

THE FINE STRUCTURE OF COCKROACH CAMPANIFORM SENSILLA

DAVID T. MORAN, KENT M. CHAPMAN, and RICHARD A. ELLIS

From the Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138, and the Neurosciences Section and Cell Biology Section of the Division of Biological and Medical Sciences, Brown University, Providence, Rhode Island, 02912

ABSTRACT

Campaniform sensilla on cockroach legs provide a good model system for the study of mechanoreceptive sensory transduction. This paper describes the structure of campaniform sensilla on the cockroach tibia as revealed by light- and electron-microscopy. Campaniform sensilla are proprioceptive mechanoreceptors associated with the exoskeleton. The function of each sensillum centers around a single primary sense cell, a large bipolar neuron whose 40 μ -wide cell body is available for electrophysiological investigation with intracellular microelectrodes. Its axon travels to the central nervous system; its dendrite gives rise to a modified cilium which is associated with the cuticle. The tip of the 20 μ -long dendrite contains a basal body, from which arises a 9 + 0 connecting cilium. This cilium passes through a canal in the cuticle, and expands in diameter to become the sensory process, a membrane-limited bundle of 350–1000 parallel microtubules. The tip of the sensory process is firmly attached to a thin cap of exocuticle; mechanical depression of this cap, which probably occurs during walking movements, effectively stimulates the sensillum. The hypothesis is presented that the microtubules of the sensory process play an important role in mechanoelectric transduction in cockroach campaniform sensilla.

INTRODUCTION

The campaniform sensillum provides a good model system with which to study sensory transduction. Campaniform sensilla on cockroach legs are sense organs which respond to stresses in the cuticle. Each sensillum includes a single, large bipolar neuron, available to physiological experimentation with intracellular microelectrodes. Preliminary studies with the electron microscope (Moran and Chapman, 1968; Moran, Chapman, and Ellis, 1969) have revealed a number of interesting cell structures which may function in nerve impulse initiation. This paper describes our recent findings in electron microscopic investigations of proprioceptive campaniform sensilla on cockroach legs.

Campaniform sensilla were originally described by Hicks (1857) on dipteran halteres; the name

“sensilla campaniformia” was suggested by Berlese (1909). They exist in many parts of the insect integument subject to strain (Snodgrass, 1935), and occur in several places on cockroach legs (Pringle, 1938). Chapman (1965) discovered that each of the large tactile spines on the tibia functions via a single, basally located campaniform sensillum. Pringle (1938) described 11 distinct groups of campaniform sensilla on *Periplaneta* legs. We have studied those of group 6, located near the proximal end of the tibia, since their location favors electrophysiological investigation with intracellular microelectrodes.

When one looks at the magnified surface of the cuticle in the vicinity of group 6, one sees approxi-

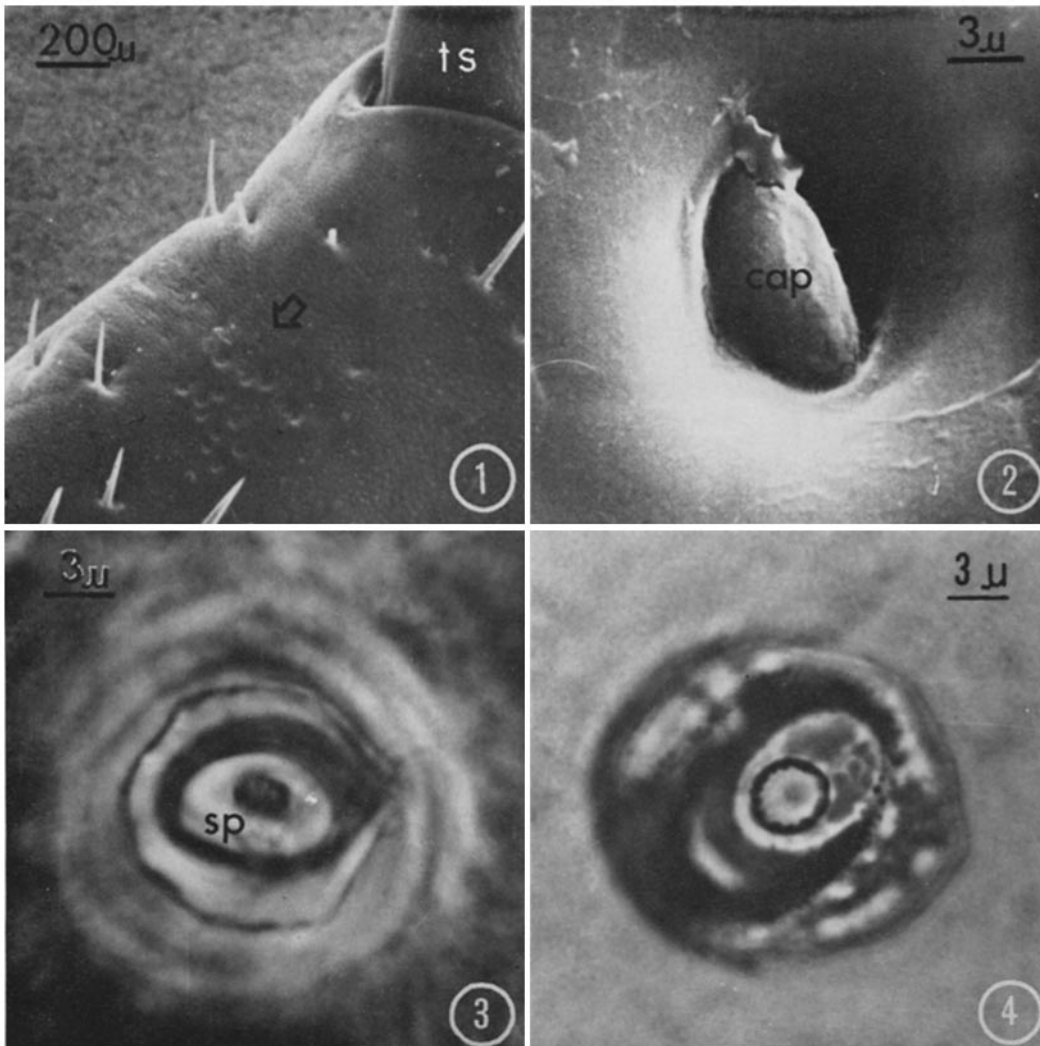


FIGURE 1 Scanning electron micrograph of tibia surface showing 18 campaniform sensilla (arrow) of Pringle's group 6. Several small tactile hairs are visible; base of large tactile spine is seen at upper right. $\times 200$.

FIGURE 2 Scanning electron micrograph of cap of single campaniform sensillum. $\times 3000$.

FIGURE 3 Phase-contrast photomicrograph of unstained whole mount of fixed cuticle and campaniform sensillum, seen from above. Sensory process is visible through the thin, translucent cap. $\times 2400$.

FIGURE 4 Cross-section of distal portion of campaniform sensillum, cut beneath cap. Central darkly-staining ring represents cuticular sheath surrounding sensory process. 1μ Epon-Araldite section stained with toluidine blue. $\times 2000$.

mately 12–18 ovoid cuticular discontinuities; each represents the cap of a campaniform sensillum, a thin piece of cuticle directly attached to an extension (a modified cilium) of the dendrite of a bi-

polar neuron. Campaniform sensilla are probably proprioceptive mechanoreceptors (Pringle, 1938). When a cockroach puts weight on its leg, the cuticle of the leg is strained. The resultant distortion of

leg cuticle is probably accompanied by relative displacement of the thin cuticular cap and the attached sensory process. These displacements lead to a train of nerve impulses which are propagated along the axon of the bipolar neuron directly to the central nervous system, where the transmission is integrated, informing the animal that there is a force on his leg.

Electron microscopy of insect mechanoreceptors began with E. G. Gray's (1960) detailed description of the fine structure of the locust ear. A number of other mechanoreceptors have been studied since Gray's pioneer work (see Thurm, 1968, for discussion). Insect mechanoreceptors associated with the cuticle appear to share several common architectural features. Their function centers around a bipolar neuron, whose axon extends toward the central nervous system, and whose dendrite extends toward the surface of the cuticle. The dendrite tip contains a basal body, from which a "9 + 0" cilium arises. In some cases, such as the locust ear, the cilium itself attaches to the modified cuticle. In other cases, such as the campaniform sensillum (Stuart and Satir, 1968; Moran et al., 1968, 1969; Chevalier, 1969), the cilium is modified to form a sensory process which inserts into the cuticle.

MATERIALS AND METHODS

Cultures of the cockroach *Blaberus discoidalis* were maintained in plastic rat cages at room temperature. Rat chow and water were continuously available; computer cards were provided for shelter. Roaches were anaesthetized with CO₂ prior to amputation of legs at the femoro-tibial joint. Karnovsky's formaldehyde-glutaraldehyde solution (Karnovsky,

1965), injected through the tibia, fixed the epidermis and campaniform organs of mature roaches well. Newly moulted animals, however, were fixed in 3–5% glutaraldehyde buffered to pH 7.3 with 0.05 M sodium cacodylate. 10 min after fixative injection, cylinders of tibia containing campaniform sensilla of Pringle's group 6 were cut, and placed in iced fixative for 1–2 hr. Tissues were washed in buffer for 10 min, postfixed in cold buffered 1% OsO₄ for 1 hr, dehydrated in a graded acetone series at room temperature, and infiltrated either with an Epon-Araldite mixture (Voelz and Dworkin, 1962) or with the low-viscosity epoxy resin recently developed by Spurr (1969). The Spurr embedding medium permitted the cutting of thin sections through the hard cuticle of tanned adults parallel to the plane tangent to the cuticle surface; this type of section was impossible to obtain with Epon-Araldite. Thin sections, cut with DuPont diamond knives on Porter-Blum MT-2 ultramicrotomes, were stained in uranyl acetate and Reynolds' (1963) lead citrate. The transmission electron micrographs were taken with RCA EMU 3D, EMU 3F, Hitachi 11-C, and Philips 300 electron microscopes. Scanning electron micrographs of gold palladium-coated specimens were taken with a JEOLCO JSM-2 scanning electron microscope, kindly made available by Dr. T. Kuwabara of the Harvard Medical School.

OBSERVATIONS

Pringle's Group 6

Pringle (1938) described 11 clusters of campaniform sensilla on the cockroach leg. The campaniform sensilla examined in this investigation originate from group 6, located at the proximal end of the tibia shown in surface view by the scanning electron micrograph in Fig. 1. In adult *B. discoid-*

Abbreviations

asc, accessory supporting cell
bb, basal body
cap, cap of campaniform sensillum
cc, connecting cilium
cut, cuticle
d, desmosome
den, dendrite of bipolar neuron
ec, enveloping cell
ecs, extracellular space
exo, exocuticle
ga, Golgi apparatus
gl n, glial cell nucleus
L-1, layer 1 of cap cuticle

L-2, layer 2 of cap cuticle
mi, mitochondrion
mt, microtubules
N, nucleus
ra, ciliary rootlet apparatus
sh, cuticular sheath surrounding sensory process
sj, septate junction
so, subgenual organ, a vibration receptor
soma, cell body of bipolar neuron
sp, sensory process
tr, trachea
ts, tactile spine
ves, vesicles

dalis, group 6 contains from 12 to 25 campaniform sensilla. First-instar nymphs, however, exhibit only two sensilla in group 6, indicating that new campaniform organs are differentiated during successive stages of development.

The surface of each individual sensillum consists of an ovoid cuticular depression containing a convex central area (Fig. 2). This domed central area, the cap of the sensillum, is a thin area of specialized exocuticle, attached directly to a modified cilium which extends from the tip of the dendrite of a bipolar neuron. When viewed from above with phase contrast, the apex of the modified cilium, the "sensory process," is visible through the transparent cuticular cap (Fig. 3). The dark ring in Figs. 3 and 4 represents the dense cuticular sheath which surrounds the sensory process. Beneath the cap, a canal containing the sensory process (Fig. 4)

tunnels obliquely through the entire thickness of the cuticle. At the base of each canal is the dendrite and cell body of a large bipolar neuron.

General Morphology of Campaniform Sensilla

The fully formed adult tibial cuticle, seen in cross-section in Fig. 5, exhibits two morphologically distinct regions. The outer exocuticle and the inner, lamellate endocuticle are both synthesized and secreted by the underlying epidermal cells. A basement membrane separates the epidermal cells from the hemocoel of the insect's open circulatory system.

When a campaniform sensillum is observed in longitudinal section (Fig. 6), the light microscope reveals a cell in the epidermis which bulks so large

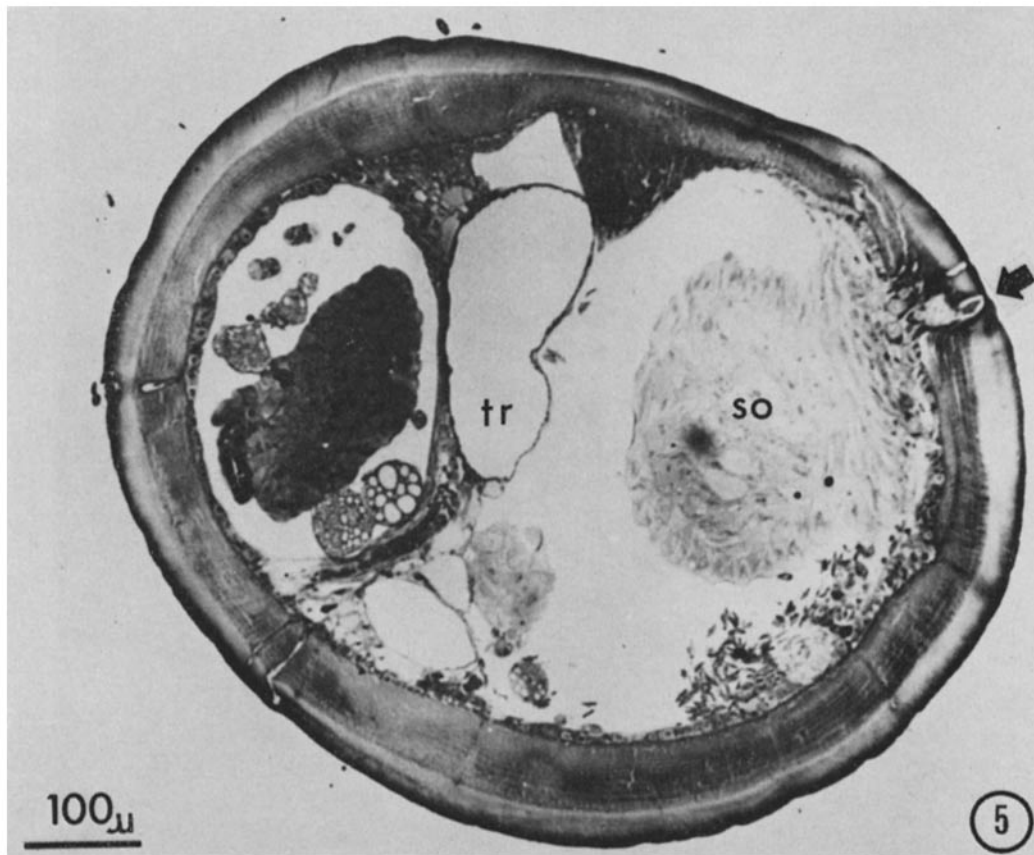


FIGURE 5 Cross-section through a cockroach leg. Arrow indicates position of campaniform sensillum. 1 μ Epon-Araldite section stained with toluidine blue. \times 150.

that it dwarfs its neighbors by comparison. This large cell is the bipolar neuron of a group 6 campaniform sensillum. The large, rounded nerve cell body can measure over $40\ \mu$ in diameter, and is sharply delineated by an intensely basophilic line formed by a sheath of glial cells which completely surround the soma and axon of the bipolar neuron. The cell body, located in a pocket of endocuticle, gives rise to a dendrite at its apex and an axon near its base. The axon emerges somewhat obliquely and courses proximally through the epidermis, surrounded by attendant glia. The dendrite, however, leaves the cell body almost vertically, and follows a slightly curved path upward through the canal in the cuticle. The distal extension of the dendrite, the modified cilium which attaches

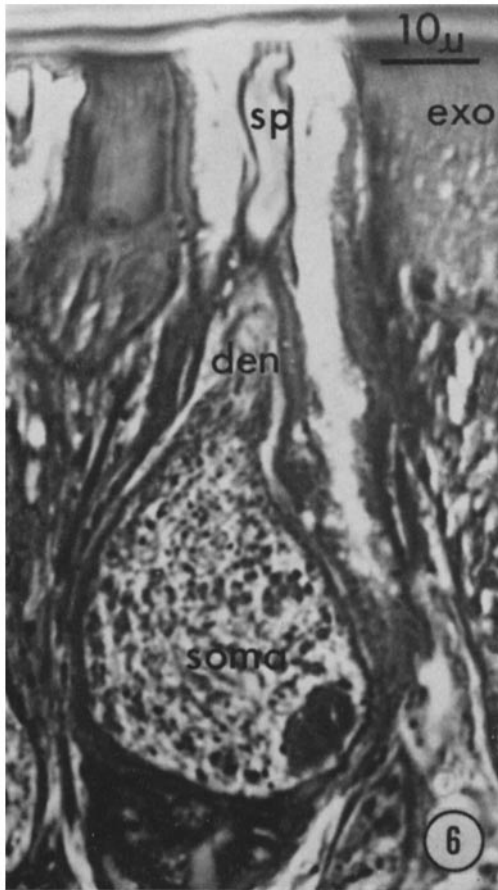


FIGURE 6 Longitudinal section through campaniform sensillum. Axon, not seen here, joins cell body of bipolar neuron out of plane of section. $1\ \mu$ Epon-Araldite section stained with toluidine blue. $\times 1300$.

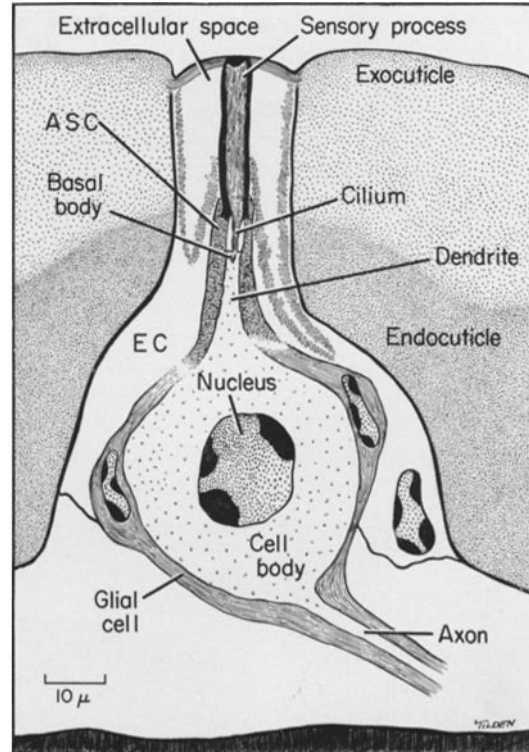


FIGURE 7 Diagrammatic representation of a campaniform sensillum.

directly to the cuticular cap at the sensillum, is encased in a dense cuticular sheath. Two modified epidermal cells, the enveloping cell and the accessory supporting cell,¹ are associated with the dendritic pole of the sensillum. The wall of the canal is lined by the enveloping cell; the accessory supporting cell surrounds the dendrite and part of the sensory process. The enveloping cell and the accessory supporting cell are partially separated by an extensive extracellular space. The cellular components of the campaniform sensillum are diagrammatically represented in Fig. 7.

The Modified Cilium

The modified cilium, which originates at the tip of the dendrite, extends approximately $20\text{--}25\ \mu$ to the cap of the campaniform sensillum (Fig. 7), and consists of two morphologically distinct regions, the "connecting cilium" and the "sensory process." The connecting cilium arises from a basal body in

¹ Terminology adopted from Stuart and Satir (1968).

the dendrite tip. The connecting cilium expands distally and becomes the sensory process, a bundle of microtubules that is surrounded by an electron-opaque cuticular sheath. The distal tip of the sensory process inserts directly into the cuticular cap of the sensillum. The connecting cilium and sensory process can be thought of as a modified cilium.

The Sensory Process

The distal tip of the sensory process, seen in longitudinal section in Fig. 8, inserts into the cap of modified exocuticle which is moved during mechanical stimulation of the sensillum. The "cap" of the campaniform sensillum is the convex area of cuticle seen at high magnification in surface view in Fig. 2.

In sectioned material, the cap is seen to be constructed from two morphologically distinct types of cuticle. The outermost layer, marked *L-1* on Fig. 8, does not exhibit any lamellae and is not penetrated by pore canals; in these two respects the outermost cuticular layer of the cap differs from the surrounding exocuticle, shown in Fig. 9. Layer 1 of cap cuticle passes over the distal tip of the sensory process, and is closely applied to the cuticular sheath which covers the vertex of the sensory process. Layer 2 of the cap cuticle lies just beneath Layer 1 (Figs. 8 and 9), and displays a markedly different image than either layer 1 or the surrounding exocuticle. Layer 2 may represent resilin, as suggested by Thurm (1964) and Che

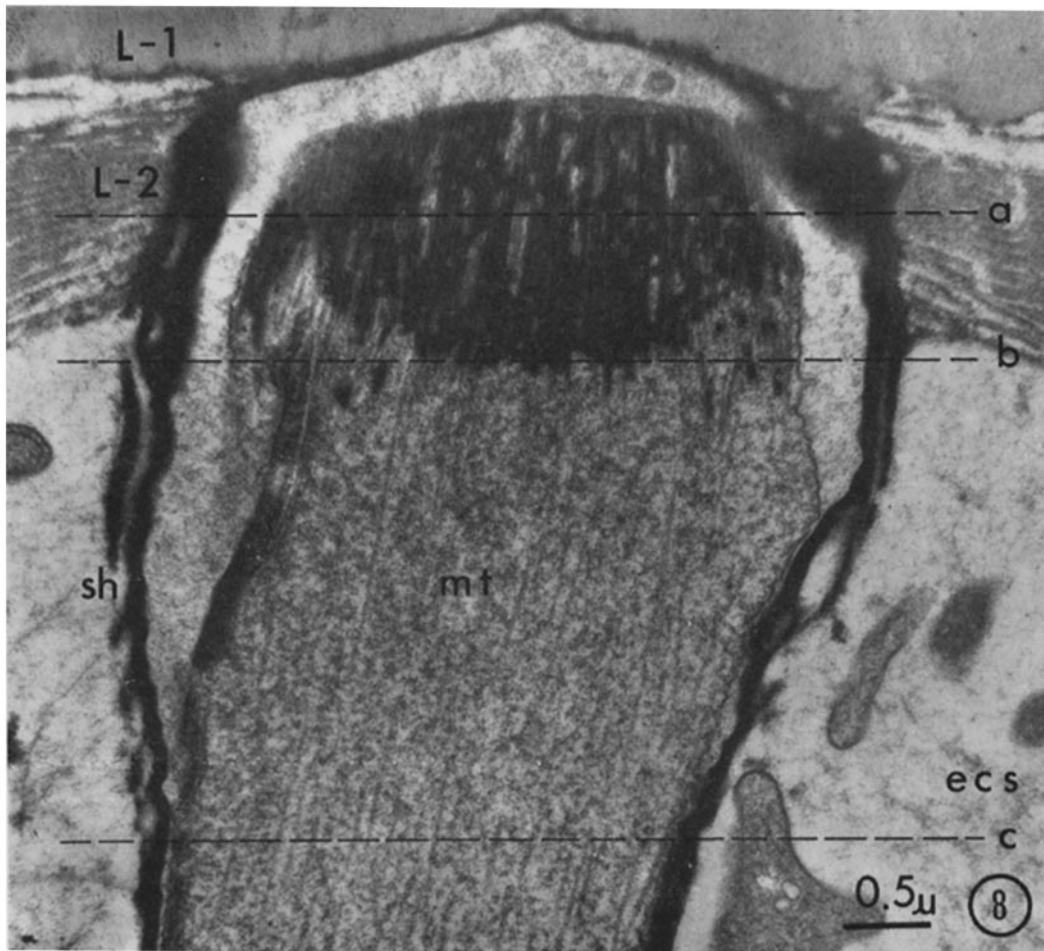


FIGURE 8 · Longitudinal section through tip of sensory process. $\times 22,000$.



FIGURE 9 Cross-section (slightly oblique) through cap of campaniform sensillum, including tip of sensory process, taken at level of dotted line *a* in Fig. 8. $\times 8700$.

valier (1969) for other campaniform sensilla. Resilin is a rubber-like protein with nearly perfect elastic properties (Weis-Fogh, 1960). If the material in Layer 2 is resilin, it may lend elasticity to the cap, allowing the cap to resume a "resting" configuration after distortion by mechanical stimulation. Cross-sections of the tip of the sensory process viewed on end (Figs. 9 and 10) show the close association between Layer 2 of the cuticle and the extracellular material of the cuticular sheath. Fig. 10 demonstrates that no line of demarcation separates the cuticular sheath from Layer 1; the sheath grades into the cuticle of the cap, indicating structural continuity between the two materials. Physical association between the cuticle of the cap and the sensory process indicates that movement of the cap during mechanical stimulation of the campaniform sensillum must be accompanied by movement of the sensory process.

The sensory process itself is a roughly cylindrical, membrane-limited bundle of 300–1000 parallel microtubules, measuring about 25 μ in length and from 2.5 to 7 μ in width. The tip of the sensory process, which inserts into cap cuticle, is flattened in shape (Figs. 8, 9, 10). In the flattened tip of the sensory process, the microtubules are surrounded by dense, amorphous, "fluffy" material (Fig. 10). The dense material, similar to that described by Chevalier in campaniform sensilla on *Drosophila* halteres, is closely associated with the walls of the microtubules. The microtubular walls, however, can be clearly distinguished from the surrounding dense material. The high-power micrograph in Fig. 10, cut at the level of dotted line *a* in Fig. 8, images the same slightly oblique thin section seen at lower magnification in Fig. 9; in Fig. 10, however, the thin section has been rotated 15° by the goniometer stage in the Philips 300 electron microscope. The resultant image shows the microtubules in the center of the sensory process tip in perfect cross-section, whereas those near the periphery appear somewhat oblique; their orientation indicates that the microtubules are fanning out away from the center of the sensory process tip. The orientation of the microtubules reflects the tapering geometry of the sensory process tip, whose shape rapidly changes from that of a flat paddle to that of a cylinder within a distance of 1 μ . Fig. 11 represents a cross-section through the lower portion of the sensory process tip indicated by dotted line *b* in Fig. 8. The sensory process has assumed its approximately cylindrical form. Small patches of dense material are still associated with the micro-

tubules at this level. Fig. 12 represents a cross-section through a campaniform sensillum, several microns beneath the tip of the sensory process cut at the level of dotted line *c* in Fig. 8. The 350 microtubules are all oriented parallel to one another and to the long axis of the sensory process. They have no specific arrangement in transverse section, and are more or less evenly distributed throughout the cytoplasmic matrix of the sensory process. Microtubules are the only organelles observed in the sensory process. Occasional large membrane-limited vesicles, however, are seen in the cytoplasm of the sensory process. A cross-section of another sensory process is shown at higher magnification in Fig. 13. This sensory process is 2.5 μ in diameter and contains 677 microtubules, 663 single microtubules and seven doublets. The inset in Fig. 13 shows one of the doublets at higher magnification; like the doublets described in motile cilia, it has two adjacent microtubules that share a common wall at their point of lateral association. These doublets were photographed at various angles of tilt with a goniometer stage, thus eliminating the possibility that the doublet images are artifacts resulting from obliquely sectioned single microtubules.

The outer limiting membrane of the sensory process is surrounded by the extracellular material of the cuticular sheath. At some points, association between the sensory process cell membrane and the cuticular sheath is intimate; in other places, however, an extracellular space separates the two. No consistent pattern of association was observed between the sensory process and the cuticular sheath; the morphology of the cuticular sheath also varies greatly among different campaniform sensilla. The sensory process in Fig. 13 is typical in that the cuticular sheath is irregularly folded; the inner aspect of each fold defines an extracellular space.

The Dendrite and Connecting Cilium

The dendrite of the bipolar neuron (Fig. 14) extends from the cell body toward the cuticular surface for a distance of approximately 20 μ , at which point its 3–4 μ -wide tip constricts sharply. A basal body lies near the center of the cup-shaped dendrite tip. Small ciliary rootlets arise near the basal body, and extend downward for several microns into the dendrite cytoplasm. The outer limiting membrane of the distal region of the dendrite is closely associated with the plasma

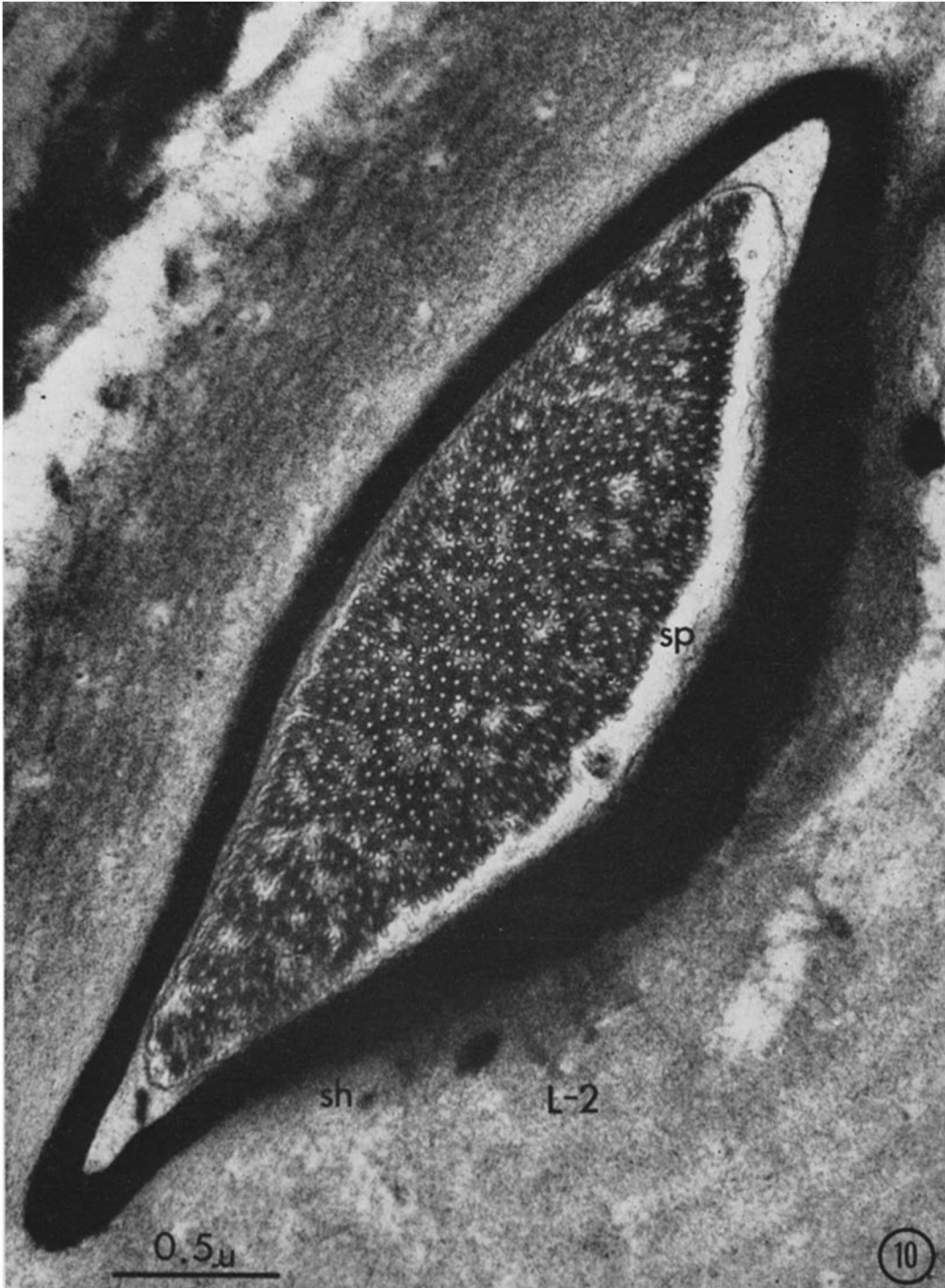


FIGURE 10 Cross-section through the tip of sensory process. This micrograph was taken from the same slightly oblique section seen in Fig. 9; here the section has been rotated 15° with the goniometer stage of the Philips 300 electron microscope. The 730 closely packed microtubules are surrounded by dense, amorphous material. $\times 44,500$.

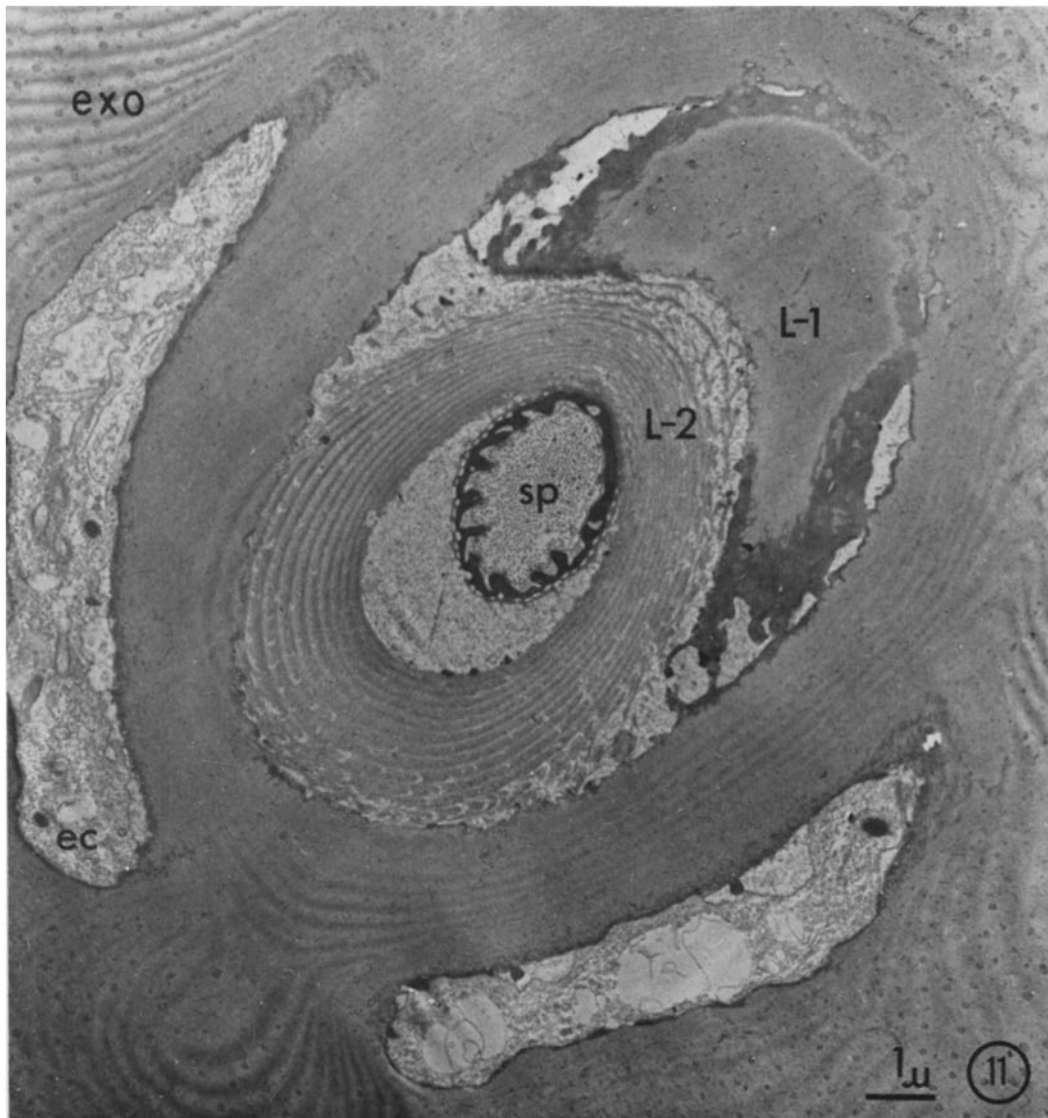


FIGURE 11 Cross-section (slightly oblique) through campaniform sensillum cut near tip of sensory process, at level of dotted line *b* in Fig. 8. Small patches of dense material are occasionally associated with microtubules of the sensory process, which, at this level, is approximately symmetrical. $\times 9000$.

membrane of the accessory supporting cell by both desmosomes and septate junctions (Fig. 14). The dendrite tip contains numerous microtubules oriented parallel to its long axis. Several mitochondria are present. Small, round vesicles, 0.05–0.2 μ in diameter, are numerous. Clusters of 200–250 A electron-opaque particles, which probably represent ribosomes, are concentrated beneath the basal body in the area surrounding the rootlet appara-

tus. The basal body gives rise to the 9 + 0 connecting cilium (Figs. 14, 15, 16), containing nine peripheral doublets but lacking the central pair characteristic of motile cilia. As in other ciliated campaniform sensilla (Stuart and Satir, 1968; Chevalier, 1969), the outer member of each peripheral doublet lacks the “arms” identified by Gibbons (1967) as the site of dynein ATPase in motile cilia. The connecting cilium is completely

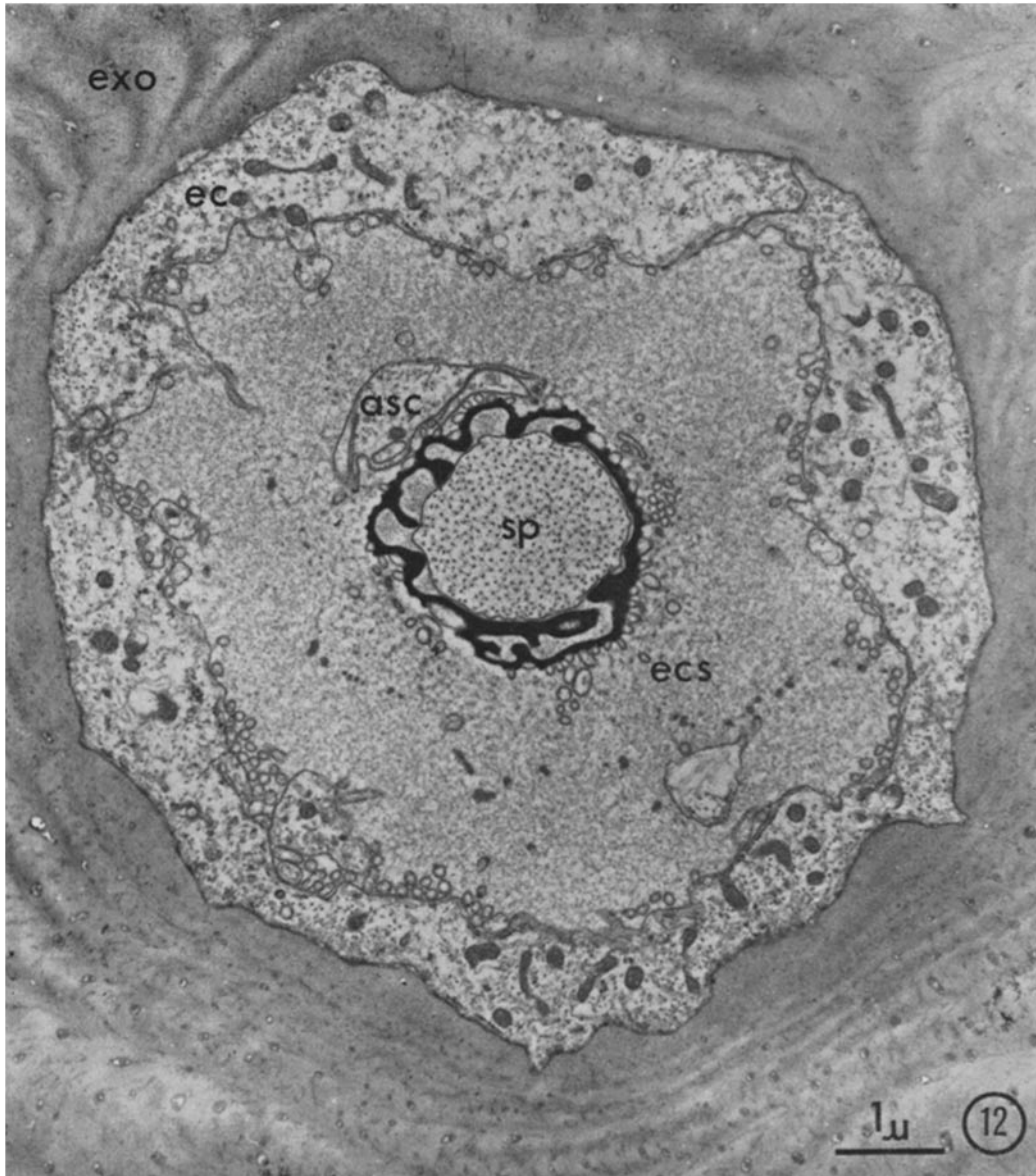


FIGURE 12 Cross-section through campaniform sensillum cut at level of dotted line *c* in Fig. 8. This sensory process contains 350 microtubules. $\times 13,000$.

surrounded by an extracellular space, which separates the cilium from the nearby accessory supporting cell. As the modified cilium ascends from the basal body toward its distal attachment at the cuticular cap, it increases in diameter and acquires more microtubules which run parallel to its long axis. The base of the connecting cilium is 0.35μ in diameter. At 5μ above its base, the modified

cilium measures 1.5μ in diameter, is still surrounded by an extracellular space, and contains approximately 100 microtubules including the nine peripheral doublets. About 6μ beyond the basal body, the modified cilium expands to $2.5\text{--}7 \mu$ in diameter, has 300–1000 microtubules, and becomes surrounded by an electron-opaque, extracellular cuticular sheath 0.2μ in thickness (Figs.

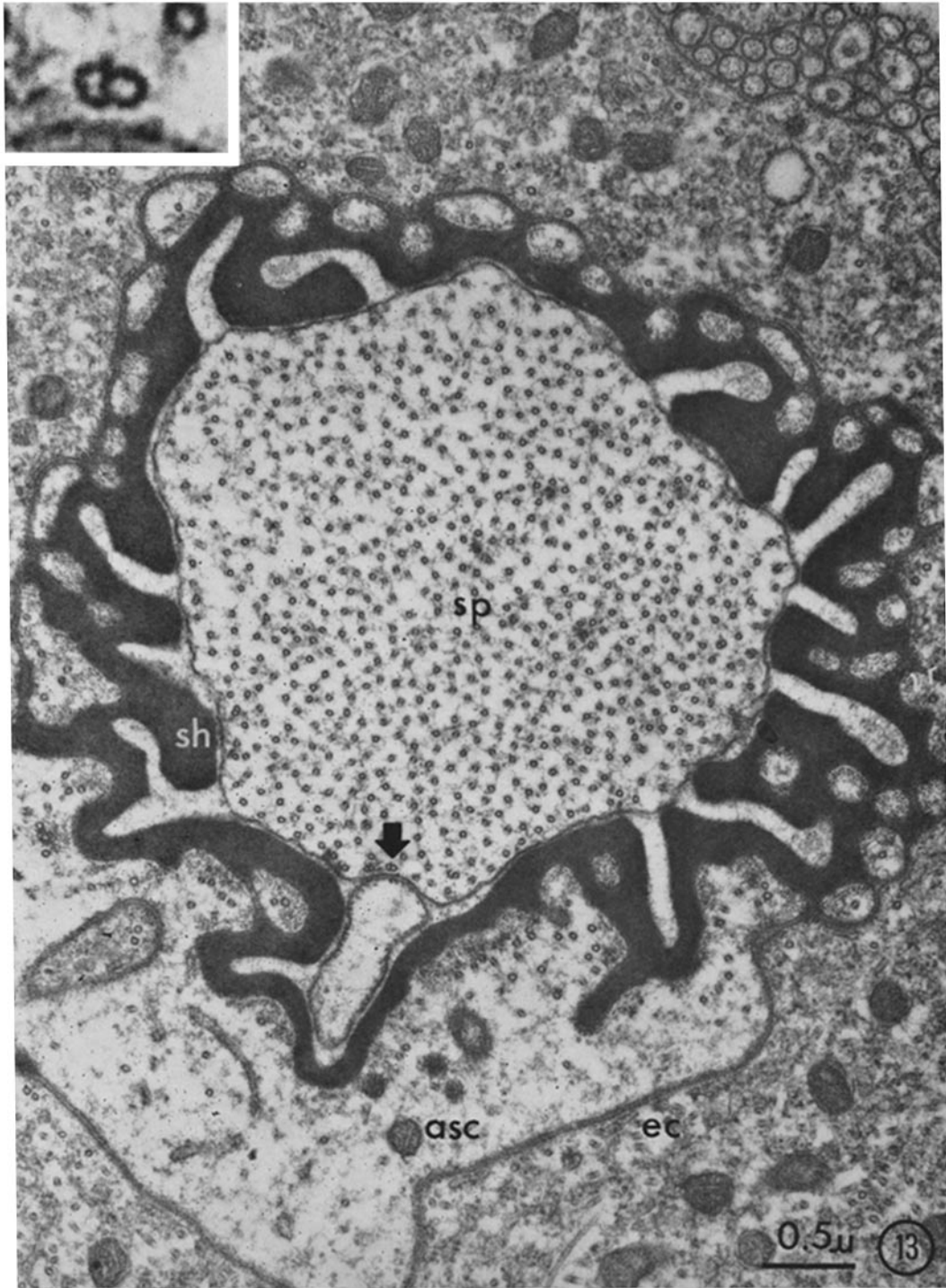


FIGURE 13 Cross-section through sensory process of newly moulted, untanned adult cockroach. This sensory process contains 663 single microtubules and seven doublets; doublet indicated by arrow appears in *inset*. $\times 27,000$.

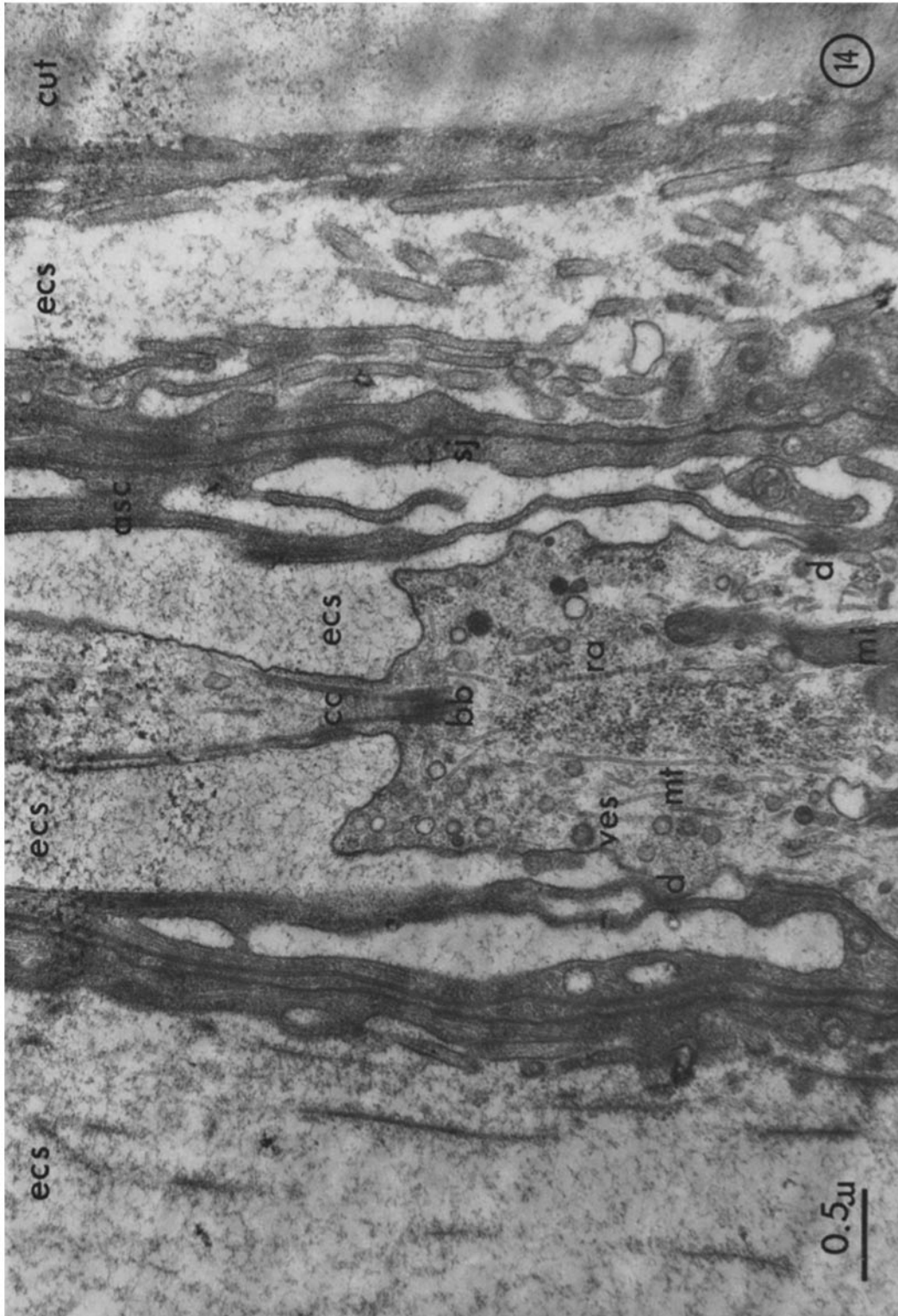


FIGURE 14 Longitudinal section through dendrite tip of campaniform sensillum. $\times 27,000$.

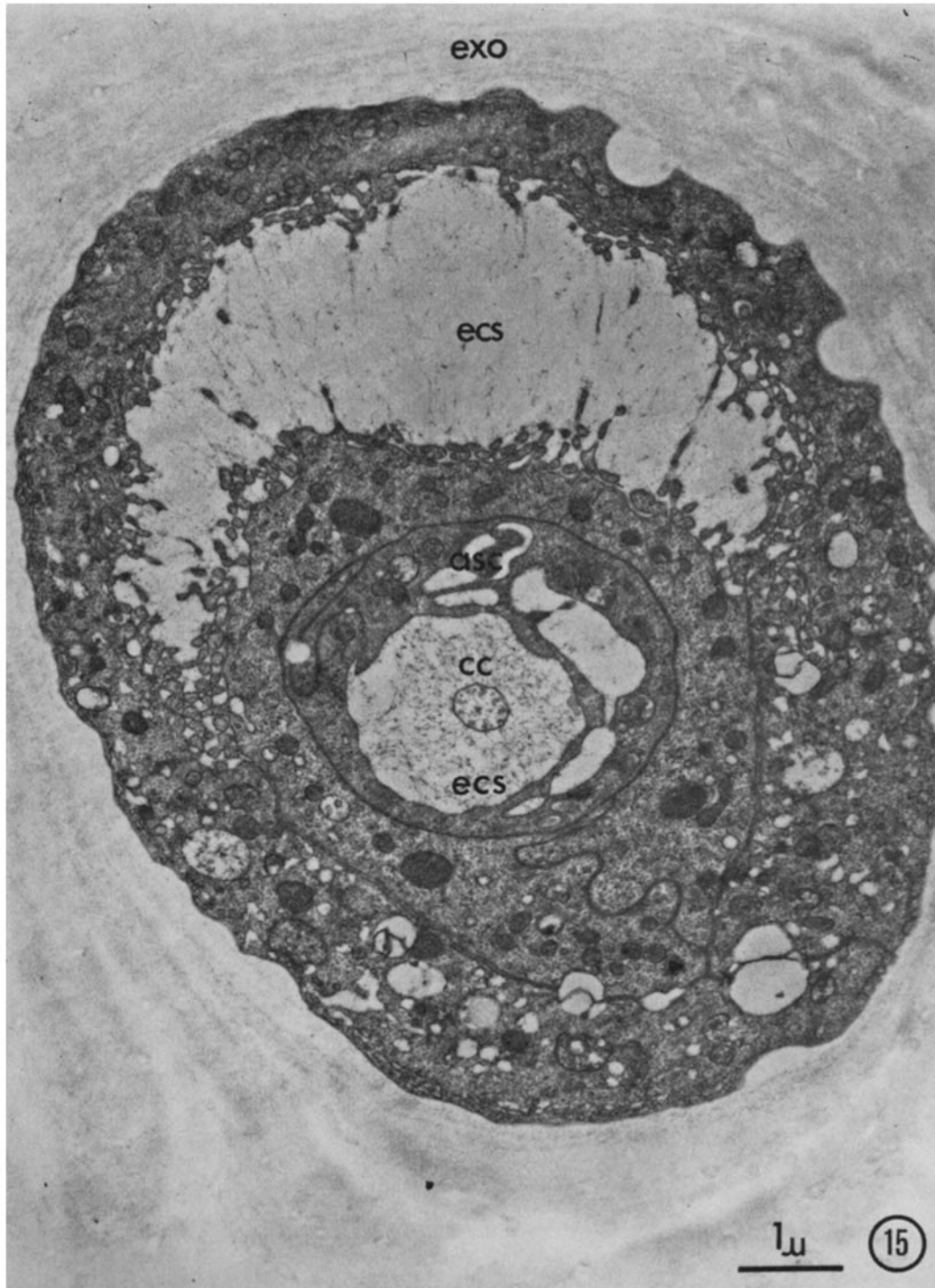


FIGURE 15 Cross-section through campaniform sensillum at level of connecting cilium. Extracellular space surrounding cilium is defined by inner cell membrane of accessory supporting cell. Enveloping cell lines canal in cuticle through which dendritic pole of sensillum passes. $\times 15,000$.

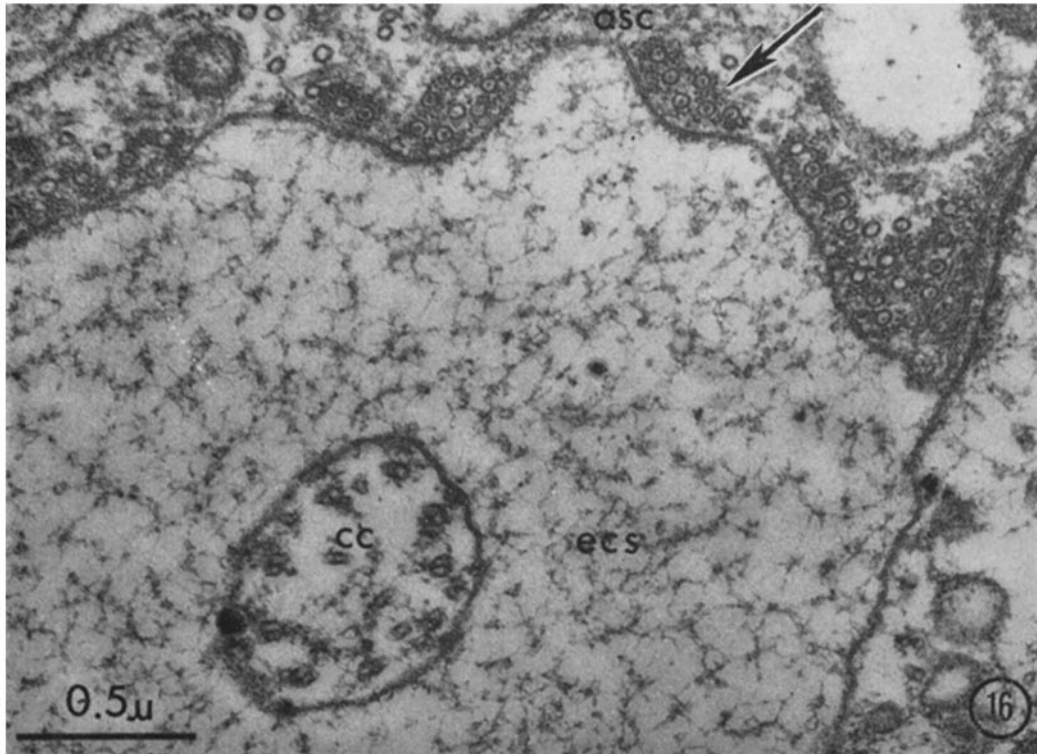


FIGURE 16 Cross-section through connecting cilium and part of accessory supporting cell. Clumps of dense-core microtubules (arrow) populate peripheral indentations of inner surface of this cell. Dense 50-A fibrils are interspersed amongst microtubules. $\times 47,000$.

14, 15), which is elaborated by the accessory supporting cell.

Accessory Cells

Two modified epidermal cells are associated with the dendrite and modified cilium; the accessory supporting cell (asc) and the enveloping cell (ec). It is most difficult to trace these cells to their point of origin in the epidermis, but their distal portions are readily observed with the electron microscope (Figs. 12, 14, 15).

The outer portion of the ec abuts directly against the cuticle, defining the canal through which the dendrite, cilium, and sensory process pass. The asc surrounds the dendrite, connecting cilium, and part of the sensory process.

The inner cell membrane of the asc defines an extracellular space which surrounds the cilium (Figs. 17, 15, and 16). This plasma membrane is indented, and between adjacent indentations are bundles of unusual microtubules (Fig. 16). Their

walls are dense; many contain dark, central rods; and most of these microtubules are surrounded by dense, 50 A fibrils. The cytoplasm of both the ec and asc is heavily populated with microtubules (Fig. 15). It is important to note that neither the enveloping cell nor the accessory supporting cell contains presynaptic vesicles. Their cell membranes do not exhibit electrically conductive tight junctions.

The Bipolar Nerve Cell Body

FINE STRUCTURE Each campaniform sensillum includes a single, large bipolar neuron whose rounded cell body measures up to 45μ in diameter. Fig. 17 depicts a portion of the nerve cell body and its glial tunic. The large, centrally located nucleus is surrounded by cytoplasm whose ground substance appears pale, suggesting that the bipolar nerve cell may be highly hydrated. The cytoplasmic matrix is, however, rich in organelles. Ribosomes are especially abundant. Some ribo-

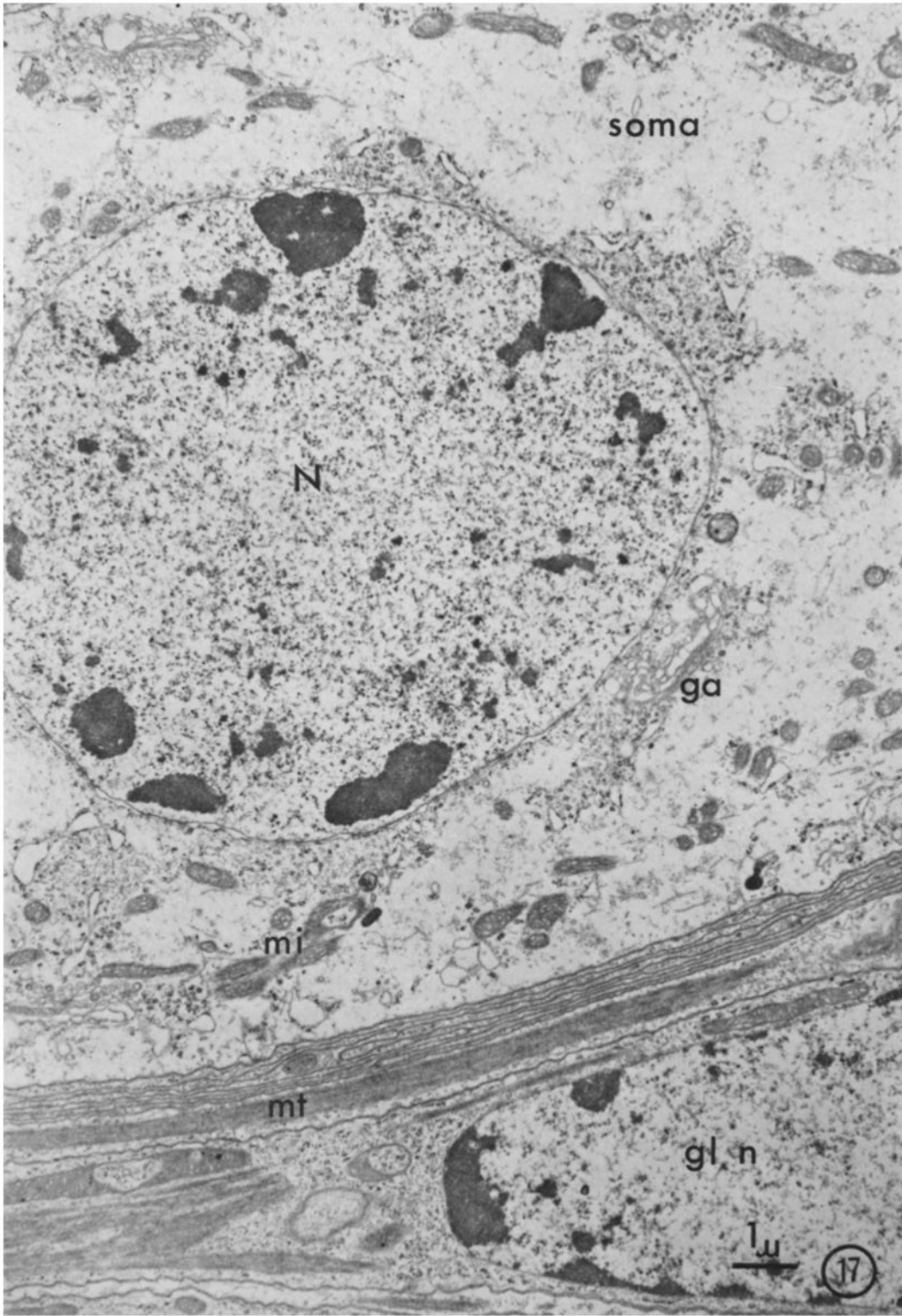


FIGURE 17 Bipolar nerve cell body and associated glia. $\times 10,000$.

somes are associated with scattered cisternae of the endoplasmic reticulum, but most are free, indicating that the nerve cell is actively synthesizing proteins for its own structural and metabolic use. Smooth-membraned cisternae of the Golgi complex, which appear crescentic in section and closely apposed, are frequently observed. Numerous mitochondria, most of whose cristae are longitudinally oriented, are scattered randomly. The neuroplasm is well supplied with ubiquitous microtubules, which appear to course individually throughout the cytoplasm in all directions.

DISCUSSION

Campaniform sensilla on cockroach legs are good sense organs with which to study mechanisms of sensory encoding. Our morphological studies show each sensillum to be a "simple" mechanoreceptor with a single, large bipolar neuron. Electrophysiological studies (Moran and Chapman, 1968) demonstrate the bipolar neuron to be readily accessible to micropipet penetration.

The modified epidermal cells associated with the bipolar neuron, e.g. the enveloping cell and the accessory supporting cell, do not contain synaptic vesicles and do not appear to communicate with the nerve cell membrane via electrically conductive tight junctions. Furthermore, a space or the cuticular sheath always separates the ec and asc from the modified cilium. Thus we conclude that these accessory cells do not participate directly in the bioelectric processes of sensation. The bipolar neuron appears to be a primary sense cell, a neuron that individually performs the functions of stimulus reception and transduction as well as impulse transmission.

Given the bipolar neuron as a primary sense cell, several important questions arise: What event stimulates the cell? What part(s) of the cell acts as the transducer, and how does the transducer divert the stimulus energy into that of a train of nerve impulses? In short, how does the campaniform sensillum work? The campaniform sensillum is a good model system with which to investigate these important processes of sensory transduction.

The Stimulus

When a cockroach shifts weight onto a leg, the leg cuticle is strained and the thin cap of the campaniform sensillum is displaced (Pringle, 1938). We do not know whether the cap moves up or down during normal activity in the living animal.

Chapman and Pankhurst (1967) have recently shown, however, that mechanical depression of the cap of a campaniform sensillum stimulates the sensillum to produce a train of nerve impulses detectable in the afferent neuron. Given that vertical pressure can effectively stimulate a campaniform sensillum, what part(s) of the neuron are affected by the mechanical stimulus?

The Cell Membrane as Transducer

Since mechanical depression of the cap serves to stimulate the sensillum, we assume the generator site to be one in which appreciable deformation occurs as the cap of the sensillum moves. Although our electrophysiological efforts have not yet identified a generator current source, it seems likely that the cell membrane of the dendritic region may fulfill this function. In terms of contemporary electrophysiology, the simplest hypothetical mechanism of mechanoelectric transduction maintains that passive conductance of the generator membrane to one or more available ions is a function of membrane strain. Strain may increase conductance, creating an end plate-like current sink whose generator current electrically excites the axon. Our morphological studies indicate potentially critical regions in the outer limiting membrane at the cap, the microtubule-packed sensory process, the cilium, the cup-shaped dendrite tip, and the cylindrical wall of the dendrite itself. What is needed to test these possibilities is either precise localization of a generator current source and/or a means of recognizing how deformation is distributed on the cell membrane. Both these quantities appear technologically inaccessible at present.

Microtubules and Sensory Transduction

The tip of the sensory process is attached to the cuticle of the cap of the campaniform sensillum; thus depression of the cap must move the top of the sensory process.

The presence of 500 or more parallel microtubules in the sensory process at the site of stimulus reception leads us to suggest that microtubules play an important role in mechanoelectric transduction in cockroach campaniform sensilla. Experiments are currently underway to test this hypothesis.

Evidence is rapidly mounting that microtubules are intimately related to cytoplasmic movement (Porter, 1966). Furthermore, chemical agents such as colchicine and vinblastine, which depolymerize

microtubules, stop chromosome movements at mitosis, and stop pigment migration in chromatophores (Porter and Junquiera, in preparation). Microtubules causing cytoplasmic movement must convert chemical energy into mechanical motion; that is, they are mechanochemical engines. It is tempting to speculate that mechanical compression of the microtubule-packed sensory process tip may, by forcibly displacing microtubules, drive these mechanochemical engines backwards, liberating chemical energy conducive to membrane depolarization.

It appears equally likely, however, that microtubules play a passive, structural role in mechanoreception in campaniform sensilla. The longitudinally oriented bundle of parallel microtubules in the sensory process may act as a rod-like structure which conducts the force of the mechanical stimulus exerted on the cuticular cap to a point such as the dendrite tip many microns from the stimulus site. Chevalier (1969) has pointed out quite correctly that the effect of compression of the cuticular cap of *Drosophila* haltere campaniform sensilla would be restricted by the geometry of the sensillum to the distal process of the dendrite. Satir (1968), in discussing his sliding filament model for ciliary motility, suggests that displacement of the microtubules at the tip of a cilium may cause bending at its base; hence the input information, mechanical displacement of the cap, may be transferred to the dendrite of the bipolar neuron of a campaniform sensillum. If the role of the modified cilium is to direct a force to the dendrite, then we would expect to find structures associated with transduction in the dendrite tip. Fig. 11 shows that the dendrite tip contains a basal body and associated ciliary rootlets, several longitudinally oriented mitochondria, and numerous 0.05–0.2 μ round, membrane-limited vesicles. Nadol and de Lorenzo (1969) note that many nonciliated mechanoreceptors, such as stretch receptors in the lobster, have high populations of small round vesicles (clearly different from synaptic vesicles) and mitochondria in areas probably related to mechanoelectric transduction. Nadol suggests that the vesicles may play an active role in transduction, and that “. . . dendritic mitochondria may function as ionic ‘sponges’ of those ions that have caused the local depolarization of the generator potential.” Thurm (1968) has shown that the large, motile abfrontal gill cilia of the lamellibranch mollusc exhibit mechanosensitivity; he calculates

that this mechanosensitivity resides in a region immediately beneath the cell membrane in the vicinity of the basal bodies.

The notion that the microtubules of the sensory process act as a stiff rod which physically conducts the force generated by cap displacement to the dendrite tip is mechanistically attractive. Its feasibility as a working model for transduction is, however, open to question on morphological grounds. If the microtubules function as a stiff translational rod, we would expect them to be continuous structures connecting the stimulus site with the dendrite tip. Our studies show this not to be the case. The vast majority of sensory process microtubules do not span the entire distance between the sensory process tip and the dendrite. The distal region of the modified cilium has 200–1000 microtubules, whereas the connecting cilium adjacent to the basal body has only 18 (the nine peripheral doublets).

The presence of seven doublets and 663 single microtubules in the sensory process in Fig. 13 raises certain questions about the origin of these two populations of microtubules. The dense, amorphous material which surrounds the microtubules at the tip of the sensory process resembles similar material consistently observed at known sites of microtubule origination (Porter, 1966) such as the centriole (Kalnins and Porter, 1969), the kinetochore (Robbins and Gonatas, 1964), and the phragmoplast of dividing plant cells (Hepler and Jackson, 1968). The presence of dense amorphous material at the tip of the sensory process suggests that the 350–1000 single parallel microtubules grow from the tip of the modified cilium toward its base. The nine doublets which extend from the basal body through the connecting cilium and up into the sensory process, however, probably originate from the basal body. Slifer and Sekhon (1969) have suggested that the single microtubules in mechanoreceptive tactile hairs on earwig antennae arise from the doublets of the ciliary 9 + 0 complex by microtubular “branching.” This is clearly not the case in microtubules in the sensory process of the cockroach campaniform sensillum seen in Fig. 13, in which 663 single microtubules and seven doublets coexist in the same plane of section. We have never observed microtubular “branching,” and would suggest that Slifer and Sekhon’s observations represent an optical artifact resulting from overlap of adjacent 250 A microtubules in a relatively thick (800 A) section.

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The reader is referred to the recent article by D. S. Smith (*Tissue and Cell*. 1969. 1:443) describing the fine structure of campaniform sensilla on blowfly halteres.

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