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Microtubules and Sensory Transduction

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ABSTRACT Cockroach legs bear tactile spines equipped with campaniform sensilla—mechanoreceptors associated with the cuticle—which function by a single bipolar neuron from whose dendrite tip extends a modified cilium packed with 350–1000 parallel cytoplasmic microtubules. These microtubules, which can be chemically disassembled with colchicine and vinblastine, are intimately associated with the site of mechanical stimulation. Treatment of living sensilla with colchicine and vinblastine abolishes their ability to respond to mechanical stimulation 1–2 hr after drug application. Loss of function is accompanied by large-scale disassembly of microtubules in the modified cilium. The experimental evidence strongly suggests that microtubules play an important role in the process of sensory transduction in campaniform sensilla.

The purpose of this study is to investigate the role of microtubules, if any, in mechanoreception. We selected campaniform sensilla on cockroach legs as an experimental system because they are accessible for investigation and have numerous microtubules at the site of stimulus reception. Each sensillum functions with only one bipolar neuron. This primary sense cell singly handles the functions of stimulus reception, transduction, and impulse transmission. The dendrite tip, like that of other ciliated mechanoreceptors, contains a basal body from which extends a long, microtubule-packed, modified cilium. The tip of the modified cilium, called the sensory process, is a membrane-limited bundle of 350–1000 parallel microtubules that attaches directly to the site of mechanical stimulation on the cuticle (Figs. 1 and 2). In order to determine whether microtubules are necessary components for mechanoreception in campaniform sensilla, we investigated the effects of chemical disassembly of microtubules. Our experimental evidence shows that chemical disassembly of the microtubules of the sensory process by colchicine or vinblastine is accompanied by loss of mechanosensitivity of sensilla.

MORPHOLOGY OF CAMPANIFORM SENSILLA

Understanding of the experiments reported below necessitates a brief description of the morphology of campaniform sensilla [for a complete description, see Moran, Chapman, and Ellis (1)]. Each cockroach leg has numerous large tactile spines on the tibia. The spine, a stiff, hollow, conical spike of cuticle, inserts into a flexible socket that is readily distorted whenever the spine is touched from any direction. Chapman (2), using the combined techniques of electrophysiology and histology, has shown that each tactile spine derives its mechanosensitivity from a single campaniform sensillum located in the cuticle at the front of the base of the spine. Because of the geometry of the spine and the placement of its campaniform sensillum, touching the tactile spine effectively stimulates the sensillum to produce a burst of nerve impulses.

Fig. 1 is a diagrammatic representation of the component parts of a campaniform sensillum. Each sensillum receives and transmits its excitation by means of a single bipolar neuron whose large, round cell body is located in a pocket of endocuticle. Its axon converges with axons of other sensory receptors to form the leg nerve, which runs up through the middle of the femur en route to the central nervous system. Chapman and Nichols (3) have conclusively demonstrated that each axon maintains its individuality throughout the length of the leg nerve. At the distal end of the bipolar neuron, the dendrite is associated with the cuticle. Its tip contains a basal body connected to a modified cilium. The base of this modified cilium, called the connecting cilium, displays nine peripheral doublets, yet lacks the central pair characteristic of the motile

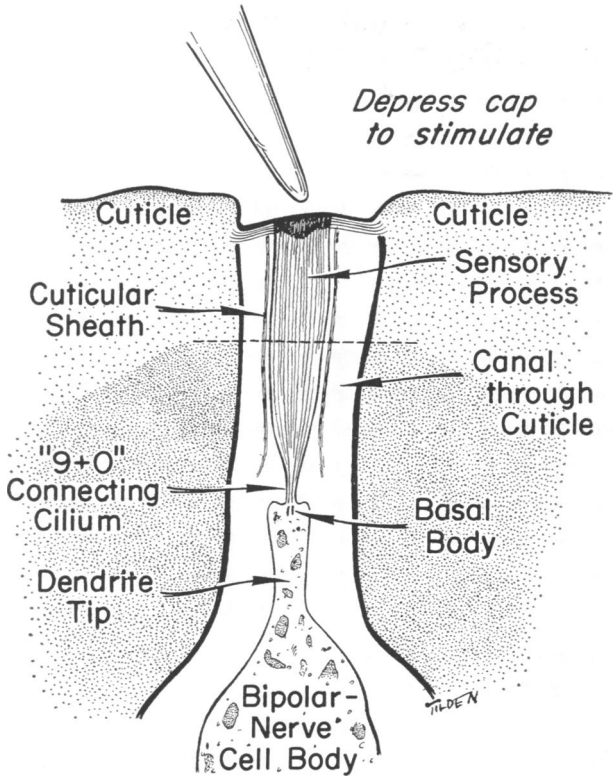

FIG. 1. Diagrammatic representation of a campaniform sensillum.

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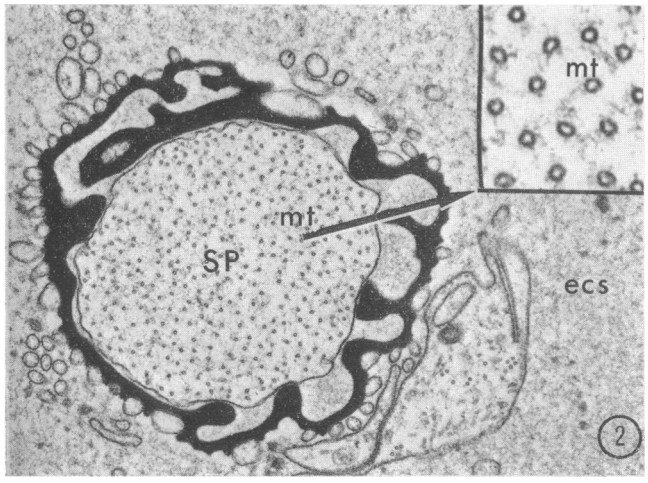


FIG. 2. Electron micrograph of a cross section through the sensory process of a campaniform sensillum. Membrane-limited sensory process (*sp*) is surrounded by darkly-stained cuticular sheath and densely populated by microtubules, shown at a high magnification in the inset. Magnification = ×18,700 (inset = ×83,350).

cilia. The distal portion of this structure, called the sensory process, is a membrane-limited bundle of 350–1000 parallel microtubules. A typical sensory process, cut and viewed in cross section, is seen in Fig. 2. This sensory process contains 350 microtubules. These tubules, the only organelles visible, are relatively uniformly distributed throughout the cytoplasm of the sensory process. The inset displays these microtubules at high magnification. Strands of moderately electron-dense material, which may represent fixed components of the matrix of the sensory process, are associated with the walls of the microtubules. This material, whose nature is presently unknown, may prove to be of mechanistic significance. The tip of the sensory process is closely associated with the thin cuticular cap of the sensillum. Depression of the cap triggers a burst

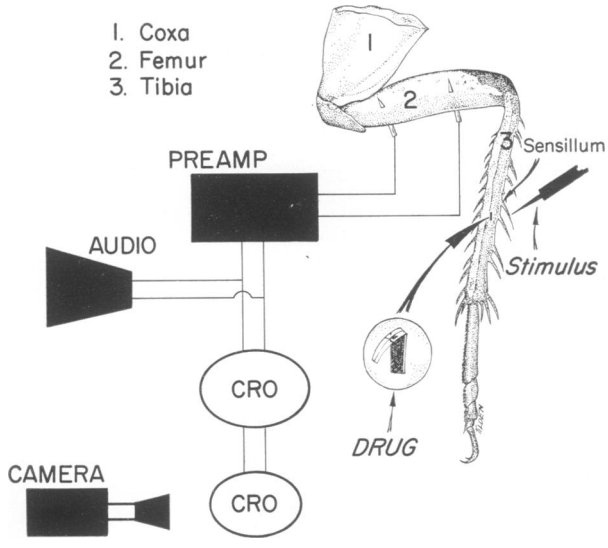


FIG. 3. Diagram of cockroach leg prepared for electrophysiological recording. When the tactile spine is gently touched with a dissecting needle, the campaniform sensillum at the base of the spine is stimulated to produce a burst of spikes detected by the insect pins that pierce the femur near the leg nerve.

of impulses that can be recorded at the leg nerve (see Figs. 3 and 4). The sensory process, attached to the point of stimulus reception, appears to depend on physical displacement for its stimulation. The presence of hundreds of microtubules (a distinct specialization of this sensory ending) suggests that these microtubules play an important role in mechanoreception in campaniform sensilla.

MATERIALS AND METHODS

Legs of the cockroach *Blaberus giganteus* were amputated at the coxa, sealed with vaseline to prevent desiccation, and mounted by the femur upon a pair of insect pins that served as recording electrodes (Fig. 3); the method of Chapman (2) was used. The two pins were connected to a Tektronix 122 preamplifier and displayed on an oscilloscope. The responses, also monitored by loudspeaker, were recorded with a Grass camera via a slave oscilloscope. With this setup, potentials ranging from 100 to 300 μV could be recorded from individual axons of the leg nerve for several hours (Figs. 3 and 4). If the tip of a tactile spine was touched with a hand-held dissecting needle, a burst of responses was elicited down the axon of its campaniform sensillum (Fig. 4); this method was used in the experimental work to define the functional state of the campaniform sensillum. The strength of mechanical stimulation was kept as uniform as possible. Since stimulation was manual, however, no attempt was made to quantitate the frequency of the responses as an index of the functional state of the sensilla. Instead, two extreme conditions were considered: (*a*) the normal condition, defined by responses judged similar to responses obtained prior to drug application, and (*b*) the nonfunctional condition, defined by an absence of responses regardless of the strength of mechanical stimulation. After a given leg had been satisfactorily prepared for recording, one of the following solutions was carefully placed over one tactile spine (hereafter called the experimental spine): (*a*) 10^{-2} M vinblastine sulfate (Velban, Eli Lilly Co.) in 1% dimethyl sulfoxide [$(Me)_2SO$] in saline; (*b*) 5×10^{-2} M colchicine in 1% $(Me)_2SO$ in saline; (*c*) 4 μg/ml of tetrodotoxin‡ in 1% $(Me)_2SO$ in saline; (*d*) 1% $(Me)_2SO$ in saline. A small "window" was made near the experimental spine, to permit the solution to diffuse through the tissues of the leg to the sensillum, by removal of a piece of leg cuticle (Fig. 3). High concentrations of colchicine and vinblastine were applied to promote diffusion of the drug through the viscous hemolymph. Since the drugs became diluted as they diffused

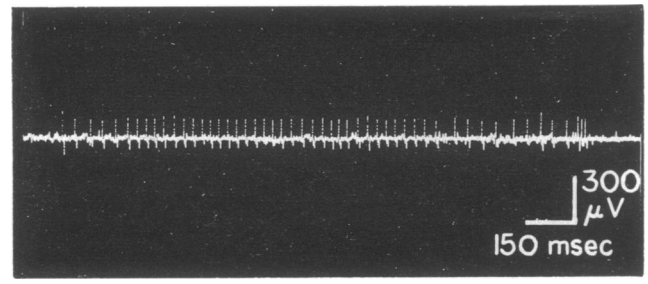
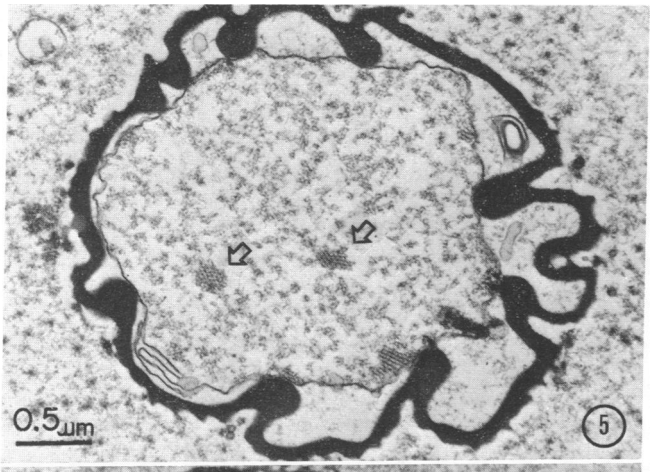


FIG. 4. Typical burst of spikes produced by mechanical stimulation of campaniform sensillum of the tactile spine.

‡ Tetrodotoxin, the active principle of pufferfish poison, is a known neurotoxin that inhibits action potentials by blocking the increase in sodium conductance that occurs during normal depolarization [Narahashi *et al.* (4)].

through the blood to the nerve cells, their actual effective concentration is unknown. $(Me)_2SO$, a well-known penetrating agent, was used to further accelerate drug diffusion. After application of the drug, the experimental spine was stimulated at regular intervals; its activity was compared to untreated control spines located *distal* to the experimental spine on the same tibia. Axons of control spines passed through the area of the leg exposed to the drugs en route to the central nervous system. These axons served as internal controls with which to detect direct effects of colchicine and vinblastine on axonic conduction.

At the completion of each physiological experiment, experimental and control spines were removed by dissection, fixed in cacodylate-buffered 3% glutaraldehyde, post-fixed in 1% osmium tetroxide, dehydrated in a graded acetone series, and embedded in the low viscosity epoxy resin originated by Spurr (5). Thin sections, cut with a DuPont diamond knife on a Porter-Blum MT2-B ultramicrotome, were mounted on Formvar-covered LKB slot grids, double-stained with uranyl acetate and Reynolds (6) lead citrate, and photographed with a Hitachi 11-C electron microscope operated at 50 KV.



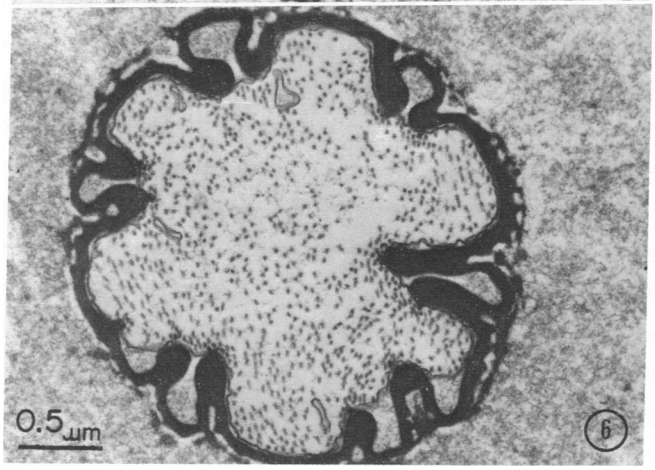


FIG. 5. Electron micrograph of a cross section through the sensory process of sensillum rendered nonfunctional by vinblastine. Microtubules exhibit considerable disassembly; arrows indicate typical crystals of microtubule protein complexed with vinblastine. Magnification = ×20,000.

FIG. 6. Cross section through the sensory process of untreated control sensillum on the same leg as that in Fig. 5. The sensillum functioned normally, and microtubules are intact. Magnification = ×20,000.

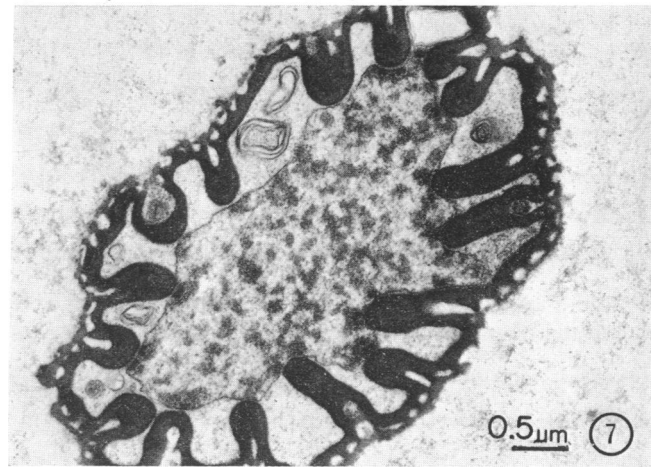


FIG. 7. Cross-section through the sensory process of campaniform sensillum rendered nonfunctional by colchicine. No intact microtubules are evident. Magnification = ×17,000.

OBSERVATIONS

The ability of campaniform sensilla of tactile spines to respond to mechanical stimulation disappears from 1 to 2 hr after exposure to colchicine or vinblastine, drugs known to disassemble microtubules (Borisy and Taylor (7); Bensch and Malawista (8)). Examination of experimental spines with the electron microscope shows the sensory-process microtubules to have undergone considerable disassembly. Fig. 5 represents a cross section through the sensory process of a campaniform sensillum exposed to vinblastine. This sensillum, which responded normally at the onset of the experiment, became nonfunctional 2 hr after administration of vinblastine. The sensory-process microtubules are no longer intact. Several crystals representing microtubule subunits complexed with vinblastine [Bensch and Malawista (8)] are evident (*arrows*). A control sensillum located distal to the experimental spine functioned normally throughout the experiment. The microtubules of its sensory process appear intact (Fig. 6).

Colchicine similarly abolished the ability of campaniform sensilla to respond to mechanical stimulation. Examination of thin sections shows that loss of mechanoreceptive capacity in colchicine-treated campaniform sensilla was accompanied by extensive disassembly of microtubules in the modified cilium. Fig. 7 is an electron micrograph of a colchicine-treated sensillum, which became nonfunctional 2 hr after drug application. Its sensory-process microtubules are no longer intact. The control sensillum, which remained functional throughout the experiment, retained its normal complement of microtubules and displayed a normal cross-sectional image similar to those of Figs. 2 and 6.

Experimental spines treated with tetrodotoxin ceased responding to stimulation from 2 to 10 min after drug application. Control spines located distal to the experimental spine on the same tibia, whose axons passed through the area in the leg exposed to the drug, were rendered nonfunctional 15–25 min after drug application.

Exposure of sensilla to 1% $(Me)_2SO$ in saline had no detectable effect either on their ability to respond to mechanical stimulation or on their fine structure.

Dendrites (proximal to the modified cilium) and cell bodies of experimental and control sensilla were examined with the electron microscope. Experimental and control cells exhibited

similar fine-structural features, except that microtubules were absent from the cell body and dendrite of experimental cells and present in the same areas of control cells. The plasma membrane and cytoplasmic membrane systems were intact in experimental cells; no structural damage was evident.

DISCUSSION

Our experimental findings strongly suggest that microtubules are necessary components in the process of mechanotransduction in campaniform sensilla. It could be argued, however, that colchicine and vinblastine, in addition to disassembling microtubules, may interfere with the metabolic machinery of the bipolar neuron at the level of the dendrite, cell body, and/or axon. We consider these possibilities unlikely for two reasons. First, vinblastine and colchicine represent separate molecular species that are *independently* capable of disassembling microtubules [Olmsted *et al.* (9)]. Both drugs are compatible with viability in a number of cell types. The probability that both drugs would interfere with the physiological competence of the bipolar neuron; and that their toxicity would be manifest over similar time-courses in a number of different experiments, is small. Second, campaniform sensilla of control tactile spines distal to the experimental spine have axons that pass through the area of the leg exposed to drugs. Such control spines showed no loss of function in experiments with colchicine or vinblastine. This can be interpreted in one of two ways: either (*a*) the drugs do not interfere with axonic conduction, or (*b*) the drugs do not contact the axon, which is ensheathed by an elaborate protective tunic of glial cells. Of these two possibilities, (*a*) is most likely since tetrodotoxin, known to abolish action potentials, blocked axonic conduction in control spines distal to the experimental spine.

A possible role for microtubules in nerve-impulse initiation has been suggested by Palay *et al.* (10). These investigators have speculated that the fasciculated microtubules in the initial segment of the axon of various neurons in the central nervous system of the rat may be related to the initiation of the action potential. The sensory process, dendrite, cell body, and axon of our experimental system all contain microtubules subject to disassembly by colchicine and vinblastine; hence, the effect of microtubular disassembly on sensory transduction may be exerted at several levels within the bipolar nerve cell. It should be noted, however, that in campaniform sensilla the distribution of microtubules is unique. An extremely high concentration of microtubules exists in the sensory process, where the population achieves a density of 70 tubules per square micrometer. Microtubules are the *only* cytoplasmic organelles in the sensory process (Figs. 2 and 6), and the sensory process is attached to the site of stimulus reception. It seems probable that colchicine and vinblastine abolish the ability of campaniform sensilla to respond to mechanical stimulation by disassembling the microtubules in the sensory process.

Since the sensory-process microtubules appear to be necessary components for normal function of campaniform sensilla, the question arises; what role do microtubules play in mechanotransduction?

Microtubules as translation rods

All neurons can act as stretch receptors; that is, mechanical deformation of the nerve cell produces electrical responses. The modified cilium establishes the sole structural connection between the stimulus site and the dendrite tip. The several hundreds of microtubules that comprise the bulk of the mass of the modified cilium may act as stiff translation rods which, when pushed by the stimulus, serve to deform the cell membrane of the dendrite tip, thus generating an electrical response.

The modified cilium as a generator

Various mechanoreceptors, such as campaniform sensilla, the locust ear (11), honey bee hair-plate receptors (12), cockroach subgenual organs (13), and vertebrate lateral-line organs, employ modified cilia at the receptor site. Horridge (14) has observed intracellular action potentials associated with the beating of motile cilia in centophore comb plate cells, and states; "It seems reasonable to conclude that the depolarization of the cell sets off the beat of the cilia of that cell." If membrane depolarization can activate motile cilia, it is reasonable to suggest—as did Gray and Pumphrey (15)—that physical movement of a nonmotile cilium can effect local membrane depolarization. If so, why do campaniform sensilla incorporate such great hypertrophy of ciliary microtubules into their structure? It is interesting to speculate that the 350–1000 parallel microtubules of the sensory process function as mechanochemical engines driven backwards by the force of the stimulus, creating conditions favorable to formation of generator current. It is possible that compression of the sensory-process microtubules effected by the mechanical stimulus causes release of bound ions from the tubules themselves or the neighboring cytoplasmic matrix. Alteration of ion balance within the sensory process might favor local current flow across the plasma membrane surrounding the sensory process. The vast proliferation of microtubules in the sensory process may serve to increase the "gain" of the mechanoreceptor.

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