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SCANNING ELECTRON MICROSCOPIC OBSERVATIONS OF THE CANINE INNER EAR

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Abstract

The sensory epithelia of the inner ear of the dog have been investigated using scanning electron microscopy (SEM). The surface appearance of the cristae ampullares of the semicircular canals and of the macula utriculi are very similar to other mammalian species. The crista ampullaris of the anterior vertical semicircular canal is divided by a non-sensory septum cruciatum, found in cats and rats but not, for example, in man. The vestibular sensory cells possess two distinct types of stereocilia, one is thick and rigid appearing, the other is thin and limp. Neither type of stereocilium is restricted to a particular hair cell type.

From SEM views of the undersurface of the tectorial membrane of the cochlea we show evidence that some inner hair cell stereocilia may be attached to the tectorial membrane. This observation is made only in middle to upper cochlear regions (those subserving transduction of low frequencies of sound).

KEY WORDS: Sensory organs, inner ear, dog, cochlea, vestibular, scanning electron microscopy.

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Introduction

The ultrastructure of the sensory surfaces of the inner ear as seen with the scanning electron microscope have been described for many mammals (e.g., review by Lewis et al., 1985). However, we are not aware of descriptions of canine cochlear and vestibular organs based on scanning electron microscopy.

The dog has been used as an experimental model in various studies relating to hereditary deafness and vestibular function, however, morphological descriptions of the inner ear sensory organs have been confined to light microscopic observations of either sectioned material or surface preparations (Branis and Burda,1985; Igarashi et al., 1972; Anderson et al., 1968).

Whilst the general features of mammalian inner ears are similar, there is often some detailed anatomical variation between species. The purpose of this present study was to expand on the morphological knowledge of canine inner ear and to provide a broader species basis of anatomical information relating to the vestibular and cochlear systems.

Materials and Methods

The five dogs used in this study are a Samoyed cross breed. They were cage and indoor-run raised and ranged in age from seven to twelve months at the time of death.

At euthanasia the animals were decapitated and the cranial block containing the temporal bone removed. The otic capsule was opened, the facial nerve canal reduced and the stapes removed. Fixative was perfused via the round and oval windows. Fixation was made using either a mixture of 4% paraformaldehyde/1% glutaraldehyde or 2% glutaraldehyde, in either case with a phosphate buffer at pH 7.4. Following primary fixation specimens were washed in buffer then postfixed in buffered 1% osmium tetroxide. The membranous labyrinth was dissected free in 70% ethanol. Following dissection specimens were either dehydrated, critical point dried then sputter coated with gold or prepared by the OTOTO technique (Hunter-Duvar and Mount, 1978) and then critical point

dried and examined in a JEOL JSM35 at 12-20keV depending on the preparative method used.

Results and Discussion

Vestibular Sense Organs

We have examined the crista ampullaris of each semicircular canal and the sensory epithelium of the otolith organs, in particular the macula utriculi. Figure 1 shows the crista ampullaris for the anterior-vertical and lateral-horizontal semicircular canals in relation to the utricle, the floor of which accommodates the macula utriculi. Also opening into the utricle, but not shown in Figure 1, is the ampulla of the posterior vertical semicircular canal. The crista ampullaris of this canal is shown in Figure 2. In both figures the gelatinous cupula which normally overlies the crista has been removed to reveal the upper surface of the sensory epithelium. The concentration of sensory cells on the periphery of the crista ampullaris is greater than in the central regions as can be observed in Figure 2. The anterior crista is divided in the midline by a protruding ridge of non-sensory epithelium called the septum cruciatum (Figure 3). This is found in some other mammalian species (e.g. cat and rat) but not man.

Figure 4 is a higher magnification view of the junction of the sensory epithelium and an area of flat irregularly shaped interlocking cells of the so called planum semilunatum which extends up the lateral wall of the ampulla (over a half moon shaped area). Each hair cell has one long, thin kinocilium and a number of stereocilia of varying height which bundle together to form a rigid appearing body. This rigidity serves to couple the movements of the overlying cupula to the apical surface of the hair cell.

Also evident in Figures 3 and 4 are spherical structures which may be a product of the secretory nature of the supporting cells (Wersall,1956; Kessel and Kardon,1979; Hunter-Duvar,1983) and may function in cupular maintenance. However, their appearance may be artifactual relating to the time delay in fixation after death which ranged from 20-45 minutes for these specimens. The supporting cells and sensory hair cells are positioned on a fibrous basement structure which accommodates a capillary network and also afferent and efferent nerve axons. This supporting structure is shown in Figure 5 which images a fracture through the crista ampullaris in a region near to the sensory epithelium proper called the transitional epithelium. Here the epithelial cells are similar to supporting cells but smaller and more cylindrical.

In the dog there are two morphologically distinct populations of vestibular stereocilia. Figure 6 illustrates the stereocilia of two hair cells of the crista. The cell on the right has stereocilia of a greater diameter and a seemingly greater rigidity than the cell on the left which has thinner, limp appearing stereocilia. These two types of stereocilium are associated with both Type I and Type II sensory cells as described by Wersall (1956). Figure 7 demonstrates a Type I and Type II cell both of which have thick, rigid stereocilia. In Figure 8 several Type I cells and a Type II cell have the thin, limp stereocilia.

Figure 1: Relative positions of the crista ampullaris of the anterior vertical (AV) and lateral horizontal (LH) semicircular canals and the utricle containing the macula utriculi (MU). The relatively dark region surrounding the macula is the dark cell region (DC). Bar = 500μm.

Figure 2: Crista ampullaris (CA) of the posterior vertical semicircular canal (SCC). A - ampulla wall; NF - nerve fibres to sensory epithelium. Bar = 100μm.

Figure 3: Crista ampullaris of the anterior vertical semicircular canal. The sensory epithelium (SE) is divided by the non-sensory area of the septum cruciatum (SC). TC - transitional cells; DC - dark cells. Bar = 100μm.

Figure 4: Junctions between sensory epithelium (SE) and the irregularly shaped interlocking cells of the planum semilunatum (PS) which extends up the lateral wall of the ampulla. Bar = 50μm.

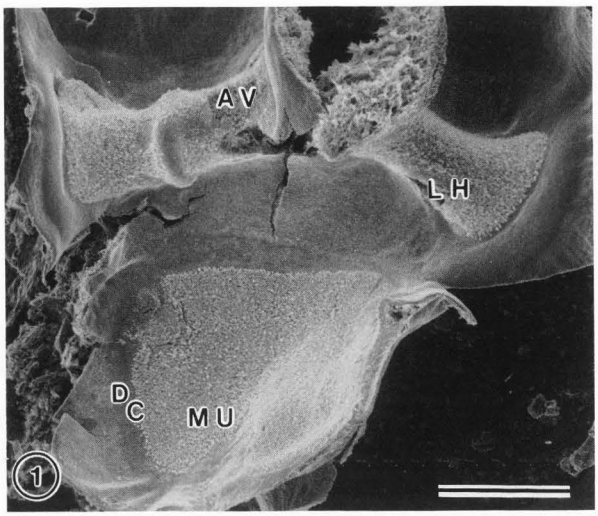
Figure 5: Fracture through the crista in the transitional epithelial area (TE). Supporting cells (SC) are aligned over a fibrous basement structure containing plexus of capillaries (C) and also afferent and efferent nerve fibres (NF). SE - sensory epithelium. Bar = 25μm.

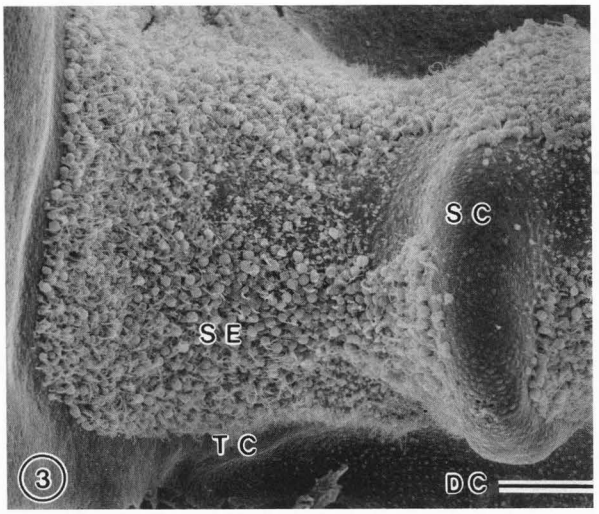
The macula utriculi is one of the otolith organs responsible for detecting the absolute position of the head with respect to gravitational effects. Necessary for this function are otoconia and gelatinous masses which normally overlay the sensory hair cells. In Figure 9 the otoconial membrane and the intermediate mesh of the macula have been removed to reveal the surface of the sensory epithelium. This macula is generally concave. The posterior border has a large 'V' shaped indentation; the other edges are relatively straight, with small 'V' shaped indentations at the anterior corners.

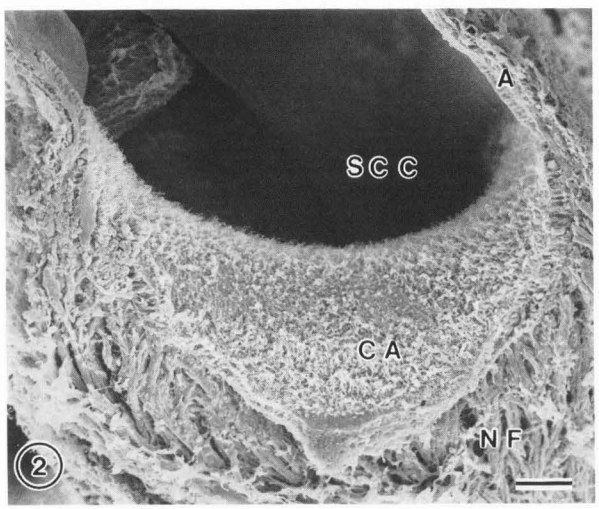
Figure 10 illustrates the "organ pipe" bundle of stereocilia and kinocilium. These bundles have an organized orientation such that the single kinocilium is facing towards the central area of the macula, the so called striola. The striola and the orientation of the hair cell bundles are represented diagrammatically in Figure 11.

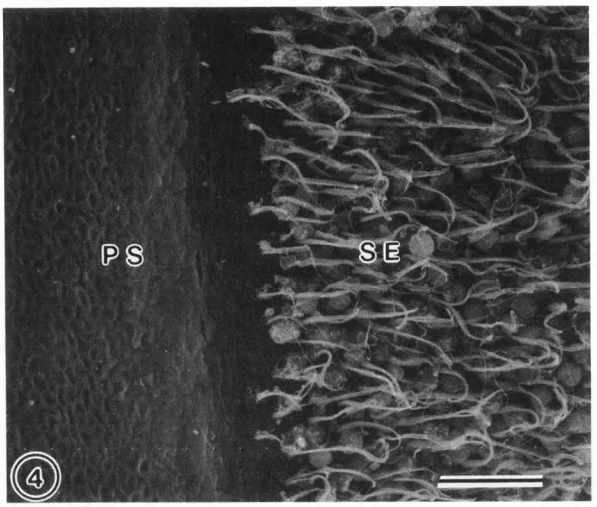
Figure 12 is a higher magnification indicating the specific orientation of sensory cells at the striola. The macula sensory cells are populated by the two types of stereocilium as are the crista sensory cells described above.

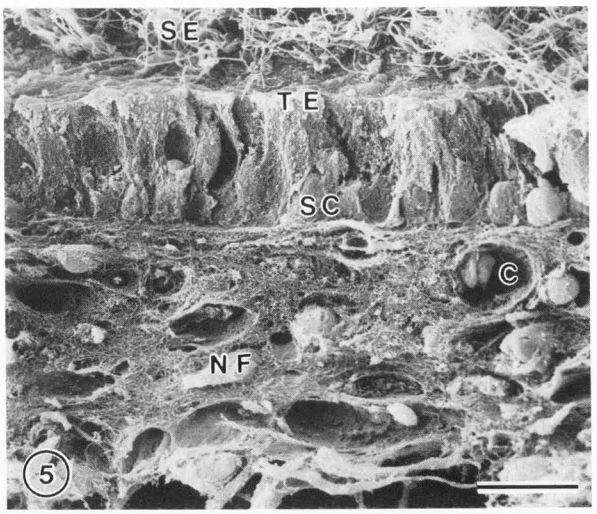
The bundles of stereocilia and the kinocilium are attached into an intermediate mesh which underlies the gelatinous layer with its embedded calcium carbonate otoconia. Figure











13 shows the structure of this otoconial mass. On the top surface are free otoconia; they appear to be singular compared with others more deeply embedded in the gelatinous layer which are smaller and grouped in clusters. In general, the shapes of the otoconia are similar to that found in all other mammalian otolith organs, cylindrical but with faceted sides, particularly at their apical ends (e.g. Lindemann, 1969).

Returning to Figure 1, a region surrounding the sensory epithelium appears slightly darker than other epithelial surfaces. This is the so called dark cell region. It is common to find fragmented otoconia in this area, and here it is supposed that excess or damaged otoconia are absorbed (Lim, 1973).

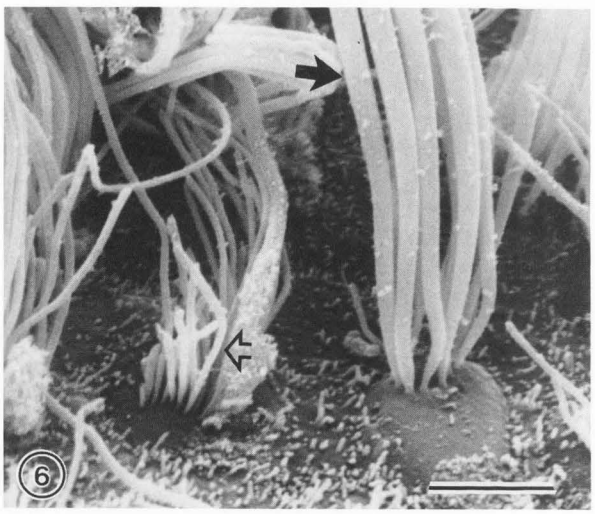


Figure 6: Sensory cells of the crista ampullaris. Two distinct populations of stereocilia are present, a thicker, rigid appearing type (solid arrow) and a thinner, limp appearing type (open arrow). Bar = 10μm.

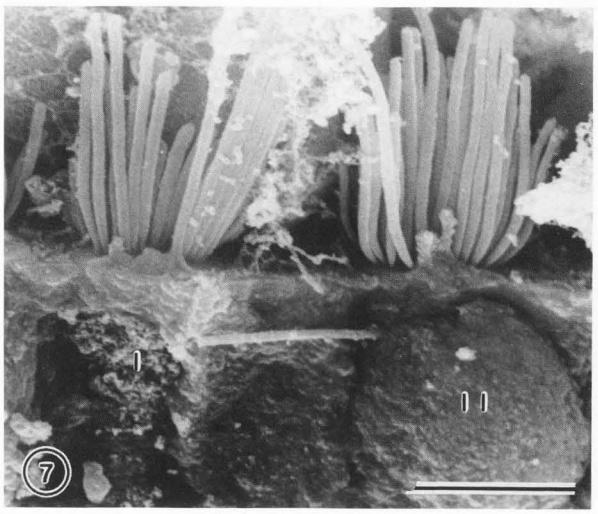


Figure 7: A Type I (I) and Type II (II) sensory cell of the crista. Both cell types have thick, rigid stereocilia. Bar = 10μm.

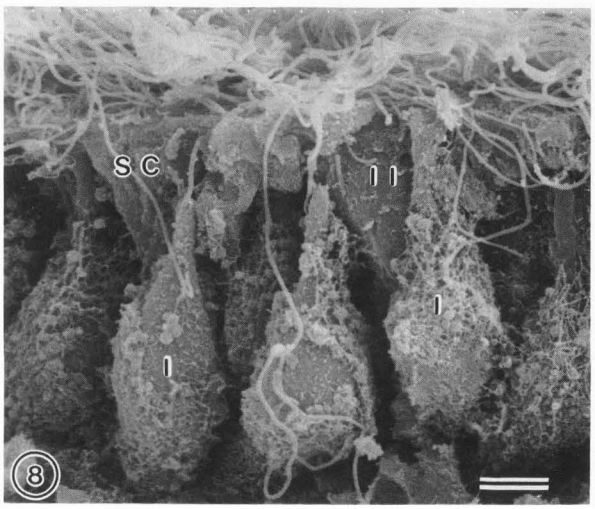


Figure 8: Fracture through the central region of crista ampullaris showing several Type I (I) hair cells and a Type II (II) hair cell. Both cell types have thin, limp stereocilia. The cilia are in a disordered state resulting from fixation artifact. SC - supporting cell. Bar = 10μm.

The Cochlea

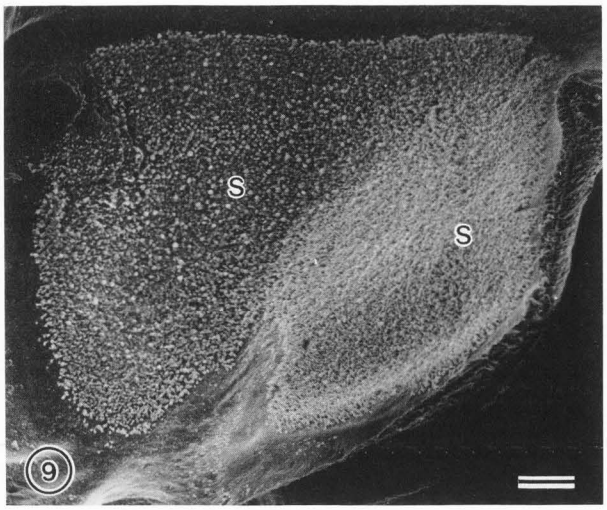
The sensory cells of the organ of Corti form one row of inner and three rows of outer hair cells as in most other mammalian species (Figure 14). In the apical turns of the cochlea there are occasional supernumary outer hair cells but not organized enough to be termed a fourth row. Throughout the length of the cochlea, the tallest of the outer hair cell stereocilia are normally embedded into the underside of the tectorial membrane (in Figure 14 this has retracted during preparation to reveal the sensory epithelium). Figure 15 shows a view of the underside of the tectorial membrane and the imprints of the outer hair cell stereocilia. In the basal and lower middle turns of the cochlea, trabeculae normally form an attachment between the tectorial membrane and the inner hair cell area, these can be observed in Figure 15.

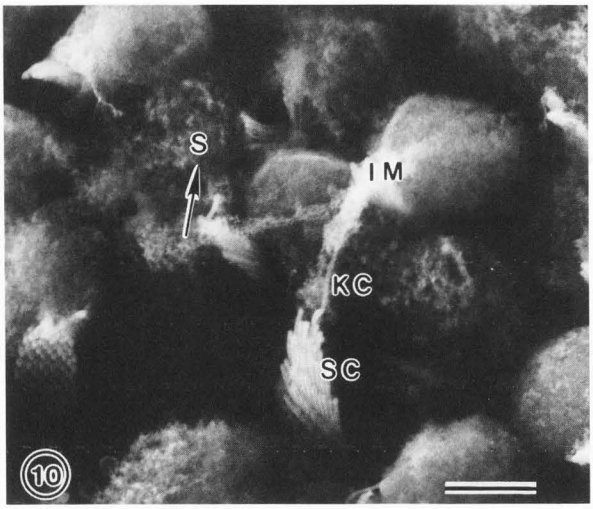
Apically from the upper middle turn, the tall outermost stereocilia of the inner hair cells are embedded in the tectorial membrane as illustrated in Figure 16. It cannot be stated with certainty if this is normal or if it is an artifact of preparation (Hoshino and Kodama, 1977; Lim, 1977).

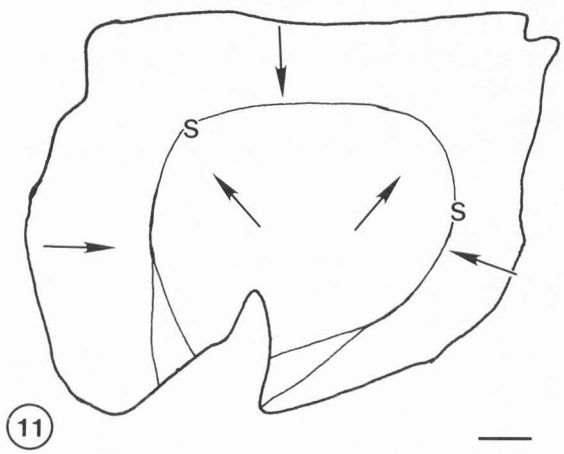
Figures 17 and 18 are stereoscopic views of inner and outer hair cells from the basal turn of the cochlea. In Figure 17 some filamentous material can be seen linking the tips of stereocilia. This is presumably derived from the tectorial membrane. Figure 18 emphasizes the relief of the reticular membrane and apical surfaces of the outer hair cells. Note also the difference in orientation of stereocilia in the third row (lower row in figure) of outer hair cells. A filamentous material similar to that in Figure 17 can be seen linking the stereocilia.

Summary

In most instances, the morphological ultrastructure of the sensory portion of the canine inner ear is similar to features which may be found in other mammals. The presence of two distinct populations of vestibular stereocilia warrant further investigation to determine their functional significance.







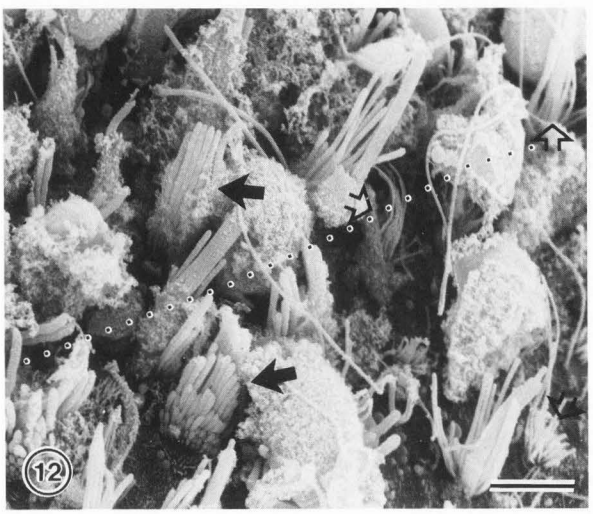


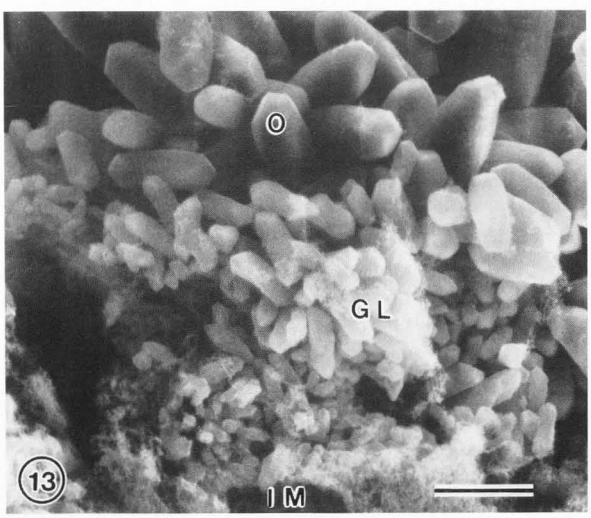
Figure 9: Macula utriculi as seen after removal of otoconial mass. S - striola. Bar = 100μm.

Figure 10: "Organ-pipe" arrangement of stereocilia (SC) and kinocilium (KC) of macula utricle sensory cell. Arrow indicates direction of striola (S). Portions of the intermediate mesh (IM) are attached to the cilia. Bar = 5μm.

Figure 11: Schematic diagram of the macula utriculi indicating the position of the striola (S) and the directional orientation of the tallest stereocilia and kinocilium (arrows). Bar = 100μm.

Figure 12: Macula sensory cells in the area of the striola (dotted line). Two types of stereocilia are present, thick, rigid (solid arrows) and thin, limp (open arrows). Bar = 10μm.

Figure 13: The otolithic membrane consists of otoconia (O) of varying size and the otoconial mass of the gelatinous layer (GL) which sits above the intermediate mesh (IM). Bar = 5μm.



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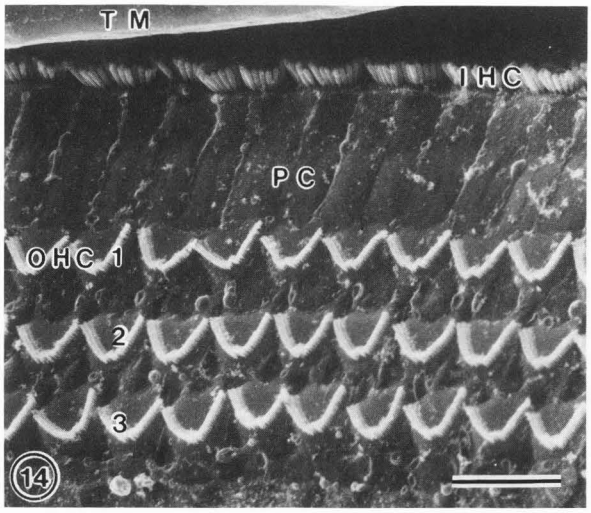

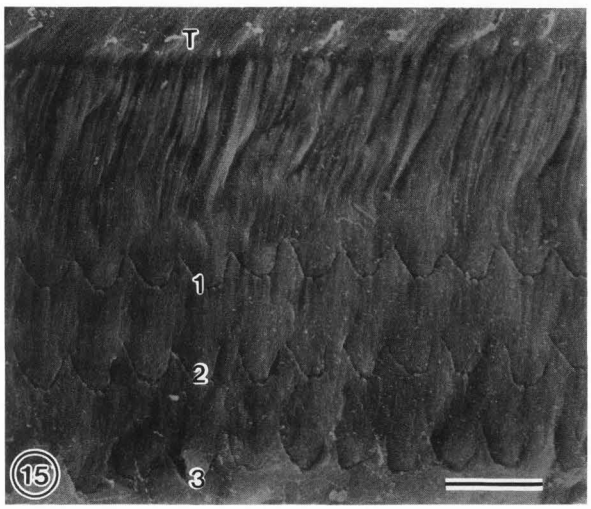

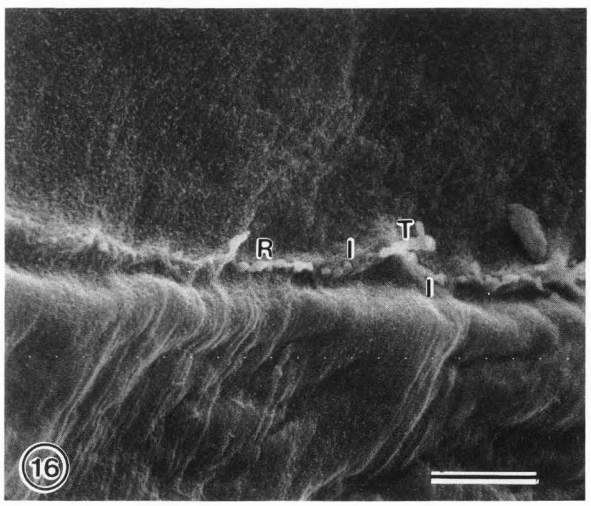

Figure 14: Surface view of the sensory epithelium of the organ of Corti after retraction of the tectorial membrane (TM). The apical surfaces of inner hair cells (IHC) and three rows of outer hair cells (OHC 1,2,3) are supported in part by pillar cells (PC). There are only stereocilia in the cochlea. Bar = 10μm.

Figure 15: The underside of the tectorial membrane from the upper basal turn of the dog cochlea. 1,2,3 - imprints of outer hair cell stereocilia; T - trabeculae. Bar = 10μm.

Figure 16: Underside of the tectorial membrane from the upper middle turn. The tallest stereocilia of the inner hair cells have been attached to the tectorial membrane. R - remnants of implanted stereocilia; I - imprints; T - trabecula. Bar = 17μm.

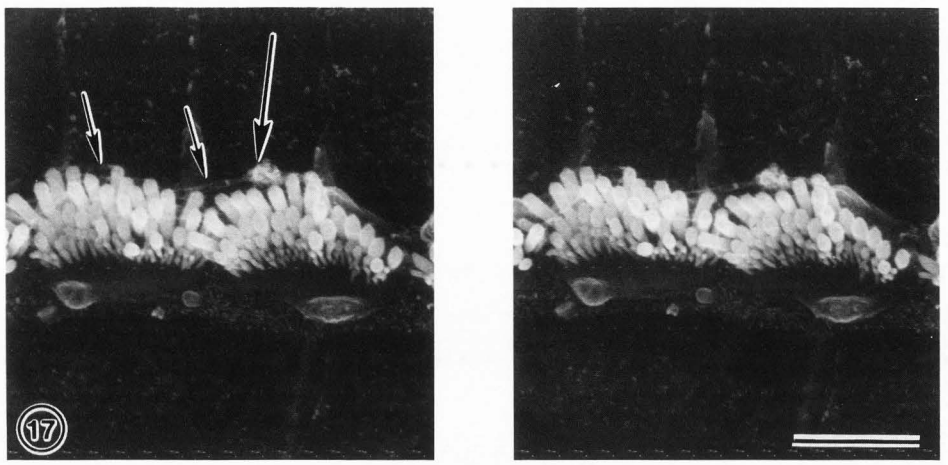

Figure 17: Stereoscopic view of inner hair cell stereocilia showing fibrils (small arrow) and the gelatinous material (large arrow) connecting the stereocilia and the tectorial membrane. Bar = 5μm.

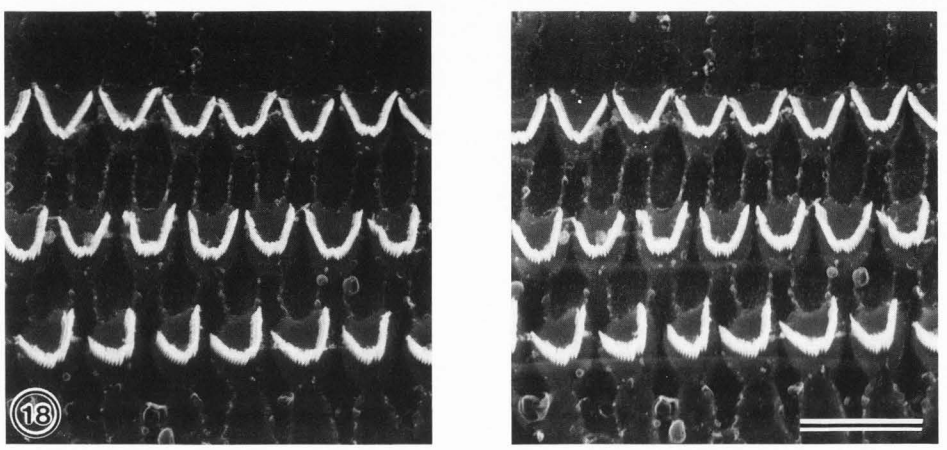

Figure 18: Stereoscopic view of outer hair cell stereocilia showing remnants of attachments to each other and the tectorial membrane. Bar = 10μm.

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Discussion with Reviewers

D.A. Cotanche: How did you keep the tectorial membrane from retracting so that you could get the view of its underside shown in Figure 15?
Authors: The tectorial membrane was dissected from the cochlea after critical point drying and mounted with its underside facing upwards.

M. Mulroy: How much time passed between the death of the animals and the perfusion of fixative through the scalae? Is there any correlation between the duration of that time and the number of floppy stereocilia and on the blebs on the apical surface of the hair cells?
What is the nature of the artifact which caused the floppy stereocilia seen in Figures 5 and 8?
D.A. Cotanche: The blebbing on the hair cells is most probably caused by the delay before fixation. We have been able to eliminate these blebs by immersing the cochlea in an oxygenated buffer solution during dissections leading up to fixation. Also, the limp stereocilia are probably caused by fixation in glutaraldehyde. The stereocilia remain much more rigid when osmium is used as the original fixative, although I don't know if this is possible in the dog cochlea.
Authors: Both the floppy stereocilia and the apical swellings of cells are almost certainly due to the fixation procedure. The material used in this study was obtained at autopsy from dogs which had been sacrificed as part of another project. Consequently, time to fixation was longer than ideal and perfusion of the inner ear with osmium was impractical.

D.A. Cotanche: In Figure 11 you indicate a wedge-like broadening of the striola at the posterior ends of the macula. What is the orientation of the cells within this area?
Authors: At its posterior ends the striola

becomes an area of varying orientation of sensory cells such that a straight line cannot be defined. The orientation of the stereocilia on individual hair cells is also disorganized in this region. Figure 19 illustrates a hair cell in which the tall stereocilia are off-centred and the stereocilia are not arranged orderly by height (compare to Figure 10).

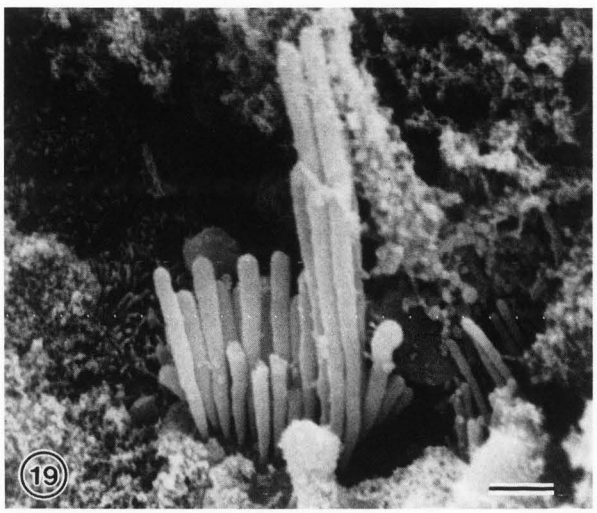

Figure 19: Sensory cell from the posterior end of the macula utriculi striola. Stereocilia are off-centred and disorganized. Compare to Figure 10. Bar = 2μm.

M. Mulroy: What criteria did you use to decide that the stereocilia tufts shown in Figure 4 are rigid?
Authors: The description is an assumption, based on the appearance of the tufts following preparation for SEM observation.

M. Mulroy: Did you see any filaments between the tips of adjacent stereocilia in a tuft similar to the "tip links" described in other mammalian cochlear hair cells?
Authors: No, we found no indication of tip links or of any basal horizontal linkage of stereocilia in either the vestibular or cochlear hair cells. The only stereocilia connections noted were the filamentous mesh-work shown in Figures 17 and 18.

D.A. Cotanche: You mention that the dark cells may be involved in the resorption of otoconia. Are these the same dark cells that are thought to play a role in ion transport and generation of the endocochlear PD in other cochlear epithelia?
Authors: Yes, the vestibular dark cells are thought to be involved in production of the endolymphatic potential and ion transport mechanisms as are the dark marginal cells of the cochlear stria vascularis.