

## Sensory nerve endings in the rat cheek mucosa: an electron microscopic study

### Terminações nervosas sensitivas na mucosa da bochecha de ratos: estudo ao microscópio eletrônico

CORRESPONDENCE TO:  
II-SEI WATANABE  
Departamento da Anatomia  
Instituto de Ciências Biomédicas da USP  
Av. Prof. Lineu Prestes, 2415  
05508-900 - São Paulo - SP  
e-mail: watanabe@usp.br

1- Departamento de Anatomia do Instituto  
de Ciências Biomédicas da Universidade  
de São Paulo, São Paulo - SP

Ii-sei WATANABE<sup>1</sup>, Marcelo Cavenaghi Pereira da SILVA<sup>1</sup>,  
Marcia Consentino KRONKA<sup>1</sup>

#### SUMMARY

The sensory nerve endings of the rat cheek mucosa were studied using the transmission electron microscopy. The specimens were fixed in modified Karnovsky solution and embedded in Epon resin. The sensory nerve endings showed a central terminal axon containing numerous mitochondria, neurofilaments, microtubules and clear vesicles. The proximal part of corpuscle revealed the cytoplasmic extensions of lamellar cells and the perineural cells. The fine bundles of collagen fibers are identified in the interlamellar spaces and the external part of corpuscle. Numerous concentric lamellae showed caveolae, interlamellar spaces filled with amorphous material, desmosome-type junctions between adjacent lamellae and the inner lamellar cells and the axoplasmic membrane. These fine structures are important to recognise and understand the morphological characteristics in the oral mucosa.

**KEY-WORDS:** Nerve endings. Lamellated corpuscle. Cheek mucosa. Transmission electron microscopy. Rat.

#### INTRODUCTION

The peripheral sensory nerve endings are mainly localized in the deep portion of the connective tissue and they are responsible for the monitoring of internal and external stimuli. These sensory nerve endings can be classified according to the ultrastructural characteristics of the terminal nerve and surrounding structures arrangement. In the oral mucosa, the first part of the digestive system, special attention was taken to the fine structure of nerve endings on periodontal structures like <sup>1, 10, 17, 18</sup> and periodontal ligament<sup>3, 2, 7, 6</sup>. On the other hand, Watanabe and Yamada<sup>19</sup> and Halata et al.<sup>4</sup> reported the fine structure of nerve endings in the hard palate, Watanabe and Yamada<sup>20</sup> in the soft palate, Tachibana et al.<sup>13</sup> and Watanabe and Ide<sup>16</sup> in the rat lip.

The present paper describes the fine structure of sensory nerve endings in the rat cheek mucosa with special attention to the lamellated corpuscles, their terminal axon and surrounding cells.

#### MATERIAL AND METHOD

Six Wistar rats were anaesthetized with sodium pentobarbital and perfused through the left ventricle of the heart with modified Karnovsky fixative solution containing 2.5% glutaraldehyde and 2% paraformaldehyde in a 0.1 M

sodium phosphate buffer (pH 7.4). The cheek mucosa was removed and immersed in the same fixative during 3 h at 4°C. The specimens were rinsed with 0.1 M sodium phosphate buffer and postfixed in 1% OsO<sub>4</sub> during 2 h at 4°C. Then, the samples were immersed in 5% uranyl acetate aqueous solution for 2 h at room temperature, dehydrated with a graded series of ethanol and embedded in Epon resin 812.

Semi-thin sections were cut in LKB ultramicrotome using glass knives, mounted and stained with 2% toluidine blue for light microscopy study. The ultra-thin sections of the selected areas were cut with Reichert Ultracut Ultramicrotome using diamond knife. The grids were counterstained with uranyl acetate<sup>22</sup> and lead citrate<sup>11</sup> and examined in a JEOL 1010 transmission electron microscopy.

#### RESULTS

In the rat cheek mucosa, several myelinated neurons and organized sensory corpuscles beneath to the epithelium were observed. The myelinated sensory axons lost their myelin sheet and entered to the organized corpuscles. They were separated from other elements like fibroblasts and capillaries by bundles of collagen fibers (Fig. 1).

The organized sensory corpuscles found called lamellar corpuscles because the terminal axon was involved by numerous concentric cytoplasmic lamellae (Fig. 2).

The terminal axon was located in the central portion of the corpuscles and in the lateral part of the terminal axon may be observed axoplasmic protrusions which continue laterally as lateral clefts to the periphery (Fig. 2).

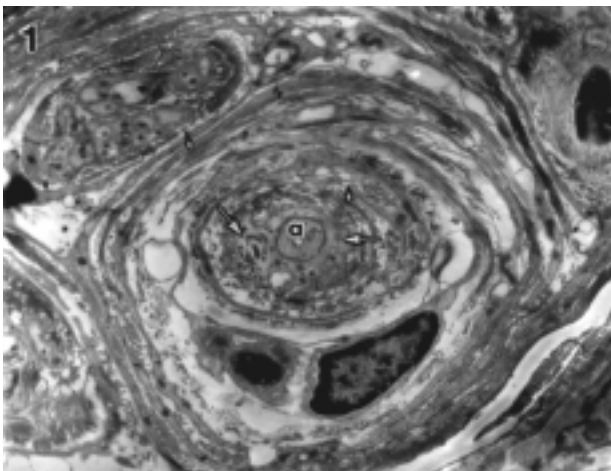
At the periphery, a capsule was formed by cytoplasmic extensions of perineural cells and bundles of collagen fibers disposed in various directions (Fig. 3).

At higher magnification, the axoplasm of axons, presented scattered mitochondria (Fig. 4 and 5) whose cristae mitochondriales were clearly seen (Fig. 4 and 5). The axoplasm also contained neurofilaments and microtubules (Fig. 4 and 5). In the axoplasmic protrusions were observed several clear vesicles and scattered mitochondria (Fig. 5).

The cytoplasm of lamellar cells contained several caveolae (Fig. 6 and 7) which are invaginations of the cytoplasmic membrane and the interlamellar spaces were filled with amorphous material and fine collagen fibers filaments. Desmosome-type junctions were observed between lamellar cells (Fig. 6) and between the terminal axon and adjacent lamellae (Fig. 7). Multivesicular corpuscles were also observed in Fig. 7.

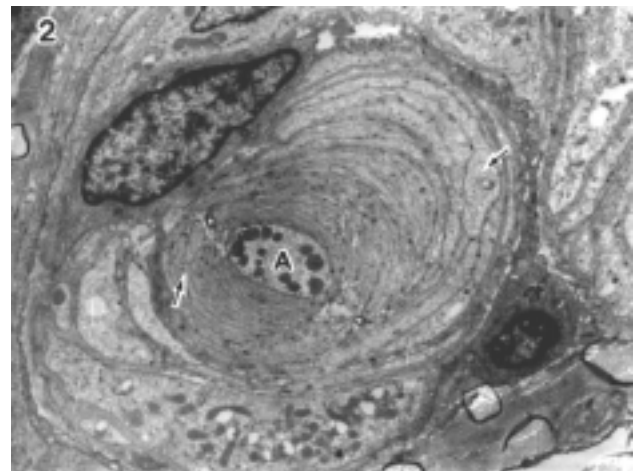
## DISCUSSION

The fine structure of lamellated corpuscles observed



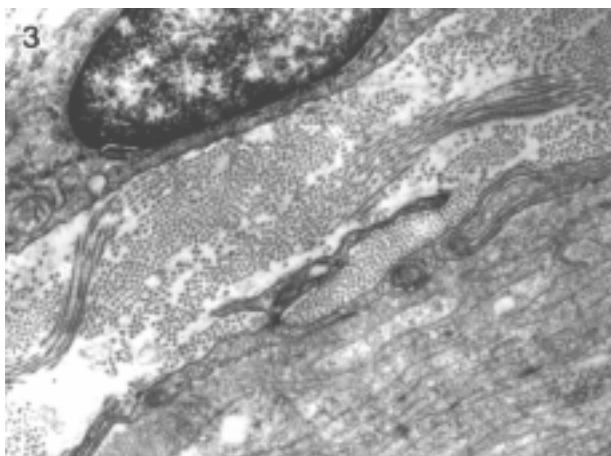
**Figure 1**

Transmission electron microscopy image of a proximal part of the sensory nerve endings of rat cheek mucosa. Shows a central terminal axon (A), bundles of collagen fibers (small arrows) and cytoplasmic extension of capsular cells (large arrows). X 6,000



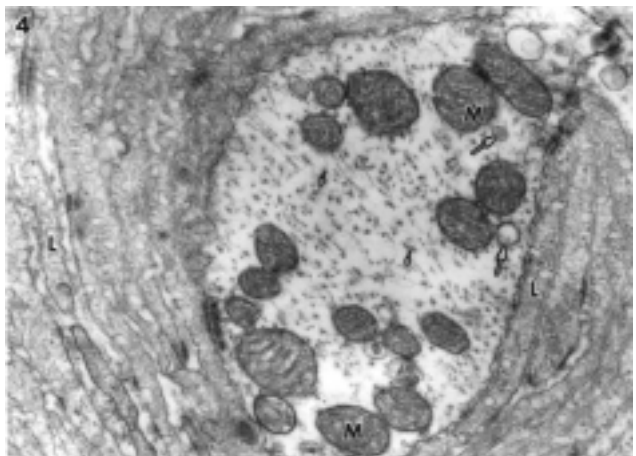
**Figure 2**

TEM image of transversal section of a lamellar corpuscle showing the terminal axon (A), the cytoplasmic lamellae (large arrows) and the lateral cleft (small arrow) can be observed. X 6,000



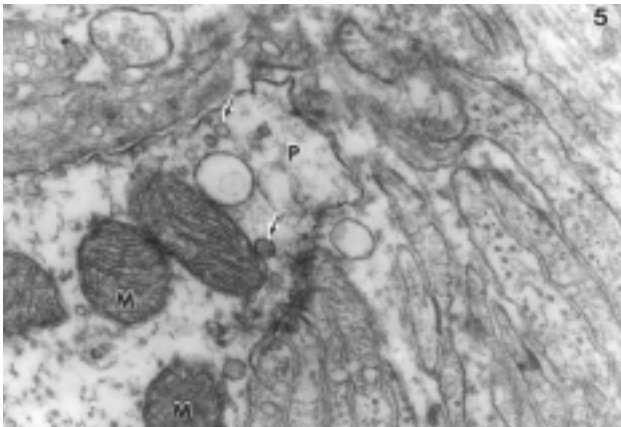
**Figure 3**

At higher magnification, TEM image reveals the bundles of collagen fibers oriented in several directions and surrounding the corpuscle. X 21,000



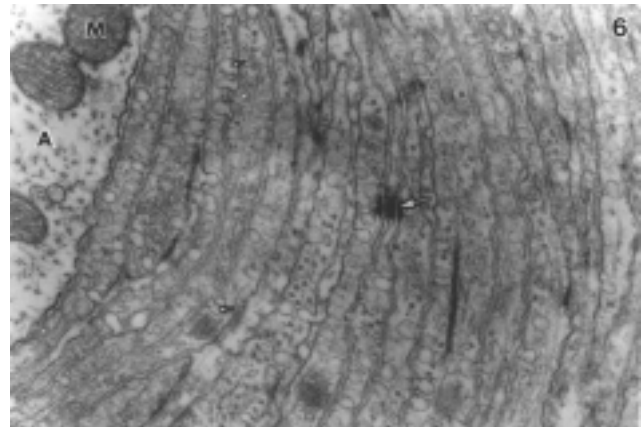
**Figure 4**

The terminal axon contains scattered mitochondria (M), neurofilaments (small arrows), clear vesicles (large arrows) and lamellae (L). X 36,900



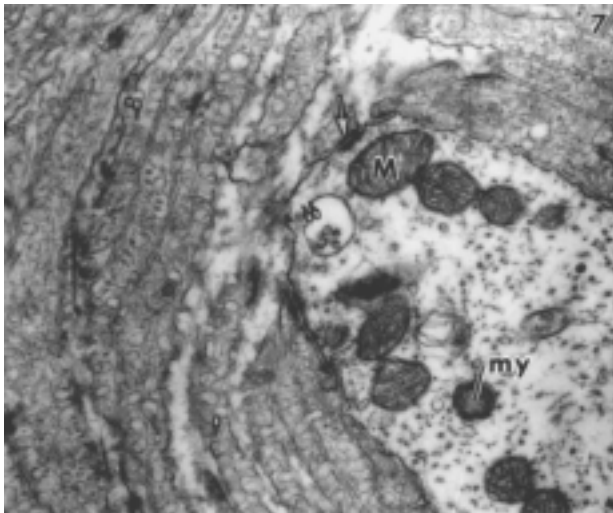
**Figure 5**

At higher magnification, the axoplasmic protrusion (P), several mitochondria (M), and small clear vesicles (arrows) can be seen. X 55,000



**Figure 6**

At higher magnification shows the interlamellar spaces containing the amorphous substance. The terminal axon (A) shows the mitochondria (M) and the lamellae present caveolae (small arrows) and desmosome-type junctions between them (large arrow). X 43,600



**Figure 7**

At higher magnification, the presence of mitochondria (M) with their cristae, multivesicular corpuscle (two arrows), desmosome-type junctions between the axoplasmic membrane and adjacent lamella (large arrow) and the caveolae (small arrows) are shown. X 30,000

in our study were similar to those reported by Martinez and Pekarthy<sup>10</sup>; Watanabe and Yamada<sup>18</sup>, in the rat gingiva and Toyoshima et al.<sup>14</sup>, in primate fungiform papillae. They were composed by several concentric lamellae and a terminal axon in the center.

This corpuscle is an organized sensory nerve ending. Recent articles classified this corpuscle according to the ultrastructural and functional differences. Malinovsky<sup>8</sup>, included lamellar corpuscles in the class III which comprised rapidly adapting endings whose dendritic zone are associated with elements of ectoderm origin.

Our study revealed a single unmyelinated terminal axon in the center of the corpuscle, which was also reported

by Jarayaraj et al.<sup>5</sup> in the sheep oral mucosa. On the other hand, Watanabe<sup>15</sup> revealed in the gingiva of man and *Cebus apella* monkey and Tachibana et al.<sup>13</sup> in the vermilion border and mucosal areas of the rat lip, observed accessory unmyelinated axons in addition to the main axon among the inner core. The terminal axon also presented a large number of mitochondria and small clear vesicles particularly in the base of axoplasmic protrusion<sup>5, 21, 13, 16</sup>.

It is believed that the axoplasmic protrusion of Paccinian corpuscles play a significant role in the process of transduction<sup>12</sup>. Tachibana et al.<sup>13</sup>, in the fungiform papillae of monkeys observed the presence of distinct finger like projections of the terminal axons which may provide advantageous information regarding the position and shape of food in the oral cavity during mastication.

The cytoplasm of lamellar cells contained several invaginations (caveolae) or clear vesicles. Based on the incidence of small clear vesicles, Malinovsky<sup>8</sup> classified corpuscles with abundant incidence of vesicles, medium incidence of vesicles and low incidence of vesicles. According to that, the cytoplasmic projections of lamellae could be classified as abundant incidence of vesicles.

Between lamellae, there is an interlamellar space filled with amorphous material and fine collagen fibers filaments. Watanabe and Ide<sup>16</sup>, in the rat lip, demonstrated cholinesterase activity in the periaxonal spaces between the terminal axon and adjacent lamellae as well as in the interlamellar spaces which may be related to metabolic or nutritional aspects, the trophic effects of lamellar cells on receptors or some relationship with the process of transduction. Although, it was also reported that the clear vesicles were only stationary pictures of the dynamic metabolic action involving transport, intake and elimination of substances onto the surface of cytoplasm<sup>9</sup>.

Several authors, Martinez and Pekarthy<sup>10</sup>; Watanabe and Yamada<sup>18,20</sup>; Tachibana et al.<sup>13</sup> and Halata et al.<sup>4</sup> reported the presence of desmosome-type junctions between lamellar

cell and the terminal axon and adjacent lamellae which may be related to the mechanical stability of the corpuscle or/and connect adjacent lamellae.

## RESUMO

As terminações nervosas sensitivas da mucosa da bochecha de ratos foram estudadas utilizando-se microscopia eletrônica de transmissão. As amostras foram fixadas em solução de Karnovsky modificada e inclusas em resina do tipo Epon. As terminações nervosas sensitivas mostraram um axônio central terminal contendo numerosas mitocôndrias, neurofilamentos, microtúbulos e vesículas claras. A porção proximal dos corpúsculos revelaram projeções citoplasmáticas de células lamelares e células perineurais. Delgados feixes de fibras colágenas foram identificados nos espaços interlamelares e na região externa dos corpúsculos. Numerosas lamelas concêntricas apresentavam caveolas, espaços interlamelares preenchidos por material amorfo, desmossomos do tipo juncional entre lamelas adjacentes e células lamelares internas e membrana axoplásmica. Essas estruturas delgadas mostram-se importantes na identificação e estudo das características da mucosa oral.

**PALAVRAS-CHAVE:** Nervos sensitivos. Corpúsculos lamelares. Mucosa. Microscopia eletrônica de transmissão. Rato.

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