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The sensory border of the land planarian *Bipalium kewense* (Tricladida, Terricola)

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ABSTRACT. The lunular headplate of *Bipalium kewense* is limited all around its margin by a distinct sensory organ, consisting of a row of papillae, intercalated with ciliated pits. We have investigated the detailed morphology of this sensory region by means of scanning and transmission electron microscopy, as well as, immunohistochemistry. Epithelial cells of the papillae have insunk nuclei and a microvillar border. The dense cytoplasm contains a system of smooth, elongate, vesicles. Monociliated ends of multipolar dendrites come to the surface of the headplate through the epithelial cells. Pear-shaped ciliated pits insert among the papillae. These pits are also provided with sensory dendrites having longer cilia, that are interpreted as chemo-receptors. The distal ends of receptors at both locations are provided with belt-like septate junctions, especially evident following lanthanum impregnation. Immunohistochemical experiments by indirect immunofluorescence have localized substance P in the ciliated receptors, thereby confirming their sensory nature.

KEY WORDS: Platyhelminthes, sensory border, ultrastructure, immunohistochemistry, substance P.

INTRODUCTION

Most sensory elements of flatworms have a rather simple construction (monociliary receptors), although some more elaborate forms exist (Bedini et al., 1975; Ehlers, 1985; Rieger et al., 1991; Wright, 1992). Epithelial ciliated receptors are free endings of neurons, whose perikarya are located in the parenchyma, making contact with the brain (Welsch & Storch, 1976). The functions ascribed to these various receptors remain for the greater part speculative, other than the eyespots and ocelli.

The land planarian *Bipalium kewense* bears a flattened headplate bordered by numerous papillae, supposedly having sensory functions. To our knowledge, ultrastructural aspects of these papillae have received little attention (Storch & Abraham, 1972; Curtis et al., 1983). Given the unique character of this headplate and its presumed involvement in the detection of prey (Hyman, 1951; Bullock & Horridge, 1965), a detailed study of these receptors, including immunohistochemistry, seemed desirable. Recently, terrestrial triclads have become a subject of great concern in certain locations as predators of earthworms, and also due to a high proliferative rate (e.g. Ogren, 1995).

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MATERIAL AND METHODS

Specimens of *Bipalium kewense* Moseley, 1878, most of them over 10 cm in length, were collected locally at the University campus and in Bariri, state of São Paulo, under boards, leaves and flagstones. They were reared within closed pots in the laboratory, fed every two weeks with live earthworms. Only the cut off headplates were used for the present experiments, the remaining bodies being left to regenerate.

Light microscopy (LM)

The heads were fixed in 4% paraformaldehyde (PF) in 0.1M phosphate-buffered saline (PBS), pH 7.4 at 4ºC, for 6h, embedded in Historesin and cut at 3 μm; sections were stained with haematoxylin / eosin.

Immunohistochemistry

The indirect immunofluorescence technique of COONS et al. (1955) was used. Heads fixed as above for 4 h, were cryoprotected in phosphate buffered sucrose, pH 7.4, then embedded in Tissue Tek, sectioned at 8-10 μm at -20ºC, transferred to gelatin-coated glass slides, allowed to dry and frozen at -70ºC. The sections were thawed and immersed in PBS (phosphate buffered saline) with 1% bovine serum albumin (BSA) and 0.2% Triton X-100, at room temperature, for 2 h, then incubated with the primary antibody (anti-rabbit substance P, Sigma, 1:200), for 48 h at 4ºC. Sections were rinsed in PBS and further incubated for 2 h with the secondary anti-rabbit IgG antibody conjugated to fluorescein isothiocyanate (1:50) (FITC-Jackson Immunoresearch Lab.). Sections were rinsed again in PBS, mounted in 80% glycerol plus 2.4% antifade (Dabco - Sigma), in 0.1M PBS and stored in the dark, until examination under a Zeiss-510 confocal scanning laser microscope.

Transmission electron microscopy (TEM) and Tracing experiments

Fixatives used were: a) 2.5% glutaraldehyde:1.5% paraformaldehyde (GTA:PF) followed by 1% OsO_4 or b) 3% GTA containing 0.1% $CaCl_2$, followed by 2% OsO_4; c) fixation in cacodylate buffered 3.5% GTA; post-fixation in 2% OsO_4 containing 3% lanthanum nitrate in s-collidine buffer, pH 7.6; dehydration, and Epon embedding (REVEL & KARNOVSKY, 1967); d) fixation in 3% GTA + 0.5% cetylpyridinium chloride for 2h, followed by 1% OsO_4 + 1% lanthanum nitrate in s-collidine buffer, pH 8.0, 2h. (SHEA, 1971). Embedding was in Epon resin. Ultrathin sections were stained with uranyl acetate and lead citrate. The electron microscopes used were: Siemens Elmiskop 101 operated at 100 kV, Philips CM-200 (at 200 kV) and Jeol 100 CX-II (at 80 kV).

Scanning electron microscopy (SEM)

Fixation at 85ºC in 2.5% GTA, pH 7.2-7.4 (SEWELL & CANNON, 1995). After cooling to room temperature, the pieces were dehydrated, critical point-dried, and coated with gold. A JSM 840-A microscope was used.

RESULTS

The sensory margin of the headplate of *B. kewense*, consists of a regular row of flattened papillae (Figs 1, 2). In contrast to the overall ciliated surface of the head, this sensory margin is covered by only microvilli and secretory droplets (Figs 2,3). The individual papilla measures about 15 x 25 μm (Fig. 2). Tufts of cilia (Fig. 3) occur at the entrance to a number of alternating pits, which vary between 20-40 μm in depth, depending on the plane of sectioning and/or contraction state of the head (Figs 4,5). Many uniciliated receptors protrude at the surface of the papillae, and can be recognized among the secretory droplets as stiff rods, about 1-2 μm high (not shown). Immunostaining experiments for the neuropeptide substance P demonstrated reactive sites all along the sensory border of the headplate (Fig. 5)

Epithelial cells covering the papillae have an expanded distal cytoplasm and the cell body insunk well beyond the basal membrane and muscular layer. Nuclei are found below the pits (Fig. 4); this arrangement is also evident in Fig. 6. The papillae are covered by microvilli and traversed by many multipolar neurons (Figs 6,7). The microvilli exhibit a glycocalyx that stains selectively with cetylpyridinium-lanthanum (Fig.10). The cytoplasm of epithelial cells is always dense, in any fixation method (compare Figs 6, 11). This high density was reinforced when accidental penetration of lanthanum occurred, revealing a characteristic population of clear vesicles (Figs 6, 7,11), scattered at random throughout the cytoplasm, except for a thin marginal layer (Figs 7, 10). Most vesicles are elongate or tubular, with dilated ends, about 200 x 45 nm, and are usually empty. On face view some vesicles seem disk-shaped. At higher magnification, a definite limiting membrane is resolved in them (Fig. 12). It has not been possible to further characterize these elements.

Sensory receptors located at the surface of the papillae are uniciliated ends of multipolar dendrites, circular in cross section, containing small mitochondria and microtubules (Figs 6, 7). Many dendrites traverse a single epithelial cell, each one establishing with it, at the distal end, a belt-like, septate junction of the pleated type (Figs 8, 9). Similar junctions occur around receptors concentrated at the bottom of the pits. Receptors found at the surface of the papilla have short "9+2" cilia, apparently stiff, showing a system of thin unstriated fibrils running from the basal body (Fig. 8). A similar construction is found for receptors of the pits, except that in this case the ciliary shaft is longer. In both locations, the junctions were clearly enhanced with lanthanum (Figs 7, 8, 9).

Legends to the figures (see opposite page)

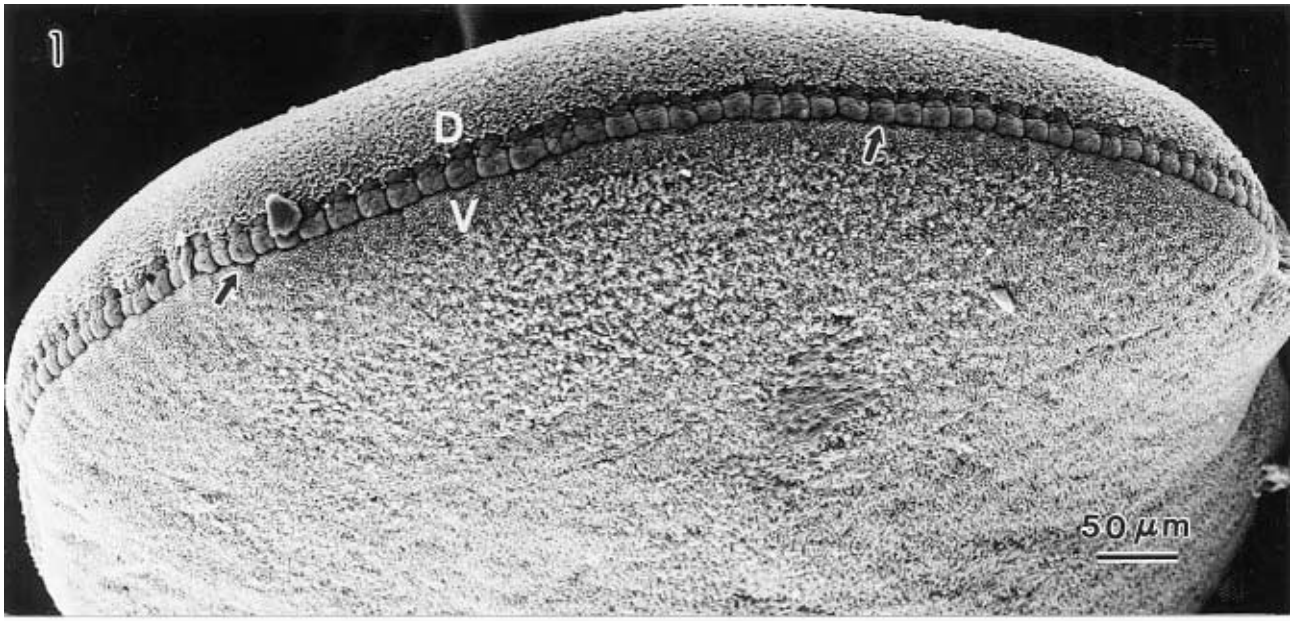
Fig. 1. – Whole view of the sensory border (arrows) delimiting dorsal (D) and ventral (V) faces of the headplate. SEM.

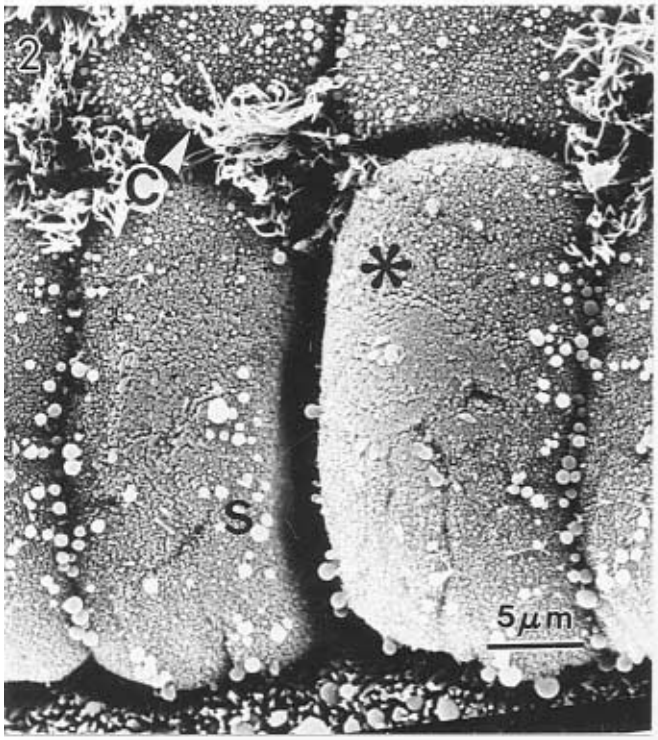
Fig. 2. – Surface view of papillae. Note the pebbled appearance of microvillar covering (*) and many secretory droplets (S); tufts of cilia (C, arrowhead) at the entrance of pits. SEM.

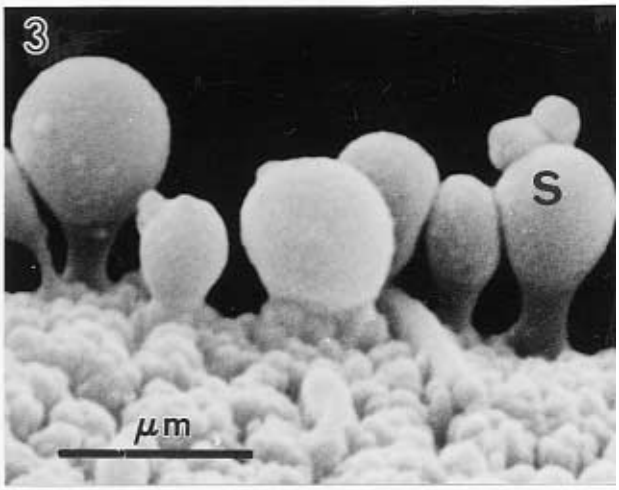
Fig. 3. – Enlarged view of secretory droplets (S), presenting a short stalk. SEM.

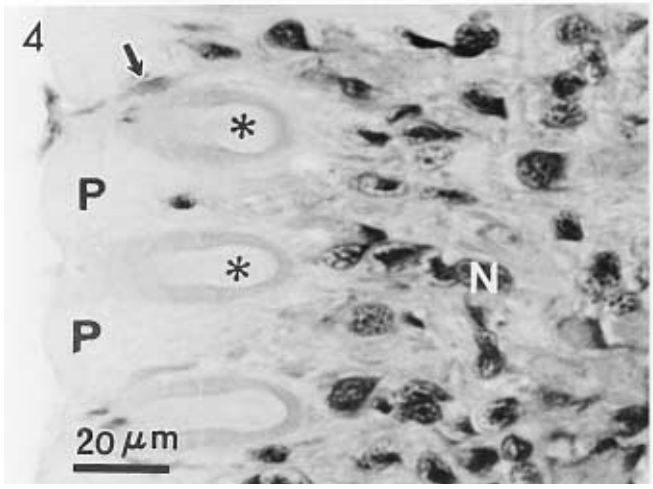
Fig. 4. – Section through the sensory margin, showing alternate arrangement of papillae (P) and ciliated pits (*). Parenchymal nuclei (N). Arrow points to secretion trail. LM section, stained with HE.

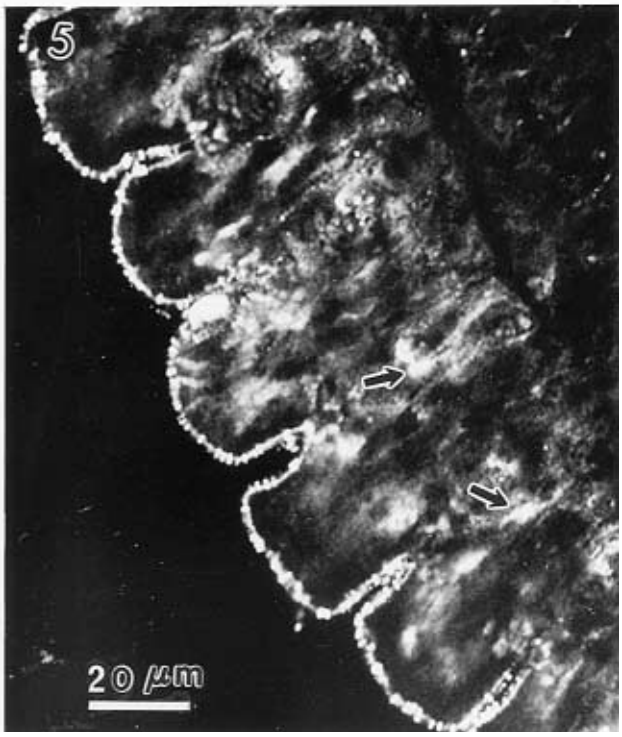
Fig. 5. – Section equivalent to Fig.4, to illustrate reactive sites for substance P along the surface of the papillae, as well as on sensory neurons (arrows). Confocal laser scanning.

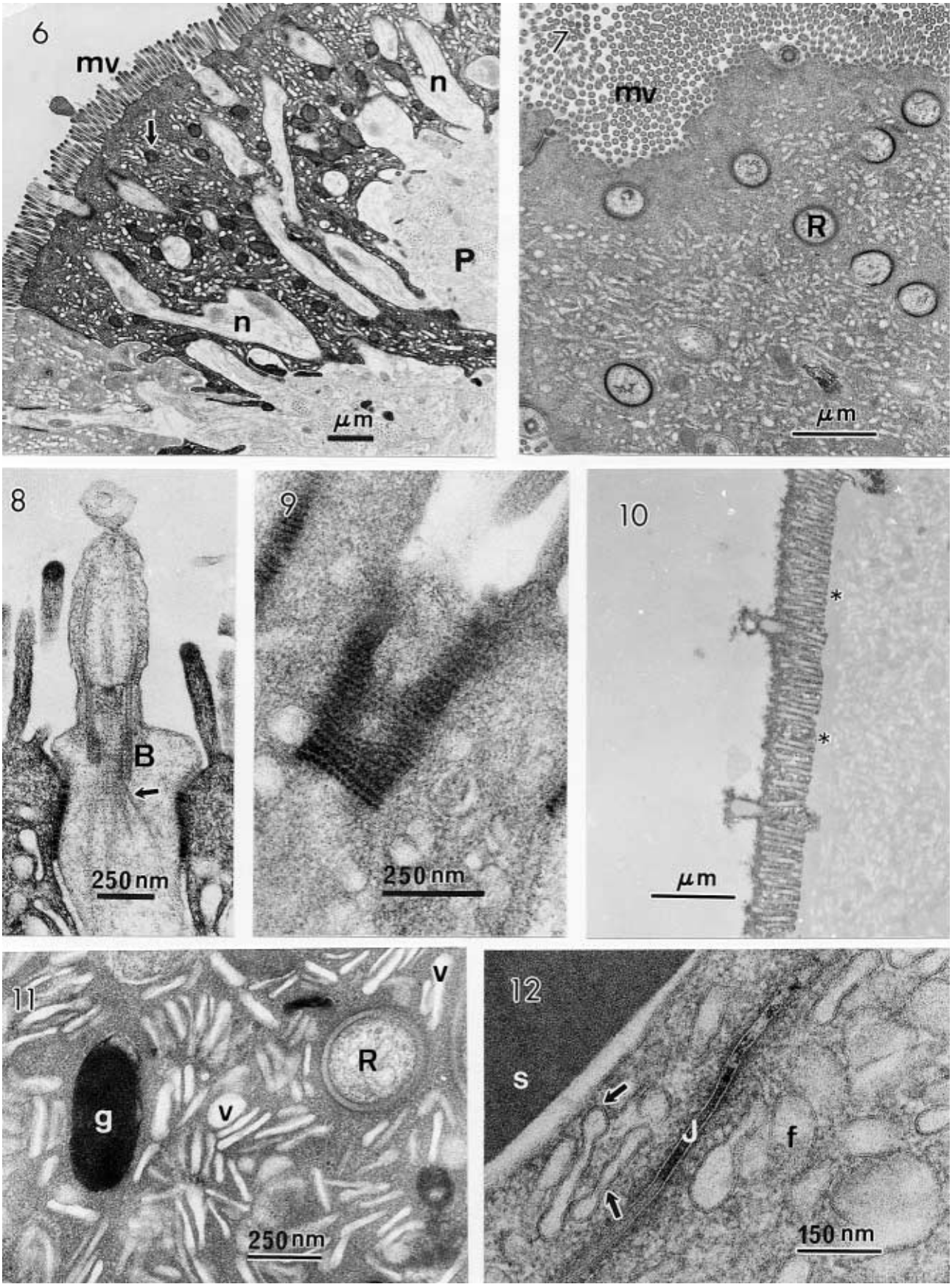

1
D
V
50 μm


2
C
*
S
5 μm


3
S
μm


4
*
P
*
N
P
20 μm


5
20 μm


6
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DISCUSSION

Electron microscopic studies on terrestrial triclads are not numerous (STORCH & ABRAHAM, 1972; BAUTZ 1977; STORCH & WELSCH 1977; CURTIS et al., 1983; MINMIN et al., 1992; MCGEE et al.,1997), especially considering the wide distribution of such worms (WINSOR, 1983). The present study confirms the sensory function of, and adds ultrastructural details to the receptors originally described by STORCH & ABRAHAM (1972). Lanthanum impregnation showed the path of sensory neurons across the epithelium, also evidencing the septate junctions engaged in cellular (mechanical or electrical) coupling (see GREEN, 1984). The occurrence of the peptide substance P all along the border of the head, and along the neurons, undoubtedly means that the papillae integrate a sensory organ, connected to the nerve plate. The reactive sites correspond to the surface receptors and to the processes of neurons extending toward the parenchyma of the headplate. It is interesting that sensory receptors of the catenulid *Stenostomum leucops* also contain substance P (WIKGREN & REUTER, 1985); the neuropeptide is also present around the eyes of *B. kewense* (FERNANDES, 2000). In particular, as seen in our experiments, the immunoreactivity demarcates the profile of all ciliated receptors.

The presence of a brush-border of microvilli on the outer surface of a land dwelling worm is somewhat surprising. As in other systems, microvilli provide a significant increase on the surface area of the epidermis (ALBERTS et al, 1994). These cytoplasmic extensions are made rather rigid due to a filamentous cytoskeleton. Its glycocalyx coat would conceivably provide the chemical component involved in signal reception, necessary for the impulse transduction (THURM, 1983; HUFNAGEL, 1992). This glycocalyx contains acidic muco-substances, likely complexed to a protein (SHEA, 1971).

Dense epithelial cells containing vesicular elements were not observed in other regions of *Bipalium kewense*, but they occur in the auricular epithelium of *Dugesia tigrina* (personal observations; see also MACRAE, 1967). The vesicles could correspond to a sort of smooth reticulum, maybe retaining Ca^{2+} ions or some elusive material not preserved by our preparative methods; they could even represent just a storage site for smooth membranes. The contents of the vesicles might contribute to the bright autofluorescence seen under the confocal microscope, in whole heads. Similar vesicles, though having a dense core, are present in the rostellar tegument of *Hymenolepis nana* (KUMAZAWA & YAGYU, 1988). Epithelial cells of *Geoplana pasipha*, another land planarian studied by us, also contain smooth vesicles that incorporate ruthenium red (unpublished observations).

The headplate, a characteristic feature for the genus *Bipalium sp.*, is a muscular organ, richly innervated and involved in the search for food (HYMAN, 1951; FERNANDES, 2000). Specialized devices for sensing chemical and mechanical stimuli are therefore expected to occur in the headplate. It is likely that the uniciliated receptors found at the surface of the papillae have tactile function, whereas those located in the recessed pits would be chemical receptors. Unfortunately, the ultrastructural approach to such identification is neither sufficient nor trivial, even in Protozoa (HUFNAGEL, 1992); it would depend on the identification of transduction molecules, ion channels, etc. It seems that each component of a receptor, however simple in construction it may be, is responsible for eliciting one step of the sensorial/excitatory function (THURM, 1983). Septate junctional complexes like those of *Bipalium kewense* are widespread among invertebrates, providing the pathway for mechanical or electric coupling between sensory dendrites and epithelial cells (GILULA et al., 1970; THURM et al., 1983).

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Fig. 6. – Section through a papilla. Dense epithelial cells are penetrated by nerve endings (n). Parenchyma (P); mitochondria (arrow); microvilli (mv). Lanthanum-impregnation. TEM.

Fig. 7. – Grazing section through the epithelial border. Septate junction around each receptor (R) was permeated by La^{3+}. Microvilli in cross sections (mv). TEM.

Fig. 8. – Longitudinal section of a ciliated receptor. The "9+2" cilium is short, attached to a basal body (B) and associated, unstriated fibrils (arrow). The ciliary shaft is slightly swollen, forming a balloon at the tip. TEM.

Fig. 9. – Tangential section through a junctional belt around a receptor, following Lanthanum impregnation. Note the wavy arrangement of the septa. TEM.

Fig. 10. – Brush border-like microvilli from epithelial cell of a papilla. Glycocalyx selectively stained according to Shea (1971). Homogeneous apical cytoplasm (*) contains no vesicles. TEM.

Fig. 11. – Epidermal cell fixed in GTA/OsO_4. Many elongate vesicles (v) stand out on dense ground cytoplasm. Secretion granules (g); receptor (R). TEM.

Fig. 12. – Cytoplasmic vesicles in profile and on face view (f) exhibit a neat unit membrane (arrows). Septate junction (J); secretion granule (s). TEM.

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