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Evidence for active role of cilia in sensory transduction

(neurobiology/sense organs/mechanoreceptors/chordotonal organs/axoneme)

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ABSTRACT **Combined high-voltage electron-microscopic and electrophysiological studies strongly suggest that cilia play an active role in sensory transduction in the grasshopper proximal femoral chordotonal organ (FCO), a ciliated mechanoreceptor. The FCO of pro- and mesothoracic legs of *Melanoplus bivittatus* contains a group of several hundred chordotonal sensilla arranged in a near-parallel bundle and slung between the proximal femur and the knee joint. Both flexion and extension of the tibia stimulate the FCO, which appears to measure the femoro-tibial angle. The FCO's U-shaped response curve indicates that progressive flexion or extension from the resting joint angle of 90° increases the response frequency of individual receptors and recruits additional units as well. Since the FCO is a purely tonic mechanoreceptor, it is possible to fix FCOs during maximum and minimum states of stimulation and electron-microscopically observe changes in the receptor's fine structure. The most conspicuous change is the production of a pronounced bend at the base of the sensory cilia in chordotonal sensilla of maximally stimulated femoral chordotonal organs.**

A wide variety of sense organs center their functions on receptor cells equipped with a cilium or ciliary derivative. Their widespread occurrence in sense organs suggests cilia participate in sensory reception. Nearly 2 decades ago, Gray and Pumphrey (1) investigated the fine structure of the locust ear, observed ciliated receptor cells (arthropods had been presumed to be devoid of somatic cilia), and said, "It seems that, when a ciliated effector is transformed into a receptor in the course of ontogeny, the fundamental structure of the cilium sometimes persists; and its retention in these cases suggests that, whatever role is ascribed to it in ciliary activity, it can play the same part but in a reversed sense in the receptor process."

Since Gray and Pumphrey's pioneer work, two opposing points of view have arisen regarding the role of cilia in sensory transduction. One viewpoint holds that sensory cilia have an active role in transduction; the other maintains that the cilia serve as passive plungers (2). We are working with ciliated mechanoreceptors (3–8), and undertake this investigation to answer the question: do cilia play an active role in sensory transduction? That is, does ATP-dependent doublet displacement leading to active sliding occur in response to mechanical stimulation, or does the cilium serve as a passive plunger that distorts a transducer membrane?

Although circumstantial evidence has given rise to much speculation (see Wiederhold, ref. 2, for review), little direct evidence exists to answer the question at hand. What is needed is a sense organ in which cilia can be readily observed in stimulated versus unstimulated receptors so that ultrastructural changes in ciliary conformation can be correlated with the presence and absence of bioelectrical activity.

Such a system is offered by the grasshopper proximal femoral chordotonal organ (3, 9), hereinafter called the FCO. The FCO has several hundred ciliated mechanoreceptors called chordotonal sensilla arranged in near-parallel, is quite amenable to ultrastructural and physiological investigation, and can be fixed in different states of excitation so changes in ciliary configuration can be observed by electron microscopy.

This report summarizes the structure of the FCO, describes its relevant physiology, and presents data that describe ciliary alterations observed by high-voltage electron microscopy in maximally and minimally stimulated femoral chordotonal organs.

Abbreviation: FCO, femoral chordotonal organ.

MATERIALS AND METHODS

Physiology. Pro- and mesothoracic legs from adult grasshoppers (*Melanoplus bivittatus*) were severed at the coxa after slight CO_2 anesthesia, sealed with silicone grease to prevent desiccation, and glued intact onto a recording table. The femora were mounted with their ventral and frontal surface on the edge of the table so the tibia could be moved freely, as in the normal animal. The relative position of the FCO can be seen in Figs. 1 and 2. Two movable pins (about 100 μm thick) mounted in a Prior micromanipulator were inserted in the positions indicated in Fig. 2. The pins were connected to a Tektronix R5103N dual beam storage oscilloscope through a 5A22N differential amplifier. Audio monitoring was provided, as well as a connection to a tape recorder for data storage. The events concurrent with the electrical signals were monitored in a second channel of the tape recorder through a microphone next to the experimenter. The leg was moved manually by means of a thread tied to the tibia, and the femoro-tibial angle was measured with a portable protractor. With practice, fairly stable velocities could be obtained. No monitoring of the leg's movement was fed into the second trace of the oscilloscope; voice monitoring was found to be sufficiently accurate for the description below. The stored data were processed directly from the tapes into a PDP-12A computer, using the Decus Library Program no. 12-15 HISTO-12 from Digital Equipment Corp. This program was designed to do histogram analysis from analog signals fed directly into the computer and windowed by an adjustable threshold. The results were displayed as a frequency histogram on the computer screen, while the mean results and sampling parameters were written in the teletype.

High-Voltage Electron Microscopy. Pro- and mesothoracic legs from two grasshoppers were fixed with the femoro-tibial angle set at either 90° (minimum stimulation of FCO) or 20° (maximum stimulation). To speed fixation, legs were set at the desired angle, affixed to a platform, and injected with Karnovsky's (10) fixative. After fixation, tissues were rinsed briefly in buffer, post-fixed in buffered 2% OsO_4, dehydrated in an acetone series, and embedded in Spurr's (11) resin. Thick (1 μm) longitudinal sections were cut through the FCO *in situ* in the embedded femur using a diamond knife in a Porter-Blum MT-2B ultramicrotome, collected on slot grids following the method of Rowley and Moran (12), double-stained in uranyl acetate (15 min) and full-strength Reynolds (13) lead citrate (15

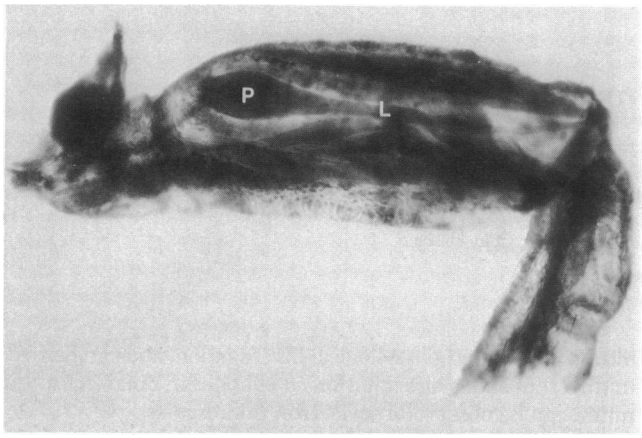

FIG. 1. Whole mount of the femur and proximal tibia of the mesothoracic leg of a second instar grasshopper nymph showing the proximal FCO (P) and ligament (L) *in situ*. Magnification = ×80.

min), and photographed at an accelerating voltage of 1000 kV with the JEM-1000 high-voltage electron microscope at the Department of Molecular, Cellular, and Developmental Biology, University of Colorado.

RESULTS

Morphology. The ultrastructure of the grasshopper proximal femoral chordotonal organ (FCO) has been described elsewhere in detail (3, 9) and will be reviewed here only to orient the reader.

The FCO is longitudinally situated within—and extends the entire length of—the femur in pro- and mesothoracic legs. As seen in the whole mount in Fig. 1 and the drawing in Fig. 2, the FCO is attached proximally to the dorsal cuticle of the femur and distally to cuticular specializations of the femoro-tibial joint. Mechanoreceptive elements, the chordotonal sensilla, lie in the belly of the organ (at the position of the P) and are arranged in near-parallel with their cilia pointing toward the knee. The cilia insert into extracellular caps attached to modified microtubule-packed epidermal cells that, taken together, form the ligament L. The attachment geometry is such that *both flexion and extension of the tibia pull the ligament;* the ligament is slack when the femoro-tibial angle is 90°.

Each chordotonal sensillum within the FCO is innervated by

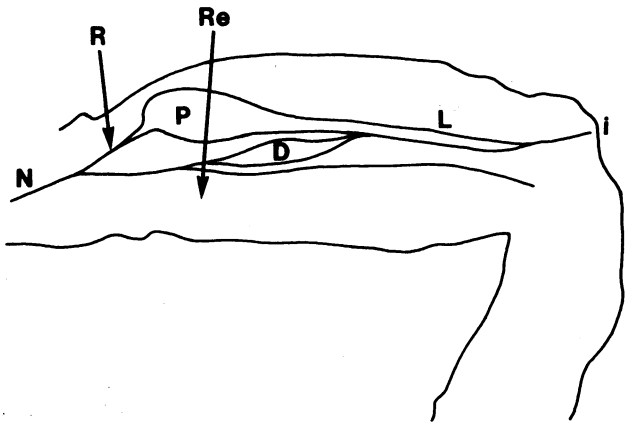

FIG. 2. Tracing of Fig. 1 showing position of reference (Re) and recording (R) electrodes that allow us to discriminate between outputs of proximal (P) and distal (D) FCOs. N, nerve; L, ligament; i, ligament insertion.

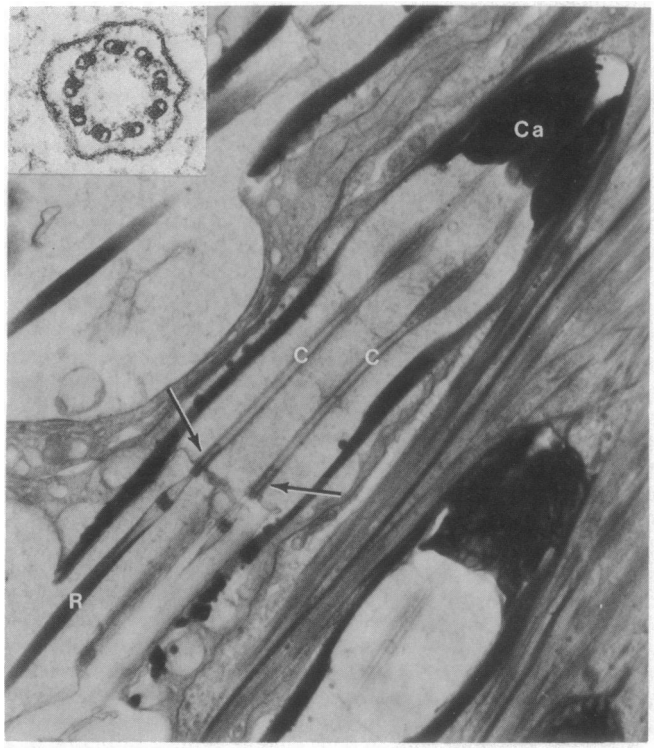

FIG. 3. High-voltage electron micrograph of a longitudinal section through the paired dendrites and cilia of a chordotonal sensillum from a minimally stimulated FCO. Note the ciliary bases are straight. R, rootlet in distal dendrite; C, cilium; Ca, cap; arrow indicates ciliary necklace. Inset shows cross-section through cilium; note (dynein?) arms attached to A-subfibers. Magnification = ×5,800 (inset = ×50,000).

a pair of bipolar neurons (Fig. 3). Each bipolar neuron sends an axon to the appropriate thoracic ganglion of the central nervous system and a dendrite toward the site of stimulus reception. The dendrite tip contains a pair of basal bodies that lie in tandem. The proximal basal body is associated with a conspicuous rootlet apparatus that extends through the dendrite well into the cell body. The distal basal body is coextensive with a modified "9+0" cilium equipped with a prominent dilatation that tapers down as the cilium enters the cap. When viewed in cross section, the ciliary axoneme has a "9+0" pattern of organization. A conspicuous pair of arms, identical in appearance and position to the dynein arms of motile cilia, is present (see inset, Fig. 3).

Physiology. The electrical output of the proximal FCO consists solely of tonic units. A maximum spike activity occurs at two positions; total extension and total flexion.

A representative set of results is shown in Figs. 4 and 5. In Fig. 4 the frequency of spikes is plotted against the femoro-tibial angle. In the lower curve 3 units of large spike size (70 μV) were sampled using a high threshold in the computer. In the upper curve a minimum threshold was used and it thus represents the total response frequency including all units of more than 30 μV. In both cases there is an increase in frequency at the extremes of extension and flexion, the response to flexion being slightly more vigorous. The steepness of the total response (upper curve) is due not only to the increase in frequency of the units activated (lower curve) but also to the increase in the *number* of units recruited as the angle deviated from the middle range. Since the spike sizes were fairly uniform, it was not possible to obtain data for single units.

In Fig. 5 the tendency of the units to adapt their rate of dis-

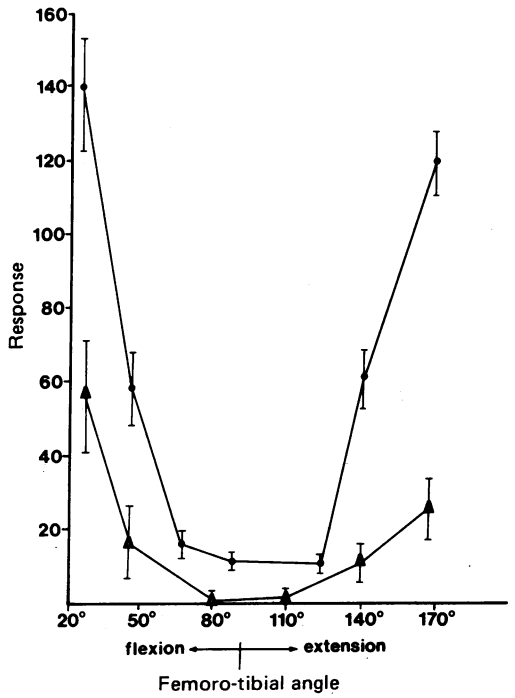

FIG. 4. Response curve from the proximal FCO of one grasshopper leg. Vertical bars show ± 1 SD. Lower curve (△): data from units of largest spike size. Upper curve (●): data sampled with minimum threshold, i.e., total response of the nerve. Response: spikes per second.

charge when maintained at a constant femoro-tibial angle is displayed. It is evident that, regardless of the femoro-tibial angle, little adaptation over time takes place. In other words, the units behave tonically, and maintain their rate of firing for as long as the mechanical stimulation is maintained. No other classes of units were found in the proximal FCO.

The fact that electrical output from the proximal FCO is purely tonic is consistent with the morphological uniformity of the organ's receptors and their symmetric arrangement. This functional characterization contrasts with that of the distal FCO, where phasic and tonic units have been observed, and supports Burns' conjecture that the output of the proximal FCO provides the central nervous system with information about the femoro-tibial angle (13).

Some controversy exists about the nature of the adequate stimulus for chordotonal sensilla. Howse (14) suggests "flexion at the scolopale-cap junction" is the adequate stimulus, whereas Young (15) maintains stretching of the ciliary apparatus is stimulatory. In the grasshopper FCO, the chordotonal sensilla are arranged as a series of concentric cones. All the receptors lie slightly off-axis from the midline of the ligament. The receptors at the periphery make a steeper angle (25°) with the ligament long axis than those at the center. Thus, pulling the ligament, which stimulates the organ, imparts both lateral and longitudinal components of displacement upon the cilia of the chordotonal sensilla. Therefore both lateral and longitudinal ciliary displacements could contribute to the adequate stimulus. The magnitude of the longitudinal displacement component is greater than that of the minute lateral component, which we estimate to be an angular deviation of <1–10°.

Ultrastructural Changes in Cilia of Stimulated Sensilla. The facts that the physiological activity of the FCO is a function of the femoro-tibial angle and the FCO is a tonic receptor allow us to fix FCOs in known states of physiological activity and look for corresponding ultrastructural changes in their sensory cilia.

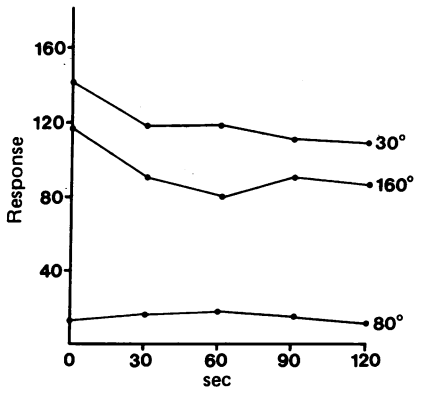

FIG. 5. Adaptation with time of the tonic units' response displayed in Fig. 4 (dots). The tibia was set at several angles; responses were averaged at different times up to 120 sec.

Furthermore, the plastic-penetrating capability of the JEM-1000's 1 MV electron beam favors the use of 1 μm thick sections that permit us to capture a statistically significant number of sensory cilia in suitable orientation for study. To that end, serial sections were cut through three FCOs from legs fixed with knee-joints set at 20° (maximally stimulated) and two FCOs fixed at 90° (minimally stimulated). Each large (1 mm^2) section was a 1 μm thick longitudinal slice through an entire FCO. Survey micrographs were taken at 3000× so all chordotonal sensilla within a given section could be included in two or three photographic fields. Each negative was placed beneath a Wild binocular dissecting microscope for observation. Two unbiased observers, each unaware of the physiological status of the receptors at hand, carefully examined each suitably sectioned cilium and scored it as "bent" or "straight" at the base.

We have examined about 200 longitudinally sectioned sensory cilia, and find the most consistent ultrastructural change consistent with physiological activity to be *bending at the base* of cilia of stimulated sensilla. Fig. 3, for example, depicts two typical "straight" cilia from a minimally stimulated FCO. Fig. 6, on the other hand, is a high voltage electron micrograph of a typical "bent" cilium from a maximally stimulated FCO. The distal basal body and attached ciliary base are tilted, and the cilium is clearly "bent" at the ciliary necklace.

In counting "bent" cilia, we specifically looked for images such as that in Fig. 6. The results of our observations, tabulated in Table 1, show that 63% of the cilia are *bent* in *maximally stimulated* FCOs, whereas only 14% of the cilia are bent in minimally stimulated organs.

DISCUSSION

In motile cilia, ciliary activity is manifest in *bending*, and bending results from force generated by dynein arms that causes active sliding to occur between component doublets of the axoneme (16). This bending occurs *locally* as the wave of active sliding is propagated. If cilia play an active role in mechanoreception, we would expect to see them bend locally in response to mechanical stimulation. The present study clearly shows the sensory cilia of the grasshopper FCO *do bend* locally in response to mechanical stimulation. This local bend cannot be explained by a passive action of the mechanical stimulation upon the axoneme, because such a passive action would lead the axoneme to be curved throughout its length. In Figs. 6 and 7 it is seen that the bend occurs only locally near the ciliary base, while the rest of the axoneme remains straight. We therefore conclude that cilia play an active role in sensory transduction in the FCO.

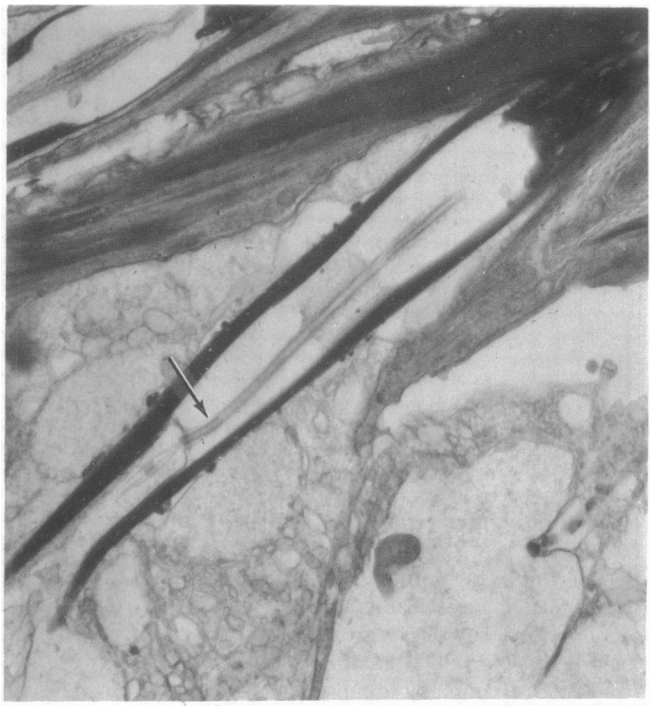

FIG. 6. High-voltage electron micrograph of a longitudinal section through a chordotonal sensillum of a *maximally* stimulated FCO. Note the base of the cilium is bent at the ciliary necklace (arrow). Magnification = ×5,800.

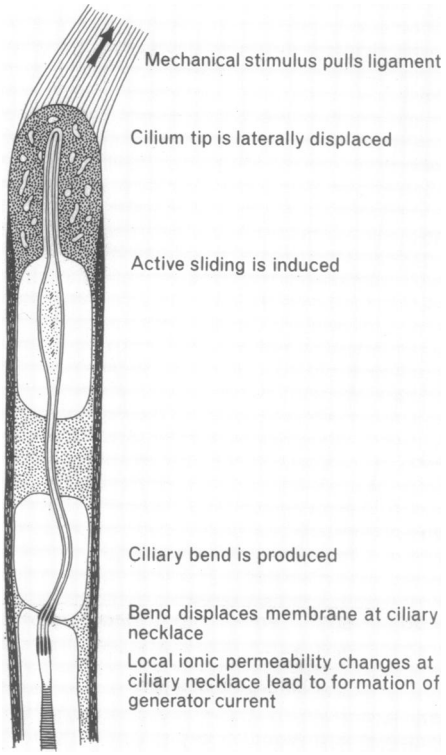

FIG. 7. A model for the active role of cilia in mechanoreception.

How, then, does the ciliary bending relate to sensory transduction? We have the following sequence of events, illustrated in Fig. 7:

(*1*) When the ligament is pulled, the cilium tip is laterally displaced.

(*2*) Mechanical displacement of the cilium tip induces active sliding between adjacent doublets of the axoneme.

(*3*) Active sliding produces ciliary bending.

(*4*) The bend is propagated to the movable distal basal body at the base of the cilium.

(*5*) Bending of the ciliary base distorts the cell membrane in the region of the ciliary necklace, causing local changes in ionic permeabilities leading to the production of generator current.

The sequence of events suggested for sensory cilia is known to occur in motile cilia. We make one basic assumption: that mechanical stimulation can induce active sliding in sensory cilia. It is generally accepted that mechanical displacement causes sliding—and sliding causes bending—in motile cilia. Thurm, for example (17), has shown that resting motile abfrontal gill cilia of the European mussel *Mytilus edulis* respond to a 2°–15° mechanical displacement with an active stroke.

Table 1. Data from counts of 262 cilia in maximally and minimally stimulated FCOs

Stimulation	Cilia		
	Bent, no.	Straight, no.	Bent, %
Maximal	79	46	63
Minimal	16	101	14

$P \ll 0.001$ that the % cilia bent is the same for maximally and minimally stimulated FCOs.

More recently, Tamm (18) has established the mechanosensitivity of motile comb plate cilia of ctenophores. From the physiological and ultrastructural studies presented in this report, we see bends in mechanically stimulated cilia. The existence of structural components common to both sensory and motile cilia—i.e., nine outer doublets with A-subfibers that bear arms—indicates the simplest explanation for bend formation is that the link between stimulation (i.e., lateral displacement) and bending in FCO cilia is active sliding, as is the case for motile cilia (19).

In a previous description of the FCO (3), we suggested its sensory cilia respond to mechanical stimulation with an active stroke. An active stroke is a coordinated bend-propagation employed by motile cilia in which dynein arms provide the force for sliding (20), and radial spokes that interconnect the doublets with the central sheath (21) transduce sliding into bending (22). The FCO sensory cilia have (dynein?) arms, yet lack spokes, central sheath, and the central pair. Therefore, we believe they are incapable of an active stroke, but *are capable of active sliding*. Our model requires sliding as a sufficient condition for producing a bend at the base of the cilium. It is interesting to note that recent application of catastrophe theory to the dynamics of ciliary motility offers the mechanical prediction that a small displacement of the tip of a sensory cilium will create a large amount of sliding that will, in turn, induce a considerable bend at the base of the cilium (23).

Bending the base of the cilium will deform the ciliary necklace. The ciliary necklace (24) is a region of modified membrane periodically attached to the axonemal doublets just distal to the basal body. We concur with the suggestion of Gilula and Satir (24) that the ciliary necklace may participate in mechanoelectric transduction through permeability changes induced by alterations in membrane particle patterns that result from ciliary bending. This notion is supported by transmission electron microscopic images that show the FCO sensory cilia

are endowed with large and conspicuous ciliary necklaces, whereas those of campaniform sensilla—mechanoreceptors that use the tips of their modified cilia for transduction (25, 26)—are relatively small.

In summary, then, this study shows that cilia of the proximal femoral chordotonal organ from the grasshopper develop conspicuous bends at their bases in response to mechanical stimulation. This strongly supports the hypothesis that cilia play an active role in sensory transduction in the FCO. Thus it seems that evolution has taken a primitive mechanochemical engine, the cilium, and placed it at the tips of certain mechanoreceptive dendrites where, made available to directed physical displacements, it can be "run in reverse."

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1. Gray, E. G. & Pumphrey, R. J. (1958) "Ultra-structure of the insect ear," *Nature* **181,** 618.
2. Wiederhold, M. L. (1976) "Mechanosensory transduction in 'sensory' and 'motile' cilia," *Annu. Rev. Biophys. Bioeng.* **5,** 39–62.
3. Moran, D. T., Rowley, J. C., III & Varela, F. G. (1975) "Ultrastructure of the grasshopper proximal femoral chordotonal organ," *Cell Tissue Res.* **161,** 445–457.
4. Moran, D. T. & Rowley, J. C., III (1975) "The fine structure of the cockroach subgenual organ," *Tissue & Cell* **7,** 91–105.
5. Moran, D. T. & Rowley, J. C., III (1975) "High voltage and scanning electron microscopy of the site of stimulus reception of an insect mechanoreceptor," *J. Ultrastruct. Res.* **50,** 38–46.
6. Moran, D. T., Chapman, K. M. & Ellis, R. A. (1971) "The fine structure of cockroach campaniform sensilla," *J. Cell Biol.* **48,** 155–173.
7. Moran, D. T. & Varela, F. G. (1971) "Microtubules and sensory transduction," *Proc. Natl. Acad. Sci. USA* **68,** 757–760.
8. Chapman, K. M., Duckrow, R. B. & Moran, D. T. (1973) "Form and role of deformation in excitation of an insect mechanoreceptor," *Nature* **244,** 453–454.
9. Slifer, E. H. & Sekhon, S. S. (1975) "The femoral chordotonal organs of a grasshopper, Orthoptera, Acrididae," *J. Neurocytol.* **4,** 419–438.
10. Karnovsky, M. J. (1965) "A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy," *J. Cell Biol.* **27,** 137A.
11. Spurr, A. R. (1969) "A low-viscosity epoxy resin embedding medium for electron microscopy," *J. Ultrastruct. Res.* **26,** 31–43.
12. Rowley, J. C. & Moran, D. T. (1975) "A simple procedure for mounting wrinkle-free sections on Formvar-coated slot grids," *Ultramicroscopy* **1,** 151–155.
13. Burns, M. D. (1974) "Structure and physiology of the locust femoral chordotonal organ," *J. Insect Physiol.* **20,** 1319–1339.
14. Howse, P. E. (1968) "The fine structure and functional organization of chordotonal organs," *Symp. Zool. Soc. London* **23,** 167–198.
15. Young, D. (1970) "The structure and function of a connective chordotonal organ in the cockroach leg," *Philos. Trans. R. Soc. London Ser. B* **256,** 401–426.
16 Satir, P. (1974) "How cilia move," *Sci. Am.* **231,** No. 4, 44–52.
17. Thurm, U. (1968) "Steps in the transducer process of mechanoreceptors," *Symp. Zool. Soc. London* **23,** 199–216.
18. Tamm, S. L. (1973) "Mechanisms of ciliary coordination in ctenophores," *J. Exp. Biol.* **59,** 231–245.
19. Satir, P. (1968) "Studies on cilia. III. Further studies on the cilium tip and a 'sliding filament' model of ciliary motility," *J. Cell Biol.* **39,** 77–94.
20. Gibbons, I. R. (1967) "The organization of cilia and flagella," in *Molecular Organization and Biological Function,* ed. Allen, J. M. (Harper & Row, Publishers, New York), pp. 211–237.
21. Warner, F. D. & Satir, P. (1974) "The structural basis of ciliary bend formation," *J. Cell Biol.* **63,** 35–63.
22. Sleigh, M. A., ed. (1974) *Cilia and Flagella* (Academic Press, New York).
23. Varela, F. J., Rowley, J. C., III & Moran, D. T. (1976) "The control of ciliary movements: an application of the cusp catastrophe," *J. Theor. Biol.*, in press.
24. Gilula, N. B. & Satir, P. (1972) "The ciliary necklace: A ciliary membrane specialization," *J. Cell Biol.* **53,** 494–509.
25. Thurm, U. (1974) "Mechanisms of electrical membrane responses in sensory receptors, illustrated by mechanoreceptors," in *Biochemistry of Sensory Functions,* ed. Jaenicke, L. (Springer-Verlag, Berlin-Heidelberg-New York), pp. 367–390.
26. Moran, D. T., Rowley, J. C., III, Zill, S. & Varela, F. J. (1976) "The mechanism of sensory transduction in a mechanoreceptor: functional stages in campaniform sensilla during the molting cycle," *J. Cell Biol.* **71,** 832–847.