Nerve Endings in the Vermilion Border and Mucosal Areas of the Rat Lip*

Tamiko TACHIBANA, Yasunori SAKAKURA, Kiyoto ISHIZEKI and Tokio NAWA

Department of Oral Anatomy (Prof. T. NAWA), Iwate Medical University School of Dentistry, Morioka, Japan

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Summary. Organized mechanoreceptors in mucosae and vermilion borders of rat lower lips were studied by light and electron microscopy. Cholinesterase histochemistry was applied to whole-mount preparations of mucosae for the light and electron microscopic identification of mechanoreceptors. Three types of lamellated corpuscles (a simple corpuscle; a coiled, simple corpuscle; and a Meissner-like corpuscle), and a unique, organized, bush-like assembly of free nerve terminals were identified. The simple corpuscles were found exclusively in the vestibular mucosa facing the incisor teeth. In contrast, bush-like endings were confined to the vestibular mucosa and to the lateral, eminent mucosa that faced the diastema. Furthermore, coiled simple corpuscles and Meissner-like corpuscles were localized in the boundary zone between the vestibular and lateral eminent mucosae and in the vermilion border. From a functional viewpoint, it is of interest that different areas of the rat lip contain different morphological patterns of mechanoreceptors.

Mechanoreceptors are, in general, classified morphologically according to the pattern of the arrangement of nerve terminals and non-neural components associated with them. It is possible that the structures of such non-neuronal elements in a mechanoreceptor may define the receptive modality of its axon terminals (IGGO, 1976; IGGO and OGAWA, 1977). Therefore, it is important to determine the ultrastructural characteristics of the mechanoreceptors in various regions of the skin and mucosa in the assignment of functional significance to a given receptor.

Earlier light microscopic studies have suggested that there is a large diversity in the morphology of sensory nerve endings in the oral mucosa (DIXON, 1961, 1962; MALINOVSKY, 1966; HYODO, 1979; YAMAMOTO, 1981; YAMAMOTO and SAKADA, 1981, 1985; SAKADA, 1983). However, few ultrastructural data on organized endings, have been available to date although free nerve endings and Merkel cell-neurite complexes have been studied extensively (see review: HOLLAND, 1984).

The lower lip of rodentia possesses two different mucosal areas: the vestibular mucosa, which faces the incisor teeth, and the lateral eminent mucosa, which faces the diastema. It is believed that these two mucosal areas have different sensitivities to mechanical stress. Therefore, the present light and electron microscopic study was designed to focus on the morphology of various organized nerve endings and their patterns of distribution in these areas of the mucosa, as well as in the neighboring glabrous skin (vermilion border) of the rat lip.

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To visualize entire profiles of mechanoreceptive corpuscles, whole-mount preparations of labial mucosae were stained by ChE-histochemistry.

MATERIALS AND METHODS

Adult male Wistar rats beyond 8 weeks of age were used. They were anesthetized with sodium pentobarbital and killed by intraventricular perfusion with a fixative that contained 1.5% glutaraldehyde and 2.5% paraformaldehyde, in a 0.05M cacodylate buffer, pH7.2. Then the lower lip was submitted to the following analyses by light and electron microscopy.

Light microscopy
The skin, muscle and submucosa were removed from the lower lip with fine scissors and forceps. The remaining mucous membrane was cut open at the boundary between the vestibular mucosa and the lateral eminence with fine scissors, then incubated in the medium of Karnovsky and Roots containing butyryl thiocholine (KARNovsky and ROOTS, 1964), for about 1 hr at room temperature. Some of the mucous membrane samples containing ChE-positive corpuscles were submitted to the following procedure for analysis by electron microscopy: the samples were whole-mounted on glass slides with the epithelial side facing upwards, after the myelinated nerve fibers had been stained with 0.1% OsO₄.

Electron microscopy
The lower lip was divided into three portions: the lateral eminence, the vestibular mucosa and the transitional portion between the skin and mucosa (vermilion border). Tissues were cut sagittally (vestibular mucosa and vermilion border) or horizontally (lateral eminence) into small blocks and immersed in the fixative described above for 2 hr at 4°C. The specimens were then fixed in 1% OsO₄ and embedded in Epon, after dehydration with a graded series of ethanol dilutions. The mucosal membranes, stained with the medium of Karnovsky and Roots, were also fixed in 1% OsO₄ and embedded in Epon to verify the correspondence between the light and electron microscopic findings.

Ultrathin sections, partly semiserial, were cut and observed under a JEM-100B electron microscope, after staining with uranyl acetate alone, or with uranyl acetate followed by lead citrate.

RESULTS

Light microscopy
Many ChE-positive corpuscles arising from myelinated nerve fibers were identified in the whole-mount preparations (Fig. 1a). Three different types of corpuscles were discerned.

The first type was characterized by a ChE-positive bulb with slightly serpentine and elongated profiles (Fig. 1a,b). These corpuscles were confined to the vestibular mucosa. Occasionally, the corpuscles had more than two branched bulbs in one corpuscle (Fig. 1b). These bulbs are thought to be the inner cores of the simple corpuscles.
The second type was characterized by a cluster-like appearance (Fig. 1a, c). These corpuscles were localized in the mucosa of both the vestibulum and the lateral eminence. From their appearance, they were thought to be the so-called bush-like endings which have previously been identified in murine lips by the use of methylene...
blue staining (YAMAMOTO and SAKADA, 1981, 1985). The first and the second type corpuscles were evenly dispersed throughout the vestibular mucosa. There were a number of thin nerve bundles containing both the simple corpuscles and the bush-like endings (Fig. 1a).

The third type of ChE-positive corpuscles showed complicated glomerular profiles (Fig. 1d). They were observed preferentially in the connective tissue papillae of the lateral eminence, of the boundary between the lateral eminence and vestibulum, and of the vermilion border.

Fig. 2. Electron micrograph of a simple corpuscle in rat vestibular mucosa (cross section). AT axon terminal, IZ inner compact zone of inner core, OZ outer clear zone of inner core, CP perineural capsule. ×7,900
Fig. 3. Part of a cross section of a simple corpuscle, with two branched inner cores and accessory nerve terminals (*). ICT axon terminals in the inner cores, LC laminar cells, CP perineural capsule. Note that the accessory nerve terminals have their own laminar investments (insert). ×9,200, insert: ×17,000
Electron microscopy

1. Simple corpuscles
Electron microscopy revealed that the corpuscles that had ChE-positive bulbs in the vestibular mucosa were, indeed, the simple corpuscles. A typical one was composed of an axon terminal, a lamellated inner core that surrounded it, and a perineural capsule (Fig. 2). The axon terminal was discoid in shape, and contained many mitochondria plus a small number of clear vesicles (Fig. 2). By contrast, the cytoplasmic lamellae, characterized by numerous caveolae, surrounded the axon terminal concentrically (Fig. 2). These cytoplasmic lamellae were extended from the lamellar cell bodies located on the margin of the inner core. These characteristics are comparable to those of the simple corpuscles previously described (see reviews: Andres and Von Düring, 1973; Halata, 1975; Chouchkov, 1978).

Fig. 4. Part of a bush-like ending in rat vestibular mucosa. Sections of nerve terminals and fibers (*) and their original myelinated nerve fiber (MN) can be seen. SC Schwann cell, FB fibroblast, EP epithelium. ×6,600
In addition to the typical simple corpuscle, two atypical variations were found. One contained more than two profiles of inner cores within a corpuscle. This atypical corpuscle seemed to correspond to the corpuscles with branched inner cores found in whole-mount preparations. The other atypical variation was unique in containing accessory unmyelinated axons in addition to the main axons that were covered by normal inner cores (Fig. 3). The accessory axons had very thin calibers and their terminals penetrated into the interlamellar spaces of the outer zone of the inner cores that surrounded the main axon terminals (Fig. 3). The accessory axon terminals contained an abundance of mitochondria, and a small number of clear vesicles, as was the case for the main axon terminals in the inner cores (Fig. 3).

2. Bush-like endings

Many groups of large and bulboid axon terminals were found beneath the epithelium of the vestibular and lateral eminent mucosae (Fig. 4). From the specificity of their localization and the intensity of their ChE activity, these grouped nerve endings are considered to correspond to the bush-like endings in the whole-mount preparations.

The bush-like endings were localized in papilla-free areas, close to the overlying

![Figure 5](image_url)

**Fig. 5.** Enlargement of a nerve terminal of a bush-like ending. Thin cytoplasmic laminae (SCL) extending from a Schwann cell (SC) incompletely cover the axon terminal. Note that the basal lamina of the Schwann cell laminae contains an abnormal number of layers (arrows). ×16,000
epithelium. There were no perineural structures around the grouped nerve endings. However, it was noticed that dense collagen bundles usually surrounded each axon terminal of the nerve endings (Fig. 4, 5).

The axon terminals of the bush-like endings, which were round, oval, or Y-shape in the sections perpendicular to the mucosal surface, contained many mitochondria (Fig. 4, 5). The terminals were covered by a layer of thin, Schwann cell cytoplasm.

Fig. 6. A coiled simple corpuscle in the boundary between the vestibular mucosa and the lateral eminent mucosa of the rat lip. Four sections of inner cores can be seen in the corpuscle. Many free nerve endings are localized between the corpuscle and the overlying epithelium. LC laminar cell, N nerve fiber or nerve terminal, FB fibroblast. ×3,400
that was rich in caveolae (Fig. 4, 5). The overlay of Schwann cell cytoplasm was interrupted in places, usually at the lateral poles of the elliptic profiles of the axon terminals (Fig. 4, 5). At these sites, axolemma was in direct contact with the basal lamina that surrounded the Schwann cell cytoplasm (Fig. 5). Especially conspicuous was the basal lamina of the Schwann cell cytoplasm, which comprised many layers in some areas (Fig. 5). Schwann cell bodies were localized between the nerve terminals (Fig. 5) and extended their cytoplasmic lamellae toward the axon terminals.

Fig. 7. A Meissner-like corpuscle in the vermilion border of the rat. Stacks of flat nerve terminals and laminar plates extending from a Schwann cell (SC) can be seen in the inner core. × 4,400
3. **Glomerular corpuscles**

Various complex corpuscles, having lamellar investments that surrounded axon terminals, were found in the lateral eminence, in the boundary between the lateral eminence and the vestibulum, and in the vermilion border. They were comparable in form to the glomerular corpuscles which were identified by light microscopy in the whole-mount preparations. They were classified into two categories on the basis of the arrangement of their cytoplasmic lamellae: a) coiled, simple corpuscles; and b) Meissner-like corpuscles. In a few instances, however, it was difficult to determine to which category a particular corpuscle belonged.

a) **Coiled, simple corpuscles**: These corpuscles were characterized by plural axon terminals, each of which was covered by a symmetrical lamellar investment (Fig. 6). The ultrastructure of the axon terminals and the cytoplasmic lamellae that surrounded them were similar to those of the simple corpuscles (Fig. 3). However, the coiled, simple corpuscles were clearly distinguishable from the simple corpuscles which had branched inner cores, by the fact that the number of profiles of inner cores varied in relation to the sectioning level of a given corpuscle.

The coiled, simple corpuscles were found in the connective tissue papillae of the lateral eminence, of the boundary between vestibular and lateral eminent mucosae and of the vermilion border. The corpuscles were seen at various levels of the connective tissue papillae, sometimes immediately adjacent to the overlying epithelium. The corpuscles in the lateral eminent mucosa usually had a typical perineural capsule, but those in other portions were covered with only a thin layer of fibrocytes (Fig. 6). Small, free, nerve endings were occasionally found between the coiled, simple corpuscle and the overlying epithelium.

b) **Meissner-like corpuscles**: Meissner-like corpuscles were characterized by stacks of cytoplasmic lamellae and discoid axon terminals (Fig. 7). Unlike the coiled, simple corpuscles, the cytoplasmic lamellae surrounding the axon terminals of these corpuscles were arranged in an asymmetrical pattern. The number of axon profiles varied, depending upon the sectioning level of a given corpuscle. Furthermore, lamellar cells were usually localized on the periphery of the corpuscle (Fig. 7).

The Meissner-like corpuscles were predominantly found in the boundary zone between the vestibular and lateral eminent mucosae and in the vermilion border. They were located in the connective tissue papillae, usually fitting themselves in the shallow depressions of the overlying epithelium (Fig. 7). They had no perineural capsules, but were covered by a discontinuous layer of fibrocytes in a manner similar to that of the coiled, simple corpuscles in the same areas (Fig. 6, 7).

**DISCUSSION**

The morphological classification and nomenclature of cutaneous sensory receptors are still controversial. HALATA (1975) divided them into three categories: Type I, with endings localizing in epidermis; Type II, with endings having bulboid nerve terminals; and Type III, with endings having lamellated inner cores. In this classification, the so-called dermal free endings, lanceolate endings, Meissner corpuscles and Ruffini corpuscles are all categorized as Type II, while Krause simple Endkolben and Vater-Pacini corpuscles are categorized as Type III. On the other hand, CHOUCHCOV (1978) proposed to classify cutaneous nerve endings into two categories: unencapsulated and
encapsulated receptors, by the presence or absence of a perineural capsule. He placed the free nerve endings in glabrous skin and lanceolate endings in hairy skin into the category of unencapsulated receptors. However, he subdivided encapsulated receptors into the following three subgroups, according to the pattern of the Schwann cell investments surrounding the axon terminals: receptors without lamellated inner cores (e.g., the Ruffini corpuscle), receptors with asymmetrical lamellated inner cores (e.g., the Meissner corpuscle), and receptors with symmetrical lamellated inner cores (e.g., the Pacinian corpuscle).

In the present study of the rat labial tissues, we have identified four types of organized nerve endings and have tentatively named them the “bush-like ending”, “simple corpuscle”, “coiled simple corpuscle”, and “Meissner-like corpuscle”. The “bush-like ending” was so named after the work of previous authors (YAMAMOTO and SAKADA, 1981, 1985), on the basis of the light microscopic appearance of the corpuscles in the whole-mount preparations. As the bush-like endings have no special capsule, they can be placed in CHOUCHCOV’s unencapsulated receptor category (1978). Although other corpuscles all have lamellated inner cores, only the simple corpuscle has a typical perineural capsule. Therefore, we propose that these corpuscles should be classified as “lamellated corpuscles”, if necessary.

The ultrastructure of nerve terminals in the bush-like endings is similar to those of free nerve endings (CHOUCHCOV, 1978; HALATA and MUNGER, 1986). Therefore, unless whole-mount preparations or serial sections are analysed, the nerve terminals can be incorrectly identified as isolated, free endings. It is noteworthy that the bush-like endings are found exclusively in the mucosal areas of the lip. Similar corpuscles have been found in the labial mucosae of mice (YAMAMOTO and SAKADA, 1981), but have never been found in canine lips (personal observation). SAKADA (1983) reported that the complex, unencapsulated endings in feline oral mucosa (the microscopic appearance of which, in our opinion, closely resembles the bush-like endings), showed a slowly adaptive response to mechanical stimuli. The axon terminals of the bush-like endings are embedded in the dense collagenous matrix in the same manner as the axon terminals in the Ruffini corpuscle, which have been well documented to be slowly adapting mechanoreceptors (CHAMBERS et al., 1972). Thus, it is conceivable that the bush-like ending in the rat labial mucosa may be concerned with slowly adapting mechanoreception. In this connection, it is interesting to note that few Merkel cell-neurite complexes, which also serve as slowly adaptive mechanoreceptors, are seen in the labial mucosal epithelium of the rat (not shown).

Simple corpuscles have so far been called by various names in literature: the mammalian end-organ (WINKELMANN, 1962), Pacinian corpuscle (MALINOFSKY, 1966), simple encapsulated corpuscle (HALATA, 1975), Krause simple Endkolben (CHOUCHCOV, 1978) and so on. The simple corpuscle has been considered to serve as a rapidly adapting mechanoreceptor (IGGO and OGAWA, 1977). There are three ultrastructural variations on the simple corpuscles of the rat labial mucosa. Of these three, the most interesting is that of having accessory axon terminals in the interlamellar spaces of inner cores. Although the thin calibers of the accessory axons suggest that they may be related to C-fibers, more precise reconstructive studies are necessary before we can reach any firm conclusion.

The term “coiled, simple corpuscle” was first used by HALATA and MUNGER (1983) for the glomerular endings in primate oral mucosae. Since then, compound corpuscles that contained similar coiled inner cores have been identified in the canine oral mucosa (TACHIBANA et al., 1987). The present report is the second to describe the presence of
this type of corpuscles in non-primate oral mucosa. The ultrastructure of the inner cores of these corpuscles resembles very much that of simple corpuscles. Hence, rapidly adapting mechanoreception may be a property of the coiled, simple corpuscles as well as of the simple corpuscles (Halata and Munger, 1983).

Primate labial mucosa and the vermilion border of the lip contain Meissner and/or coiled simple corpuscles (Munger, 1973, 1975; Halata and Munger, 1983). Although murine toe pads contain Meissner-like corpuscles (digital corpuscles, Idé, 1976), no other subprimate mammals contain Meissner corpuscles in the skin (Malinovsky, 1986). Thus, we conclude that the rodentia are unique in that they have mechanoreceptors with a primate-like morphological pattern.

Although lamellated corpuscles are generally concerned with rapidly adapting mechanoreception, different morphological types may be endowed with their own functional parameters. Therefore, it is noteworthy that different areas of rat lip contain different morphological patterns of lamellated corpuscles. This fact seems to reflect a region-specific sensitivity.

The morphology of mechanoreceptors varies according to classes of animals (Malinovsky, 1986). From the phylogenetic point of view, it is of interest that the rat lip contains both the primate and non-primate types of lamellated mechanoreceptors. Furthermore, it is notable that there are a few lamellated corpuscles with features intermediate between those of the Meissner-like and coiled simple corpuscles, suggesting a phylogenetically close relationship between these two types of corpuscles.

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