Nematode Chemosensilla: Form and Function[1]

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Abstract: As an introduction to a symposium of nematode chemoreception, the anatomy of nematode chemosensilla, their distribution on plant parasitic nematodes, and their possible functional roles is briefly reviewed. Comparison of nematode chemosensilla with those of other animals shows their greater resemblance to olfactory primary sense cells of vertebrates. Although the sensory process is obviously derived from a cilium, the absence of many ciliary features is noted. Retention of the ciliary necklace may be important functionally. A simple model is proposed, wherein binding of stimulant molecules to receptors in the membrane of the cilium-derived process results in entry of Na^+ and Ca^{++} (the latter via the ciliary necklace) to produce a receptor potential that spreads along the dendrite to the cell body where action potentials continue along the short axon to synapses. *Key words:* sensilla, chemoreception, ciliary necklace.   Journal of Nematology 15(2):151-158. 1983.

Nematologists have long recognized that many aspects of nematode biology are the result of responses to chemical attributes of their environment. Croll and Sukhdeo (4) presented a repertoire of 22 activities which in their opinion cover the spectrum of nematode behaviors. Of these, 14 are very likely in part, or entirely, responses to chemical signals. Similarly, Doncaster and Seymour (6) gave a scheme for the hatching of *Meloidogyne* eggs and penetration of their larvae into roots that indicates a major role for chemoreception. At the same time, there has been a resurgence of interest in sensilla of nematodes, largely through electron microscope studies that have given us greater insights into their structure and likely function. These anatomical aspects have been reviewed recently with respect to plant nematodes by Coomans and DeGrisse (3) and from a broader comparative view by myself (21). I refer to these reviews for details that follow.

I wish to introduce this symposium with some discussion on the current status of knowledge of structure and function of nematode chemosensitive sensila and to suggest a model for their function. Subsequent papers will consider the biochemistry of olfaction, methods used to study chemotactic behavior of nematodes, a possible mechanism whereby chemosensory capacity may be enhanced in some nematodes, and an appraisal of potential for interfering with nematode chemoreception in order to modify behavior patterns.

SENSILLA ANATOMY

Basic features: The most complete documentation of nematode sensilla comes from serial section reconstruction studies published by Ward et al. (18) and Ware et al. (19) on the cephalic sense organs of *Caenorhabditis elegans*. The basic features of sensilla shown here were similar to those seen earlier in other nematodes and have been confirmed in later descriptions. Yet some minor differences in patterns have been indicated between sensila in different regions of the body and/or in species belonging to the classes Secernentea or Adenophorea. Most nematode sensilla are associated with body cuticle and as such can be called cuticular sensilla; they sense the external environment. Other sensory units of rather different structure occur internally and hence are simply identified as *internal receptors;* they sense mainly internal body factors.

In general, cuticular sensilla consist of one or more sensory neurons associated with two nonneuronal cells (Fig. 1). The outermost nonneuronal cell has been termed the "socket cell" and is considered to be responsible for forming whatever cuticular modifications are a part of the sensillum.
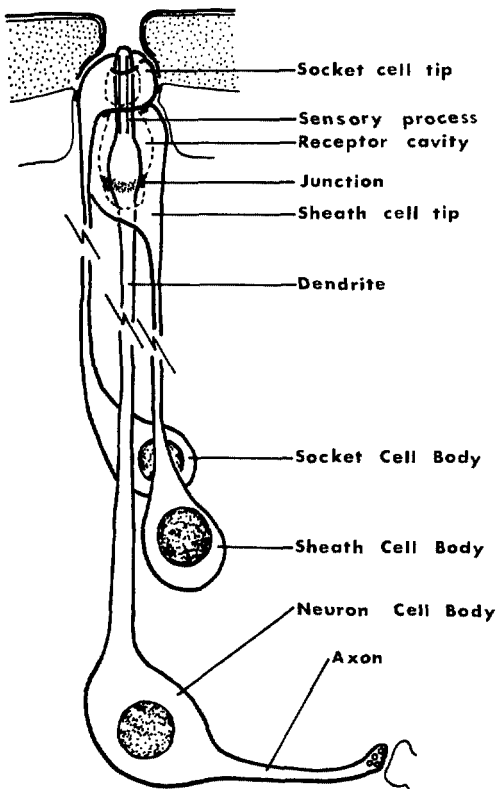
Fig. 1. Diagram of the anatomy of a nematode's chemosensillum.

The innermost nonneuronal cell, the "sheath cell," is sealed to the socket cell and to the tip of the sensory neuron, forming an extracellular chamber, the "receptor cavity," around the neuron tip and its sensory process. The sheath cell probably produces and regulates the contents of this receptor cavity. Although the socket and sheath cells are considered to be modified hypodermal cells, they are rather unusual in form. Within the region of the peripheral sensillum, the socket cell is seen only as a small unit of cytoplasm underlying the cuticle and wrapped around the sensillum's sensory process. Few cell organelles occur in it, but it is continuous with a tenuous cell process that extends into the body cavity of the worm and ultimately connects to a cell body including the nucleus. The socket cell body of cephalic sensilla in Secernentean nematodes lies just anterior to the circumesophageal nerve ring, perhaps 80–100 um posteriorly. The sheath cell is of similar form, although the cytoplasm in the sensillum region is not wrapped around

the sensory process but, rather, is penetrated by it. Its cell body lies near that of the socket cell. The sensory neuron or neurons are bipolar nerve cells whose cell bodies are a part of ganglia of the central nervous system. Axons connect these cell bodies, via synapses, to other neurons, while the forward projecting dendrite associated with the sensillum terminates in an expanded tip from which arises one or more cilium-derived sensory processes.

Anatomical variations: This basic plan has been most completely documented for cephalic cuticular sensilla of Secernentean nematodes (Fig. 2). However, in Adenophoreans the development of sheath and socket cells may differ. In *Xiphinema americanum* the sheath cell body of amphids lies at the same level as the expanded tips of sensory neurons at the base of the sensillum. Dorylaim nematodes also have numerous sensory body pores, and here the socket cell is part of the adjacent hypodermal chord. Since this cell is in contact with a considerable amount of cuticle, it is clearly functioning as a general hypodermal cell, forming body cuticle during molting and probably regulating body permeability, as well as functioning for the sensillum. The sheath cell of body pores also lies close to the sensory component, along the inner surface of the hypodermal chord.

Functional identification: Chemosensilla in nematodes have been identified by comparative anatomy, genetic studies, and microsurgery. In some respects, arthropod chemosensilla may be a close parallel to nematode chemosensilla, as they also must sense through an external cuticle. Here, combined anatomical and electrophysiological studies suggest that most chemosensory units have pores through the cuticle allowing direct access of external chemicals to the enclosed sensory processes. Furthermore, the sensory process may be relatively simple, containing only neurotubules. These features are evident in nematode sensilla identified as chemosensitive. Studies on mutant strains of *Caenorhabditis elegans* that do not respond normally to various chemicals have shown abnormalities in the sensory neurons of sensilla judged as chemosensory by anatomical features (11). Re-
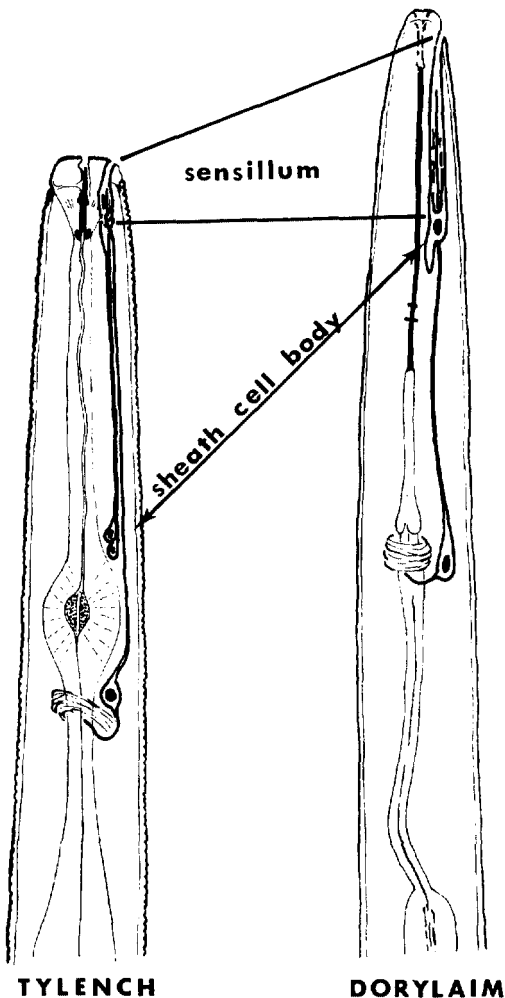
surface of the pore opening through the cuticle, while the other ends just beneath the cuticle. Criconematids have only one sensory neuron whose process extends into the pore. Aphelenchida have inner labial sensilla with two neurons as described for tylenchs, but their four cephalic sensilla also include one neuron whose sensory process extends to a pore in the cuticle and is likely chemosensitive.

In the tylenchs pores of the inner labial sensilla occur in various positions around the oral opening. In more primitive species these pores may be in grooves or on short papillae just external to the mouth (e.g., *Macrotrophurus* and *Ditylenchus*), while in some more specialized parasites they may open just inside the oral opening in the prestoma. For example, in *Rhadopholus* the lateral inner labial sensilla open to the prestoma while the remainder of them open to the outside; in *Rotylenchus* all inner labial sensilla open to the prestoma.

It is likely that such placement of pores allows discrimination or selection of stimuli drawn into the buccal cavity from more external factors. In *Hemicycliophora* and *Macroposthonia* the inner labial neurons approach the prestoma but are not exposed to the outside by a pore. Coomans and De Grisse (3) suggest that this reduction in sensory capacity may be correlated to the longer stylet that draws food from deep in the root more removed from the head surface.

Amphids have long been considered as chemosensory. They generally have a larger opening through the cuticle than do inner labial sensilla. In tylenchs and aphelenchs there are usually seven neurons whose sensory processes extend into the amphidial canal and approach the amphidial pore. Other neurons give rise to dendritic processes that project into the sheath cell. Endo (7) has found in *Heterodera glycines* that these neurons extend through the sheath cell and expand in elaborate sheets as the "outer accessory receptors." In addition, another neuron ends in the base of the receptor cavity. It possesses one or two cilium-derived processes and many finger-like projections (sometimes referred to as microvilli) that interdigitate with the inner surface of the amphidial sheath cell. The

Fig. 2. Diagramatic comparison of sensillum structure in tylenchids and dorylaimids. Note the more anterior location of the sheath cell body.

cently, Davis and Dusenbery (5) found that damage to cephalic region of *C. elegans* by a laser microbeam resulted in alteration in responses to chemicals.

DISTRIBUTION OF CHEMOSENSILLA ON PLANT PARASITIC NEMATODES

Cuticular sensilla: Sensilla of the head region include six inner labial sensilla, six outer labial sensilla, and four cephalic sensilla, as well as the lateral paired amphids. In the Tylenchida both inner labial sensilla and amphids are undoubtedly chemosensitive. In most species studied inner labial sensilla contain two sensory neurons, each ending with a single sensory process. One of these processes extends close to the

numbers of these "microvilli" vary greatly between species, and they increase in number from larvae to adults. Much of this neuron tip is thus enclosed by the sheath cell, but it is also open to some degree to the receptor cavity. It seems unlikely that these neurons interact significantly with external stimulants, since they lie deep within the sensillum and since the inner end of the cuticular amphidial canal constricts and minimizes the space around the sensory processes distal to these neurons. It is more likely that the sensory processes that are limited to the receptor cavity may monitor the environment of this area or the state of the sheath cell membranes. Those that project beyond the sheath cell may be classed as internal cephalic receptors perhaps sensitive to light or other forms of electromagnetic radiation.

Adenophorea exhibit a greater degree of chemosensory capacity than do Secernenteans. All 16 of the sensilla at the head of dorylaimids have characteristics of chemosensilla with relatively larger pores opening to the outside. In addition, each sensillum generally has more sensory neurons. Thus, *Xiphinema americanum* has four neurons in each inner and outer labial sensillum and two or three neurons in the cephalic sensilla. Similarly, amphids of most Adenophorea studied have larger openings and contain more sensory neurons. In *Xiphinema americanum* there are 14 neurons that give rise to 19 sensory processes. The elaborately developed neuron that is associated with the sheath cell in Secernentean amphids is absent. But in *X. americanum* there is one small neuron whose tip enters the receptor cavity and bears one short sensory process which terminates well inside this area.

Sensilla of the tail region vary greatly with the sex. Unfortunately, much less attention has been paid to their structure. Paired lateral phasmids occur in both sexes, but only in the Secernentea. Phasmids of *Caenorhabditis elegans* contain two sensory neurons ending in a small simple receptor cavity (16). The phasmidial pore in Hoplolaimids is unusually large and contains a dense fibrous material.

Males have variously developed caudal papillae and in some species a copulatory bursa including rays ending in a sensillum with a pore through the cuticle. The sensilla of bursal rays in male *Caenorhabditis elegans* include two neurons ending in a small receptor cavity. Caudal papillae of *Aphelenchoides* have been shown to have neurons likely to respond separately to chemical and mechanical stimuli. More prominent chemosensilla in males are those in the spicules. In *Tylenchulus* each spicule includes a single sensillum with two sensory neurons. One neuron bears a long sensory process that ends at a pore in the tip of the spicule, while the sensory process of the second neuron ends within the receptor cavity. In *Pratylenchus* and *Heterodera* two pores occur at the tip of each spicule, but details of internal structure are only sketchy. In *Caenorhabditis elegans* each spicule has two pores each associated with a sensillum containing a single neuron.

Internal receptors: In contrast to these cuticular sensilla, internal receptors sense internal factors of the body. They have been recognized only recently and most are suggested to be mechanosensitive, controlling organ activity through proprioceptive feedback. However, in the Longidorids the esophageal nervous system includes regions where neurites have many short finger-like processess oriented toward the esophagus lumen. At least in *Xiphinema diversicaudatum* a centriole has been found in adjacent neuron cytoplasm. As these differentiations are so closely associated with the food canal, Robertson (14) suggested that they might be gustatory. In *Aphelenchoides* a neuron encloses the stylet shaft where the stylet cuticle is permeated by fine reticulate canals. Shepherd et al. (15) suggest that this may similarly be a gustatory region.

COMPARISON OF NEMATODE, VERTEBRATE, AND ARTHROPOD CHEMOSENSILLA

The nematode's sensory neuron is bipolar with a dendrite that carries information from the sensillum to a cell body located at the level of the nerve ring and a short axon that conveys the information to other elements of the central nervous system (Fig. 3). The sensory cell is clearly a primary sense cell. Arthropod chemosensitive sensilla, while also being primary sense

VERTEBRATE   **NEMATODE**   **ARTHROPOD**
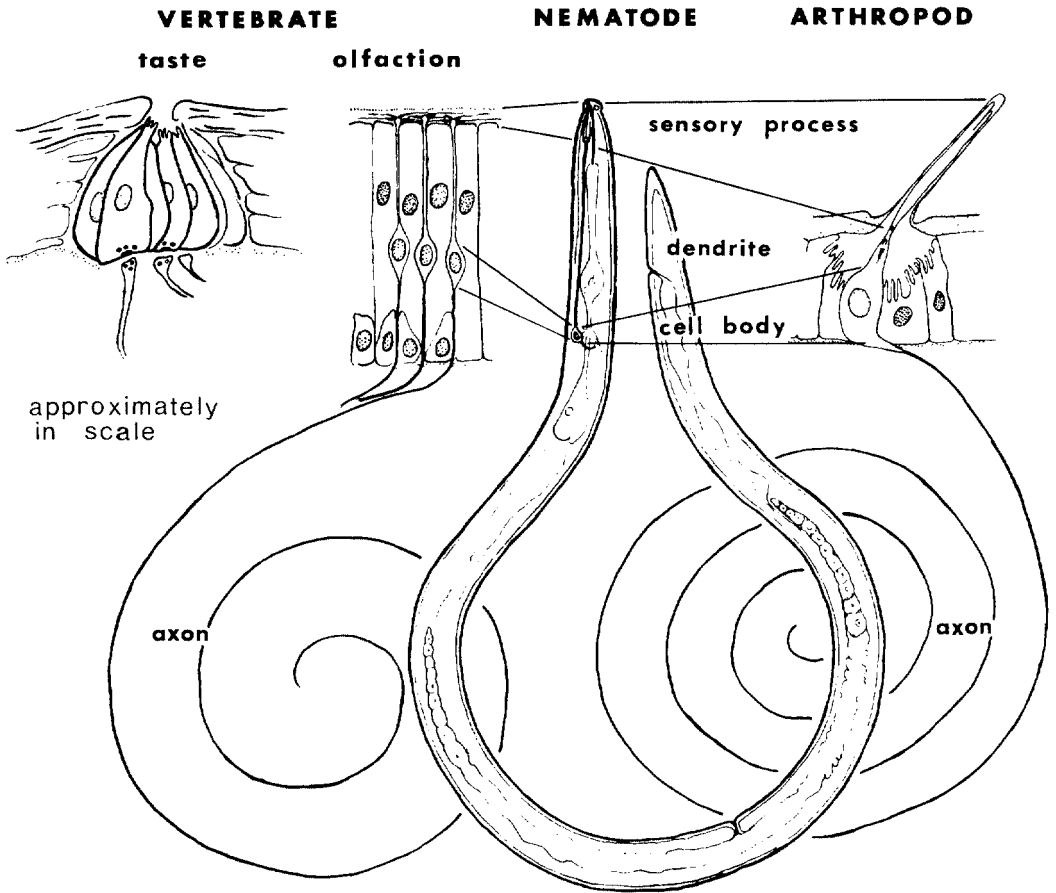
taste   olfaction

Fig. 3. Comparative illustrations of the anatomy of chemosensilla in nematodes, vertebrates, and arthropods.

cells, have their cell body located peripherally, close to the sensillum with a long axon (up to some centimeters in length) connecting to other elements in the central nervous system. The sensillum includes one or more cilium-derived sensory processes that extend into a perforated cuticular peg. This portion is much longer than the comparable sensory process in the nematode and the dendrite is very short. Vertebrate chemoreceptors include both primary sense cells (responsible for olfaction) and secondary sense cells (responsible for taste). The latter are specialized epithelial cells that are chemosensitive but communicate to the central nervous system via interneurons with which they synapse. These secondary sense cells bear microvilli, but not cilia, on their exposed surfaces. The vertebrates' olfactory sense cells are long thin bipolar neurons. Their cell bodies lie in the mid level of the olfactory epithelium. The height of this epithelium is about equal to the length of the dendrite of the nematode's sensory neuron. The dendrite ends in an expanded bulb from which cilia project into a layer of mucous overlying the epithelium. The axon (perhaps a centimeter long) extends from the epithelium to the olfactory bulb of the brain. It seems that the nematode's sensory neuron more closely resembles the primary sensory neurons of the vertebrates' olfactory epithelium but with a much shorter axon.

A MODEL FOR THE FUNCTION OF NEMATODE CUTICULAR CHEMOSENSILLA

If we consider the nematode's sensillum from the perspective of arthropod and vertebrate sensilla where extensive electrophysi-

ological data have been correlated to their morphology, it is possible to formulate a model for their function.

Chemical stimulants undoubtedly gain access to the sensillum via the cuticular pore (Fig. 4). The sheath cell of the sensillum produces a material comparable to insect sensillum liquor or vertebrate mucous that may be a polyanionic glycoprotein responsible for protecting and/or nourishing the sensory process or may be involved in maintaining appropriate ionic levels. Secretion of glycoproteins may involve the endoplasmic reticulum and Golgi in the sheath cell while ion transport is likely the role of prominent infoldings of the sheath cell membrane limiting the sensillum's receptor cavity. Stimulating molecules may interact
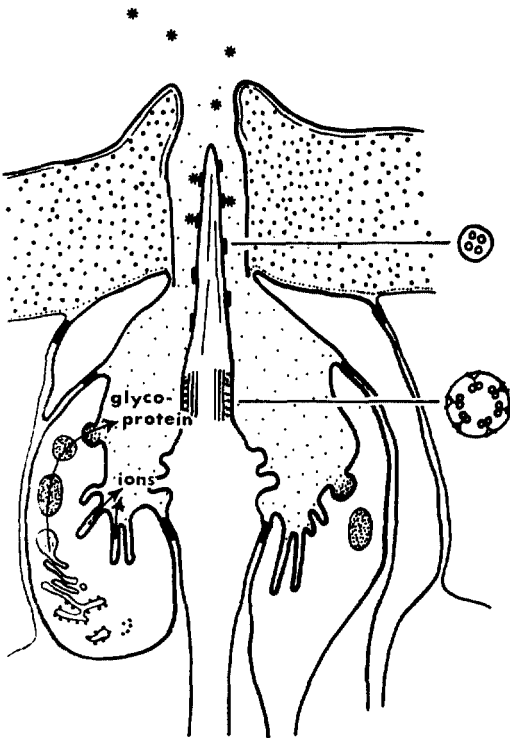

Fig. 4. Diagram of a nematode chemosensillum showing the cuticular pore through which stimulating molecules (*) may enter and bind with receptor proteins in the membrane (thickenings) of the sensory process. Singlet microtubules are indicated in the offset cross sectional diagram of the process. Doublet microtubules linked to the cell membrane characterize the ciliary necklace region also shown in a cross sectional diagram. Sheath cell cytoplasm surrounding the sensory process and tip of the sensory dendrite likely secretes ions and glyco-proteins into the receptor cavity.

with these secretions temporarily while en route to the membrane of the sensory process, or the material may serve only to maintain aqueous channels to the membrane surface. Here the stimulant probably binds with a specific receptor that may be a protein membrane component. The receptor spectrum likely determines the specific sensitivity of the sensillum. Ward (17) suggested that nine types of receptors would be needed in amphids and inner labial sensilla to account for chemosensitivity of *C. elegans*. The result of receptor-stimulant interaction is a change in permeability of the membrane allowing an influx of sodium ions and giving an electric membrane potential referred to as the receptor potential. In arthropods and vertebrates such receptor potentials are limited to the general area of the sensillum, while information is transferred to the central nervous system as action potentials along the sensory axon. In the arthropods it seems likely that action potentials originate somewhere in the region of the cell body (10). In the vertebrate olfactory epithelium receptor, potentials have been recorded throughout the entire height of the olfactory epithelium, while action potentials travel along the axons (13). Although the sensory cell processes of nematodes may be somewhat finer in diameter and the cell body smaller, it is likely that receptor potentials generated in the nematode sensillum could spread along the entire sensory dendrite and perhaps even along the axon to directly affect other elements of the central nervous system. Alternately, action potentials may be generated in the cell body region and spread along the short axon to the synapse. I suspect this latter would be more likely.

The part of the sensillum most directly exposed to stimulating molecules, and where the initial reactions undoubtedly occur, is the cilium-derived sensory process. Its membrane is the likely site where stimulants are bound to receptors and receptor potentials are generated. What anatomical features of the sensory process may be key in this role? Cilium-derived sensory processes in different animal groups show varying degrees of resemblance to motile cilia. I suggest that the nematode's sensory process exhibits a reduction to a least common

denominator that may indicate the crucial components. A comparison of the anatomy of motile cilia and nematode sensory processes may be instructive.

Both the cilium and sensory processes are membrane limited and contain microtubules. In motile cilia microtubules are arranged at nine doublets, with ATPase dyneine proteins associated with the A tubule, plus two central tubules connected to the doublets by radial fibrils. In most sensory processes microtubules are singlets, with doublets occurring only at the base of the process. However, some doublets may extend some distance into the process. If so, they lack dyneine arms; motility is therefore unlikely. The number of doublets may vary greatly from four to perhaps more than 50 (in some animal parasites). At the base of the sensory process, doublets are arranged in a circle and are linked to the peripheral membrane by Y-shaped fibrils identical to the morphology of the ciliary necklace of motile cilia. Within the tip of the sensory dendrite, a number of ciliary organelles may be present or absent. A basal body composed of radially splayed triplets of microtubules has not been found in nematode sensilla. Instead, some species have densities equatable with microtubule organizing centers. Although banded rootlets may be present in nematode sensory dendrites, they are frequently absent. Sensory dendrites often contain prominent mitochondria, yet nematode sensory dendrites often lack them. Similarly, vesicles are sometimes present in the dendrite but often absent. Thus, the component of the motile cilium that is conserved in nematode sensory process, and is perhaps common to all cilium-derived sensilla, is the ciliary necklace. Here there are morphologically distinguishable links between the axonemal microtubules and the cell membrane. Perhaps of greater significance is the presence of globular subunits in the membrane itself at this point (9). Anderson and Hein (1) demonstrated anionic binding capacity in this region of the cilium, strengthening Gilula and Satir's suggestion (9) that the subunits of the ciliary necklace might be sites of selective ion permeability. Fischer et al. (8) raised the possibility that the subunits might be Ca pump proteins.

Boisvieux-Ulrich et al. (2) see the ciliary necklace of motile cilia as "forming a boundary between two different states of cellular membrane." In mechanoreceptors the ciliary necklace has been seen as a significant region for stimulus induction (12, 20). In the nematode chemosensillum the ciliary necklace could be important in generation of the receptor potential.

A model for function of the nematode sensillum and sensory neuron then could be summarized as in Figure 5. Receptors for chemostimulants likely occur in the membranes of the sensory process. Reactions between these proteins and stimulants likely
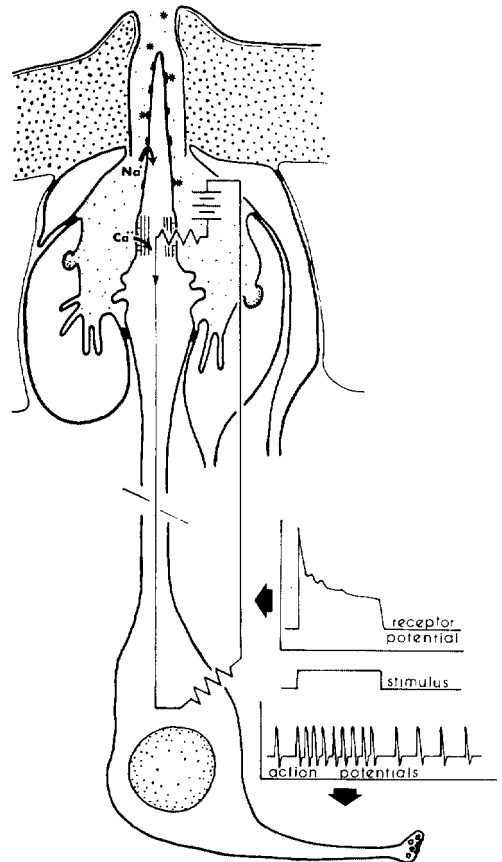

Fig. 5. Diagramatic representation of a model of chemosensillum function. Upon binding of stimulant molecules to receptors of the sensory process, a Na^+ influx likely occurs and Ca^{++} may enter via ion pumps in the membrane of the ciliary necklace region. This produces an inflowing of electric current and production of a receptor potential in the dendrite that propagates toward the cell body. Action potentials are likely generated in the cell body region and spread along the axon to a terminal synapse.

result in an influx of Na^+, and perhaps Ca^{++} ions as well via the ciliary necklace, giving a change in resistance across the membrane and a receptor potential that spreads down the dendrite to the region of the cell body where action potentials may be generated and transmitted via the axon to other elements of the central nervous system.

Thus, in the last decade we have made great advances in understanding the anatomy of nematode sensilla. In conclusion, there still remain several aspects of these sensory structures that require attention. Can we really make meaningful correlations between anatomical variations and ecological differences such as feeding habits and host-seeking activities of different nematodes? Can electrophysiological techniques be refined and applied to test the model for sensillum function suggested here? Can we undertake biochemical studies to identify receptor proteins in nematode chemosensilla? Can we use chemical cues to confuse or decoy nematodes from plants we wish to protect?

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