

Nervous System of Ascidian Larvae: Caudal Primary Sensory Neurons

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Summary. In larvae of *Diplosoma macdonaldi* one sensory nerve extends along the dorsal midline of the tail and another extends along the ventral midline. Each nerve is composed of 50–70 naked axons lying in a groove in the base of the epidermis, and each projects to the visceral ganglion. The cell bodies of the caudal sensory neurons occur in pairs within the epidermis, and are situated along the courses of the nerves. A single cilium arises from an invagination in the soma of each neuron, passes through the inner cuticular layer of the tunic and enters a tail fin formed by the outer cuticular layer. We propose that these cells are mechanoreceptors. The caudal sensory system is similar in representative species of ten families of ascidians.

A. Introduction

The central nervous system of ascidian larvae consists of a rudiment of the adult cerebral ganglion and neural gland (the neurohypophysis), a fluid-filled sensory vesicle, an associated visceral ganglion, and a hollow, dorsal nerve cord that extends into the tail from the visceral ganglion (Elwyn 1937; Cloney 1978). Motor axons lie in two lateral grooves in the ependyma of the dorsal cord, adjacent to the caudal muscle cells they innervate (Cloney 1978; Cavey and Cloney 1976; Tannenbaum and Rosenbluth 1972; Mackie and Bone 1976). The ultrastructure of the sensory organs associated with the sensory vesicle has been described by Eakin and Kuda (1971), Barnes (1971, 1974) and Torrence (1980).

In contrast to the central sense organs mentioned above, the arrangement and structure of peripheral sensory cells in ascidian larvae is poorly known. Grave (1934) observed sensory ganglia associated with the anterior adhesive papillae in *Botryllus niger* by light microscopy. We have identified papillar primary sensory cells in several species (Cloney and Torrence 1982). Putative

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sensory nerves running in grooves in the bases of epidermal cells of the tail have been reported in larvae of several species (*Boltenia villosa*, Cloney 1969; *Distaplia occidentalis*, Cavey and Cloney 1972; *Diplosoma macdonaldi*, Cavey and Cloney 1976; *Aplidium* (*Amaroucium*) sp., *Ciona intestinalis* and *Dendrodoa grossularia*, Mackie and Bone 1976). Neuronal somata have not been identified in the tail of any species, although Mackie and Bone made a special search for them. It has been assumed that the axons in the caudal sensory nerves, like those in the motor nerves, arise from somata in the visceral ganglion. It has also been suggested that the sensory neurites in larvae of *Aplidium* and *Ciona* may pass between caudal epidermal cells to enter the tunic (Mackie and Bone 1976).

In this paper we describe the ultrastructure of the caudal sensory system in the larva of the compound ascidian *Diplosoma macdonaldi*. The first detailed account of peripheral sensory structures in an ascidian larva and the first report of neuronal somata in the tail are included. We show that the axons in the caudal sensory nerves arise from peripheral, primary sensory neurons resident in the caudal epidermis. The sensory endings that invade the tunic in this species are modified cilia arising from these sensory cells.

B. Materials and Methods

Colonies of *Diplosoma macdonaldi* (Herdman 1886) were collected from floats near the Friday Harbor Laboratories, Friday Harbor, Washington. Swimming larvae released by mature colonies were pipetted into a fixative composed of 2.5% glutaraldehyde, 0.2 M Millonig's phosphate buffer (pH 7.6) and 0.14 M sodium chloride (Cloney and Florey 1968). The osmolality was about 960 mOsm. After 1–2 h in this primary fixative, specimens were rinsed in a solution containing 0.2 M Millonig's phosphate buffer (pH 7.6) and 0.3 M sodium chloride and postfixed for 1 h in 2% OsO_4 buffered with freshly mixed 1.25% sodium bicarbonate adjusted to pH 7.2. Specimens were rinsed in distilled water, dehydrated in ethanol and embedded in Epon with propylene oxide as an antemedium. One micrometer sections for light microscopy were stained with an alkaline solution of azure II and methylene blue. Ribbons of thin sections for electron microscopy were collected on parlodion and carbon coated copper grids and stained with saturated aqueous uranyl acetate and lead citrate before examination with a Philips EM 300 microscope.

We also looked for components of the caudal sensory system in larvae of nine other species by electron microscopy (unreferenced data, Table 1). These specimens were fixed as described above or with the secondary fixative alone.

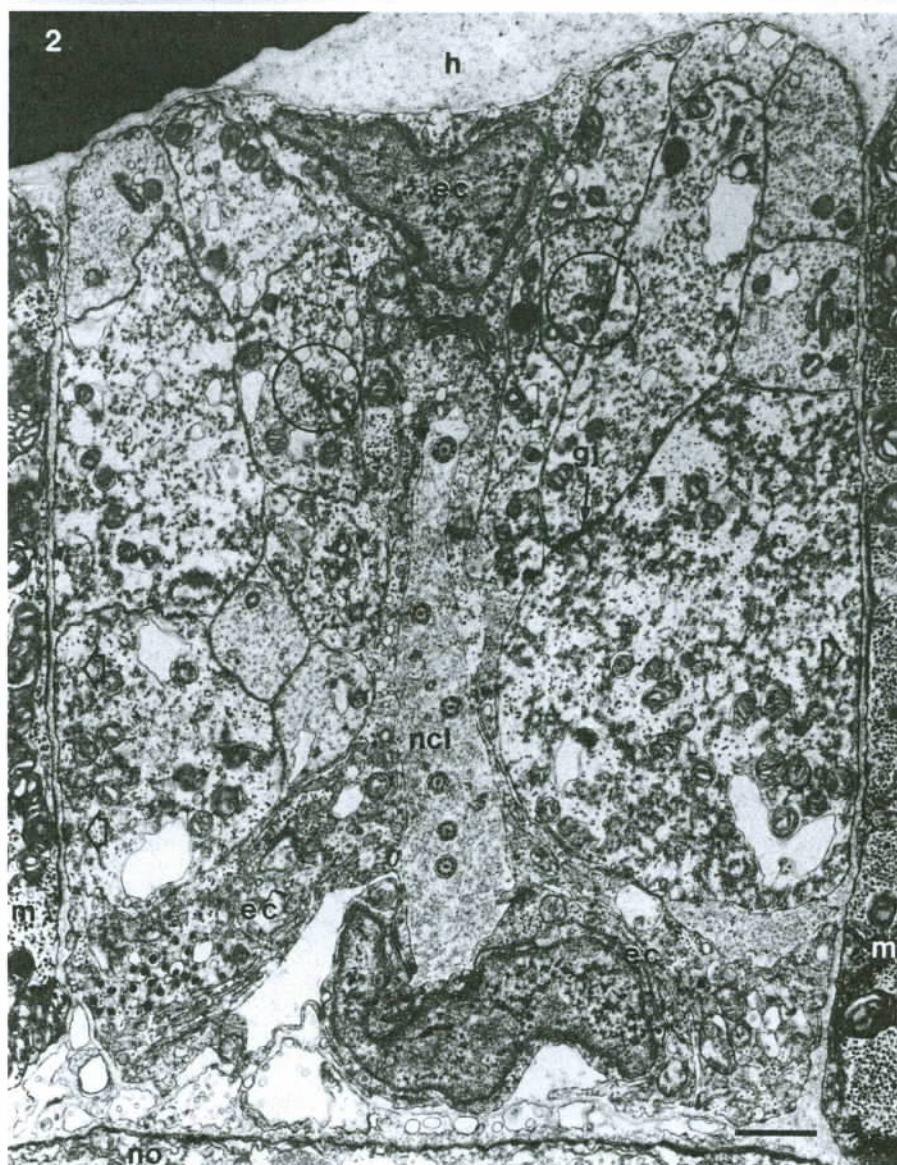
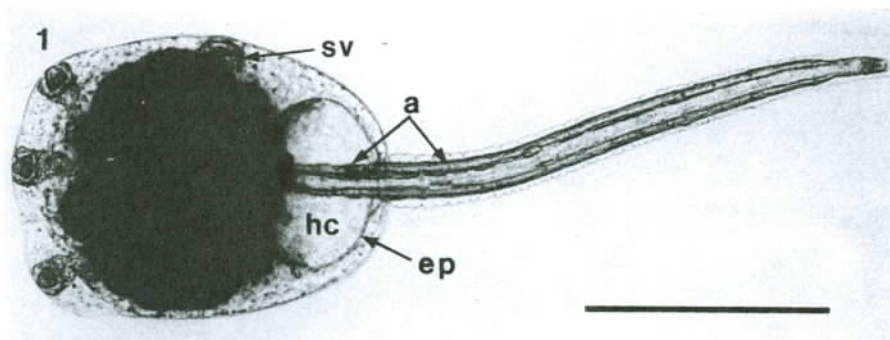
C. Results

1. Larval Tail Morphology

The general morphology of the tail of *Diplosoma macdonaldi* (Figs. 1, 3) has been described by Cavey and Cloney (1976) and is similar to that of other

Fig. 1. Larva of *Diplosoma macdonaldi*, left side. The sensory vesicle (sv) marks the dorsal side of the trunk. Tail fins lie in a plane perpendicular to this micrograph. Bright field microscopy. $\times 65$; bar = 0.5 μm

Fig. 2. Transverse section of the dorsal nerve cord of the tail, dorsal side uppermost and notochord (no) below. Ciliated ependymal cells (ec) line the central neurocoel (ncf). Arrows indicate neuromuscular junctions. Axo-axonal synapses are circled. Two axons are joined by a gap junction (gj). Typically there is one more axon on one side than on the other. $\times 10,800$; bar = 1 μm



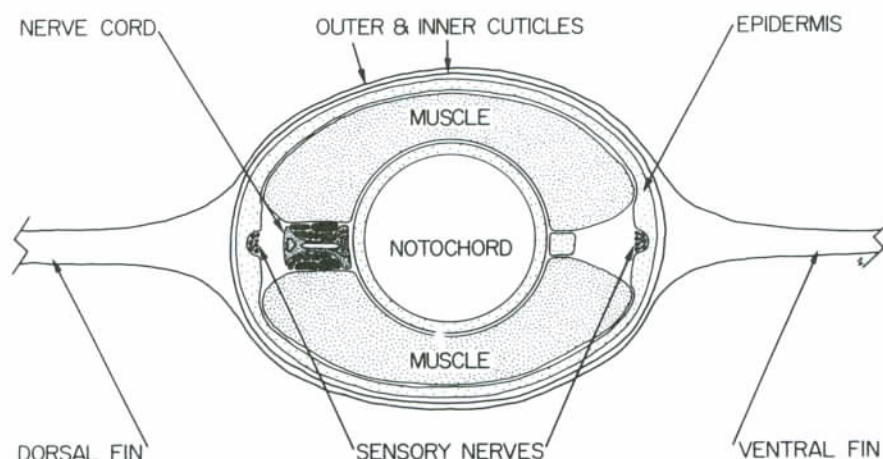


Fig. 3. Schematic transverse section of the larval tail. Nerve fibers drawn solid black

compound ascidian larvae (Cloney 1978). During embryogenesis the tail rotates 90° counterclockwise so that the dorsal nerve cord is on the left side relative to the trunk. In this paper, the nerve cord will define the dorsal side of the tail and anatomical directions will be designated with respect to the axes of the tail unless otherwise specified.

The central, hollow notochord, a hydrostatic skeleton, is flanked on the right and left by longitudinal bands of striated muscle (Fig. 3). An endodermal strand lies ventral to the notochord. Ependymal cells of the dorsal nerve cord embrace intraepithelial motor axons in two lateral channels (Figs. 2, 3). These organs comprise the axial complex of the tail. The epidermis surrounding the axial complex is a simple squamous epithelium. At the base of the tail the epidermis reflects away from the axial complex to encompass a large hemocoelic chamber in the posterior trunk (Fig. 1). Both the anterior end of the axial complex and the visceral ganglion lie free in this chamber where the visceral ganglion gives rise to the dorsal nerve cord of the tail (Figs. 4, 5). At metamorphosis the axial complex is thrust into a coil in this chamber by a contraction of the caudal epidermis (Cloney 1978).

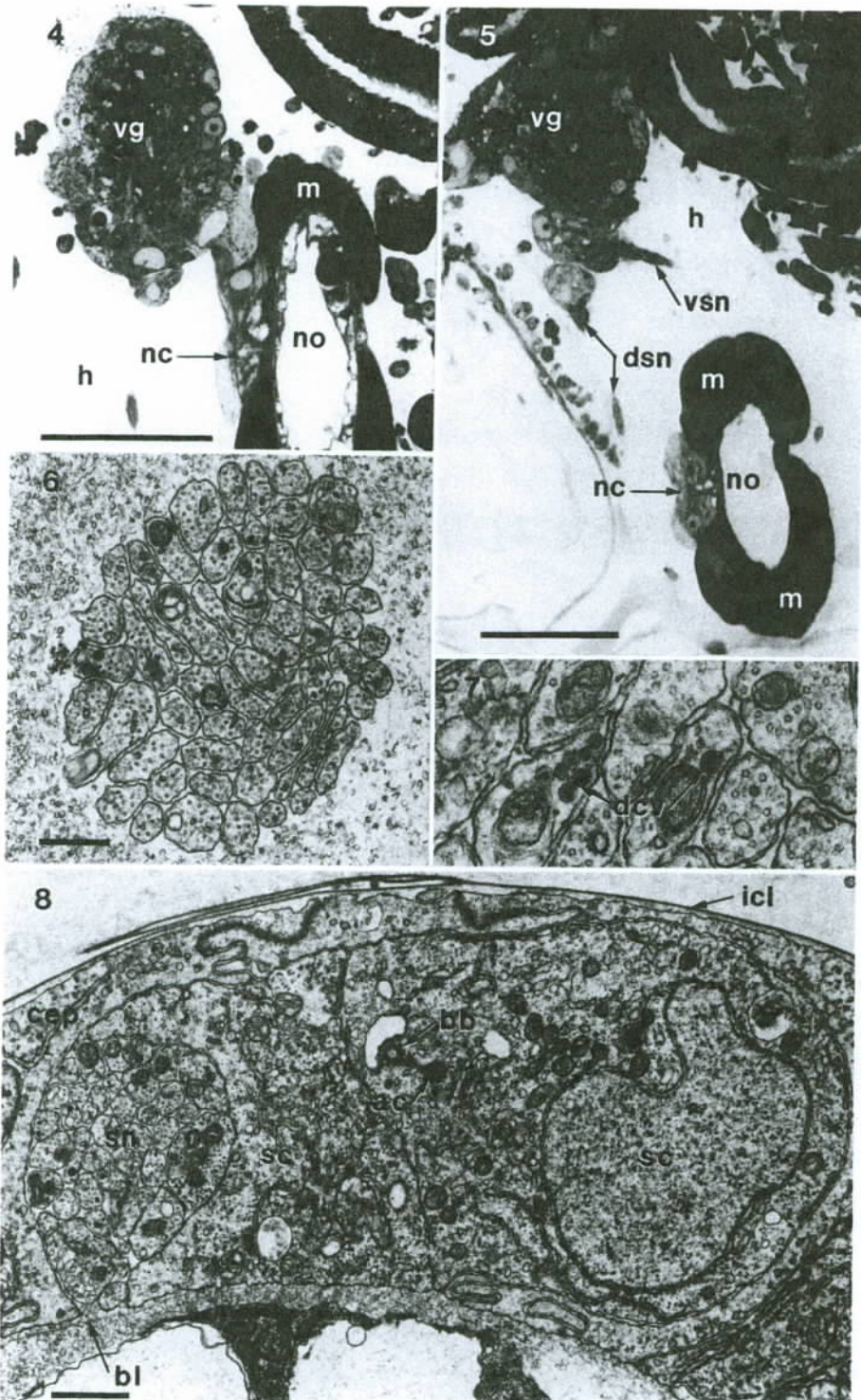
Fig. 4. Near-sagittal section (frontal with respect to trunk) of the axial complex of the tail and visceral ganglion (*vg*). The dorsal (motor) nerve cord (*nc*) arises from the ganglion. $\times 480$; bar = $50\ \mu\text{m}$

Fig. 5. Section to the right of Fig. 4 (dorsad with respect to the trunk) showing entry of dorsal (*dsn*) and ventral (*vsn*) sensory nerves into the visceral ganglion. $\times 380$; bar = $50\ \mu\text{m}$

Fig. 6. Transverse section of a sensory nerve lying free in the posterior hemocoelic chamber of the trunk. $\times 19,800$; bar = $0.5\ \mu\text{m}$

Fig. 7. Transverse section of part of a sensory nerve illustrating dense-cored vesicles (*dsv*). $\times 37,400$

Fig. 8. Transverse section of the caudal epidermis. Adjacent to the sensory nerve (*sn*) is a pair of sensory cells (*sc*). All are overlain by common epidermal cells (*cep*). $\times 11,400$; bar = $1\ \mu\text{m}$



The entire larva is covered by a secreted tunic, composed of two transparent layers of cuticle separated from each other and from the epidermis by layers of fine filaments in an electron-lucent matrix. The outer cuticle forms the fins of the tail and is entirely cast off at metamorphosis (Cloney 1978). The inner cuticle becomes the outermost surface of the juvenile.

2. Caudal Sensory Nerves

There are two sensory nerves in the tail of *Diplosoma*, one running along the dorsal and one along the ventral midlines (Fig. 3). Both are situated in grooves in the basal surface of the epidermis (Fig. 16) or between epidermal cells (Fig. 8). Each nerve is composed of a bundle of axons lying above the basal lamina, and is thus within the epithelium. Where the epidermis diverges from the axial complex at the base of the tail, the sensory nerves leave the epithelium and pass through the hemocoel as naked bundles of axons (Figs. 5, 6). The dorsal sensory nerve runs along the dorsal side of the axial complex to enter the visceral ganglion slightly above the origin of the caudal motor nerves (Fig. 5). The ventral sensory nerve extends along the ventral side of the axial complex. Near the anterior end of the axial complex, it turns across the right side of the axial complex (the dorsal side with respect to the trunk) toward the visceral ganglion. The ventral sensory nerve enters the visceral ganglion near the entry of the dorsal sensory nerve (Fig. 5).

The naked axons of the sensory nerves are small, ranging from 0.2 to 1.2 μm in diameter. Along with microtubules, mitochondria and smooth endoplasmic reticulum, they often contain dense-cored vesicles 50–100 μm in diameter (Fig. 7). There are approximately 50–70 axons per nerve at the base of the tail. This number may differ between the dorsal and ventral sensory nerves of one individual. The number of axons in each sensory nerve declines progressively in more distal sections.

Fig. 9. Sagittal section of the caudal epidermis. The axon (*ax*) of a sensory cell (*sc*) projects anteriorly in the sensory nerve (*sn*). $\times 9,400$; bar = 1 μm

Fig. 10. Putative synapse between a sensory cell body (*above*) and an adjacent axon in the sensory nerve (*below*). $\times 25,500$

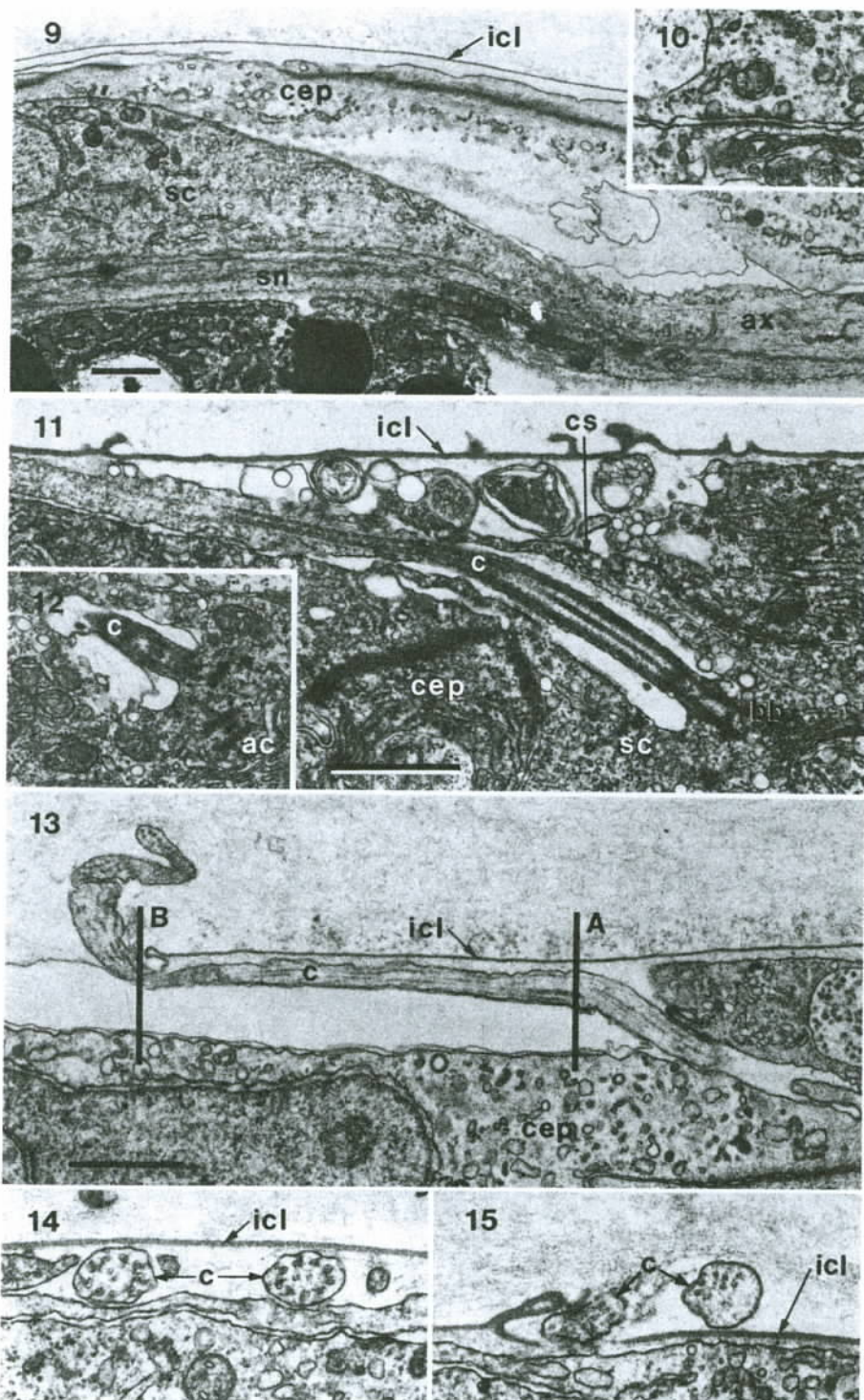
Fig. 11. Sagittal section of the apical surface of the caudal epidermis. A single cilium (*c*) projects posteriorly from a sensory cell (*sc*). Broad projections of the cell (not microvilli) ensheath the cilium (*cs*). $\times 17,800$; bar = 1 μm

Fig. 12. Sagittal section of a caudal sensory cell showing the accessory centriole (*ac*). $\times 16,600$

Fig. 13. Sagittal section of the apical surface of the caudal epidermis. This sensory cilium (*c*) arose from a neuron beyond the right edge of the micrograph. $\times 15,900$; bar = 1 μm

Fig. 14. Transverse section of a pair of sensory cilia (*c*) at a position equivalent to line A in Fig. 13. $\times 35,600$

Fig. 15. Transverse section of a pair of sensory cilia (*c*) at a position equivalent to line B in Fig. 13. $\times 35,600$



3. Epidermal Sensory Neurons

a) Somata. The axons of the caudal sensory nerves arise from cells lying in the caudal epidermis immediately adjacent to the nerves (Figs. 8, 9, 17) and run anteriorly, toward the central nervous system. The cells are paired (Fig. 8) and are found at somewhat irregular intervals along the tail. Dorsal and ventral cells do not occur in register. Thus the system shows no indication of a segmental arrangement. The somata of the caudal neurons are flattened ovoids and measure approximately $4 \times 6 \times 9 \mu\text{m}$. Each contains a single nucleus, numerous mitochondria, a small amount of rough endoplasmic reticulum, a scyphate Golgi apparatus and one or more prominent secondary lysosomes (Figs. 8, 9, 16). A few dense-cored vesicles similar to those in the axons are usually also observed. Microtubules are prominent in the axon hillock (Fig. 9) and sometimes in the ciliary sheath (Fig. 11, *vide infra*). In electron micrographs the neurons often appear darker than the surrounding common epidermal cells because of a higher concentration of free ribosomes or glycogen particles. Structures that resemble poorly developed chemical synapses are occasionally observed connecting a cell body and an adjacent axon (Fig. 10) or two adjacent axons in the nerve. Gap junctions have not been observed between any sensory neuron and any other cell.

The epidermis is a simple epithelium in which the apices of all cells border the tunic. However, it is characteristic of the caudal sensory neurons that they are almost entirely surrounded by adjacent common epidermal cells; they are exposed to the tunic only over a small area (Figs. 8, 9, 11, 16, 17). Along with their location in the midline, their submerged position in the epithelium has proven to be a reliable means of identifying these cells in *Diplosoma*.

b) Sensory Endings. A single cilium arises in a deep pocket in the apical surface of each caudal sensory neuron (Figs. 11, 17). A cylindrical extension of the cell reaches to, and somewhat beyond, the surface of the epithelium as a sheath around the ciliary shaft. It is joined to the surrounding cells by a zonula occludens. The cilium and sheath are the only parts of a neuron that contact the tunic.

The basal body of a sensory cilium lies at the bottom of the apical pocket, below the level of the zonula occludens. An accessory centriole is often observed adjacent to the basal body (Figs. 8, 12). Ciliary rootlets have not been observed. Sensory cilia run posteriorly from the cell bodies, between the epidermis and the inner cuticle (Figs. 11, 13, 14, 16, 17) before passing through the inner cuticle (Figs. 13, 15, 17) and penetrating the caudal fins (Figs. 16, 17).

At the base of a cilium and for most of its course below the inner cuticle, the axoneme is composed of a typical array of $(9 \times 2) + 2$ microtubules (Fig. 14). However, approximately where a cilium passes through the inner cuticle, the microtubules become disarranged and usually cease to form doublets (Figs. 15, 16). As a consequence of this disarray, the processes that are found in the fins are not readily recognizable as cilia. Every process that we have traced through serial sections has proven to be a cilium. We have not observed any



Fig. 16. Transverse section of the caudal epidermis and part of the overlying fin. A pair of cilia (*c*) lie below the inner cuticular layer (*icl*) and project into the fold of the outer cuticular layer (*ocl*) that forms the fin. $\times 11,200$; bar = $1\ \mu\text{m}$

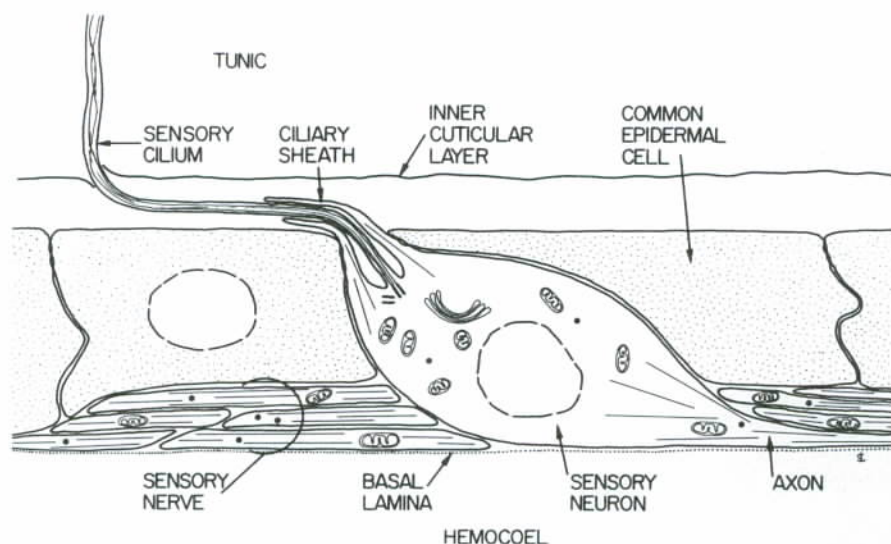


Fig. 17. Schematic sagittal section of a sensory neuron in the caudal epidermis of *Diplosoma macdonaldi*. Anterior is to the right.

process in *Diplosoma* that can be interpreted as a neurite passing directly into the tunic from a sensory nerve.

In serial transverse sections of the tail we have consistently observed the sensory cilia to be paired (Figs. 14, 15, 16). Paired cell bodies may be somewhat staggered along the nerve; however, a pair of cilia arise close together, pass through the inner cuticle together (Fig. 15) and run together into the fin (Fig. 16).

D. Discussion

The evidence that the cells we have described are sensory cells may be summarized as follows. The cells have axons that project to the central nervous system. They are not associated with any potential effector organ. The cilia arising from the neurons themselves are unlikely to be motile because of the disorganization of their axonemes. On the other hand cilia, variously modified, are common appendages of sensory cells and are usually proposed to be the actual sensory endings (Bullock and Horridge 1965; Laverack 1968, 1974; Altner and Prillinger 1980).

There are no behavioral or physiological observations that indicate the modality of this system but the structure of the cells with their long, thin processes lying in the narrow, cuticular fins, suggests that they are mechanoreceptors. Thus the system might provide proprioceptive information during swimming or might trigger swimming. It is probably not involved in generating the basic swimming rhythm because the tails of several species (e.g., *Boltenia*, Cloney 1961; *Dendrodoa*, Mackie and Bone 1976) continue to "swim" after isolation from the trunk, and we have observed no peripheral connection between the caudal sensory and motor nerves.

Table 1. Components of the caudal sensory system observed in all ascidian families with known larvae. Blanks indicate insufficient information. Unreferenced data represent our own observations

	Epi- dermal nerves	Cell bodies	Sensory cilia
Polycitoridae			
<i>Archidistoma ritteri</i>	+	+	+
<i>Clavelina huntsmani</i>	+		+
<i>Distaplia occidentalis</i>	+ ^a		+
Polyclinidae			
<i>Aplidium</i> sp.	+ ^b		
<i>Aplidium constellatum</i>	+	+	+
Didemnidae			
<i>Diplosoma macdonaldi</i>	+ ^c	+	+
Cionidae			
<i>Ciona intestinalis</i>	+ ^b		
Asciidae			
<i>Ascidia callosa</i>	+	+	
Correllidae			
<i>Corella inflata</i>	+		
Perophoridae			
<i>Ecteinascidia turbinata</i>	+	+	
Styelidae			
<i>Styela plicata</i>	+	+	
<i>Dendrodoa grossularia</i>	+ ^b		
Pyruridae			
<i>Boltenia villosa</i>	+ ^d		
Molgulidae			
<i>Molgula occidentalis</i>	+	+	

^a Cavey and Cloney 1972

^b Mackie and Bone 1976

^c Cavey and Cloney 1976

^d Cloney 1969

The caudal sensory neurons of *D. macdonaldi* larvae are strikingly similar to the cupular sensory cells of adult *Ciona* (Bone and Ryan 1978). The cupular sensory cells are also epidermal neurons. Their presumptive sensory endings are long cilia that arise from deep, apical invaginations. As in the tail of *Diplosoma*, the sensory cilia in the cupular organ have $(9 \times 2) + 2$ microtubular axonemes near the cell body but these become disarranged more distally. The major difference between the two cell types is the presence in the cells of the cupular organ of a prominent electron-lucent apical zone that is absent from the caudal sensory cells. Bone and Ryan (1978) have provided evidence that the cupular organ is a mechanoreceptor responding to water-borne vibration.

Dorsal and (usually) ventral epidermal sensory nerves have been identified in the larval tails of species representing ten families of ascidians (Table 1). We have observed sensory neurons similar to those in *Diplosoma* in several of these species (Table 1, Fig. 18) although sensory cilia have not yet been observed in all. We infer that this is the common organization of the caudal sensory system in ascidian larvae. There are some differences in details. *Molgula* and *Dendrodoa* have a dorsal sensory nerve but lack a ventral nerve. The epidermal neurons in *Molgula* may lack cilia. The sensory nerves in *Ecteinascidia* lie in the hemocoel, subjacent to the epidermis rather than within it. A more

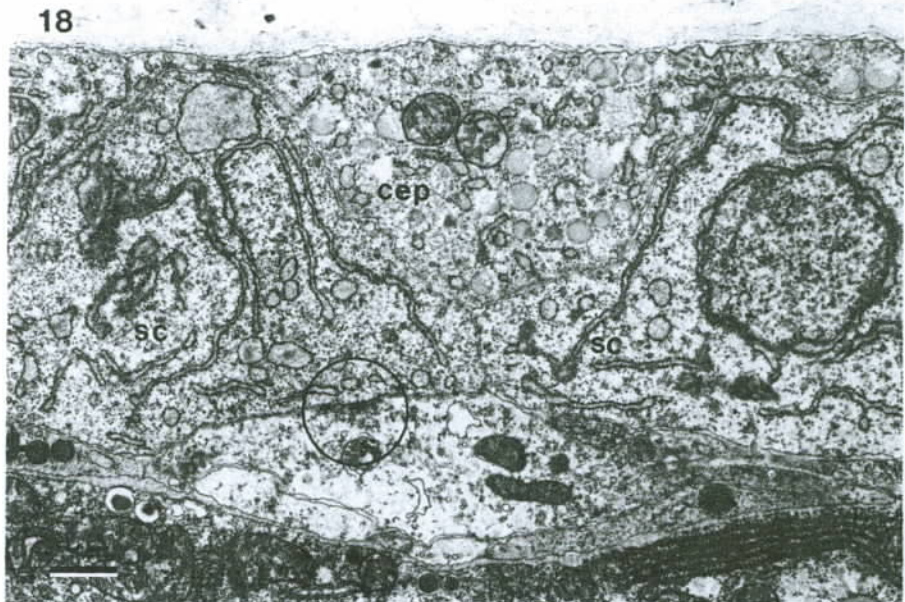


Fig. 18. Mid-sagittal section of the caudal epidermis of a larva of the solitary ascidian *Molgula occidentalis*. Sensory cells (*sc*) are overlain by common epidermal cells (*cep*). The caudal sensory nerve lies below the sensory cells. An axo-somatic synapse is circled. $\times 8,900$; bar = $1 \mu\text{m}$

extensive comparison would undoubtedly reveal more variations but those observed so far do not obscure the basic pattern.

The caudal sensory system presents an intriguing developmental question. It has been well established in arthropods that peripheral sensory fibers find their way to the central nervous system by following pre-existing nerves. The initial connections are made over short distances in the embryo and elongate during subsequent growth of the embryo (reviewed by Anderson et al. 1980). In embryos of *Ascidia callosa*, however, there are no observable axons in either the caudal motor or sensory nerves during the early stages of tail elongation (Cloney 1978 and unpublished). Peripheral cells that resemble the caudal sensory neurons of *Diplosoma* are identifiable in these embryos. The ingrowth of the first sensory axons must occur over a considerable distance. Perhaps the epidermis provides a guiding pathway.

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Abbreviations

a axial complex of the tail
ac accessory centriole
ax axon
bb basal body

bl basal lamina
c cilium
cep common epidermal cells
cs ciliary sheath

<i>dsv</i>	dense-cored vesicles	<i>nc</i>	dorsal nerve cord
<i>dsn</i>	dorsal sensory nerve	<i>ncl</i>	neurocoel
<i>ec</i>	ependymal cells	<i>no</i>	notochord
<i>ep</i>	epidermis	<i>ocl</i>	outer cuticular layer of the tunic
<i>gj</i>	gap junction	<i>sc</i>	sensory cell
<i>h</i>	hemocoel	<i>sn</i>	sensory nerve
<i>hc</i>	hemocoelic chamber	<i>sv</i>	sensory vesicle
<i>icl</i>	inner cuticular layer of the tunic	<i>vg</i>	visceral ganglion
<i>m</i>	caudal muscle	<i>vsn</i>	ventral sensory nerve

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