Ultrastructure of the ciliated cups of a synaptid holothuroid,
*Leptosynapta galliennei* (Echinodermata)

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**Abstract.** The medial epidermal surface of the buccal tentacles of the synaptid holothuroid, *Leptosynapta galliennei*, bears 5–30 ciliated cups. Each cup consists of an epidermal elevation with a central invagination forming a small pit. Whereas the epidermis of the outer lining of the cups is similar to that on the adjacent tentacle, the epidermis lining each pit consists of ciliated cells gathered together in a central bulb and surrounded by elongated support cells. The basal parts of ciliated cells are tapered and, together, form a nerve bundle entering the intradermal nerve plexus of the tentacle. We suggest that the ciliated cells and thus the entire ciliated cup has chemosensory function. It is further suggested that the cups allow the holothuroid to test the properties of the surrounding water and could also provide information on the palatability of collected food material.

**Additional key words:** Sensory organs, Synaptidae

Sensory structures in echinoderms usually consist of scattered sensory cells that are occasionally organised into organs. Such organs are well developed in the synaptid holothuroids where they are said to compensate for the lack of podia (for review, see Hyman 1955; Reese 1966; Smiley 1994). Many synaptid species have photo-receptor organs (i.e., the ocelli of *Opheodesmosa spectabilis*, Yamamoto & Yoshida 1978), mechanoreceptor organs (i.e., the statocysts of *Leptosynapta inhaerens*, Ehlers 1997) and supposed chemo-receptor organs (i.e., the ciliated cups of *Leptosynapta inhaerens*, Cuenot 1891). The ciliated cups have been extensively studied in the species *Leptosynapta inhaerens* by classical light microscopy (e.g., Cuenot 1891; Clark 1907) but there have been no ultrastructural accounts of these organs reported to be sensory (Cuenot 1891; Clark 1907; Heding 1928; Hyman 1955; McKenzie 1988). Cuenot (1891) and Clark (1907) reported that the cups are elevations of the tentacular epidermis surrounding a ciliated pit, and that a nerve plexus connects each cup to the main tentacular nerve. The aim of the present paper is to describe the fine structure and discuss the function of these cups in light of ultrastructural detail in the burrowing species *Leptosynapta galliennei*.

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**Methods**

Adult *Leptosynapta galliennei* (Herapath 1865) were hand-collected at low tide in the “Anse du Poul- du” near Roscoff (Brittany, France) in September 1995. They were transported to the marine biology laboratory of the Mons University and kept alive in a closed-circuit marine aquarium (13°C, 33‰ salinity).

For light microscopy, buccal tentacles and ciliated cups were removed from individuals previously anaesthetized for 1 h in a marine solution of propylene phenoxytol (Nipa Laboratories, UK, see Hill & Reischmidt 1976). They were fixed in Bouin’s fluid, embedded in Paraplast, and cut into 5-μm thick sections. Sections were stained with Masson’s trichrome and Mayer’s hemalum coupled with phloxine and light green (Ganter & Jollès 1969–1970). Histochemical observations were performed using Alcian Blue (pH 2.6) and the periodic acid-Schiff (PAS) techniques for the detection of mucopolysaccharides (Ganter & Jollès 1969–1970).

For scanning electron microscopy (SEM), cups were fixed in Bouin’s fluid for 24 h (Lahaye & Jangoux 1985). They were dehydrated in a graded ethanol series, dried by the critical point method (using CO₂ as transition fluid), mounted on aluminum stubs, coated with gold, and observed with a JEOL JSM 6100 scanning electron microscope.

For transmission electron microscopy (TEM), cups were fixed by immersion in 3% glutaraldehyde
in cacodylate buffer (0.1 M, pH 7.8) for 3 h at 4°C. They were then rinsed in cacodylate buffer and post-fixed for 1 h in 1% osmium tetroxide in the same buffer. After a final buffer wash, they were decalcified according to the method of Dietrich & Fontaine (1975), dehydrated in a graded ethanol series, and embedded in Spurr’s resin. Sections were cut on an LKB V ultramicrotome, contrasted with uranyl acetate and lead citrate, and observed with a Zeiss EM 10 transmission electron microscope.

Water currents around sensory cups were investigated in vitro (light microscopy) using single tentacles immersed in seawater containing suspended carmine particles (5 mg/ml).

**Results**

**External morphology**

The mouth of *Leptosynapta galliennei* is surrounded by twelve pinnate buccal tentacles of almost equal size. Each tentacle measures ~0.4 cm in length when extended, and is comprised of a stem from which 3–6 pairs of distal lateral branches (“pinnules”) and a single, long, apical filament arise (Figs. 1, 2). On the inner proximal parts of the tentacle stems (i.e., the surface facing the mouth), 5–30 ciliated cups occur (Fig. 1). The cups measure about 60 μm in diameter and 70 μm in height (Fig. 2). Apically, each cup is invaginated, forming a small pit up to 12 μm deep. The
lateral wall of the pit is smooth while its bottom is heavily ciliated; the cilia may protrude slightly beyond the edge of the pit (Fig. 3). Observations using suspended carmine particles failed to reveal water currents at the tip of the ciliated cups; thus, the cilia of the pit appear to be non-motile.

**Fine structure**

The ciliated cups consist of epidermal elaborations; these are small elevations with a central pit in which the epidermis is specialised (Figs. 4, 5). The connective tissue underlying the epidermis follows the contour of the cup (Fig. 4); it is composed of scattered collagen fibres embedded in an extracellular matrix, in which no cellular components have yet been identified. The outer epidermal lining and connective tissue of the cup are continuous between the cups and their equivalents in the tentacle stem.

The epidermis, measuring 50 μm at the most, progressively changes in cellular composition from the tentacle stem to the deepest part of the ciliated cup. The epidermis of the stem and of the outer lining of
the cup measures ~50 \( \mu \text{m} \) thick, although it is much thinner in some areas. It is made up of support cells and scattered interspersed mucocytes (Figs. 4–10). At the point where the epidermis invaginates, the mucocytes are lost and the invaginated epidermis is made entirely of support cells that organise around a cluster of ciliated cells (Figs. 4–7, 11–13). Epidermal cells are bound together by apico-lateral junctional complexes consisting of a zonula adherens and subjacent septate desmosome (Fig. 12). They rest on a basal lamina to which they are anchored by hemidesmosome-like structures. A thin cuticle (0.5 \( \mu \text{m} \)) overlies the entire epidermis of the cup except the pit aperture. Underneath the cuticle is a narrow subcuticular space (1 \( \mu \text{m} \)) which is crossed by the microvilli of the epithelial cells (Figs. 10A, 12). Bacteria occur in the subcuticular space (Figs. 8, 12).

The support cells differ in shape with position on the cup. In the outer lining of the cup, they have a classical T-shaped structure: a thin extended apical area, a neck, and an enlarged nucleus-containing body. At the edge of the pit, the support cells progressively change from T-shaped to club-shaped cells; the support cells in the pit possess an elongated neck which forms a kind of collar surrounding a central cluster of ciliated cells (Figs. 4, 6). The support cells of the outer lining contain bundles of filaments that link the connective tissue fibers and the apical extensions of the cells by way of hemidesmosomes (Figs. 4, 10A-B). Except for these filaments, the cytoplasm of all support cells is similar. It contains numerous vesicles measuring ~0.2 \( \mu \text{m} \) in diameter, enclosing an electron-lucent material. There are also membrane-bound granules of various sizes and a few mitochondria (Figs. 9, 10).

Mucocytes only occur in the epidermis of the tentacle stem and outer lining of the cup, where they are scattered between support cells. They are round to egg-shaped cells, and their cytoplasm is densely packed with vacuoles (Figs. 7, 8). These are about 0.4 \( \mu \text{m} \) in diameter and contain Alcian Blue and PAS positive materials.

Within the cup, ciliated cells are gathered together in a central bulb surrounded by support cells (Figs. 4, 5, 6). The ciliated cells have a narrow neck, an enlarged nucleus-containing central part, and an elongate and tapered basal process (Figs. 4, 11–13). These processes converge to form a nerve up to 100 \( \mu \text{m} \) long and 15 \( \mu \text{m} \) in diameter that enters the lateral nerve of the tentacle (Figs. 4, 5, 14). Each cell bears a single apical cilium (~10 \( \mu \text{m long} \)) surrounded by a ring of ~10 microvilli. The cilia have a regular 9 \( \times \) 2 + 2 arrangement of microtubules, and are associated with a prominent ciliary rootlet extending through the apical two thirds of the cell (Figs. 4, 11, 12). The cytoplasm contains numerous spherical membrane-bound granules; these measure ~0.25 \( \mu \text{m} \) in diameter and contain an electron-dense material. The apical cytoplasm also harbours many large mitochondria (Figs. 11, 12). At the place where the ciliated-cup nerve differentiates, the basal lamina of the epidermis is in continuity with the basal lamina of the lateral nerve.

**Discussion**

The buccal tentacles of *Leptosynapta galliennei* possesses cups that consist of a cluster of ciliated cells surrounded by tall elongate support cells. The ciliated cells are morphologically similar to other echinoderm sensory cells described by Holland (1984); they are columnar and bear a basally directed process or axon which contacts a lateral nerve of the tentacle. As shown by McKenzie (1988), this nerve is connected to the main nerve of the tentacle which itself contacts the perioesophageal nerve ring. There is, thus, a clear connection between the ciliated cells of the cup and the central part of the nervous system of the individual.

The particular arrangement of the sensory cells within the cups is reminiscent of the sensory-secretory epidermal elevations that occur throughout the integument of synaptid holothurians (Cuénot 1891; Clark 1907; Hyman 1955). Those elevations consist of a basal circle of gland cells that surround a slightly projecting sensory bud. The latter is made up of ciliated neuroepithelial cells whose basal parts are tapered, forming together a nerve fibre entering the intradermal nerve plexus (Cuénot 1891; Clark 1907; Hyman 1955).
The ciliated cups of a synaptid holothuroid
1955). According to Clark (1907), these structures are tactile (mechano-receptor) organs.

Though ciliated cups occur in a number of species belonging to different genera of apodous holothuroids (Clark 1907; Hyman 1955), their structure was investigated only to the light microscope level and only in Synapta inhaerens. Although there have been no studies on chemosensory activity in the feeding behaviour of synaptids, the cups were suggested to test the surrounding medium (Cuenot 1891; Clark 1907). According to Cuenot (1891), the cilia beat continuously and thus produce a steady water current through the ciliated pit where chemicals would be detected. Our observations failed to reveal water currents at the tip of the cup. Nonetheless, our ultrastructural observations clearly show that the cups contain typical sensory cells and thus, there is a little doubt that these cups serve as sensory organs; whether the cups are mechano-receptor or chemo-receptor organs is, however, uncertain. According to Cobb (1987), it is not possible to separate these two modalities of perception ultrastructurally.

From the feeding behavior of *L. galliennei*, it is tempting to suggest that the ciliated cells are chemoreceptors. *L. galliennei* is a burrowing holothuroid which assumes an “L-shaped” posture in the sediment, with the oral tentacles projecting onto the substratum with their inner surface facing the water column (Féral 1985). The ciliated cups are thus exposed to surrounding water current and could function as chemoreceptor organs, perhaps in triggering the feeding activity of the individual. *Leptosynapta galliennei* is known to eat all kinds of detritus that can be removed from the substratum (Féral 1985). When feeding, *L. galliennei* picks up food particles on the sticky outer surfaces of the tentacles (McKenzie 1988); a loaded tentacle then bends towards the mouth, and the outer surface with attached particles comes into close contact with the proximal inner surface of the opposite tentacle, where ciliated cups occur. Thus, the ciliated cups could provide information on the palatability of collected food material. The results of this study emphasize the need for studies on sensory abilities in the feeding behaviour of synaptids, which would contribute to our understanding of the functional capabilities of the ciliated cups and other sensory structures in the tentacles of these holothuroids.

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**Fig. 11–14.** *Leptosynapta galliennei*. Fine structure of a ciliated cup. **Fig. 11.** Longitudinal section through the apical part of the central bulb. **Fig. 12.** Detailed view of the apex of ciliated cells. **Fig. 13.** Detailed view of the basal part of ciliated cells. **Fig. 14.** Longitudinal section through the ciliated cells processes. Basal lamina (B), bacterium (BA), cilium (C), ciliated cell (CC), connective tissue (CO), ciliated cell processes (CCP), ciliary rootlet (R), granule (G), mitochondrion (M), microvilli (MV), nucleus (N), septate desmosome (SD).