the PGP 9.5 antibody. Each axon terminal of this type was covered by non-immunoreactive Schwann sheaths, which were further surrounded by layers of the basal lamina with varying thickness (Fig. 7). Cytoplasmic projections, referred to as the axonal spines of Ruffini endings, extended through the discontinuities of the Schwann sheath, reaching almost 1 μ m in length. Mitochondria in the Ruffini axon terminals were generally evenly distributed in the axoplasm. The immunoreactive products of GAP-43 were localized exclusively in the cytosolic region of the Schwann sheath, and were not associated with the axon terminals of the Ruffini endings (Fig. 9e).

Changes after extraction of opposite molars

By 2 weeks after extraction, the periodontal ligament underwent a dramatic decrease in thickness, becoming associated with vessel occlusion and fiber pattern disruption (Fig. 4). However, few changes were noted in the morphology and distribution density of nerve elements in the regions of the ligament as compared with the controls (Fig. 4).

At 4 and 8 weeks after operation, the Ruffini endings appeared to locate throughout the narrow areas

Fig. 7. Electron micrograph of a PGP 9.5 immunoreactive periodontal Ruffini ending of control rats at the 8th week. Axon terminal (AT) enriched with mitochondria is filled with electron-dense immunoreactive substances and covered by a pair of Schwann sheaths (SS) associated with thick layers of the basal lamina (BL). Arrow indicates a PGP 9.5 immunoreactive microprojection extending through the sheath slit. $\times 12,000$

of the periodontal ligament, ranging from the central zone to the alveolar bone-related zone (Figs. 4, 9b). Some PGP 9.5 and/or GAP-43 immunoreactive endings were also located in the vicinity of the cementum (Figs. 4, 9b). At higher magnifications, a variety of changes were noted in the light microscopic profiles of PGP 9.5 or GAP-43 immunopositive Ruffini endings as displayed in Figures 6 and 9d. Ultrastructural changes were also noted in the Ruffini endings at the 4th week after extraction, including a thickening of the axon terminals associated with the uneven distribution of mitochondria that had shifted to the axonal membrane of the swollen terminals (Fig. 8a). Deposition of a moderately electron-dense, amorphous structure in the center of the essentially organelle-

Fig. 8. Electron micrographs of PGP 9.5 immunopositive nerve endings in the periodontal ligament of experimental rats at the 4th (**a**, **b₁**, **b₂**), 8th (**c**), and 16th (**d**, **e**) week after the extraction of opposing teeth. **a.** Slightly thickened Ruffini ending showing the eccentric localization of mitochondria (arrows) along the axonal membrane and lack of other organelles. Arrowhead indicates a thickened microprojection. $\times 13,000$. **b₁.** (continued on the next page ^)

free axoplasm was occasionally encountered in highly swollen terminals (Fig. 8b₁, b₂). In almost all cases, the area surrounding the amorphous structure showed little penetration by the embedding medium (Epon 812), and hence was easily torn by the electron beam (Fig. 8b₁, b₂). At the 8th week after extraction, some axon terminals showed elongated axonal spines highly immunoreactive to PGP 9.5 emerging from the slits of the Schwann sheath. These spines were much longer than those of controls ($0.74 \pm 0.21 \mu\text{m}$), reaching 1.6–2.2 μm in length (Fig. 8c). Around the elongated axonal spines, small rounded immunoreactive structures, presumably cross-sections of the elongated spines, were also found among the collagen fibers (Fig. 8c).

By the 16th week after extraction, Ruffini endings became unrecognizable near the cementum surface and tended to be confined to the central and alveolar-related zones of the ligament, where an increased number of sinusoidal vessels were located (Fig. 4). Extensive invaginations of the Schwann sheath deep into the expanded axon terminals were often encountered in these nerve terminals (Fig. 8d, e). Similar invaginations could be seen 4 and 8 weeks after surgery.

Throughout the experimental period, the axoplasm of periodontal Ruffini endings was never immunoreactive to GAP-43. Schwann sheaths of these endings, however, showed positive reactions to this protein, although the intensity varied considerably among the individual terminals including those without recognizable reactions (Fig. 9f, g).

DISCUSSION

Movement of mandibular first molar after extraction of opposing teeth

The tooth undergoes unimpeded eruption if the antagonistic tooth—and ensuing occlusal force—is removed. Despite the significant changes in spatio-temporal relationship between the upper and lower jaws due to bone growth during the experimental period, it was confirmed in this study that there was no direct contact between the lower molars and the

gingiva of the opposing jaw throughout the experimental period, since there were neither impressions left nor ulcerative lesions in the gingiva of the upper jaw facing the lower molars. Moreover, the average vertical distance of eruption of the lower 1st molars during the post-operative period of 16 weeks measured in the present study was smaller than the average crown height of the extracted upper 1st molars. Histochemical analyses of TRAP and ALPase activities indicated that the hypofunctional molars also drift distally, similar to normally functioning molars (Fig. 3). Such a movement of the mouse molar reportedly requires the continuous remodeling and turnover of periodontal ligament fibrils at the cementum-related and alveolar bone-related zones (DEPORTER and TEN CATE, 1980). Consequently, in occlusal hypofunction, the Ruffini endings likely undergo constant remodeling and/or adjustment to the changing ligament environment, despite the removal of mechanical stimuli.

Influence on vascular and neural elements

In the normal periodontal ligament of rat molars, vascular and neural elements are located in the alveolar bone-related portions of the periodontal ligament (MAEDA et al., 1987; SODEYAMA et al., 1996). It is also known that vascular elements decrease in diameter and number over time in the hypofunctional periodontal ligament of rat molars (TANAKA et al., 1998). In the present study we also noted under the light microscope that periodontal neural elements, mostly Ruffini endings, undergo dramatic changes in distribution and profile in the narrowing periodontal ligament after extraction of the opposing teeth.

The ultrastructural changes of Ruffini endings in the hypofunctional periodontal ligament were characterized by the loss of cytoplasmic organelles in the axon terminals, except for mitochondria pressed to the axonal membrane. The axoplasm, now almost free of organelles, occasionally revealed deposits of amorphous structures, resembling debris from cytoplasmic organelles (Fig. 8b). PIYAPATTAMIN et al. (1999) reported similar but more severely altered periodontal Ruffini endings in the para-periodontal space area of aged rat incisors that showed a knob-

- 8b₁. Transverse view of an extensively swollen terminal showing the deposition of an electron-dense, amorphous material (*arrowhead*) and various membrane fragments in edematous axoplasm. $\times 7,500$. b₂. Enlarged view of the electron-dense amorphous material (*arrowhead*) and the artifactual hole made under the electron beam. $\times 22,500$. c. Unusual elongation of axonal microprojection (*arrowhead*) and PGP 9.5 immunoreactive vesicular structures scattered around the axon terminal (*small arrows*). $\times 11,000$. d and e. Extensive invagination (*arrows*) of Schwann sheaths (SS) into the immunoreactive axon terminal (AT). No other abnormality is seen in the axon terminal. d: $\times 16,000$; e: $\times 16,500$

Fig. 9. GAP-43 immunoreactivity in control (a, c, e) and experimental rats (b, d, f, g) in the 4th week after the extraction of opposing teeth. **a-d.** GAP-43 immunoreactive Ruffini endings in both control (a, c) and experimental (b, d) rats showing distributions and light microscopic features comparable to those of PGP 9.5 reactive elements as shown in Figures 4-6. C cementum, AB alveolar bone. a, b: $\times 170$, c, d: $\times 600$. **e.** (continued on p. 379)

like profile and totally organelle-free axon terminals. They interpreted these changes to be atrophic features caused by the absence of mechanical stimuli. In the present materials, we did not observe such extreme changes in the hypofunctional Ruffini endings, presumably due to the fact that certain mechanical stimuli to the lower molars would have not been avoided since a hard diet was administered during the experimental period. Despite the absence of the upper molars, pellet fragments may have become trapped in the narrow spaces between the lower molars and gingiva of the upper jaw during feeding, resulting in mechanical stimuli to the periodontal ligament. In fact, in some specimens in 16th week group, the width of the periodontal ligament had increased more than in the earlier periods.

Another characteristic morphological change in the hypofunctional Ruffini endings was the unusual elongation of the microprojections emerging from the slits of Schwann sheaths (Fig. 8c). Their length measured $1.6\text{--}2.2\text{ }\mu\text{m}$, much longer than the mean length, $0.74 \pm 0.21\text{ }\mu\text{m}$ ($n=32$), in the controls. Similar long microprojections ($1.5\text{--}2.0\text{ }\mu\text{m}$) have been reported in the periodontal ligament of rat molars under traumatic occlusion (SODEYAMA et al., 1996). The presence of many PGP 9.5 immunoreactive rounded structures, presumably cross sections of the microprojections (Fig. 8c), implicates the prevalence of elongated, as well as twisted, microprojections in the hypofunctional ligament.

Significance of GAP-43 expression

In the rat molar ligament, an expression of GAP-43 immunoreactivity in the axoplasm of Ruffini endings and its partial disappearance from the Schwann coverings reportedly occur during experimental tooth movement (KOBAYASHI et al., 1998). The transient expression of GAP-43 in the terminal Schwann cells and axonal profiles of the periodontal Ruffini endings also reportedly occur following nerve injury, implicating a close association of the expression of this protein with the axon-Schwann cell interaction during regeneration (YOUN et al., 1998). These experimental data suggest that the Schwann sheaths play a role in maintaining the morphology as well as the function of periodontal Ruffini endings.

The continued expression of GAP-43 immunoreactivity in certain portions of the Ruffini endings in the hypofunctional periodontal ligament demonstrated in the present observations may be related to the dynamic remodeling of periodontal fibers associated with the distal drift of the molars as already mentioned. The partial and complete disappearance of GAP-43 immunoreactivity in some Schwann sheaths of the Ruffini endings without recognizable structural abnormality (Fig. 9g) may indicate the functional alteration of the mechanoreceptors in the absence of normal functional stimuli. The morphological alterations in hypofunctional Ruffini endings, such as those shown in our experimental model, may reflect not only degenerative changes, but also the morphological adaptation of periodontal mechanoreceptors to a changing environment.

In conclusion, the present findings indicate that occlusal stimuli are essential to maintain the width of the periodontal ligament as well as the distribution and morphology of the periodontal Ruffini endings. Observing the complete process of the post-eruptive development of the tooth without occlusal stimuli, in relation to periodontal neural and vascular elements, may provide insights into the significance of the characteristic morphology and distribution of these elements, as well as factors that cause the distal drift of molars. Combined morphological and physiological studies are needed to elaborate further on the functional aspect of the observed changes.

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^9 e. Electron micrograph of Ruffini endings of the control molar ligament showing the exclusive localization of GAP-43 immunoreactivity in Schwann sheaths (SS) surrounding the immunonegative axon terminal (AT). Arrowhead indicates an axonal microprojection. $\times 7,000$. f. A Ruffini ending of an experimental molar showing GAP-43 immunoreactivity restricted to Schwann sheaths (SS). $\times 9,500$. g. A Ruffini ending without immunoreactivity to GAP-43 in either Schwann sheaths (SS) or axon terminal (AT) found in the same section as f. $\times 10,000$

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