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Mechanoreceptors in calanoid copepods: designed for high sensitivity

T.M. Weatherby[a], P.H. Lenz[b,*]

[a]*Biological Electron Microscope Facility, Pacific Biomedical Research Center, University Hawaii at Manoa, 1993 East-West Road, Honolulu, HI 96822, USA*
[b]*Békésy Laboratory of Neurobiology, Pacific Biomedical Research Center, University of Hawai at Manoa, 1993 East-West Road, Honolulu, HI 96822, USA*

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Abstract

The mechanoreceptors of the first antennae of *Pleuromamma xiphias*, a mesopelagic calanoid copepod, are critical for the detection of potential threats. These receptors exceed the physiological performance of other crustacean mechanoreceptors in sensitivity to water velocities as well as in frequency response. A study of these receptors was initiated to elucidate structure–function relationships. Morphologically, the receptors resemble the arthropod scolopidial organs by the presence of a scolopale tube. However, the rigidity of the copepod receptors greatly exceeds those described for crustaceans and other arthropods. The scolopale tube completely encloses the distal dendrites and it is firmly anchored to the cuticle. Microtubules are organized in register and arise from microtubule subfibers associated with crescent-shaped rods which extend from the basal body region to the setal socket. The distal dendrites are filled with a large number of cross-linked microtubules. Termination of the distal dendrites inside the lumen of the setae is gradual with a firm anchoring to the cuticle. A likely mechanism for mechanotransduction would involve a linkage between individual microtubules and mechano-gated channels in the dendritic membrane. The rigidity probably contributes to the high frequency sensitivity, and termination of the dendrite inside the seta increases the overall sensitivity of these receptors.

Keywords: Crustacean; Cuticular sensilla; Sensory; Mechanotransduction; Scolopidia; Zooplankton

1. Introduction

Planktonic organisms inhabit an environment characterized by a lack of cover, yet an abundance of predators. Many planktonic organisms, including most marine copepods, are small and they operate at spatial and temporal scales in the millimeter and millisecond ranges. Thus, the sensory environment of these animals is distinct from the more commonly studied arthropods. Behavioral and physiological studies of marine copepods have shown that the sensorimotor performance exceeds that for most other arthropods (Yen et al., 1992; Hartline et al., 1996; Lenz and Hartline, 1999). Presumably this reflects adaptations to these animals' unique environment. Copepods evade predators with a rapid and powerful escape response (Lenz and Hartline, 1999; Lenz et al., 2000). So high is the premium on speed that the more recently-evolved calanoids have shortened their reaction times by the acquisition of myelinated axons (Davis et al., 1999; Lenz et al., 2000).

The escape behavior depends on the detection of potential threats. In arthropods generally, exteroceptive transduction of mechanical signals involves the detection of movement of mechanosensory setae, which protrude into the surrounding medium (for review see Keil and Steinbrecht, 1984; French, 1992; Keil, 1997). In the calanoids, predators are detected via multiple mechanosensory setae located on the prominent first antennae (Gill, 1985; Gill and Crisp, 1985; Hartline et al., 1996; Yen and Strickler, 1996). In the vertebrate cochlear hair cells, mechanoreception occurs via the direct gating of transduction channels (Hudspeth and Gillespie, 1994). Similar mechanisms have been suggested for the insects (Keil and Steinbrecht, 1984; Keil, 1997), spider (Höger et al., 1997) and nematodes (García-Añoveros and Corey, 1997). In the copepods, the seta pivots on its basal hinge and this movement may be detected by mechano-gated ion channels found in the membrane of the sensory neuron. These channels may be activated through tension produced by molecules linked mechanically to a structure that moves relative to the membrane when the seta is displaced. In the insect mechanoreceptors, it has been proposed that bridges between the microtubules in the tubular body of the sensory dendrite and the dendritic membrane may serve this function (Keil and Steinbrecht, 1984). In copepods, the tubular

* Corresponding author. Tel.: +1-808-956-8003; fax: +1-808-956-6984.
E-mail address: petra@pbrc.hawaii.edu (P.H. Lenz).

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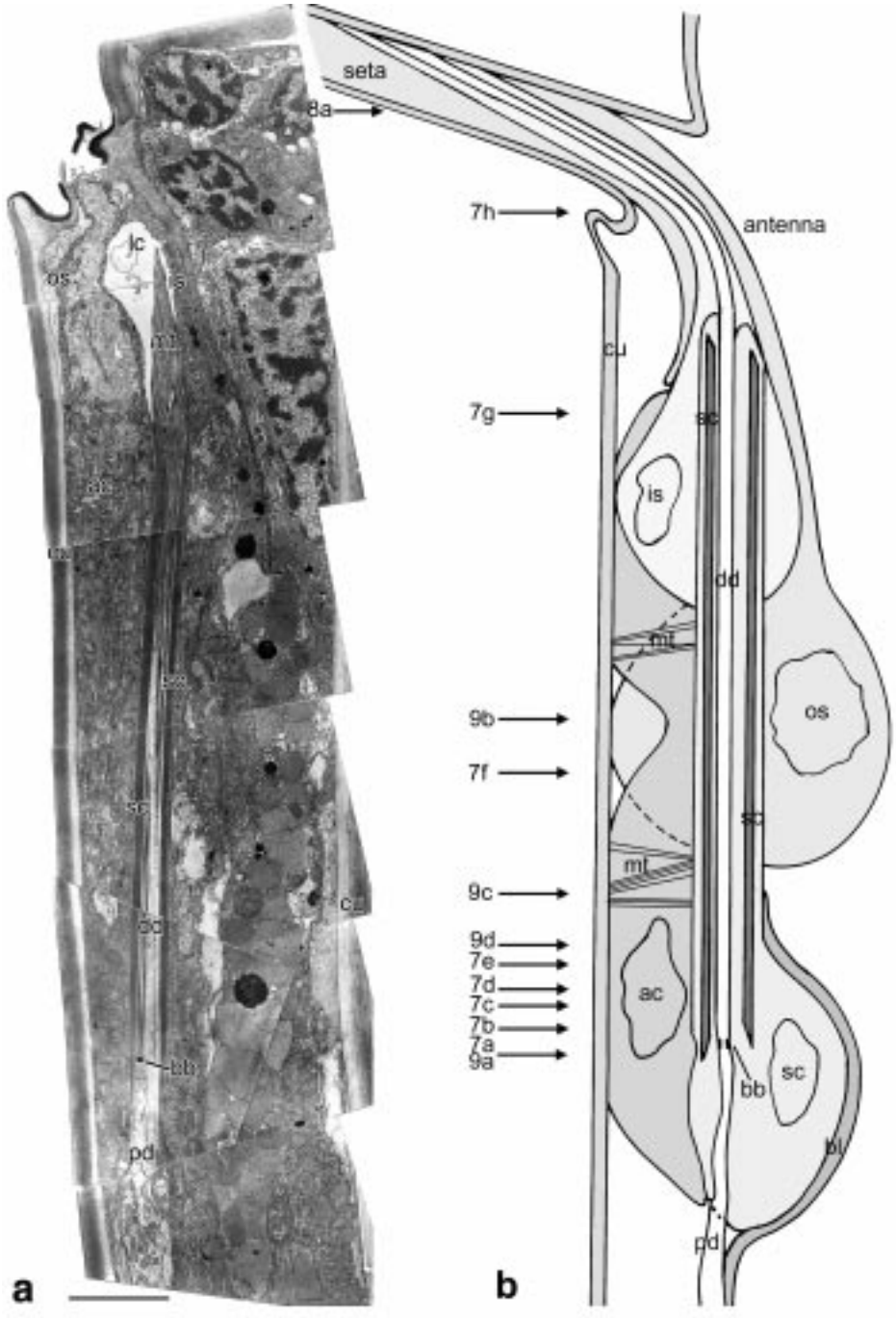

Fig. 1. Arrangement of elements of *P. xiphias* mechanoreceptors. (a) Transmission electron micrograph of a longitudinal section through antennal segment XI (nomenclature of Huys and Boxshall, 1991). (b) Schematic drawing of antennal mechanoreceptor showing relative positions of sensory dendrites and enveloping cells. Only one dendrite shown for clarity. Cross-sections shown in Figs. 7(a)–(h), 8(a) and 9(a)–(d) are indicated by arrows. Schematic drawing not to scale. (ac) anchor cell; (bb) basal body; (bl) basal lamina; (cu) cuticle; (dd) distal dendrite; (is) inner sheath cell; (lc) liquor cavity; (mt) microtubules; (os) outer sheath cell; (pd) proximal dendrite; (sc) scolopale. Bars = 5 μm.

body is absent and the termination of the sensory dendrites occurs inside the seta (Weatherby et al., 1994). Physiological sensitivity and frequency response characteristics of the copepod mechanoreceptors to hydrodynamic stimuli exceed those described for near-field sensory receptors of other aquatic organisms (Yen et al., 1992; Lenz and Yen, 1993; Hartline et al., 1996). These mechanoreceptors, although similar in the overall organization to those in other arthropods, have many unique features, which appear to correlate with their physiological properties (Strickler and Bal, 1973; Gill, 1986; Weatherby and Lenz, 1993, 1995; Weatherby et al., 1994). Structure–function relationships may be better understood by examining systems with unusual performance. How copepods have modified their

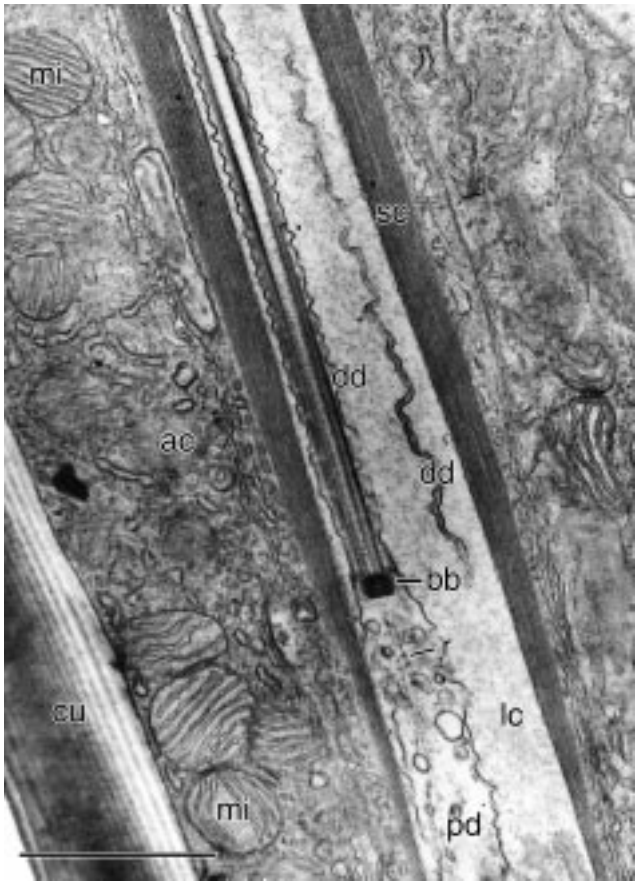

Fig. 2. Higher magnification of basal body region. In the plane of the section, one dendrite (left) and the edge of the second dendrite (right) are visible. (ac) anchor cell; (bb) basal body; (cu) cuticle; (dd) distal dendrite; (lc) liquor cavity; (mi) mitochondrion; (pd) proximal dendrite; (r) rootlet; (sc) scolopale. Bar = 1 μm.

sensorimotor system from the basic arthropod design has potentially broad implications.

2. Materials and methods

2.1. Collection

The study focused on *Pleuromamma xiphias* (Metridinidae), a mesopelagic calanoid copepod. This abundant and widespread species is a strong vertical migrator. During the day, *P. xiphias* occurs below 500 m and at night it feeds in the upper 100 m. In addition to *P. xiphias* we examined the mechanoreceptors of the first antenna of other calanoid copepods collected from around the Hawaiian Islands. *Labidocera madurae* (Pontellidae), *Undinula vulgaris* (Calanidae) and *Euchaeta rimana* (Euchaetidae) were collected in surface nettows (0.5 m diameter, 333 μm mesh) in Kaneohe Bay and off the Oahu coast. *P. xiphias* (Metridinidae) and *Gaussia princeps* (Metridinidae) were collected from the outflow of a deep pipe with a 586 m intake depth located at the Natural Energy Laboratory of Hawaii at Ke'ahole Point, Island of Hawaii. A 183-micrometer mesh net was attached to the 30.5 cm diameter, 800 m long PVC pipe and animals were removed and sorted every 6–8 h. Intact calanoids were placed into 2 or 4-l jars, maintained at either room temperature (*L. madurae*, *U. vulgaris*, *E. rimana*) or 6–8°C (*P. xiphias* and *G. princeps*) for less than 3 days prior to fixation. Only actively swimming animals with intact antennae were used for TEM.

2.2. Transmission Electron Microscopy

Methods for fixation and preparation for TEM are a modification of Weatherby (1981). Penetration of fixative into the first antenna (A1) was aided by making an incision behind the head with iridectomy scissors just prior to fixation. Animals were preserved in 4% glutaraldehyde in 0.1 M sodium cacodylate buffer with 0.35 M sucrose (pH 7.6), decalcified for 1–1.5 h in fixative with 2% disodium EDTA, then returned to buffered glutaraldehyde for 0.5 h, before being washed in buffer. Animals were post-fixed in buffered 1% osmium tetroxide and dehydrated in a graded ethanol series and propylene oxide, then embedded in LX-112 (Ladd). Serial ultrathin (90 nm) sections through the antenna, obtained with a Reichert Jung Ultracut E microtome, were double stained with uranyl acetate and lead citrate, and viewed and photographed in a Zeiss 10/A TEM and a LEO 912 EFTEM at 80 and 100 kV.

2.3. Antibody staining

For immunolabeling, specimens were fixed in 2% paraformaldehyde and 0.5% glutaraldehyde in 0.2 M Sorensons's phosphate buffer with 0.35 M sucrose, washed and dehydrated as described above, then embedded in LR White resin, and polymerized with UV light at −20°C. Eighty to 90 nm sections on nickel grids were labeled with either mouse anti-β-tubulin monoclonal antibody (Sigma) at 1:100 or with mouse anti-actin mab (Amersham) at 1:100, followed by goat-anti-mouse coupled to 10 nm colloidal gold (Ted Pella, Inc.) at 1:40.

3. Results

3.1. Overall cellular organization

The mechanosensory setae of the first antenna (A1) in *P. xiphias* are spiniform with a relatively thick cuticle (Lenz et al., 1996). Setal lengths vary from short (ca. 10 μm) to very long (>1 mm), although most are appproximately 100 μm in length (Weatherby et al., 1994; Lenz et al., 1996). Except for a few singly innervated setae of the distal tip, these spiniform setae are innervated by two large mechanosensory dendrites and several small, presumably chemosensory dendrites (Lenz et al., 1996). *P. xiphias* possesses long dendritic processes from the primary sensory neurons to the setae. A rigid arrangement of the outer

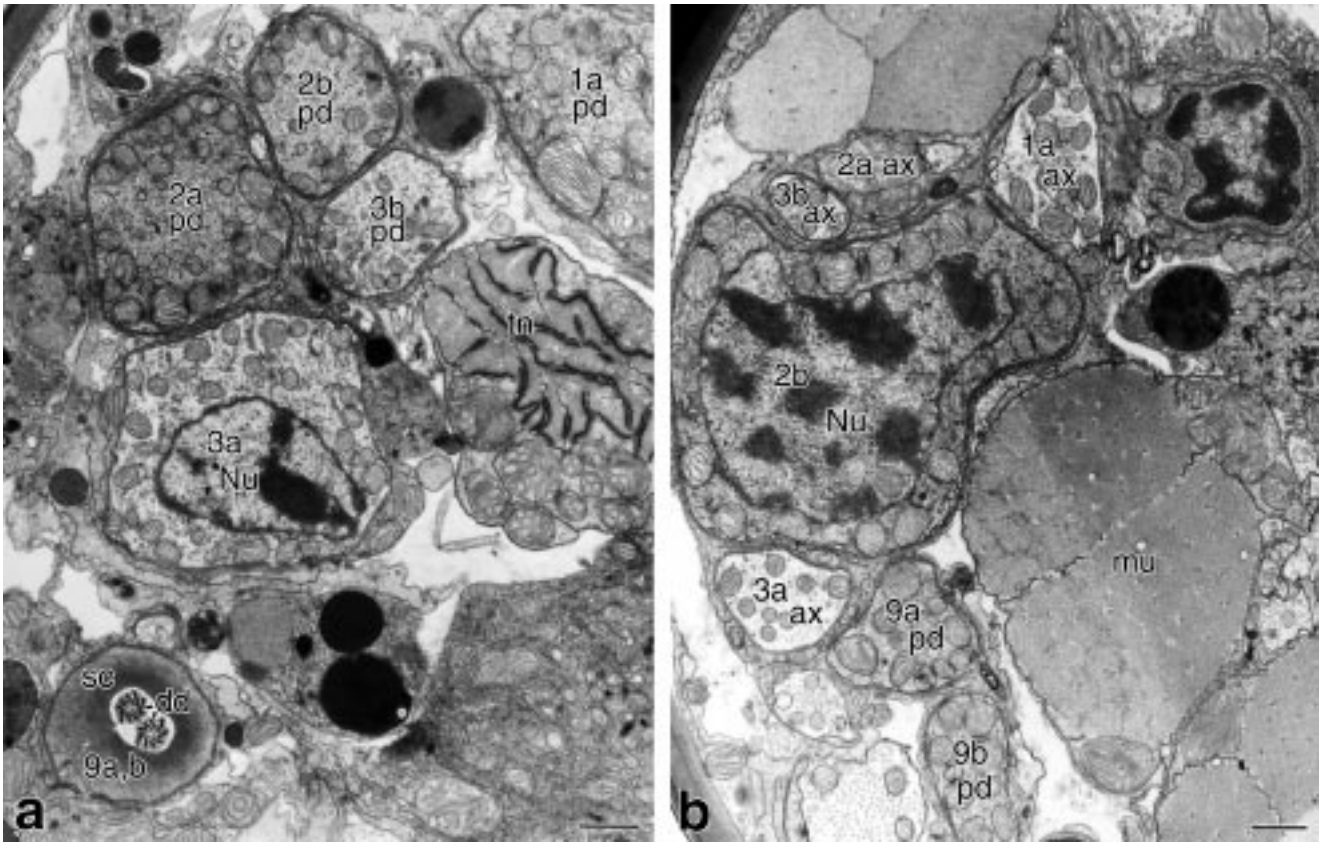

Fig. 3. Cell bodies and proximal dendrites of mechanosensory neurons. Cross-sections through the antenna tip, segment XXVI, showing sensory cells from 4 dually innervated setae. Corresponding pairs of dendrites are labeled a or b. (a) Electron micrograph showing one of the sensory cell bodies with its nucleus from seta 3, proximal dendrites from setae 1, 2 and 3, and distal dendrites from seta 9 (nomenclature of Weatherby et al., 1994). (b) Electron micrograph showing one of the sensory cell bodies from seta 2, proximal dendrites from seta 9, and axons from setae 1, 2 and 3. Cross-section taken ca. 4 micrometers proximal to (a). (ax) axon; (dd) distal dendrite; (mu) muscle; (Nu) sensory cell nucleus; (pd) proximal dendrite; (sc) scolopale cell; (tn) tendon. Bars = 1 μm.

dendrite and supporting cell characterizes its cellular organization (Fig. 1). The elongation of the distal segment provides more points of attachment for anchors both inside the A1 and the seta. The somata of the bipolar sensory cells are located in the antennal shaft well proximal to the seta they innervate. Distal to the paired somata the appearance of basal bodies in the dendrites is preceded by the proximal beginning of a tubular electron-dense scolopale (Fig. 2). As the dendrites proceed distally, they become densely packed with proliferating microtubules. Along this entire length, the distal dendrites are surrounded by a particulary well-developed scolopale tube. This tightly packed unit is firmly anchored to the cuticle of the A1 via microtubule bands arising from the anchor cell [Figs. 1(b), 9(c) and (d)]. The scolopale tube terminates near the hinge, where the microtubules and surrounding dendrites bend and enter into the lumen of the seta. Inside the seta the dendrites are enveloped by inner and outer sheath cells [Figs. 1(b) and 8]. The dendrites and their microtubules terminate by firmly attaching to the setal cuticle. The termination of the dendrites is staggered and the attachment region extends into the seta (Fig. 5).

Other species examined include *G. princeps*, *L. madurae*, *U. vulgaris*, *E. rimana*. There are differences in the external structures of cuticular sensilla in different species. Lengths of individual setae vary among species, and some calanoids have setules on their spiniform setae. The total number of setae also differs among species. However, from the sensory cell to the attachment of the distal dendrite inside the lumen of the setae, the internal structure of the mechanoreceptors is conserved. Setae are typically innervated by two mechanosensory dendrites. The dendrites are characterized by large numbers of microtubules, and each pair is enclosed by a well-developed scolopale. Proximally, the axon morphology shows two distinct patterns: in some species the axons are enveloped by multiple layers of myelin (*U. vulgaris*, *E. rimana*; Davis et al., 1999; Lenz et al., 2000), whereas in other species the axons have a few glial cells surrounding individual axons (*P. xiphias*, *G. princeps*, *L. madurae*; Davis et al., 1999; Lenz et al., 2000).

3.2. Bipolar sensory cell

3.2.1. Soma

The sensory somata are located in the A1 ca. 90 μm proximal to the setae they innervate. Cell bodies typically

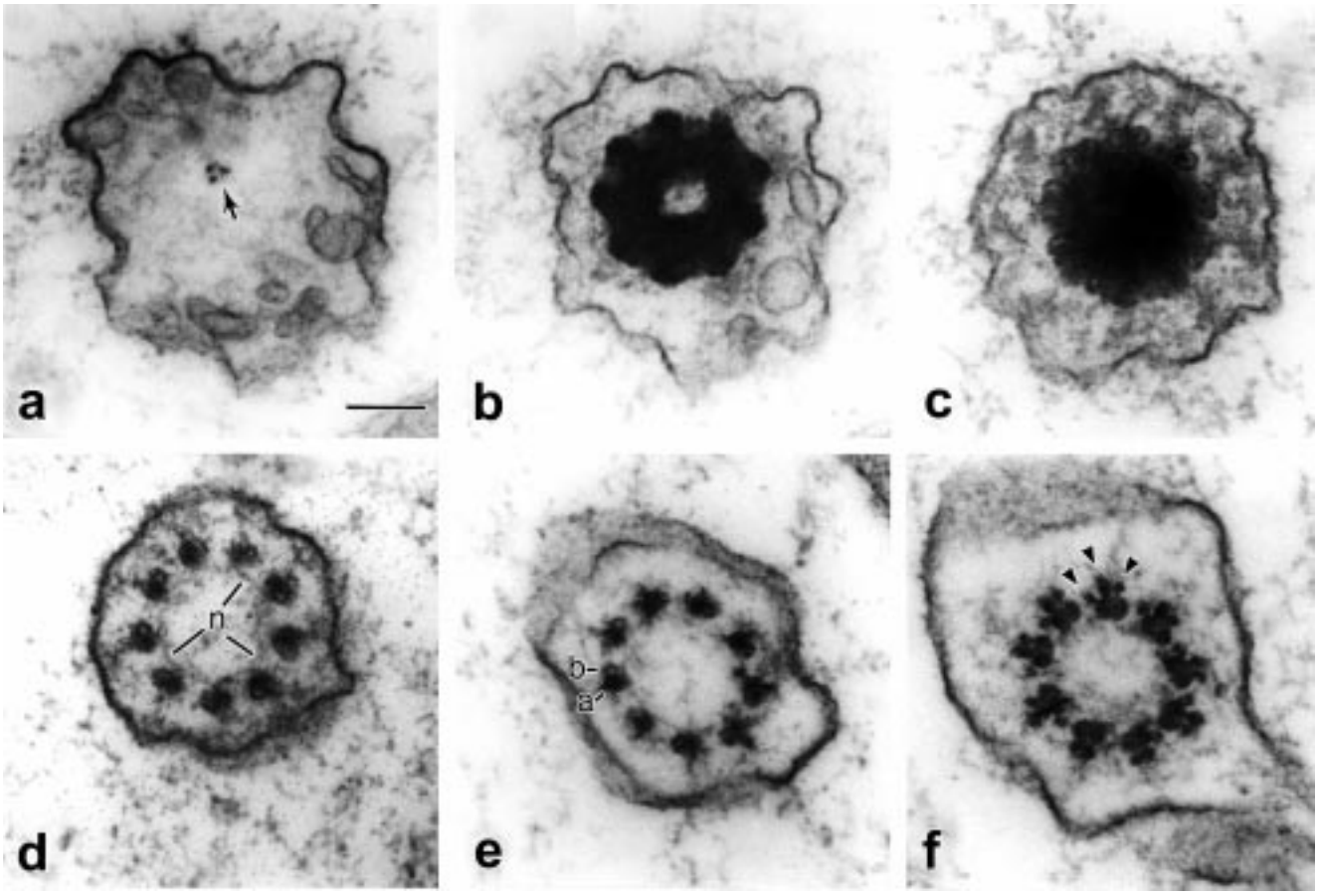

Fig. 4. Basal body and early transition region of sensory dendrite. (a) Cross-section proximal to the basal body, arrow points to three rootlet fibers. (b) and (c) Cross-sections through electron-dense basal body. (d) and (e) 9×2 microtubular circlet. (f) Cross-section ca. 800 nanometers from (b), just proximal to appearance of crescents. (a), (b) A and B microtubule subfibers; (arrowheads) electron dense arms; (n) nexin. Bar = 0.1 μm.

are ovoid to polyhedral in shape and measure ca. 10–35 μm [Fig. 3(a)]. The large nucleus is not necessarily centered within the soma. No invaginations of the cell or nuclear membrane were observed. The perikarya contain numerous large (ca. 0.5–1 μm) mitochondria with lamellar inner cristae, tubular smooth endoplasmic reticulum, ribosomal endoplasmic reticulum, free ribosomes, sparse Golgi and microtubules. Each cell soma is surrounded by a single thin layer of a sheath cell process, separated from the neuron by a narrow (60–80 nm) extracellular space which contains a moderately electron-dense, finely granular basal lamina. The basal lamina extends distally around the proximal dendrites and for a portion of the length of the distal dendrites. The cell body narrows through the axon hillock to an axon which extends proximally towards the cephalosome. Axons contain mitochondria, longitudinally-oriented microtubules, and a few electron-lucent vesicles. Axons surrounded by a single glial cell join the large posterior nerve, located near two hemocoel cavities (Lowe, 1935; Davis et al., 1999; Weatherby et al., 2000).

3.2.2. Proximal dendrite

The proximal dendrite is the dendritic process extending from the cell body to the ciliary portion [Fig. 1(b)]. These dendrites contain large (ca. 1 μm) and smaller (ca. 0.3 μm) mitochondria, smooth endoplasmic reticulum, ribosomal endoplasmic reticulum, free ribosomes, and microtubules in a finely-granular cytoplasm [Fig. 3(a) and (b)]. The proximal dendrites are surrounded by the same extracellular basal lamina and a single layer of cell cytoplasm as the soma [Fig. 3(a) and (b)]. The cell bodies and proximal dendrites are individually wrapped up to the ciliary region.

In the dually-innervated mechanoreceptors, as the paired dendrites approach the ciliary region and become apposed to one another, the basal laminae appear to fuse and become a common layer. The scolopale cell insinuates itself between the dendrites and the basal lamina, forming an irregular extracellular tubular cavity around each of the dendrites. This is the proximal beginning of the liquor cavity. It contains a pale, finely granular material [Figs. 1(a) and 2].

3.2.3. Basal body ('ciliary') and transition region

Each dendrite contains a single electron-dense basal body (Fig. 4). In the dually innervated mechanoreceptor, the basal bodies are slightly staggered, reflecting the relative positions of their cell bodies [Fig. 7(a)]. A long rootlet

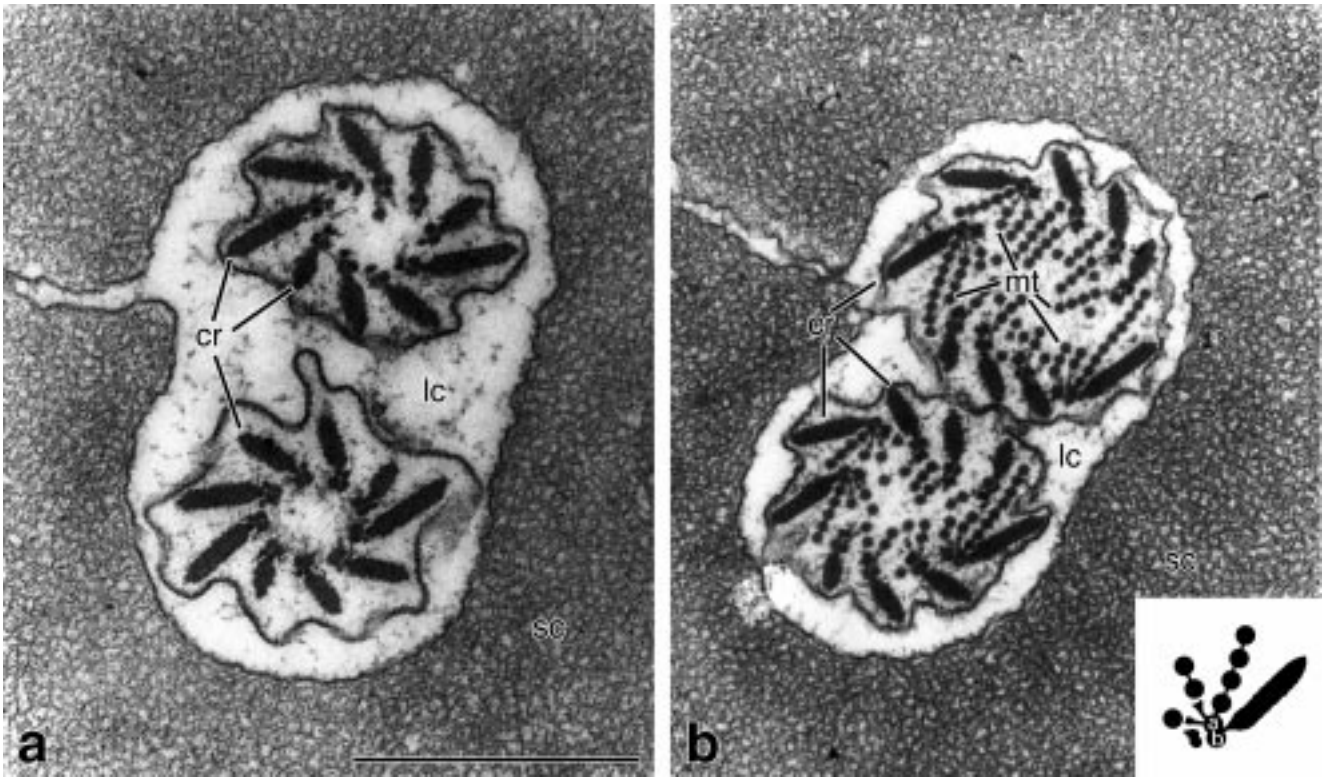

Fig. 5. Proliferation of microtubules. Cross-sections through regions distal of Fig. 4(f). (a) Crescents and A and B subfibers. (b) Budding of microtubules in register from crescents. Note fibrillar nature of scolopale. Inset: schematic drawing of microtubule doublet and crescent with rows of microtubules. (cr) crescents; (mt) microtubules; (sc) scolopale; (lc) liquor cavity. Bar = 0.5 μm.

composed of three to ten striated fibers extends proximally [Fig. 4(a)]. In contrast to the rootlets of insect as well as other crustacean mechanoreceptors, it seems fragile and lacking in anchoring ability. Distally, there is a transition between the basal body and the region where the microtubule proliferation begins in the distal dendrite [Fig. 4(b)–(f)]. First, a ciliary ring of nine microtubule doublets becomes visible [Fig. 4(d)] and electron-dense A-subfibers and B-subfibers can be discerned [Fig. 4(d) and (e)]. Three thick and one or more thin arms project from each doublet. Lateral thin arms arising from the A-subfiber are consistent with the appearance and position of nexin arms [Fig. 4(d) and (e)]. The middle thick arm lengthens and thickens to form a crescent shaped rod, which appears to project from

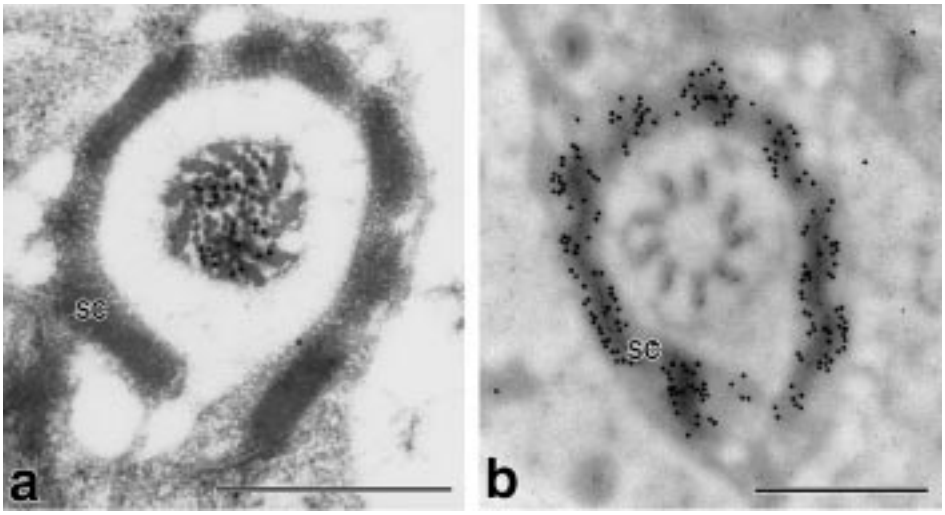

Fig. 6. Immunolabeling with colloidal gold of receptor structures. (a) Microtubules with anti-β-tubulin. (b) Scolopale tube with anti-actin. Both micrographs are from singly-innervated mechanoreceptors in the antennal tip. Bars = 0.5 μm.

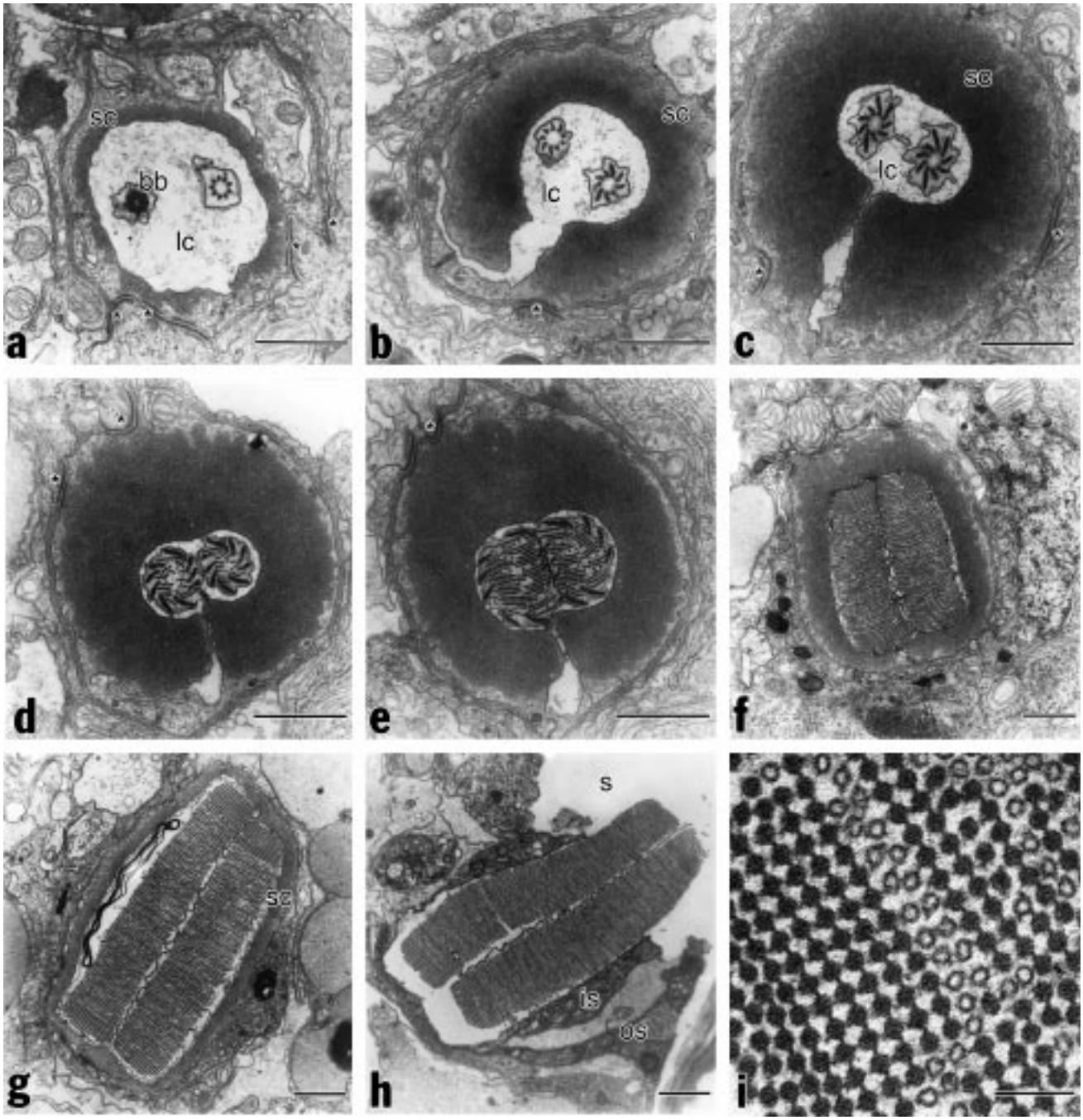

Fig. 7. Distal dendrite. Cross-sections through the dendrite from the basal body region to the setal hinge. Locations of cross-sections (a)–(h) are shown in Fig. 1. (a) Basal body and early transition region. Scolopale tube is thin, anchored by multiple hemidesmosomes to the anchor cell and the cuticle. (b)–(e) Elongation of crescents, addition of microtubules and thickening of scolopale tube as sections progress distally. (f) and (g) Clearing of some of the microtubule cores and thinning of scolopale tube. (h) Socket region of seta, where dendrites bend to enter lumen of seta. Scolopale tube has disappeared, and inner and outer sheath cells can be seen. The maximum number of microtubules occurs here. (i) Rows of microtubules near socket, showing some microtubules have lost their dense cores. Bridges occur between dense cored microtubules within, and sometimes between, rows. (bb) basal body; (is) inner sheath cell; (lc) liquor cavity; (os) outer sheath cell; (s) socket; (sc) scolopale tube; (*) hemidesmosomes. Bars (a)–(h) = 1 μm, bar (i) = 0.1 μm.

between the A- and B-subfibers [Figs. 4(f) and 5(a)]. Each crescent extends outward at an angle in a pinwheel fashion. One or more arms project inward from the A-subfibers. It is at the end of these arms that microtubules seem to be initiated and rows of 25-nm diameter microtubules with electron-dense cores radiate from each A-subfiber [Fig. 5(b), inset]. The microtubules as well as the doublets labeled with anti-β-tubulin, however, the crescents did not [Fig. 6(a)]. Two more electron-dense circular profiles can be seen lying just inward from the doublet, and microtubule

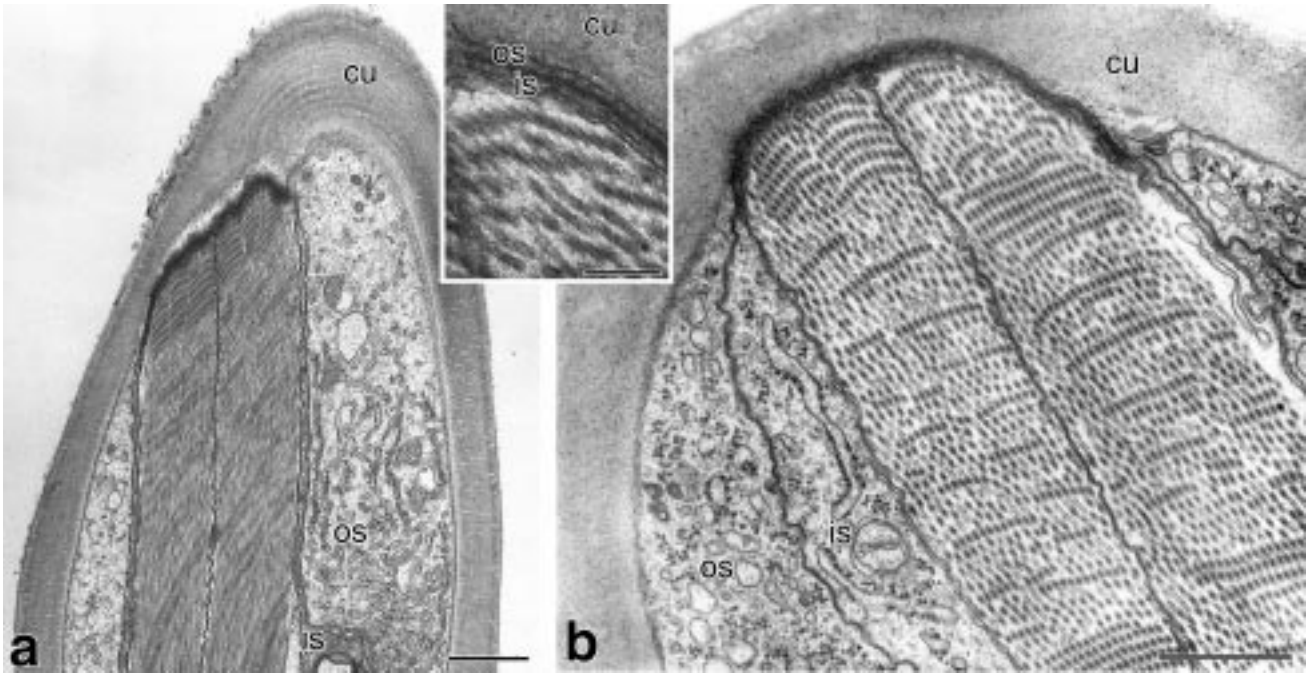

Fig. 8. Termination of the distal dendrites inside the seta. (a) and (b) and inset. The inner and outer sheath cells wrap around the microtubule-filled distal dendrites inside the seta. Staggered attachment of the dendrites to the setal cuticle occurs on the distal wall. (cu) cuticle; (is) inner sheath cell; (os) outer sheath cell. Bar (a) = 1 μm, bar (b) = 0.5 μm, bar (inset) = 0.1 μm.

initiating arms may project from them as well. However, these two structures are not always as large in diameter as microtubules.

3.2.4. Distal dendrites

Cross-sections through the distal dendrites show the progression from basal body to microtubule-filled dendrites in Fig. 7. In the paired dendrites the crescents occur in an identical orientation, with three short crescents in each dendrite matched across the membrane [Fig. 7(d)–(h)]. These crescents, which have not been described in other organisms, are a prominent feature in the cross-sections through the distal dendrites [Fig. 7(c)–(f)]. Forty microtubules are added per micrometer of distal progression and this proliferation continues until the socket region of the seta. The microtubules arising in each row are connected by thick electron-dense bridges [Fig. 7(d) and (e)]. New rows are periodically initiated and older rows detach from the arms. Bridged rows may span the entire width of the dendrite, or may project only partway from each side of the dendrite. The dendritic cross-sections elongate from a nearly circular profile to an oval and finally to a rectangular shape as the number of microtubules increases (Fig. 7). Inside the A1, the dendrites are located near the anterior edge with a short side of the rectangle nearest to the cuticular wall. In the dually innervated mechanoreceptors the longer sides of the rectangular dendrites contact each other. The overall size of the crescents diminishes distally until they disappear near the point of maximal abundance of microtubules [Fig. 7(g) and (h)].

The peak number of microtubules near the setal socket varies among setae ranging from less than 150 in the short, singly innervated mechanoreceptors of the distal tip to over 2900 microtubules per dendrite in the long, dually innervated distal setae (Weatherby et al., 1994). A peak of ca. 1000 microtubules is characteristic of the spiniform setae along the antennal shaft. The maximum number of microtubules is correlated with the length of the scolopale tube (Weatherby et al., 1994). The microtubules are packed in broken rows at a density of $590 \pm 30\ \mu m^{-2}$ [Fig. 7(f)–(h)]. Bridges of electron-dense material between microtubules within and between rows can often be discerned [Fig. 7(i)]. The microtubule rows that radiate from the longer crescents are particularly well-organized [Fig. 7(e)–(g)]. The microtubules fill the entire dendrite and no other cellular organelles are visible. Proximally, microtubules have electron-dense cores. Distally, some of the microtubules (as many as 40%) have electron-lucent centers, and occasionally a few have half-filled centers [Fig. 7(i)]. The number of microtubules with electron-lucent centers progressively increases distally and into the seta.

Prior to entering the seta, the crescents disappear and the proliferation of microtubules stops [Fig. 7(h)]. In the socket region the two dendrites bend and enter the lumen of the seta side-by-side with the narrow face of their rectangular profile on the inner and outer edges of the bend. The rows of microtubules are aligned with the side of the seta that bends (Fig. 8). The dendritic termination occurs only along the distal (inner) wall of the seta, whether anteriorly or posteriorly directed. Setae are hinged so as to be more readily deflected

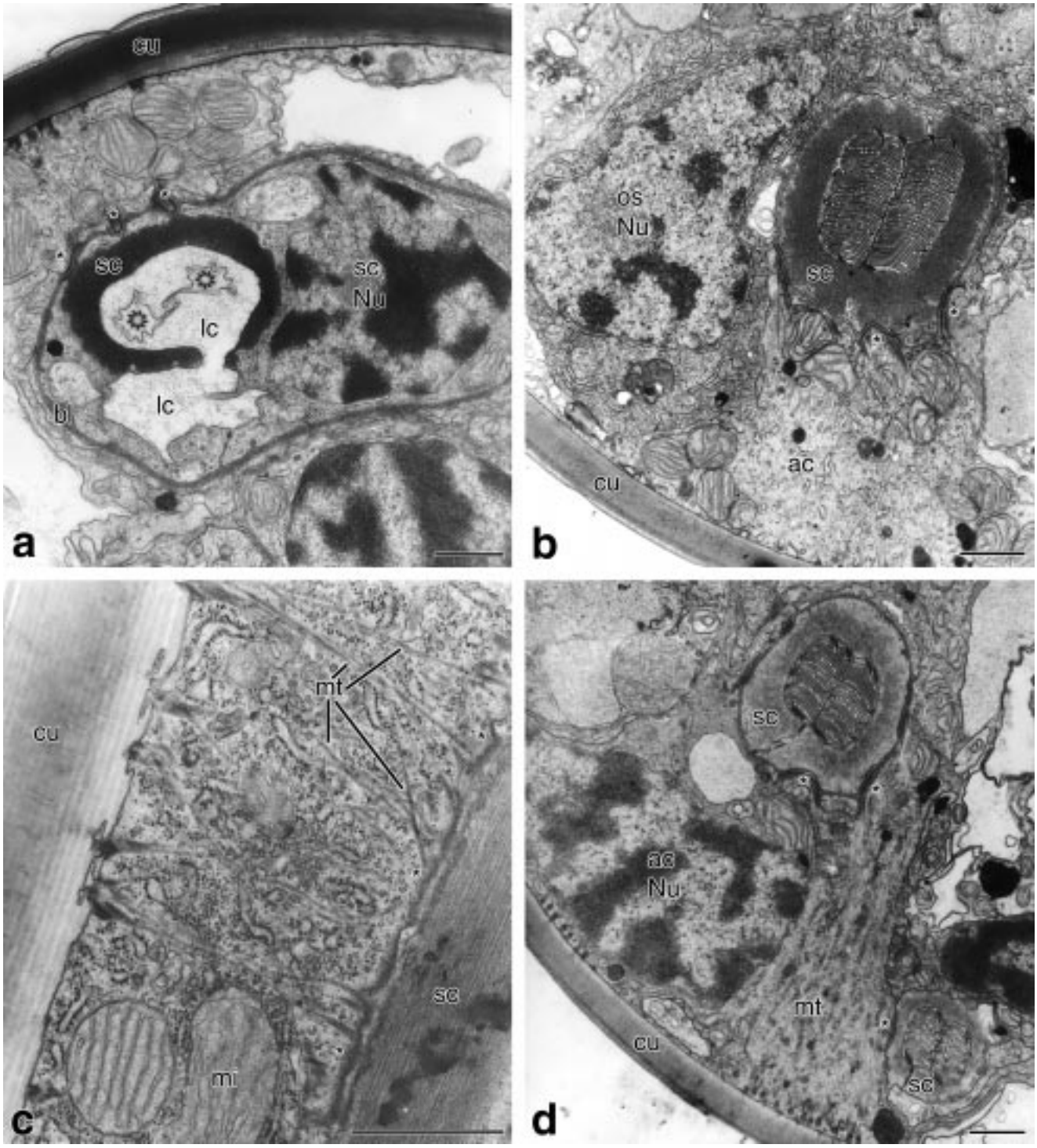

Fig. 9. Accessory cells. (a) Cross-section of distal dendrites near the basal body region showing nucleus of scolopale cell, thinning of scolopale tube, and liquor cavity. (b) Cross-section through distal dendrite showing anchor cell and nucleus of outer sheath cell. (c) Long section of anchor cell showing the microtubular bands that attach the scolopale cell to the cuticle of the antenna. (d) Nucleus of the anchor cell and microtubular band. (ac) anchor cell; (bl) basal lamina; (cu) cuticle; (lc) liquor cavity; (mi) mitochondrioan; (mt) microtubules; (Nu) nucleus; (os) outer sheath cell; (sc) scolopale; (*) hemidesmosomes. Bars = 1 μm.

in a distal than a proximal direction for large movements. The dendrites are completely surrounded by the inner and outer sheath cells, which form two layers [Fig. 8(b)]. In the attachment region, these cells are very narrow and their cytoplasm is characterized by a thin electron-dense layer (Fig. 8, inset). At the boundary between the two distal dendrites, the membranes of the sheath cells loop back. The attachment zone is also characterized by a narrowing of the cuticle with a firm anchoring between cuticle, sheath cells and dendritic membrane (Fig. 8). The rows of

microtubules along this edge terminate, and a gradual decrease in the number of microtubules is observed.

3.2.5. Accessory cells

The distal dendrites are associated with four accessory cells: scolopale, anchor, and inner and outer sheath cells [Figs. 1(b) and 9]. Each sensory seta has a scolopale and two sheath cells surrounding the dendrites. The anchor cell may be shared by several mechanosensory setae. The anchor cell is rich in some small but mostly large mitochondria, rER, free ribosomes, sER, multivesicular bodies and other vesicles, and Golgi [Figs. 2 and 9(b)–(d)]. This cell is involved in anchoring the scolopale cell to the cuticle (see below). The inner and outer sheath cells extend into the seta where they completely envelop the distal dendrites [Figs. 1(b) and 8]. The main portion (including the nucleus) of the inner sheath cell is located along the distal third of the scolopale tube. The cell contains many large mitochondria, ribosomes, rER, Golgi and small, electron lucent vesicles. Near the setal hinge, where the scolopale tube ends, a narrow process from this cell enwraps the distal dendrites. The main portion of the outer sheath cell is located along the middle of the scolopale tube [Figs. 1(b) and 9(b)]. This cell partially encircles the scolopale cell, leaving room for the anchor cell. The cell is characterized by an abundance of sER, some ribosomes and rER, and Golgi [Fig. 8(b)]. Distally, a process of the outer sheath cell extends into the lumen of the seta where it enwraps the inner sheath cell and dendrites. This cell appears to anchor the dendrites to the cuticle (Fig. 8).

3.2.6. Scolopale cell and liquor cavity

A scolopale cell is associated with each spiniform seta and its mechanosensory dendrite(s). This cell consists of a large cell body in the proximal dendrite region, an irregularly shaped cytoplasm and a prominent electron-dense tube, which is almost entirely filled with scolopale fibrils (Figs. 2, 5 and 7). The scolopale tube extends from proximal of the basal body to the socket region of the seta being innervated [Figs. 1, 2, 7(g) and (h)]. The scolopale tube is composed of a longitudinally arranged, fibrillar, electron-dense substance which labels with anti-actin antibodies, as in other crustaceans [Fig. 6(b)]. Near the basal body, the scolopale cell encloses the dendrites but it lacks the electron-dense fibrils. Distally, in the region where microtubules begin to proliferate within the dendrites, the scolopale cell is filled almost entirely with fibrils, and no other cell processes intrude (Fig. 7). Each dendrite or pair of dendrites is completely surrounded by the scolopale tube, except for a narrow opening (Figs. 5 and 7). The scolopale tube is firmly fastened to the antennal cuticle via the anchor cell [Fig. 9(c) and (d)]. There are hemidesmosomes linking the scolopale cell to the basal lamina. Similarly, hemidesmosomes connect the anchor cell to the basal lamina. These double hemidesmosomes across the basal lamina form a tight connection between the scolopale and anchor cells. Strands of microtubules extend from each hemidesmosome in the anchor cell to the cuticle [Fig. 9(c)]. These strands occur at intervals along the distal dendrite and they form a diagonal, criss-cross pattern [Fig. 1(b)]. Because the strands are not uniformly distributed along the scolopale tube, they are not visible in all planes or cross-sections [Figs. 1(a) and 7(f)]. The strands are embedded into the cuticle [Fig. 9(c) and (d)].

The distal dendrites are surrounded by a liquor-filled cavity, which is filled with moderately electron-dense floccular material (Fig. 7). This cavity is spacious near the basal body region, where the dendrites are narrow [Figs. 2, 7(a) and (c)]. As the microtubules proliferate, the dendrites enlarge and the liquor cavity becomes limited to a very narrow space between the scolopale and the dendrites. This tight apposition of a scolopale completely surrounding microtubule-packed dendrite suggests a rigid structure. Approaching the insertion point of the seta, the scolopale tube and cell end and do not extend into the setal lumen. At this point the liquor cavity is visible as a fluid-filled space in which the dendrites may be free to move to the extent that their microtubule-packed condition allows.

4. Discussion

The overall organization of the mechanoreceptors of the A1 in *P. xiphias* is similar to the cuticular mechanosensilla of other crustaceans and arthropods, but it is unique in many specific details. A prominent morphological feature is the large number of highly organized microtubules completely enveloped by a scolopale tube. However, this is not the only striking difference between these mechanoreceptors and those of other crustaceans. The physiological performance and the unusual morphological features have raised questions about structure–function relationships in these receptors. In the discussion we address: (1) comparison between the calanoid receptors and those of other arthropods; (2) mechanotransduction; and (3) structure–function considerations.

4.1. Comparison to other arthropod exteroceptors

In arthropods, including the copepods, bipolar cuticular mechanosensory neurons have dendrites with one or two basal bodies from which multiple microtubules extend towards the base of the sensory hair that they innervate (for review see Keil and Steinbrecht, 1984; Schmidt and Gnatzy, 1984; McIver, 1985). Distally, the dendrites are associated with or attached to the cuticle (Ball and Cowan, 1977; Keil and Steinbrecht, 1984; McIver, 1985). The termination of the dendrites, which usually occurs in the socket region, is presumably the site for mechanotransduction (Gaffal et al., 1975; Keil and Steinbrecht, 1984; Keil, 1997).

The cuticular sensilla in the crustaceans are scolopidial in structure. The scolopidial organs are considered to be the

primitive form of the mechanoreceptor in the arthropods for both the cuticular and sub-cuticular types (e.g. chordotonal organs; Kouyama and Shimozawa, 1982). Although copepod mechanoreceptors have a scolopale cell, their structure differs from the typical scolopidial organ. The latter is characterized by at least one dendrite with a ciliary basal body having very large rootlets (Schmidt and Gnatzy, 1984). The rootlets are in close contact with the dendritic membrane, which makes desmosomal connections to the scolopale, thus firmly anchoring the basal body region of the sensory cell (Ball and Cowan, 1977; Guse, 1978; Altner et al., 1983; Schmidt and Gnatzy, 1984; Schmidt, 1989). In *P. xiphias,* sensory dendrite basal bodies lack large rootlets, and the thin threads that compose the rootlets are not associated with the dendritic membrane. Instead, dendrite anchoring seems to occur more distally through the tight fit of the microtubule-packed dendrites inside the scolopale tube. The electron-dense structure that makes up the scolopale tube in the copepods is uniform and lacks the more granular appearance described in other crustaceans (*Acetes sibogae*: Ball and Cowan, 1977; *Neomysis integer*: Guse, 1978; *Procambarus clarkii*: Kouyama and Shimozawa, 1982; *Carcinus maenas*: Schmidt, 1989). In the latter, this granular appearance is due to the presence of microtubules in the scolopale tube (Wolfrum, 1990; Sugawara, 1996). In *P. xiphias*, microtubules are nearly absent from the scolopale tube, which is primarily composed of actin filaments. Actin filaments have also been found in insect scolopidia (Wolfrum, 1990). In many crustaceans, the scolopale cell presumably secretes the extracellular dendritic sheath or cap that ensheathes the distal dendrites (Guse, 1978; Wolfrum, 1990; Sugawara, 1996). This dendritic sheath, also found in insects, is absent in *P. xiphias*.

In the decapods, the scolopale cell, as well as several enveloping cells surround the sensory dendrites (Ball and Cowan, 1977; Espeel, 1985, 1986; Schmidt, 1989, 1990). These cells appear to be involved in the secretion of cuticular material for the hinge and sensory hair (Guse, 1978; Espeel, 1986; Crouau, 1982). In cross-section, the dendrites in some crustaceans are surrounded by multiple layers of these enveloping cells. In *P. xiphias*, there are three accessory cells in addition to the scolopale cell. The anchor cell attaches the scolopale cell to the cuticle via microtubule bands, thus firmly anchoring the scolopale cell and the dendrites. This type of attachment to the cuticle has not been reported in other crustacean mechanosensilla (*A. sibogae*: Ball and Cowan, 1977; *N. integer*: Espeel, 1985; *C. maenas*: Schmidt and Gnatzy, 1984; Schmidt, 1989). One or more sheath cells follow the dendrites into the lumen of the seta (Altner et al., 1983). In *P. xiphias*, two sheath cells surround the dendrites within the seta. These cells are involved in the distal termination of the dendrites within the lumen of the hair (see below).

Proliferation of microtubules in the distal dendrite is one of the distinguishing features between mechano- and chemosensory neurons. Similar to other crustaceans, the microtubules in the mechanosensory dendrites in *P. xiphias* increase in number from the basal body region towards the seta (Guse, 1978; Altner et al., 1983; Schmidt and Gnatzy, 1984; Espeel, 1985; Schmidt, 1989). However, the distal dendrites of *P. xiphias* differ from other crustaceans in: (1) the maximum number of microtubules exceeds that of other crustaceans by one to two orders of magnitude; (2) the microtubules have electron-dense cores; (3) the presence of electron-dense crescents that extend from the basal body to the setal hinge region; (4) the organization of the microtubules, which are in register and interconnected; and (5) the alignment of rows of microtubules parallel to the attachment to the cuticle. In addition to free microtubules within the dendrite, the insects possess a tubular body, which is a discrete structure that attaches the dendrites to the hinge (Erler 1983; Keil and Steinbrecht 1984). The microtubule-packed distal dendrites in the copepods are more reminiscent of the tubular body of the insects than of crustacean dendrites. The tubular body, composed of microtubules embedded in a fibrous matrix, is a rigid structure (Keil and Steinbrecht, 1984). The distal dendrite in the copepod appears to be similarly rigid: large numbers of microtubules organized in linked rows, with cross-bridges between rows [Fig. 7(i)].

Aquatic crustacean mechanoreceptors lack tubular bodies. Termination of mechanosensory dendrites in crustaceans may be more variable than in the insects. In the crustaceans, an extracellular sheath or cap surrounds the distal dendrites and extends to the termination of the dendrites, which can occur near the setal hinge (Ball and Cowan, 1977; Kouyama and Shimozawa, 1982; Schmidt, 1989, 1990), inside the lumen of the seta (Guse, 1978; Espeel, 1985) or near the tip of the hair (Altner et al., 1983). The cap in which the dendrites end is composed of an electron-dense plug-like material, which links them to the cuticle (Ball and Cowan, 1977; Guse, 1978; Espeel, 1985; Schmidt, 1989). In the calanoid, the distal dendrites terminate along the distal wall of the seta. Attachment to the cuticle occurs via the two sheath cells with greatly reduced cytoplasmic space, instead of the extracellular dendritic sheath. Thus, the region of putative mechanosensory transduction has a significantly different morphology in calanoids than in either other crustaceans or in insects.

4.2. Mechanotransduction

Across taxa, a special class of mechano-gated channels has been implicated in sensory mechanotransduction, the process by which a mechanical stimulus is transformed into a cellular response. From nematodes to vertebrates, mechano-gated channels are believed to be connected via linking proteins to relatively rigid structures both outside and inside of the sensory cell (García-Añoveros and Corey, 1997). Tension on the links is assumed to open the channels and the resultant current flow produces an excitatory receptor potential. In the insects, the distal termination

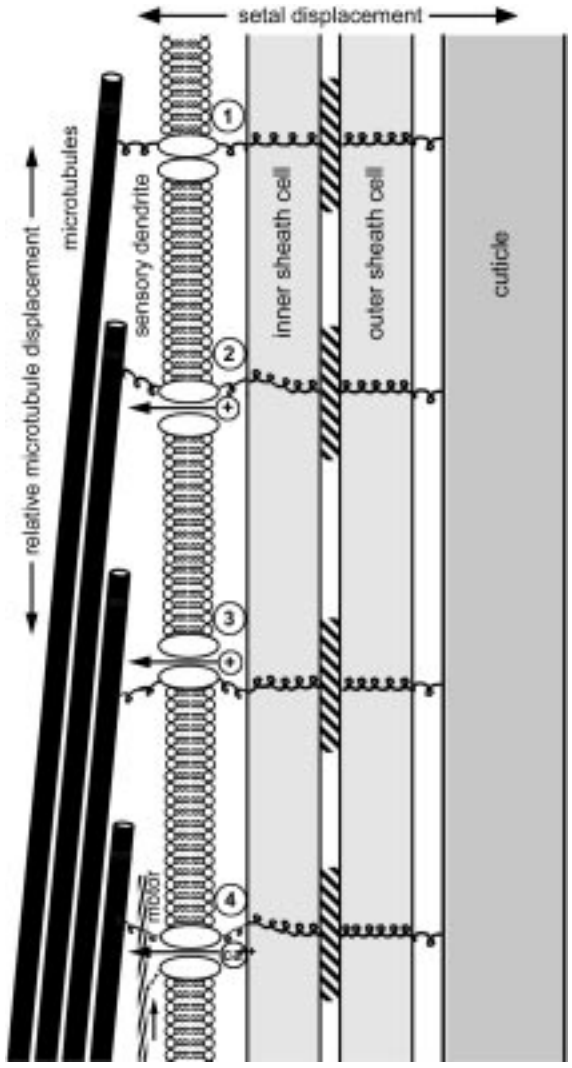

Fig. 10. Proposed model for copepod mechanosensory transduction based on ultrastructure, bidirectional sensitivity and analogy to hypothetical molecular mechanisms in other systems (Hudspeth and Gillespie, 1994; García-Añoveros and Corey, 1997; Höger et al., 1997). Movement of seta translates into a shearing movement of microtubules parallel to the cuticular wall. Mechano-gated cation channels in the dendritic membrane are opened by the tension this produces in elastic connections to the microtubules and the extracellular and intracellular bridges to the cuticle on the opposite side. Both proximal (2) and distal (3) setal movements increase tension and open channels (closed state at minimum tension represented by (1). Adaptation may occur by a molecular mechanism for tension relief (4), e.g. the hair cell 'motor'. Longitudinal view, drawing not to scale.

of the dendrite adjacent to the tubular body appears to be the most likely site of transduction (Gaffal et al., 1975; Keil and Steinbrecht, 1984; Keil, 1997). The tubular body ends abruptly within the socket region, where the microtubules are linked on the cytoplasmic side to the dendritic membrane via 'membrane-integrated cones' (MICs) (Keil and Steinbrecht, 1984). At a specialized point of cuticular contact, the 'stimulating edge', the dendritic membrane is connected externally via 'attachment filaments' to the surrounding sheath and to the cuticle (Keil, 1997). Movement of the hair produces compression of the membrane and an increase in tension at the connecting links (Keil, 1997). Dissociation of the microtubules in the tubular body decreases, but does not eliminate mechanosensitivity (Kuster et al., 1983). An analogous model including molecular detail has been hypothesized for the nematode, *Caenorhabditis elegans*. It proposes one protein (*mec*-2) as the intracellular linkage between the microtubules and the mechano-gated channel and other proteins (*mec*-1, *mec*-5 and *mec*-9) providing connections between the channel and the extracellular mantle (reviewed in García-Añoveros and Corey, 1997). Mutants lacking microtubules are mechanically insensitive (García-Añoveros and Corey, 1997). A similar model seems well-suited for the copepod mechanoreceptor (Fig. 10). The cross-linked microtubules within the dendrites would be linked to the dendritic membrane containing mechano-gated channels. Extracellularly, linkage to the cuticle may be achieved through fiber-like material cross-linking through the ensheathing cells. Periodic lines within the ensheathing cells are suggestive of the presence of such material. In contrast to the features of insect (and presumably other crustacean) mechanoreceptors which promote directional sensitivity, the microtubules and cuticle of copepod antennular setal receptors meet at a shallow angle. Movement of the seta in either direction could cause a shear of the dendritic membrane relative to the rigid microtubules. By this model, tension on the connecting links would increase for either case, explaining physiological observations that these receptors are bi-directional (Lenz et al., in preparation).

4.3. Structure–function considerations

Physiologically, *P. xiphias* mechanoreceptors show two unusual features: they can detect smaller stimuli ($<$10 nm displacement) with higher frequencies (peak displacement sensitivity near 1 kHz) than other crustaceans (Yen et al., 1992; Lenz and Yen, 1993; Hartline et al., 1996). The morphology of the receptor holds the key to the sensory physiology. The distinct morphological features that characterize the calanoid mechanoreceptors are likely to be critical for their physiological performance. The geometry of the termination of the distal dendrites inside the setal lumen, instead of at the base, enhances the physical displacement at the presumed transduction site, thus promoting high sensitivity. Attachment in the hinge area as is typical of arthropod mechanoreceptors minimizes the stretching or compression of the termination area. This suggests a system of reducing the physical movement at the site of mechanotransduction with a possible reduction in sensitivity. Since the mechanical linkages to stretch-sensitive channels permit sensitivity to movements of molecular dimensions, reduction in the physical movement may dampen background noise levels. In contrast, small planktonic calanoids, which move with the ambient water movements, require high sensitivity to detect microdisturbances within the background noise.

The rigidity of the receptor probably limits the elasticity, decreasing compliance between the movable (seta) and stationary (antennal shaft) components, thus enabling the detection of high-frequency signals. The scolopale tube acts as a supporting structure that adds rigidity to the system owing to the thickness of the tube, its tubular geometry and its length. Rigidity of the distal dendrite is further ensured by a firm anchoring of the scolopale to the cuticle wall of the antenna, as well as the abundance of cross-linked microtubules. The stiffness of the structure should enhance mechanical sensitivity, and might reduce 'dash-pot' components of the link, thus increasing relative sensitivity at higher frequencies.

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