Cowles, *Plant Physiol.* **63**, 402 (1979); R. Howard, F. Battye, G. Mitchell, *J. Histochem. Cytochem.* **27**, 803 (1979); H. Steen, E. Brye, K. Skarstad, B. Bloom, T. Godal, S. Mustafa, *Cytometry* **2**, 249 (1982).
3. R. Douglas and C. Ballou, *J. Biol. Chem.* **255**, 5979 (1980).
4. R. Langlois, A. Carrano, J. Gray, M. Van Dilla, *Chromosoma* **77**, 229 (1980).
5. P. Dean, in *Flow Cytometry*, O. Laerum, T. Lindmo, E. Thorud, Eds. (Universitetsforlaget, Oslo, 1980), vol. 4, pp. 41–44; P. Dean and D. Pinkel, *J. Histochem. Cytochem.* **26**, 622 (1978).
6. *Bergey's Manual of Determinative Bacteriology*, R. Buchanan and N. Gibbons, Eds. (Williams and Wilkins, Baltimore, ed. 8, 1974).
7. A. G. Fredrickson and G. Stephanopoulos, *Science* **213**, 972 (1981).
8. This work was performed under the auspices of the Department of Energy by the Lawrence Livermore National Laboratory under contract W-7405-ENG-48.

28 June 1982; revised 30 November 1982

Interrelated Striated Elements in Vestibular Hair Cells of the Rat

Abstract. *Unusual fixation procedures revealed a series of interrelated striated organelles in type I and type II vestibular hair cells of the rat; these organelles seemed to be less well developed in cochlear hair cells. The findings suggest that contractile elements may play a role in sensory transduction in the inner ear, particularly in the vestibular system.*

Actin is present in the hair cells of vestibular (*1, 2*) and auditory (*3, 4*) receptors. By S-1 myosin decoration methods (*5*), actin has been found in the stereocilia and their rootlets, the cuticular plate, and the electron-opaque material that forms the junctional complexes of the reticular lamina. Because myosin also has been found in apical parts of hair cells of the organ of Corti (*6*), it might be expected that actin and myosin are organized into contractile elements that take part in signal processing in the inner ear. Generally, however, S-1 myosin decoration has failed to demonstrate highly organized actin filaments in the cuticular plate (*1–4*). Only Slepecky and her colleagues (*7*) have illustrated an infracuticular, striated element containing actin (*4*). Lowenstein and Osborne (*8*) earlier described ribbonlike striated elements in vestibular hair cells of ammocoetes larvae of the lamprey extending from the cuticular plate to the cell membrane basally and ending close to synaptic sites. Their results, obtained by conventional transmission electron microscopy, prompted them to hypothesize that such striated material might be involved in transduction. Other investigators have also reported finding isolated laminated, or striated, elements in the basal part of the cuticular plate, or below it, in hair cells of both normal (*9–14*) and pathological (*9, 15*) inner ears in many species. Striated material was also abundant in hair cells of old monkeys (*12*) and elderly humans (*12, 16*), and in drug-treated animals (*13, 17*). The apparently increased incidence of such bodies under pathological conditions and as a consequence of aging seems to have overshadowed findings in normal material, and laminated bodies have generally come to be regarded as pathological entities. Nevertheless, several investigators have emphasized that striated elements are normal constituents of vestibular (*8, 10–12*) and cochlear (*7, 14*) hair cells.

We now describe our ultrastructural finding of a series of interrelated striated elements in apical parts of vestibular hair cells of the rat. Included in the series are the cuticular plate and its basal attachments to the hair cell margins, the connections of the strut array of the kinociliary basal body (*18*) to the cuticular plate, and striated organelles associated with the plasma membrane and extending below the apical junctional complexes. The latter organelles are more extensive in type I than in type II hair cells. In contrast, rat cochlear hair cells seem to lack similar striated organelles associated with the plasma membrane and to have less robust striations in the cuticular plate. Our findings indicate a broader distribution of striated elements in vestibular hair cells than was found previously. They support the concept that contractile elements may play a role in transduction in the inner ear (*7, 8, 19*), particularly in the vestibular system.

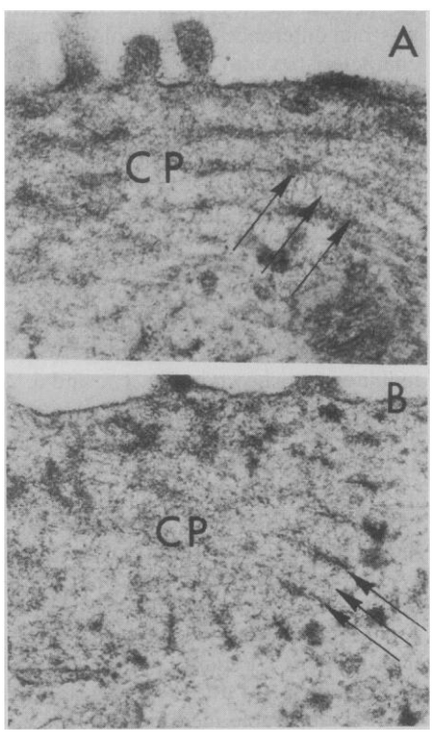

Fig. 1. (A) Striations in the substance of the cuticular plate (*CP*) of a type I utricular hair cell. (B) Striations along the lower face of the circular plate of a type II utricular hair cell. The three arrows indicate opposite electron-opaque stripes and an intermediate line. Magnification: ×43,000.

Our results were obtained in specific-pathogen-free, young adult, Sprague-Dawley rats (Charles River) during an effort to determine an optimal method for preserving inner ear tissue collected under space-flight conditions. Osmium tetroxide could not be used, because of its toxicity; tissues, once collected, might have to be stored for as long as 5 days before further processing could take place. The following method resulted in unexpected preservation of the striated organelles in hair cells. Temporal bones were rapidly removed from decapitated rats, and the oval and round windows of the cochleas were opened immediately upon immersion in 2.5 percent glutaraldehyde in 0.1*M* sodium phosphate buffer (*p*H 7.4). After fixation for 2 hours at room temperature, the tissues were stored in fresh fixative in vials at 4°C for 5 days. The tissues were then washed in buffer, fixed in 1.0 percent osmium tetroxide in 0.1*M* sodium phosphate buffer (*p*H 7.4) for 1 hour, then washed again, microdissected, and prepared by standard methods for transmission electron microscopy. Subsequent experiments demonstrated that the striated organelles were well preserved when prolonged storage was omitted. The organelles were not seen, however, after fixation in Karnovsky's (*20*) 4 percent paraformaldehyde–5 percent glutaraldehyde solution.

The striated organelles of hair cells have a repeating pattern of alternating electron-opaque stripes and broader, more electron-lucent bands. The bands are intersected by narrow, electron-opaque, intermediate lines (Figs. 1 and 2). This pattern is organizationally similar to that of striated rootlets of the kinociliary basal body in the hair cells themselves, and particularly to that described for contractile rhizoplasts in the flagellate *Platymonas subcordiformis* (*21*). It is also comparable to the pattern commonly reported for isolated laminat-

ed bodies in normal (*9, 10, 14*) and abnormal (*9, 13, 15, 17*) hair cells.

The three-dimensional organization of the striations in the highly ordered cuticular plate is not entirely resolved. The striations in the substance of the plate are slightly oblique to the apical surface of the cell, but those at the periphery are perpendicular to the edge of the plate and follow its curvature (Fig. 1). The stripes are interconnected in a complex fashion. The stereociliary rootlets seem to correspond to electron-opaque stripes and to be linked to the cuticular plate through filaments projecting toward nearby intermediate, electron-opaque lines. Such linkages could explain the actin filaments with opposing polarities observed by Flock *et al.* (*2*) between the rootlets. Furthermore, the strut array of the kinociliary basal body has connections to striated material associated with the cuticular plate. This arrangement suggests that interactions between the kinocilium and the stereocilia are possible through the cuticular plate and that such interactions might carry vectoral information.

The base of the cuticular plate is supported by a striated element anchored to spurs of electron-opaque material located at the zonula adherens (Fig. 2A). The striations are arranged parallel to the cell margin. The electron-opaque stripes in the cuticular plate and its attachments are ≃ 40 nm wide. The periodicities of the stripes vary between 110 and 130 nm in the plate and between 110 and 160 nm in the attachment zone.

Both type I and type II vestibular hair cells have striated organelles associated with the plasma membrane that extend for various distances below the junctional complexes (Fig. 2). The type I hair cell is characterized by its flask shape and is ordinarily surrounded over much of its surface by a calyx formed by the afferent nerve ending (*10, 22*). In this cell type, a striated neck element originates broadly from the spurs of electron-opaque material at the zonula adherens and then extends into the neck region (Fig. 2B), apparently following the contours of the hair cell membrane under the calyx nerve ending. The stripes are 45 to 70 nm wide and occur at 140- to 180-nm intervals. The stripes extend into the cell in a direction perpendicular to the contiguous cell membrane. The hair cell membrane often shows puckers corresponding to the positions of the stripes.

Type II hair cells are typically cylindrical, lacking a well-defined neck and calyx (*10, 22*). Instead, they have afferent and efferent bouton terminals basally. The striated organelle associated with the plasma membrane in this type of hair cell has been observed thus far only in cross sections of the utricular macula (Fig. 2B). The saccular macula and ampullary cristae have not yet been examined for its presence. This organelle appears to be less extensive than its counterpart in the type I hair cell. It originates eccentrically, apparently from spurs of electron-opaque material described above, and approaches the cell membrane to follow it internally (Fig. 2B). The organelle terminates where a type II hair cell lies close to the calyx of a type I hair cell (Fig. 2B), with an intercellular cleft only 23 nm wide intervening. The electron-opaque stripes are oriented almost perpendicularly to the cell membrane. They are ≃ 40 nm wide and occur at 130- to 200-nm intervals.

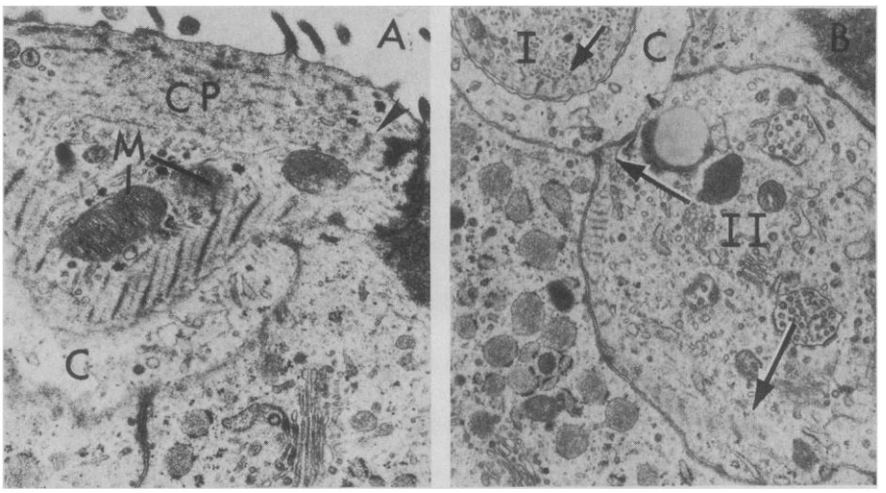

Fig. 2. Type I and type II hair cells having striated elements associated with the plasma membrane. Both the upper part (A) and the lower part (B, at upper left arrow) of an element in a type I hair cell lie under a calyx (*C*). The striated element (B, arrows) in the type II hair cell terminates where the cell membrane lies close to a calyx. In (A) the single arrowhead (upper right) points to the attachment of the cuticular plate (*CP*) to the cell margin; *M*, mitochondria. Magnifications: (A) ×16,250; (B) ×14,500.

Two factors appear to be of consequence in preserving the striated organelles of hair cells reported here: rapid primary fixation with glutaraldehyde, which cross-links actin molecules and stabilizes actin against denaturation (*23*); and fixation in osmium tetroxide in a sodium phosphate buffer. The choice of buffer is apparently critical. Osmium can fragment actin filaments even after they have been fixed in glutaraldehyde; but sodium phosphate buffers inhibit the destructive action of the osmium (*24*). Furthermore, our findings suggest that prolonged exposure to glutaraldehyde does not harm and may even enhance preservation of the striated elements of inner-ear hair cells. The presence of proteins that can bind to actin, such as tropomyosin, can also inhibit the destructive effects of osmium (*24*). This may account for the widespread finding of laminated bodies in hair cells even when optimal conditions for actin preservation have not been met. It is also possible that changes in associated proteins occur under pathological conditions and detrimentally stabilize some of the actin.

The striated elements described here for vestibular hair cells are potentially contractile and, like contractile rhizoplasts (*21*), might prove to be dependent on adenosine 5′-triphosphate and regulated by calcium ions and modulators. Future research will be required to determine the role of each element in signal processing or in transduction in the inner ear.

MURIEL D. ROSS
CAROL BOURNE
Department of Anatomy and Cell Biology, University of Michigan, Ann Arbor 48109

References and Notes

1. A. Flock and H. Cheung, *J. Cell Biol.* **75**, 339 (1977); N. Hirokawa and L. G. Tilney, *ibid.* **95**, 249 (1982).
2. A. Flock, H. Cheung, B. Flock, G. Utter, *J. Neurocytol.* **19**, 133 (1981).
3. L. G. Tilney, D. J. DeRosier, M. J. Mulroy, *J. Cell Biol.* **86**, 244 (1980).
4. N. Slepecky and S. C. Chamberlain, *Cell Tissue Res.* **244**, 15 (1982).
5. H. E. Huxley, *J. Mol. Biol.* **7**, 281 (1963).
6. J. C. Macartney, S. D. Comis, J. O. Pickles, *Nature (London)* **288**, 491 (1980).
7. N. Slepecky, R. Hamernik, D. Henderson, *Hearing Res.* **2**, 413 (1980); *Acta Oto-Laryngol.* **91**, 189 (1981).
8. O. Lowenstein and M. O. Osborne, *Nature (London)* **204**, 197 (1964).
9. D. A. Hilding and W. F. House, *Laryngoscope* **74**, 1135 (1964).
10. H. Engström, B. Bergström, H. W. Ades, *Acta Oto-Laryngol. Suppl.* **301**, 75 (1972).
11. T. Hoshino, *Acta Oto-Laryngol.* **80**, 43 (1975); J. M. Jorgensen, *ibid.* **94**, 241 (1982).
12. H. Engström *et al.*, *Adv. Oto-Rhino-Laryngol.* **22**, 93 (1977).
13. V. Jahnke, *Acta Oto-Laryngol.* **68**, 336 (1969).

14. R. S. Kimura, *ibid.* **61**, 55 (1966); H. Spoendlin, *Adv. Oto-Rhino-Laryngol.* **13**, 19 (1966).
15. I. Friedmann, T. Cawthorne, K. McLay, E. S. Bird, *J. Ultrastruct. Res.* **9**, 123 (1963); I. Friedmann, T. Cawthorne, E. S. Bird, *ibid.* **12**, 92 (1965); *Nature (London)* **207**, 171 (1965); D. A. Hilding, A. Sugiura, Y. Nakai, *Ann. Otol. Rhinol. Laryngol.* **76**, 647 (1967).
16. U. Rosenhall and B. Engström, *Acta Oto-Laryngol. Suppl.* **319**, 3 (1974).
17. I. Friedmann, J. V. Dadswell, E. S. Bird, *J. Pathol. Bacteriol.* **92**, 415 (1966); H. Spoendlin, in *Ultrastructure of the Peripheral Nervous System and Sense Organs*, J. Babel, A. Bischoff, H. Spoendlin, Eds. (Mosby, St. Louis, 1970), pp. 294–295.
18. A. Flock and A. J. Duvall, *J. Cell Biol.* **25**, 1 (1965); A. M. Kuzirian, D. L. Alkon, L. G. Harris, *J. Neurocytol.* **10**, 497 (1981).
19. M. D. Ross, *Physiologist* **25** (Suppl.), S113 (1982).
20. M. J. Karnovsky, *J. Cell Biol.* **27**, 137A (1965).
21. J. L. Salisbury and G. L. Floyd, *Science* **202**, 975 (1978).
22. J. Wersäll, *Acta Oto-Laryngol. Suppl.* **126**, 1 (1956).
23. S. S. Lehrer, *Biochem. Biophys. Res. Commun.* **48**, 967 (1972).
24. P. Maupin-Szamier and D. Pollard, *J. Cell Biol.* **77**, 837 (1978).
25. We thank J. E. Hawkins, Jr., M. Welsh, and S. Brown for their careful reading of this manuscript and T. Halstead and L. Cipriano for their encouragement. This research was supported by NASA grant NSG 9047 and NASA contract NAS2-10535.

16 August 1982; revised 30 December 1982

Bark Beetle Conversion of a Plant Compound to a Sex-Specific Inhibitor of Pheromone Attraction

Abstract. *Both sexes of the bark beetle* Dendroctonus brevicomis *convert the (+) and (−) enantiomers of the tree terpene* α*-pinene to the corresponding enantiomers of* trans*-verbenol at about equal rates. (−)-*trans*-Verbenol inhibited the response of females, but not of males, to a mixture of attractive pheromone components. Since the female initiates the attack on a pine tree, (−)-*trans*-verbenol may play a role in reducing intraspecific competition for breeding areas.*

Many species of bark beetle have pheromones that incite individuals to collect in a mass attack on a tree for the purposes of finding mates and locating suitable breeding areas; this often results in the death of the tree. Some species use an olfactory mechanism to avoid interspecific competition for hosts (*1–3*) as well as to reduce intraspecific competition (*4*). The Western pine beetle *Dendroctonus brevicomis* LeConte is a destructive species infesting ponderosa pine, the predominant forest tree in California. Male and female beetles are equally attracted to a mixture of three pheromone components: *exo*-brevicomin, produced by females; frontalin, produced by males; and myrcene, derived from tree resin (*5*).

Racemic α-pinene, a major monoterpene of the pine tree (*6*), is apparently hydroxylated by *D. brevicomis* females to form *trans*-verbenol (*7*), which accumulates in the hindguts at the beginning of colonization (*2, 8*). However, despite several attempts, no behavioral or ecological function has been established for *trans*-verbenol (*9, 10*). I have found that the (−) enantiomer of *trans*-verbenol acts to inhibit the attraction of females, but not of males, to pheromone components. Since the female begins the attack on a tree, this inhibitory response appears to play a role in regulating the density of colonization and intraspecific competition. Renwick *et al.* (*11*) reported that the cohabiting bark beetle *Ips paraconfusus* converted the (+) enantiomer of α-pinene to *trans*-verbenol and converted the (−) enantiomer to an attractive pheromone component, *cis*-verbenol. In contrast, I found that both sexes of *D. brevicomis* use both enantiomers of α-pinene to synthesize the corresponding enantiomers of *trans*-verbenol (*12*) and synthesize only trace amounts, if any, of *cis*-verbenol.

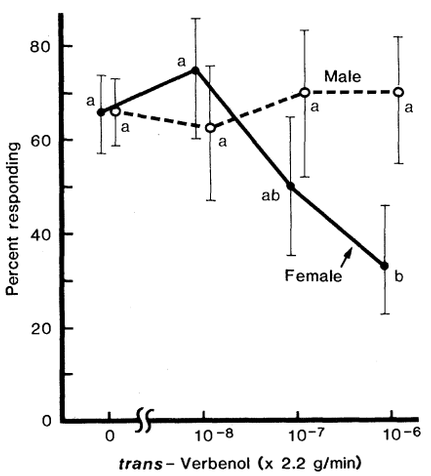

Fig. 1. Effect of increasing release rates of (−)-*trans*-verbenol on the attraction response of walking male and female *Dendrostonus brevicomis* to a 1:1:1 mixture of pheromone components *exo*-brevicomin (> 95 percent), frontalin (> 95 percent), and myrcene (> 99 percent) (Chemical Samples Company), each released at 2.2×10^{-9} g/min (18 October 1976). Points with the same letter were not significantly different ($\alpha = .01$, χ^2). The brackets represent 95 percent confidence limits for proportions.

Adult *D. brevicomis* were reared in the laboratory from the bark of larva-infested ponderosa pine trees (Sierra National Forest, California) and were used in experiments shortly after they emerged. The beetles were exposed for 18 hours to vapors of either the (+) or (−) enantiomer of α-pinene (Aldrich), purified > 99.8 percent by gas-liquid chromatography (GLC). The quantities of *trans*-verbenol, verbenone, and myrtenol in the hindguts were determined by GLC (*13*) (Table 1). The amounts of *trans*-verbenol produced from each enantiomer of α-pinene were similar in males and females, as were the amounts of myrtenol (Table 1). Females did not produce verbenone in detectable levels, whereas males produced significant amounts after being held in a jar for 18 hours at room temperature, whether or not they were exposed to α-pinene vapors. A beetle of either sex contained no more than 0.5×10^{-7} g of *cis*-verbenol (quantified by GLC) after exposure to (−)-α-pinene, not significantly different from the amount in unexposed beetles—but exposure to (+)-α-pinene resulted in about 5×10^{-7} g of a compound having the retention time of *cis*-verbenol.

Samples of *trans*-verbenol were collected in ethanol from the gut extracts (diethyl ether) by condensation on glass beads from the GLC effluent (*14*). The concentrations were quantified by GLC, and the optical rotations (± 5 percent) were obtained with an electrobalancing polarimeter (Autopol III). A comparison of the specific rotations of the (+)- and (−)-α-pinene with the specific rotations of (+)- and (−)-*trans*-verbenol produced in males ($[\alpha]_D^{22} = +105.2°, -89.5°$) and females ($[\alpha]_D^{22} = +104.9°, -91.5°$) indicated that both sexes converted both enantiomers, but each only to the corresponding enantiomer of *trans*-verbenol (*15*). The GLC-purified samples of *trans*-verbenol were then subjected to analysis by gas chromatography–mass spectrometry (V.G. Micromass 7070F mass spectrometer with computerized data system) on a 40-m capillary column (SCOT; OV101) at 115°C. The *trans*-verbenol extracted from males and females had mass spectra identical to the mass spectrum of a standard purified > 99.8 percent by GLC (Glidden Organics) (*16*).

Walking beetles were tested with a laboratory olfactometer for their response to a mixture of pheromone components containing increasing concentrations of *trans*-verbenol, $[\alpha]_D^{22} = -131°$ (Glidden Organics) (*3*). Only the response of females to pheromone components was inhibited at the highest release