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PODIAL SENSORY RECEPTORS AND THE INDUCTION OF METAMORPHOSIS IN ECHINOIDS

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Abstract: Larvae of *Strongylocentrotus droebachiensis* (Müller), *Lytechinus pictus* (Verrill), and *Lytechinus variegatus* (Leske) which are competent to metamorphose display what appears to be substratum-testing behavior prior to metamorphosis. Larvae cease swimming, partially evert the adult rudiment, and walk about examining the substratum with their five primary podia. Larvae either metamorphose or withdraw their podia and resume swimming to settle again elsewhere. Scanning and transmission electron microscopic examinations of the primary podia revealed sensory receptor cells on the rim and on a conical projection at the center of the podial sucker. Each sensory cell has a single short cilium on its apical surface and an axonal process at its base which contributes to the basiepithelial nerve plexus. Mature adults of the same species also have comparable sensory structures on their tube feet suckers. It is suggested that the sensory receptors on the primary podia of settling larvae, although they are not specialized larval structures, may be involved in the perception of tactile stimuli which have been previously demonstrated to be involved in the induction of metamorphosis.

INTRODUCTION

The distribution of benthic marine organisms is determined in part by the preferential settlement of planktonic larvae in suitable adult habitats (Crisp, 1974; Scheltema, 1974; Chia & Rice, 1978). For some animals it has been demonstrated that the morphological and physiological changes of metamorphosis can be induced by "factors" from preferred adult habitats (Meadows & Campbell, 1972; Scheltema, 1974; Hadfield, 1978). Such is probably the case for many species of echinoids. Strathmann (1978) notes that there are seven species of echinoids which preferentially settle in the presence of substrata associated with adult habitats. Cameron & Hinegardner (1974) have demonstrated for two echinoids that metamorphosis is induced by a non-particulate organic chemical cue and the contact of the larva with a surface. There have been several recent reports of the developmental processes that comprise metamorphosis in echinoids (Cameron & Hinegardner, 1978; Chia & Burke, 1978; Burke, 1980), but very little is known about initiation or control of these processes.

Reports of the settlement and metamorphosis of regular echinoids have described a characteristic form of settlement behavior in which larvae examine the substratum with the five primary podia of the adult rudiment. MacBride (1903), Cameron & Hinegardner (1974), and Strathmann (1978) have suggested that the sucker tips of the primary podia are the location of sensory organs that are responsible for the perception of the substratum-associated cues to metamorphosis. This study was

undertaken to determine if there are recognizable sensory structures on the tips of the primary podia, and if the primary podia are specialized as larval settlement organs.

MATERIALS AND METHODS

Embryos and larvae were cultured using the standard procedures outlined by Strathmann (1968) and Hinegardner (1969). Adult *Lytechinus pictus* (Verrill) were obtained from Pacific Biomarine Supply, Venice, California, U.S.A.; adult *Strongylocentrotus droebachiensis* (Müller) were collected intertidally on San Juan Island, Washington, U.S.A.; and adult *Lytechinus variegatus* (Leske) were collected from the Indian River near St. Lucie Inlet, Florida, U.S.A. Photographs of live larvae were made using a Nikon inverted microscope and a Zeiss Tessovar photomacrographic system.

Whole larvae and tube feet from adults were prepared for transmission electron microscopy (TEM) by initially fixing in a solution containing 2.5% glutaraldehyde, 0.2 M phosphate buffer (Millonig, 1961), and 0.14 M sodium chloride (Cloney & Florey, 1968). Specimens were post-fixed for 1 h at room temperature in 2% osmium tetroxide in 1.25% sodium bicarbonate (Cloney & Florey, 1968). Dehydration was by acetone exchange and specimens were infiltrated and embedded in Epon (Luft, 1961). Sections were stained with 50% ethanol saturated with uranyl acetate and lead hydroxide chelated with sodium citrate (Reynolds, 1963). Observations and micrographs were made using a Zeiss EM 9S-2 and a Hitachi HU-12 electron microscope.

Specimens were prepared for scanning electron microscopy (SEM) by fixation and post-fixation as described above for TEM. After post-fixation, specimens were rinsed in distilled water and put in a saturated, aqueous solution of thiocarbohydrazide for 15 min, after which they were rinsed and put in 2% osmium tetroxide for 13 min. After a final rinse in distilled water, specimens were dehydrated using alcohol exchange, critical point dried (CO_2), and coated with evaporated carbon and gold-palladium. Specimens were viewed and photographed with an AMR-1000 scanning electron microscope.

RESULTS

SETTLING BEHAVIOR

In larvae competent to metamorphose, the adult rudiment occupies the entire left half of the larval body for all three species studied (Fig. 1). Probably owing to the increased mass of the rudiment, competent larvae were less able to swim at the surface of the water and tended to be found on or near the bottoms of the cul-

ture dishes. The spines and podia of the rudiments of competent larvae were able to move within the vestibule and extend through its dilated opening (Fig. 1). Larvae were able to use the podia to attach themselves to the substratum and walk (Fig. 2).

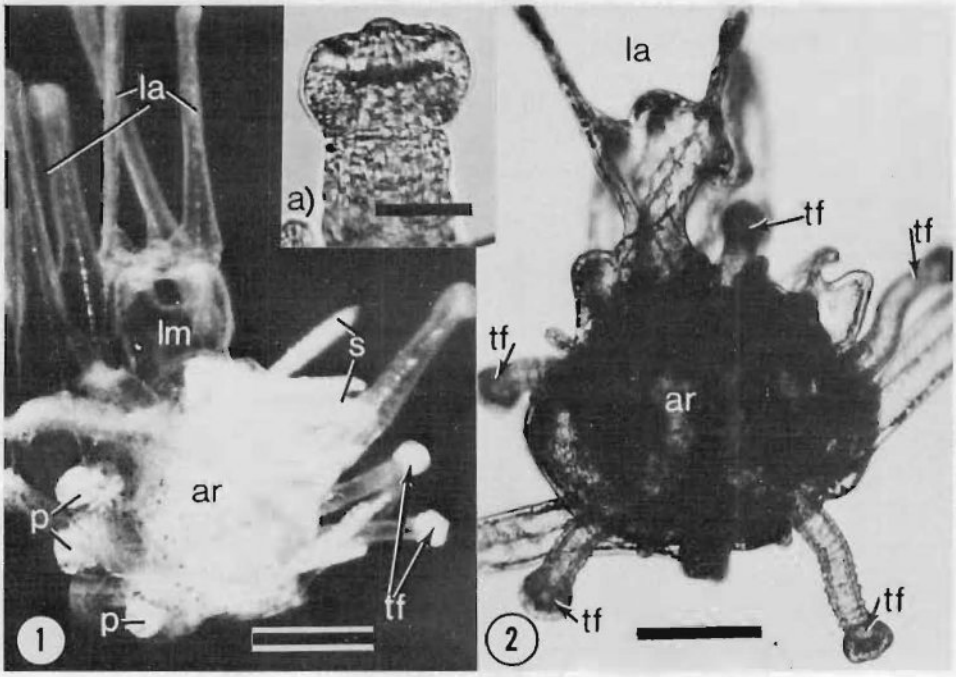

Fig. 1. Pluteus larva of *Lytechinus variegatus* which is competent to metamorphose; note the primary podia (tf) extending from the adult rudiment; bar 100 μm; a, the sucker tip of a primary podium of a competent *L. variegatus* larva; bar 25 μm.

Fig. 2. A competent larva of *Lytechinus pictus* which has settled and is using its primary podia to attach itself to the substratum (a glass dish); the larva was photographed from beneath with an inverted microscope; bar 100 μm; ar, adult rudiment; la, larval arms; lm, larval mouth; p, pedicellariae; s, adult spines; tf, primary podia.

If a suitable substratum was provided for them (rocks and shells from adult habitat) larvae usually underwent metamorphosis. However, if larvae were kept in clean glass bowls, they seldom metamorphosed. Larvae in glass bowls spent prolonged periods (15 min to several days) walking on the bottom. Attached larvae were frequently observed to retract the spines and podia of the rudiment into the vestibule and resume swimming.

STRUCTURE OF THE SUCKERS OF THE PRIMARY PODIA

Competent larvae of all three species have five primary podia evenly spaced around the perimeter of the adult rudiment (Fig. 2). The podia are capable of flexion,

contraction, and extension up to 0.5 mm. Podia are 15–35 μm in diameter, and have a disc-shaped sucker at the distal tip (Fig. 1a). The sucker is 40–45 μm in diameter with an elevated rim and a centrally located cone which projects above the rim (Fig. 1a).

The podial sucker is comprised of two tissue layers separated by a connective tissue layer (Fig. 3). The inner layer is part of the water vascular system and consists of a tapered cylinder with muscular walls which extends into the central cone of the sucker. The tip of this inner cylinder is usually filled with mesenchymal cells.

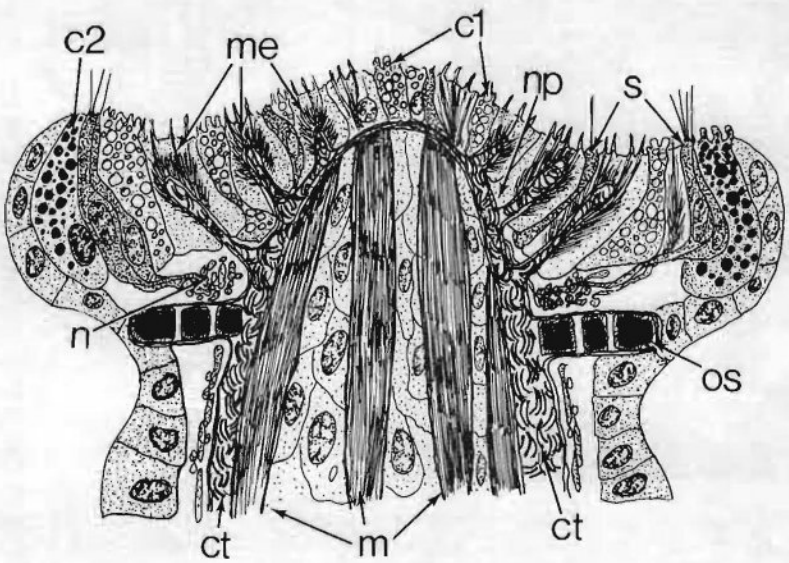

Fig. 3. A schematic drawing of a sagittal section through a primary podium of a competent larva of *Strongylocentrotus droebachiensis*: c1, Type I collocyte; c2, Type II collocyte; ct, connective tissue; m, muscle layer of water vascular system; me, myoepithelium; n, nerve ring; np, basiepithelial nerve plexus; os, ossicle; s, sensory cells.

The connective tissue layer is made up of collagen-like fibers and varies in thickness from 0.5–3 μm. At the base of the sucker, within the connective tissue layer there is a discoid, calcareous ossicle. On the sides of the central cone, the connective tissue layer extends finger-like projections into the overlying epithelium of the sucker. The sucker epithelium is a simple, pseudostratified epithelium predominantly made up of myoepithelial and secretory cells. The epithelium covering the entire podia is underlain with a basiepithelial nerve plexus. Also, at the base of the sucker is located a nerve ring associated with the distal surface of the ossicle.

The myoepithelial cells are 4–5 μm wide, up to $\approx$10 μm long and appear to surround the finger-like projections of connective tissue (Fig. 4). Arrays of filaments

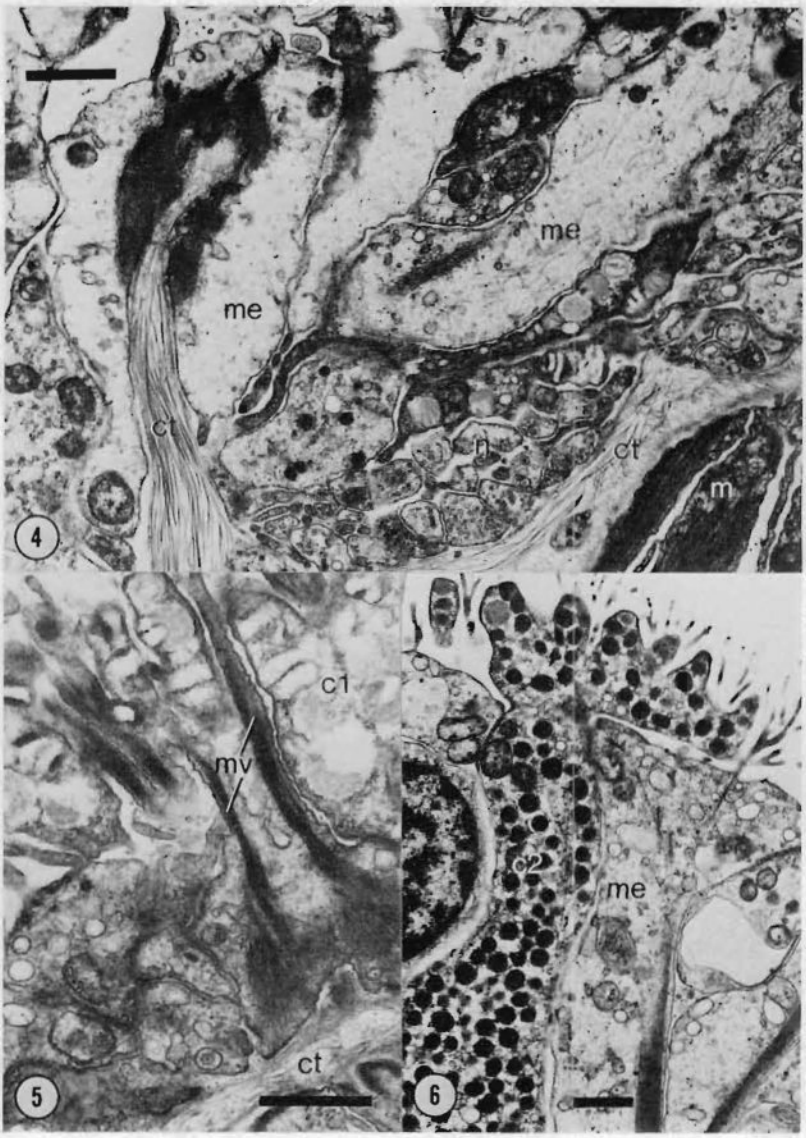

Fig. 4. Transmission electronmicrograph of the sucker epithelium of a primary podium of a competent larva of *Strongylocentrotus droebachiensis*: bar 1 μm; ct, connective tissue; m, inner muscle layer; me, myoepithelial cells; n, basiepithelial nerve plexus.

Fig. 5. Transmission electronmicrograph of a portion of the myoepithelium of the sucker of a competent larva of *Strongylocentrotus droebachiensis*: bar 1 μm; c1, Type I collocyte; ct, connective tissue; mv, microvilli of a myoepithelial cell.

Fig. 6. Transmission electronmicrograph of a region on the rim of the sucker from the primary podium of a competent larva of *Lytechinus pictus*: bar 1 μm; c2, Type II collocyte; me, myoepithelial cell.

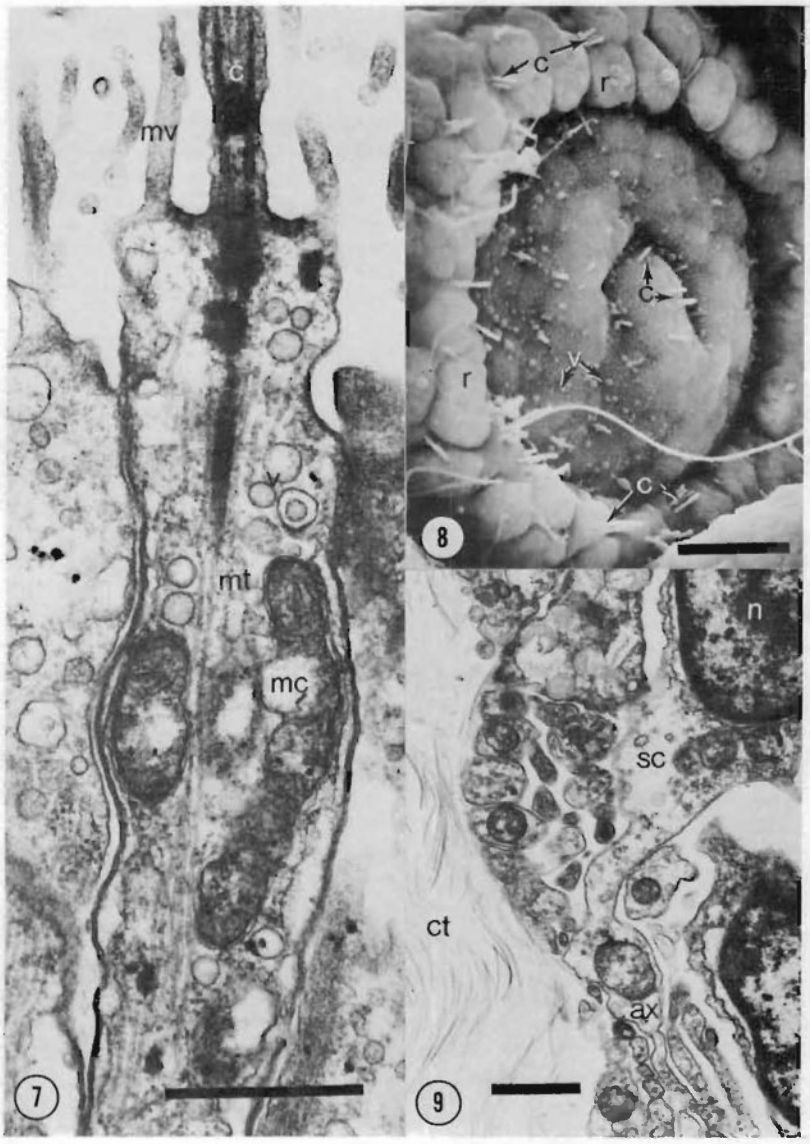

Fig. 7. Transmission electronmicrograph of the apical portion of a sensory cell from the sucker epithelium of a competent *Strongylocentrotus droebachiensis* larva; bar 1 μm; c, cilium; mc, mitochondria; mt, microtubules; mv, microvilli.

Fig. 8. Scanning electronmicrograph of the sucker of the primary podium of a *Strongylocentrotus droebachiensis* larva; bar 10 μm; c, cilia; r, rim; v, vesicles.

Fig. 9. Transmission electronmicrograph of a region of the basiepithelial nerve plexus subjacent to the sucker epithelium of a primary podium of a larva of *Strongylocentrotus droebachiensis*; note the basal axonal extension of a sensory cell contributing to the nerve plexus; bar 1 μm; ax, axon; ct, connective tissue; n, nucleus; sc, sensory cell.

oriented along the major axis of the cell extend between the connective tissue core and the apical surface of the cell. Microvilli, 1–2 μm long, on the apical surface of the cells each contain a core of filaments which are an extension of the filaments of the main portion of the cell (Fig. 5). Otherwise, the cytoplasm of the myoepithelial cells is relatively vacant containing only scattered mitochondria, microtubules, and a few vesicles.

The secretory cells of the sucker epithelium appear to be of two morphologically distinct types. Type I collocytes occur over the entire surface of the sucker and probably correspond to the secretory cells described from the tube feet suckers of holothuroids (Harrison, 1968) and asteroids (Harrison & Philpott, 1966; Souza Santos, 1966). The cells are 4–5 μm wide, 10 μm long, and are interspersed with the myoepithelial cells (Fig. 5). Type I collocytes have characteristic secretory vesicles which contain condensed flocculent material in a central band and around the periphery of the vesicle (Fig. 5). The apical surface of the Type I collocytes is comprised of numerous irregular projections packed with secretory vesicles. The Type II collocytes are restricted in their distribution to the rim of the sucker and contain numerous electron dense vesicles. The cells are 3 μm wide and up to 15 μm long (Fig. 6). The apical surface of the Type II collocytes is elaborated into projections similar to the surface projections of the Type I collocytes.

Bipolar sensory cells occur over the entire sucker epithelium. The sensory cells are clumped into groups of three to five cells on the rim of the sucker, while they occur singly over the central regions (Fig. 8). Each cell is up to 1 μm wide and 15 or 20 μm long (Fig. 7). The apical surface has a single cilium which is only 3–4 μm long, and is surrounded at its base by a collar of 0.5-μm long microvilli. In favorable sections, the basal ends of the sensory cells can be seen to taper to axonal processes, 0.1–0.2 μm in diameter, which contribute to the basiepithelial nerve plexus (Fig. 9). Mitochondria, microtubules, and 0.1-μm clear vesicles are the predominant organelles of the sensory cells.

STRUCTURE OF THE TUBE FEET SUCKERS OF ADULTS

For the three species studied, the form of adult tube feet is essentially the same as the form of the primary podia of competent larvae. However, adult tube feet are much larger; they are able to extend to ≈8 cm and are ≈0.5 mm in diameter. The histological organization of adult tube feet is the same as that of the primary podia with the notable difference that the tissues are much thicker. The epithelium of the sucker is ≈200 μm thick, and comprised of stratified, columnar cells (Fig. 10). The sucker epithelium is made up of the same cell types as the podial sucker epithelium, and the cells have essentially the same ultrastructural characteristics as those of the primary podia described above.

Sensory cells are dispersed over the entire surface of the adult tube feet suckers. The sensory cells on the rim of the sucker are clumped into groups of about 50 cells

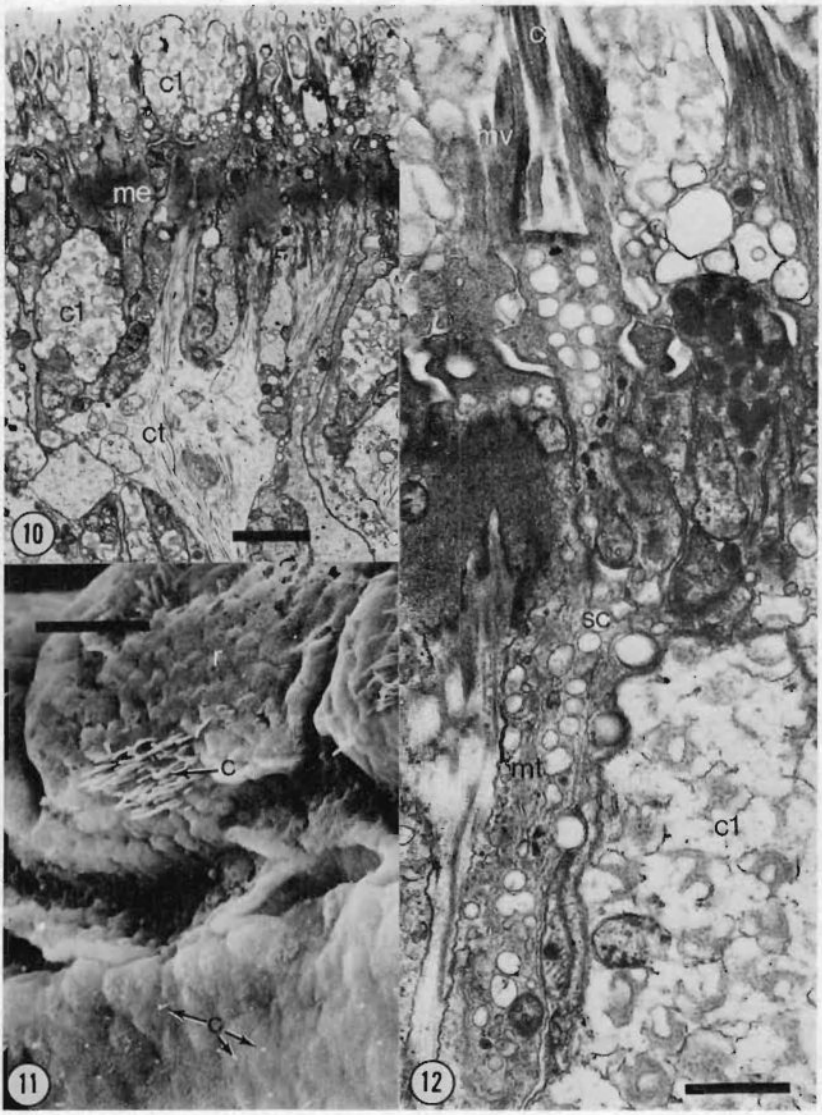

Fig. 10. Transmission electronmicrograph of the sucker epithelium of the tube foot of an adult *Strongylocentrotus droebachiensis*: bar 3 μm; cl, type I collocyte; ct, connective tissue; me, myoepithelium.
Fig. 11. Scanning electronmicrograph of a portion of a sucker, near the rim, of a tube foot of an adult *Strongylocentrotus droebachiensis*: bar 5 μm; c, cilia; r, rim.
Fig. 12. Transmission electronmicrograph of the apical portion of a sensory cell from the sucker epithelium of a tube foot of an adult *Strongylocentrotus droebachiensis*: bar 0.5 μm; c, cilium; cl, Type I collocyte; mt, microtubules; mv, microvilli; sc, sensory cell.

and the sensory cells in the central region occur singly (Fig. 11). Each sensory cell bears a single short cilium on its apical surface, while a collar of microvilli surrounds it at its base (Fig. 12). The basal end of the sensory cells extends as a thin axonal process into the basiepithelial nerve plexus. The cytoplasm of the sensory cells is characterized by microtubules, mitochondria, and clear vesicles.

Discussion

The sensory cells described here are similar to those described from other phyla (Bullock & Horridge, 1965). Specifically, the apical cilium and the basal axonal process are characteristics shared by many superficial sensory organs (Laverack, 1968, 1974; Bedini *et al.*, 1973; Bonar, 1978). The functions most commonly ascribed to superficial sensory organs of this type are mechanoreception and chemoreception (Laverack, 1968, 1974). Horridge (1965) has reviewed the modifications described from different sensory organs and suggested that it is not possible to determine the precise function of sensory organs from their structure. Similarly the sensory structures described here probably serve as receptors for some external stimuli, but it is not possible to state unequivocally the nature of those stimuli from structural evidence alone.

The ability of the larvae to come in contact with a benthic habitat, then retract their tube feet and resume swimming, suggests that the larvae are actually searching for an appropriate place to settle. Cameron & Hinegardner (1974) concluded that at least two conditions had to be met to induce metamorphosis in *Lytechinus pictus* and *Arbacia punctulata*: (1) the presence of a soluble organic chemical cue, and (2) contact of the larva with a surface. Cameron & Hinegardner (1974) found larvae in sea water containing the chemical cue require a prolonged (several minutes) contact of the podia with a solid surface for metamorphosis to proceed. In 80% of their tests, larvae held so that the podia were not allowed to touch a surface, would not metamorphose. Releasing the larvae or allowing them to contact a surface permitted metamorphosis to proceed. It is difficult to determine the location of sense organs which perceive the diffuse chemical cue, but it seems likely that the sensory structures on the tips of the primary podia may be employed at least in tactile perception of the substratum.

The observations of Cameron & Hinegardner (1974) apply to *Lytechinus pictus* and *Arbacia punctulata* and it is not certain that all echinoids have a similar set of cues to metamorphosis. Indeed, *Mellita quinquesperforata* and *Dendraster excentricus* metamorphose without the preliminary settling behavior reported here (Caldwell, 1972; Burke, unpublished observations). Yet both of these species appear to be induced to metamorphose by some substratum-associated factor from the adult environment (Caldwell, 1972; Highsmith, 1977). Neither *Lytechinus variegatus* nor *Strongylocentrotus droebachiensis* have been demonstrated to require anything more

than a surface for metamorphosis. Undoubtedly, there are several types of cue for metamorphosis in echinoids. One characteristic shared by many of them is that settlement precedes or is coincidental with metamorphosis (Hyman, 1955; Strathmann, 1978). Conceivably, echinoid larvae share some means of sensing the immediate presence of a surface; some species may rely on chemical cues, others evidently employ a tactile sense and some may require both forms of stimuli.

The sensory cells described on the podial suckers are not exclusively larval structures. There are some differences in the size and number of sensory cells on the podia of adults as compared to those of the larva, but the distribution appears to be the same for both larva and adult. The sensory cells may function primarily in the control and coordination of the sucker itself. The myoepithelium, the muscular wall of the inner layer, and the two types of collocytes may all be under nervous control which originates with sensory input from the sensory receptor cells of the podial epithelium. The control of podial function is not at all clear from the structural data available (Florey & Cahill, 1977; Cobb & Raymond, 1979). However, the observation of sensory structures on the sucker may infer that they are autonomously controlled in a manner similar to that suggested for pedicellariae (Laverack, 1968).

Barker (1978) described the structure of the organs of attachment of brachiolarian larvae of two species of asteroids and noted ciliary sense cells that he suggested are used in the recognition of substrata suitable for settlement of the larva. Brachiolarian arms are specialized larval structures that appear to function solely during the settlement of the larva (reviewed by Strathmann, 1978) and so their role in identification of substratum-associated cues seems apparent. Echinoid larvae lack structures homologous to brachioloarian arms, but the primary podia do play an analogous role during settlement and metamorphosis. The receptors on the podia probably do not function only during settlement, but appear to be more generalized sensory elements that may control podial suckers throughout the life of the urchin. Tactile stimulation of the podial nervous system when combined with other cues associated with suitable adult habitat may serve as the initial steps in the induction of metamorphosis in echinoid larvae.

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