

## **Fine Structure of the Ocellus of *Sarsia tubulosa* (Hydrozoa, Anthomedusae)**

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**Summary.** The fine structure of the ocellus of *Sarsia tubulosa* is described. The ocellar cup is formed of pigment cells and receptor cells. The receptor cells outnumber the pigment cells in almost a 2:1 ratio. Lateral extensions of neighbouring pigment cells enclose a distal region of 2 to 10 receptor cells. The receptor cell body is 5–7  $\mu\text{m}$  in diameter with an apical extension (20–60  $\mu\text{m}$  long) that reaches the ocellar cavity. A cilium (9 + 2 microtubules) arises from the distal part of the receptor cell. The ciliary membrane forms lateral microvilli. The tips of a number of cilia are swollen into large vesicles forming a “cornea”. The central region of the ocellar cavity contains extracellular electron dense homogeneous material surrounded by swollen ciliary tips and small vesicles. The close apposition between the plasma membrane covering the distal part of adjacent receptor cells as well as the adjacent ciliary shafts suggests the presence of gap junctions. The basal part of each receptor cell forms an axon. The axons of receptor cells form 3 to 4 nerve bundles that join to form the optic nerve. Synapses occur between receptor cell bodies, between axons and receptor cell bodies and among axons.

### **A. Introduction**

*Sarsia tubulosa* a common hydromedusan jellyfish having four ocelli, has attracted the attention of many workers. Hyman (1940) reported that *Sarsia sp.* will gather in a beam of light. The removal of the tentacle bulbs bearing the ocelli abolishes this behaviour. Passano et al. (1967) described the alteration of the output of the marginal pulse and swimming pacemakers upon illumination of the ocelli. Recently Weber (1982), by direct electrophysiological recording from the ocelli found that the response of *Sarsia* to light stimuli is much stronger than that of *Polyorchis*.

Linko (1900) stated that the ocelli of *Sarsia* have a “Glaskörper”. The fine structure of the ocelli of the hydromedusan *Polyorchis penicillatus* (Eakin

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and Westfall 1962; Singla and Weber 1982), *Spirocodon saltatrix* (Yamasu and Yoshida 1973; Toh et al. 1979), *Leuckartiara octona*, *Bougainvillia principis* and *Tiaropsis multicirrata* (Singla 1974), and the lens-bearing ocelli of *Cladonema radiatum* (Weber 1981a) has been described, but no such structure has been found.

The present paper describes the fine structure of the ocelli of *Sarsia tubulosa* and the synaptic organization of the photoreceptor cells.

## B. Material and Methods

Specimens of the anthomedusan *Sarsia tubulosa* (Sars 1835) were collected at Oak Bay Marina, Victoria, B.C. The medusae were anaesthetized in isotonic magnesium chloride (67.5 gms/l) diluted with an equal volume of sea water. Tentacle bulbs with ocelli were dissected and fixed in 2.5% glutaraldehyde in Millonig's phosphate buffer for one hour at room temperature. The tissue was rinsed in the same buffer and post-osmicated in 1% osmium tetroxide at 4° C for one hour. It was rinsed in distilled water, dehydrated through graded ethanols and propylene oxide, and embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate.

For scanning electron microscopy the tissue was fixed and dehydrated in the same manner as for transmission electron microscopy. It was dried in a critical point drier and coated with gold.

To study the effect of light and darkness, some specimens were placed in running sea water aquarium at a distance of 30 cm from a 22 watt fluorescent lamp (Sylvania, FC8T9-CW-RS, "cool white"). The exposure time followed their natural photoperiod. Other animals were placed in total darkness for a period of 14 h. Animals kept in the light and those maintained in darkness were fixed as whole specimens under corresponding conditions of light and dark as described above.

## C. Results

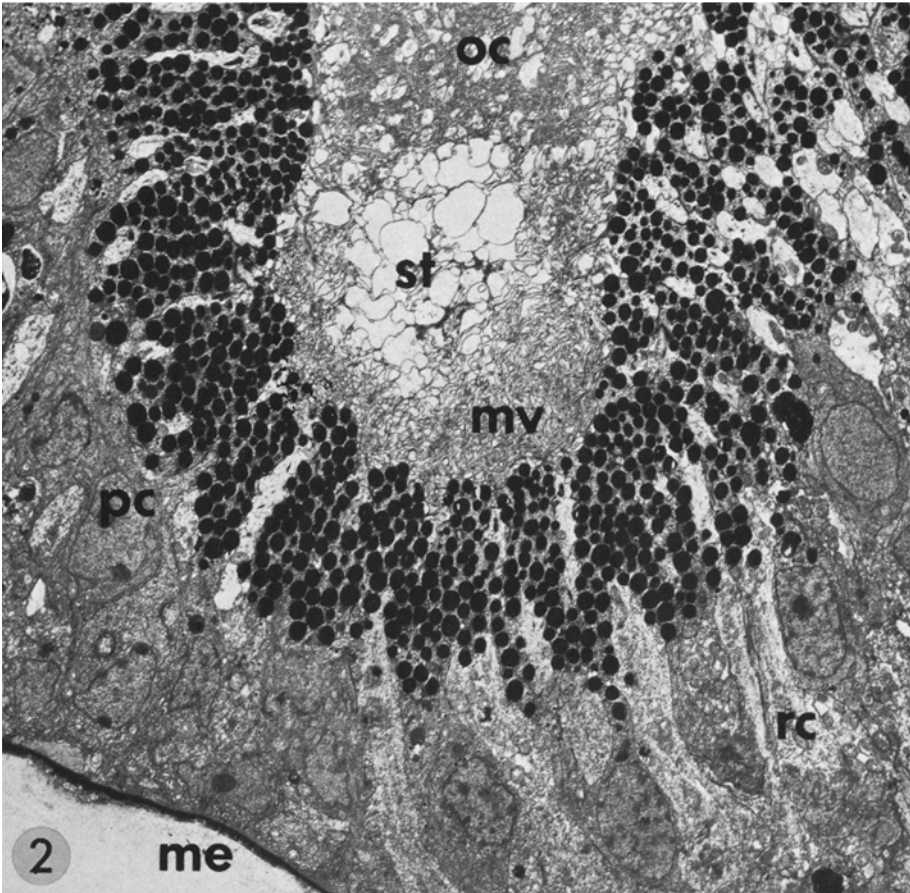
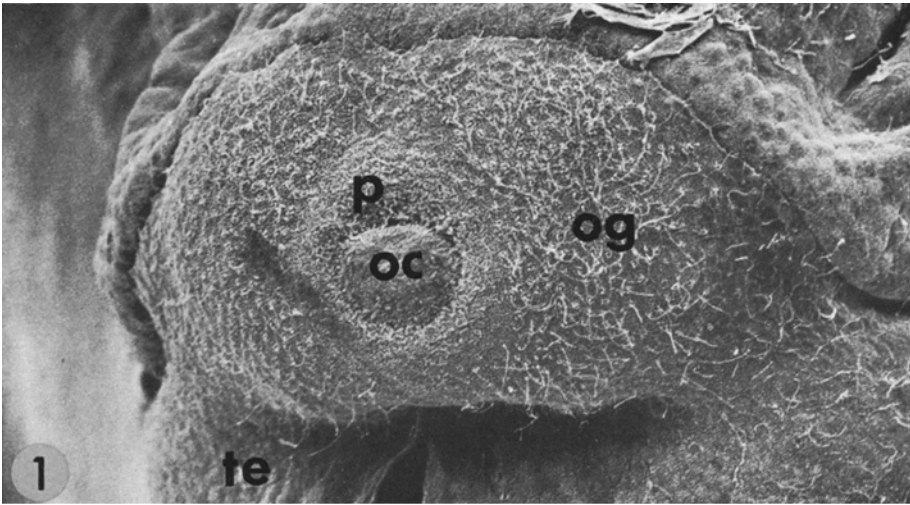
### 1. Gross Anatomy

The umbrella of *Sarsia tubulosa* is higher than wide (up to 18 mm high). The medusa has four tentacles, each with an ocellus situated on the abaxial side of the tentacle bulb. The tentacle bulb has many ciliated sensory cells (Fig. 1). Each ocellus has a convex surface. The ocellar cavity is cylindrical, about 90  $\mu\text{m}$  deep and 50 to 60  $\mu\text{m}$  wide (Fig. 2). The ocellar cavity usually faces downwards. An approximately 0.2  $\mu\text{m}$  thick mucous layer covers the distal part of the ocellus. Some of the mucus is present between the distal ends of the receptor cell cilia (Fig. 10, 19). The ocellus is formed of two types of cells: receptor cells and pigment cells.

### 2. Receptor Cells

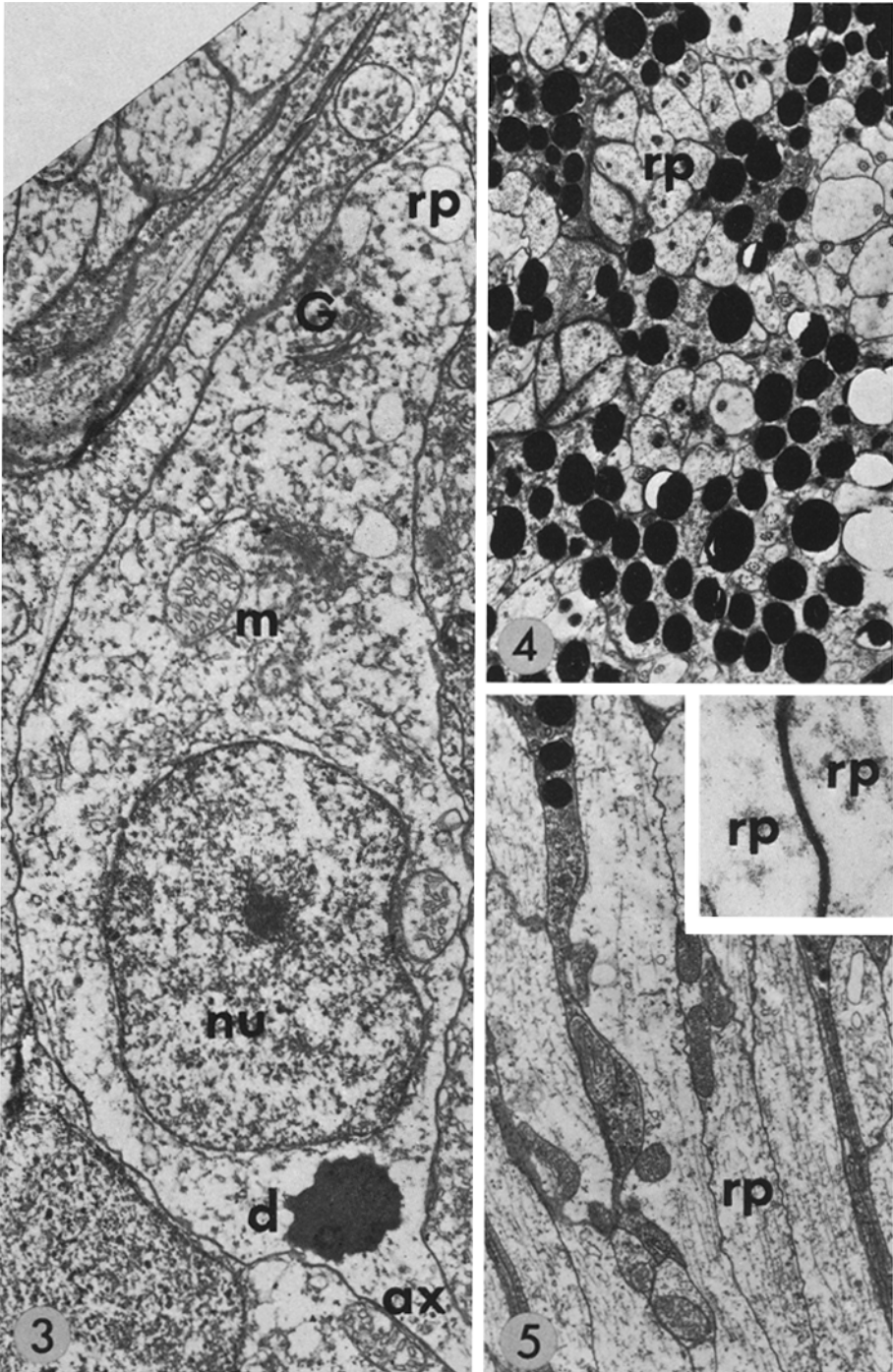
The receptor cells outnumber the pigment cells in almost a 2:1 ratio. The receptor cells occur at various levels. Most of them lie in the ocellar perimeter. A few are present between the pigment cup and the tentacular mesoglea. The receptor cells are bipolar, with the apical region extending towards the pigment cup and the proximal region forming an axon (Fig. 3).

The cell bodies of photoreceptors lie behind the pigmented cup, 20 to 60  $\mu\text{m}$  from the ocellar cavity. The cell body is 5 to 7  $\mu\text{m}$  in cross-section. It has a sometimes lobulated central nucleus, about 3  $\mu\text{m}$  in diameter. The cytoplasm



**Fig. 1.** Scanning electron micrograph of ocellus of *Sarsia tubulosa*. *oc* ocellar cavity; *og* optic ganglion; *p* pigment cup; *te* tentacle.  $\times 316.8$

**Fig. 2.** Electron micrograph of cross-section of ocellus of *Sarsia*. *me* mesoglea; *mv* microvilli; *oc* ocellar cavity; *pc* pigment cell; *rc* receptor cell; *st* swollen ciliary tip.  $\times 2,352$



**Fig. 3.** Longitudinal section of receptor cell with distal region (*rp*) and proximal axon (*ax*). *d* electron dense droplet; *G* Golgi apparatus; *m* mitochondrion; *nu* nucleus.  $\times 10,560$

**Fig. 4.** Cross-section below opening of pigment cup. Lateral extensions of neighbouring pigment cells enclose distal region (*rp*) of 2–10 receptor cells.  $\times 9,600$

**Fig. 5.** Longitudinal section of distal region (*rp*) of a number of receptor cells.  $\times 7,000$ . Inset shows a close apposition between the plasma membrane of distal region (*rp*) of adjacent receptor cells.  $\times 69,120$

is characterized by one or more electron dense droplets (about 1  $\mu\text{m}$  in diameter) and contains a number of active Golgi elements, some endoplasmic reticulum and many mitochondria. A few of the receptor cells contain a multivesicular body. A number of mitochondria are intertwined in groups of 2 to 4.

The distal part of the receptor cell varying from 0.8–2.5  $\mu\text{m}$  in thickness and 10–50  $\mu\text{m}$  in length extends toward the ocellar cavity. It has a number of mitochondria many of which are curled into groups of 2 to 4 (Fig. 6). The cytoplasm contains many microtubules and a few vesicles about 100 nm in diameter. The distal narrow part of 2 to 10 receptor cells is grouped together (Figs. 4, 5). Fig. 5 shows a close apposition between the adjacent receptor cells with hardly any intercellular space. A cilium arises from an infolding of the distal part of the receptor cell 2–3  $\mu\text{m}$  below the opening of the pigment cup and projects into the ocellar cavity (Fig. 7). The ciliary tubules have a 9+2 pattern. From the basal body a long striated rootlet extends proximally.

Within the ocellar cavity the plasma membrane covering the cilium forms lateral “microvilli” measuring about 200 nm in thickness and several micrometers in length. The thickness of the “microvilli” may vary along their length. A few of the “microvilli” divide into secondary branches (Fig. 9). A number of the “microvilli” run together for some distance (Fig. 8). The tips of the cilia are swollen into large vesicles of about 2 to 2.5  $\mu\text{m}$  in diameter (Fig. 10).

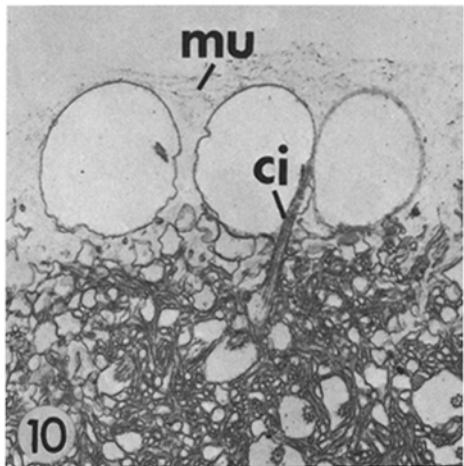
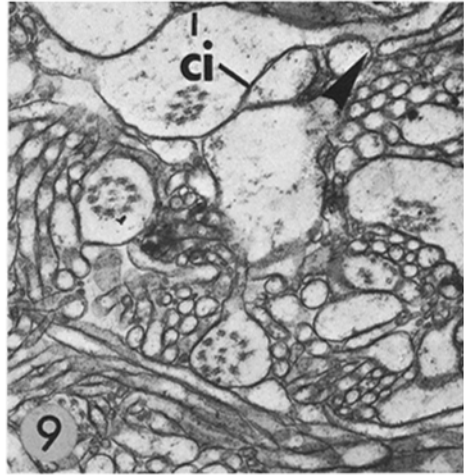
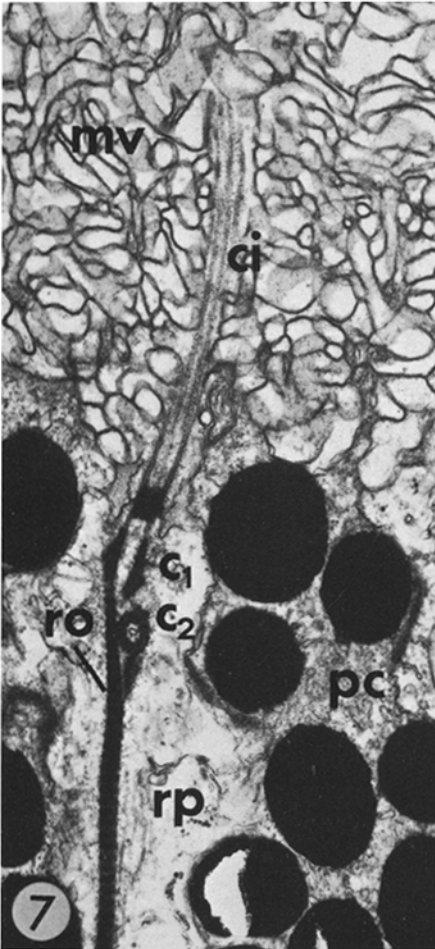
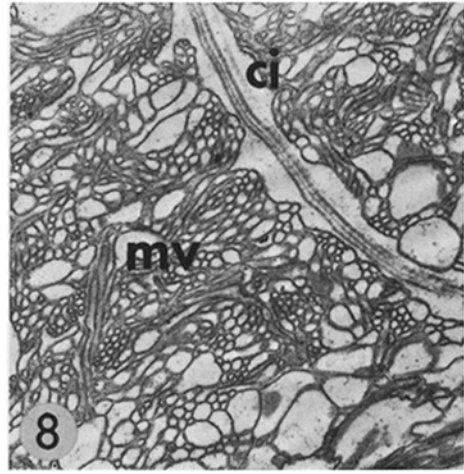
The central region deep within the ocellar cavity contains a large amount of extracellular electron-dense homogeneous material surrounded by swollen ciliary tips and 100 nm diameter vesicles (Fig. 11). The ocelli of the medusae kept in the dark and those exposed to continuous illumination have the same amount of this material. The plasma membrane of adjacent ciliary shafts in this region show close apposition (Fig. 11).

### 3. Pigment Cells

The pigment cells form the ocellar cup. Each pigment cell is 20 to 25  $\mu\text{m}$  tall, about 5  $\mu\text{m}$  in diameter and has a large basal nucleus. The cytoplasm contains rough endoplasmic reticulum, Golgi elements, mitochondria and membrane-bounded pigment granules of 0.6–0.9  $\mu\text{m}$  in diameter. Occasionally the cytoplasm has a ciliary rootlet but no cilium. The distal region of each pigment cell forms lateral extensions that join processes from neighboring pigment cells. These extensions enclose 2 to 10 receptor cells (Figs. 4, 19). Apically the pigment cells project short microvilli into the ocellar cavity (Fig. 12). A number of pigment cells form distal processes that bear lateral microvilli (Fig. 14) about 2  $\mu\text{m}$  long and 70–160 nm thick. A few of the microvilli bifurcate near their tips (Fig. 13). The microvilli of the pigment cells have denser cytoplasm than those of the receptor cell microvilli and thus can be distinguished from the latter.

### 4. Nervous System

The proximal part of each receptor cell forms an axon (1–2  $\mu\text{m}$  thick). Because the cell bodies of receptor cells are not situated in a single layer, but occur at various levels, many sections show receptor cells and axons lying side by

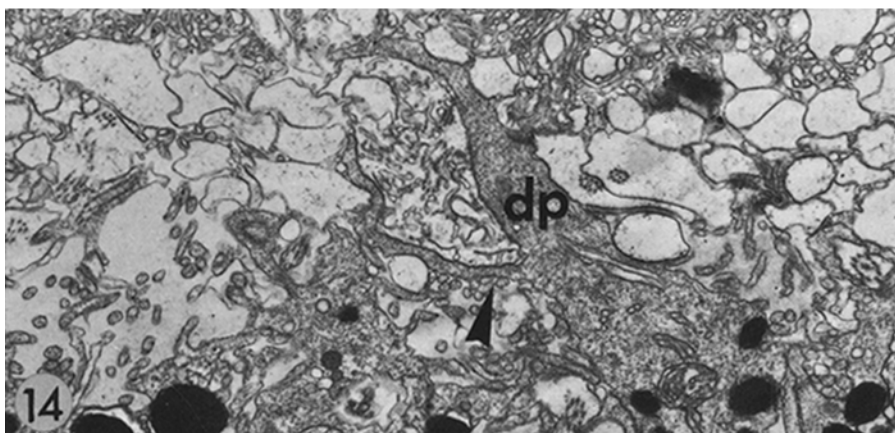
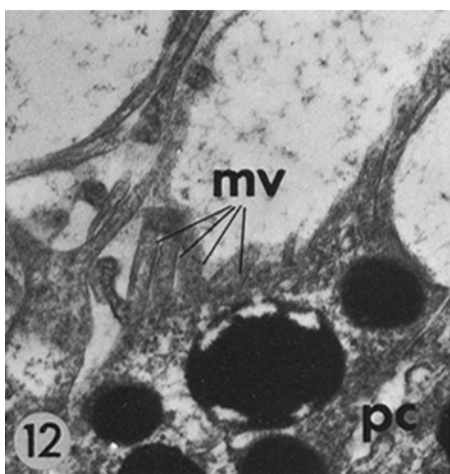
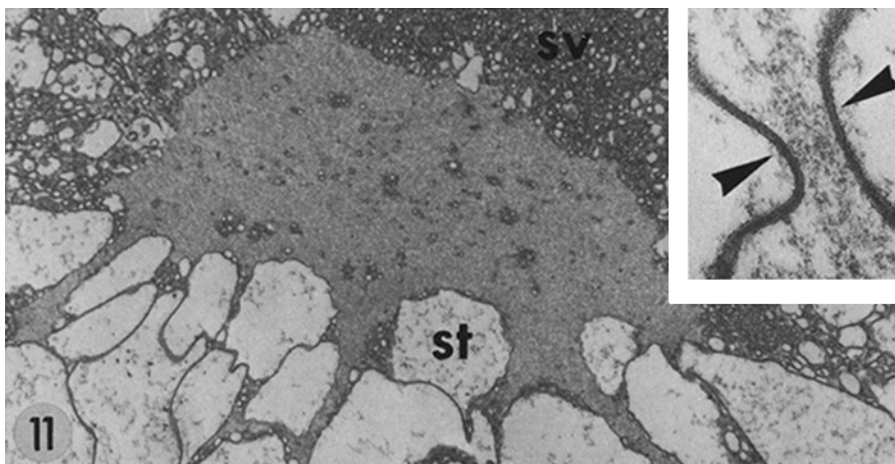


**Fig. 6.** Distal region of receptor cell with mitochondria curled into groups.  $\times 14,400$

**Fig. 7.** Longitudinal section of a distal part of receptor cell (*rp*) bearing a cilium (*ci*). *c*<sub>1</sub> and *c*<sub>2</sub> centrioles; *mv* microvilli; *pc* pigment cell; *ro* rootlet.  $\times 20,160$

**Figs. 8 and 9.** Microvilli (*mv*) of ciliary (*ci*) membranes form bundles. Fig. 9. A few of the microvilli divide into secondary branches (*arrow*).  $\times 14,400$  and  $24,000$  respectively

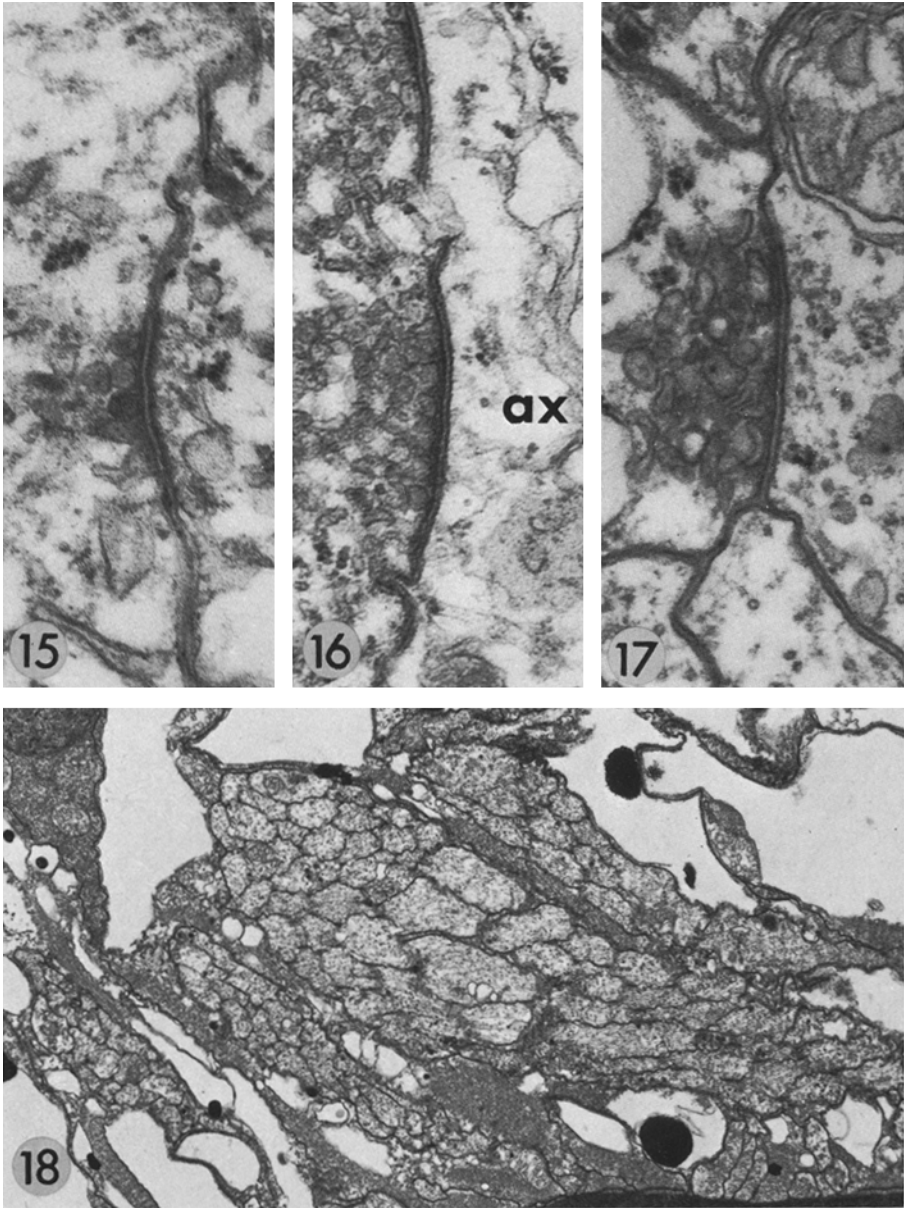
**Fig. 10.** *ci* tips of cilia swollen into large vesicles; *mu* mucous laver covering ocellar cavity.  $\times 9,600$



**Fig. 11.** Extracellular electron-dense homogeneous material surrounded by swollen ciliary tips (*st*) and small vesicles (*sv*). Inset shows close apposition between the plasma membrane of adjacent ciliary shafts.  $\times 8,352$  and  $\times 69,120$  respectively

**Figs. 12 and 13.** Distal part of pigment cell (*pc*) with apical microvilli (*mv*). Microvillus with secondary branch (*arrow*).  $\times 25,920$

**Fig. 14.** Pigment cell process (*dp*) bearing lateral microvilli (*arrow*).  $\times 10,560$



**Fig. 15–17.** Synapses between receptor cell bodies (Fig. 15), between axon (*ax*) and receptor cell body (Fig. 16) and between two axons (Fig. 17).  $\times 58,560$

**Fig. 18.** Cross-section of nerve bundles forming the optic nerve.  $\times 8,160$



side. The axons can be distinguished from the receptor cells, however, by the lack of curled mitochondria and close apposition of the plasma membrane (Fig. 19). The axons group together to form 3 to 4 nerve bundles that join to form the optic nerve (Fig. 18). In each ocellus a pair of optic nerves travels around either side of the base of the tentacle. On reaching the subumbrellar side the nerves enter the tentacular ganglion as described by Mackie (1971).

Synapses are common between the receptor cell bodies (Fig. 15), between axons and receptor cell bodies (Fig. 16), and among axons (Fig. 17). They are characterized by the presence of dense cored vesicles 65–180 nm in diameter and by thickened plasma membranes. No synapses were observed between the ciliated sensory cells of the tentacular bulb and the receptor cells or their axons.

#### D. Discussion

The receptor cells of the ocellus of *Sarsia tubulosa* are of the ciliary type (9+2). Microvilli arise laterally from the ciliary shaft similar to the receptor cells of *Polyorchis penicillatus* (Eakin and Westfall 1962; Singla and Weber 1982) and *Spirocodon saltatrix* (Yamasu and Yoshida 1973; Toh et al. 1979).

Linko (1900) reported that the ocelli of *Sarsia sp.* have a "Glaskörper". The present study shows electron dense homogeneous material and many small vesicles lying in the central region deep within the ocellar cavity. There is no difference in location, structure and amount of this material in ocelli of medusae kept in the dark or exposed to light. This suggests that the change in illumination has no bearing on its formation. The deposit of this homogeneous material has no definite shape and lies far behind the usual position of a lens in an ocellus. Similar electron dense material, however, forms a lens in the gastropod *Helix aspersa* (Eakin and Brandenburger 1967) and the lens capsule in the ocelli of a cubomedusan *Tamoya bursaria* (Yamasu and Yoshida 1976). Whereas the origin of the dense material in *Tamoya* is not known, in *Helix aspersa* it is secreted by the presumptive corneal cells and the prospective cells of the retina. In hydromedusae a primitive lens consisting of tubular material as in *Bougainvillia principis* (Singla 1974), or a crystalline lens as in *Cladonema radiatum* (Weber 1981a and b), is formed by pigment cell processes. In *Sarsia*, however, most of the pigment cells bear only microvilli without any secretory material. The origin and function of the dense material in the ocelli of *Sarsia* is not clear.

The ocelli of cubomedusan *Tamoya bursaria* (Yamasu and Yoshida 1976) are covered by a layer of corneal cells. Weber (1981a) reported that in *Cladonema radiatum*, an anthomedusan, neighbouring epithelial cells extend over the ocellus and form a cornea. In ocelli of *Sarsia* the swollen tips of the receptor cells may serve the same function (Fig. 19).

Yamamoto and Yoshida (1978) for holothurians and Eakin and Brandenburger (1979, 1980) for sea stars reported that the microvilli of the receptor cells are sensitive to light. They are deranged during continuous illumination and regenerate in the dark. In ocelli of *Sarsia* we found no difference in the structure and arrangement of receptor cell ciliary microvilli between medusae kept in the dark or exposed to continuous illumination for 14 h. Electrophysio-

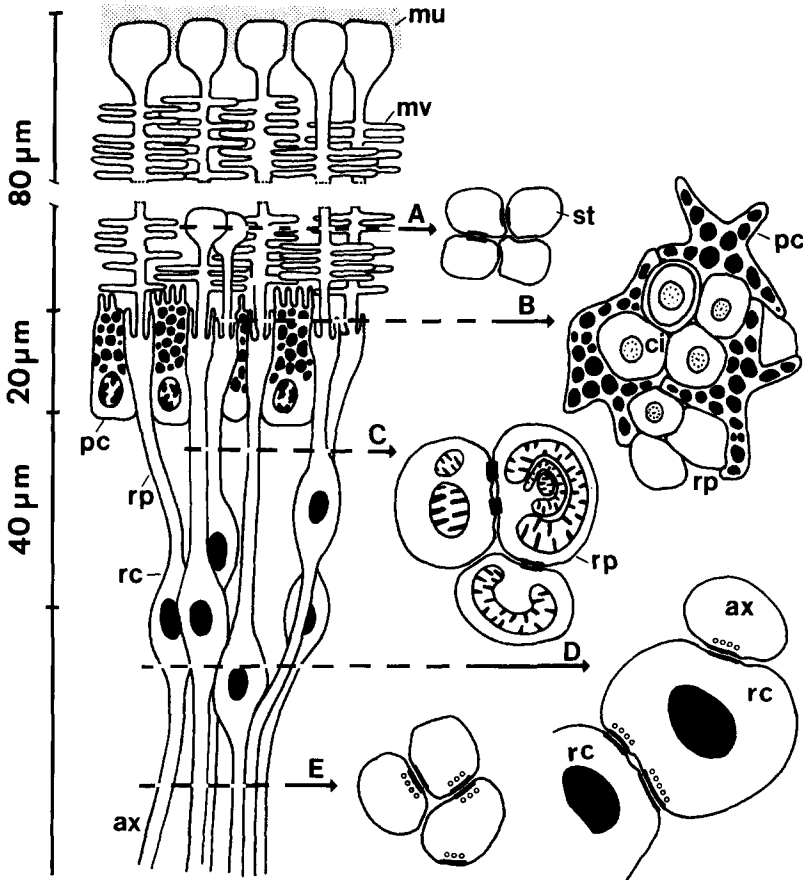


Fig. 19A-E. Diagram of receptor cell arrangement in an ocellus of *Sarsia tubulosa*. A Plasma membrane of adjacent swollen ciliary tips (*st*) form gap junctions. B lateral extensions of neighbouring pigment cells (*pc*) enclose distal region of 2-10 receptor cells (*rp*). C Gap junctions between adjacent receptor cells. D Synapses between receptor cell (*rc*) bodies, and between axon (*ax*) and receptor cell body. E Synapses among axons. *ci* cilium; *mu* mucus; *mv* microvilli

logical recordings from the ocelli of *Sarsia* (kept in the dark or exposed to continuous light) likewise show no difference in response to light stimuli. Unlike the ocelli of holothurians, which are flat, and those of sea stars, which form shallow cups, each ocellus of *Sarsia* forms a deep cup that generally faces downwards. The pigment cells forming the ocellar cup thus somewhat shield the receptor area from light penetrating from above.

The close apposition between the membranes covering cilia of receptor cells as well as between distal part of the receptor cells suggests the presence of gap junctions. Gap junctions have been described among fiber cells in the amphibian and cephalopod lenses (Jacob and Duncan 1981), in hydromedusae between the photosensory giant neurons of the inner nerve ring of *Polyorchis penicillatus* (Anderson and Mackie 1979; Spencer and Satterlie 1980), epitheliomuscular cells of *Aglantha digitale* (Singla 1978a) and the epitheliomuscular

cells of *Polyorchis penicillatus* (Singla 1978b). The gap junction provide low resistance pathways and communication channels for the transfer of molecules (Spencer and Satterlie 1980; Bennet et al. 1980; Jacob and Duncan 1981). If the gap junctions in the ocelli of *Sarsia* are functional, then each group of receptor cells could behave as in concert.

The neural organization of the anthomedusae in which the receptor cells synapse with the second order neurons has been described in *Spirocodon saltatrix* (Toh et al. 1979), *Nemopsis dofleini* (Yamamoto and Yoshida 1980) and *Polyorchis penicillatus* (Singla and Weber 1982). In *Sarsia*, however, no second-order neurons were observed. Synapses are common between receptor cell bodies, between the receptor cell body and adjacent axons and among axons forming the optic nerve. Similar synapses were described between the receptor cells and the neurons of the ephyrae of *Aurelia aurita* (Yamasu and Yoshida 1973) and between the adjacent axons of the receptor cells of *Tamoya bursaria* (Yamasu and Yoshida 1976). The present study suggests that the receptor cells of *Sarsia* are unique among hydromedusae in having both electrical and chemically mediated junctions.

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