Scanning Microscopy

Volume 6 | Number 4 Article 21

9-24-1992

Scanning Electron Microscopy of Nerve Fibers in the Dog Cochlea

Hirofumi Morita
Hamamatsu University School of Medicine

Tomoyuki Hoshino
Hamamatsu University School of Medicine

Kunihiro Mizuta
Hamamatsu University School of Medicine

Scanning Microscopy, Vol. 6, No. 4, 1992 (Pages 1105-1113)
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

0891-7035/92$5.00+.00

SCANNING ELECTRON MICROSCOPY OF NERVE FIBERS IN THE DOG COCHLEA

Hirofumi Morita*, Tomoyuki Hoshino, Kunihiro Mizuta

Department of Otolaryngology, Hamamatsu University School of Medicine
3600 Handa-cho, Hamamatsu 431-31, Japan

(Received for publication March 6, 1992, and in revised form September 24, 1992)

Abstract

The nerve fiber arrangement inside the organ of Corti in the dog was studied. Thick sections were cut serially from celloidin-embedded cochleas and observed with a scanning electron microscope (SEM). The nerve fibers in the organ of Corti were clearly exposed in sections cut in the horizontal plane. The arrangement of nerve fibers in the dog showed considerable difference from that of other species. The tunnel basilar fibers in the dog curved basalward, and took a long longitudinal course to form a broad bundle in the center of the tunnel floor. This bundle has not been found in adult animals of other species. Two distinct types of tunnel radial fibers, upper tunnel radial fibers and underpassing radial fibers, were recognized. The latter type of radial fibers ran beneath the bundle of basilar fibers, and seemed to be characteristic of the dog cochlea. From the morphological characteristics, these underpassing radial fibers were thought to be efferent in nature.

Key Words: Scanning electron microscopy, dog, organ of Corti, nerve fiber.

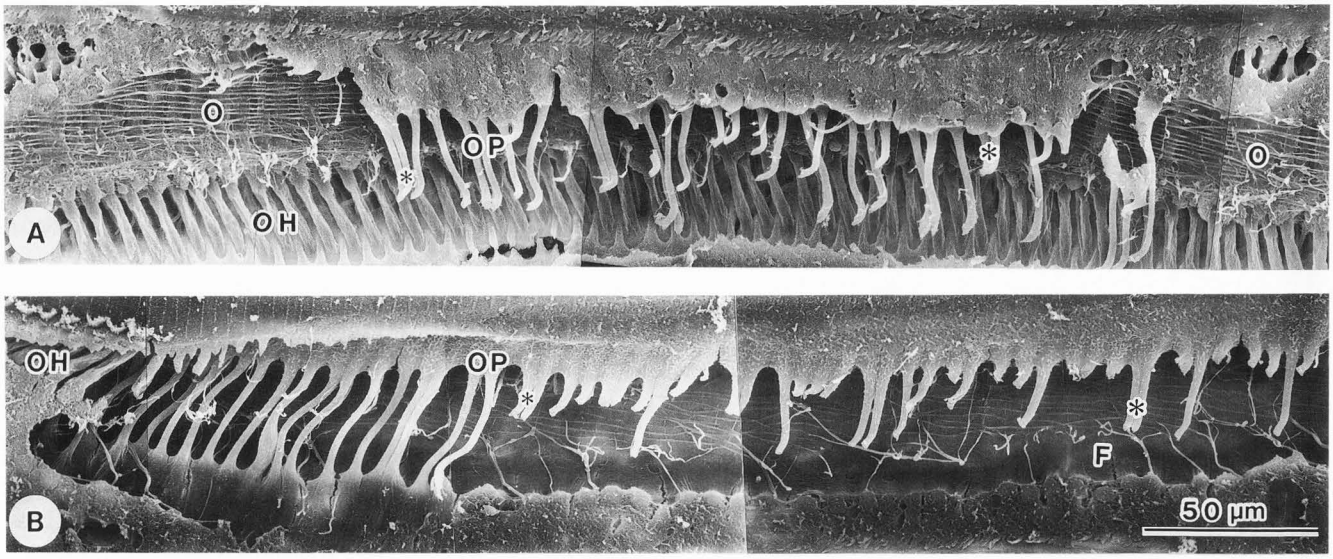
*Address for correspondence:
Hirofumi Morita
Hamamatsu University School of Medicine
Department of Otolaryngology
3600 Handa-cho, Hamamatsu 431-31, Japan
Telephone Number: 81-53-435-2252
Fax Number: 81-53-435-2253

Introduction

The innervation of the mammalian cochlea has been intensively studied by transmission electron microscopy (Spoendlin, 1969, 1972; Lim, 1980; Takasaka *et al.*, 1983), and by light microscopy using the Golgi method and horseradish peroxidase (HRP) techniques (Smith, 1975; Kiang *et al.*, 1982; Ginzberg and Morest, 1983, 1984; Brown, 1987; Simmons and Liberman, 1988a, b). Scanning electron microscopic (SEM) study is suitable for determining the three-dimensional appearance of nerve fibers in the organ of Corti, and some studies have reported on the nerve fibers in the cochlear fluid spaces (Bredberg, 1977, 1981; Harada *et al.*, 1983; Hoshino and Nakamura, 1985; Hoshino, 1990). With the previously used specimen preparation methods however, it was difficult to expose the fluid space in the organ of Corti. Recently we reported a method of SEM observation of the cochlea in thick sections cut from celloidin-embedded temporal bones (Mizuta *et al.*, 1990). This method is suitable for studying the nerve fiber arrangement in the fluid spaces of the organ of Corti. The present SEM study reports on observations of an inside view of the dog cochlea using this method and discusses the morphological characteristics of the nerve fiber network.

Materials and Methods

Six dogs, weighing 180 g, 300 g, 500 g, 800 g, 7 kg and 16 kg, were studied. The latter two were beagles and the others were mongrels. The exact birth dates of the dogs were not ascertained except for the first dog which was two days old. The 300 g, 500 g and 800 g weighing dogs were apparently young puppies. None of them showed inflammatory changes in the middle ear. After anesthesia by intraperitoneal injection of pentobarbital, the temporal bones were dissected out and 1% phosphate-buffered glutaraldehyde (pH 7.4) was perfused via the round and oval windows. The temporal bones were decalcified in 5% trichloracetic acid, dehydrated in graded ethanol, and embedded in celloidin. After hardening the celloidin, the specimens were cut in 100-150 μm sections with a sliding microtome such that



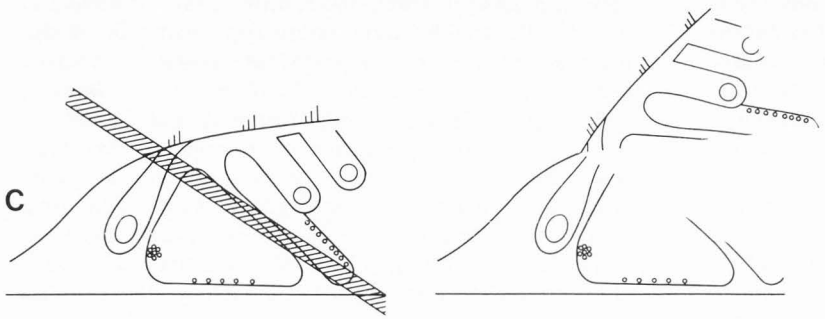


Figure 1. Both sides of a cutting plane through the organ of Corti in a dog, separated by the row of outer pillar cells (OP). Nuel's space is seen in **A** and the floor of Corti's tunnel is seen in **B**. Marked by "*", the outer pillar cells in these two figures correspond to each other. The cochlear base is toward the right. The cutting plane is schematically shown in **C**. OH: outer hair cells; O: 1st outer spiral bundle; F: tunnel floor. (800 g, upper middle turn).

Figure 2 (facing page top). Survey views of floor of Corti's tunnel in the apical turn (**A**), the upper middle turn (**B**), and the lower middle turn (**C**) of a dog. There are longitudinally running basilar fibers in the center of each tunnel floor. Note that the degree of exposure of basilar fibers to Corti lymph and the numbers of fibers composing the bundle vary along the length of the cochlea. Radial fibers passing under the spiral course of basilar fibers are seen in B and C (arrowheads), but they are not numerous in the apical turn. Upper tunnel radial fibers were cut (arrows). The cochlear base is toward the right; the modiolar side is at top. OP: outer pillar cells, T: tunnel spiral bundle. (500 g).

Figure 3 (facing page, below). Surface view of the tunnel floor in the upper basal turn (**A**) and upper middle turn (**B**). Tunnel basilar fibers run longitudinally. The cochlear base is toward the right. The modiolar side is at top. Some radially running fibers (arrows) cross underneath these longitudinal fibers. Some of the underpassing radial fibers ascend in the middle of the bundle (arrowhead). OP: outer pillar cells. (500 g).

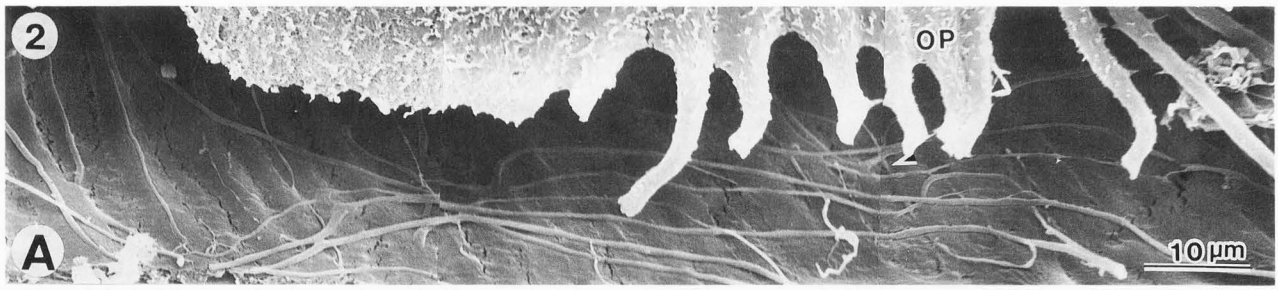
---

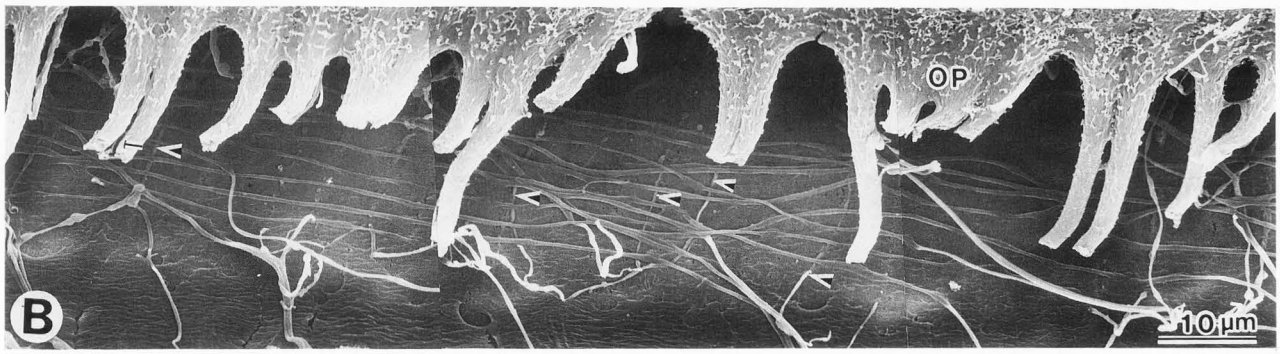
the cutting plane would be parallel to the basilar membrane. After removing celloidin in ether-alcohol (1:1) solution, the sections were dried by freeze-drying method using t-butyl alcohol (Inoue and Osatake, 1988). The sections were then mounted on metal stubs with carbon paste, coated with gold using a sputter coater (JEOL JFC-1100), and observed under a scanning electron microscope (HITACHI S-800).

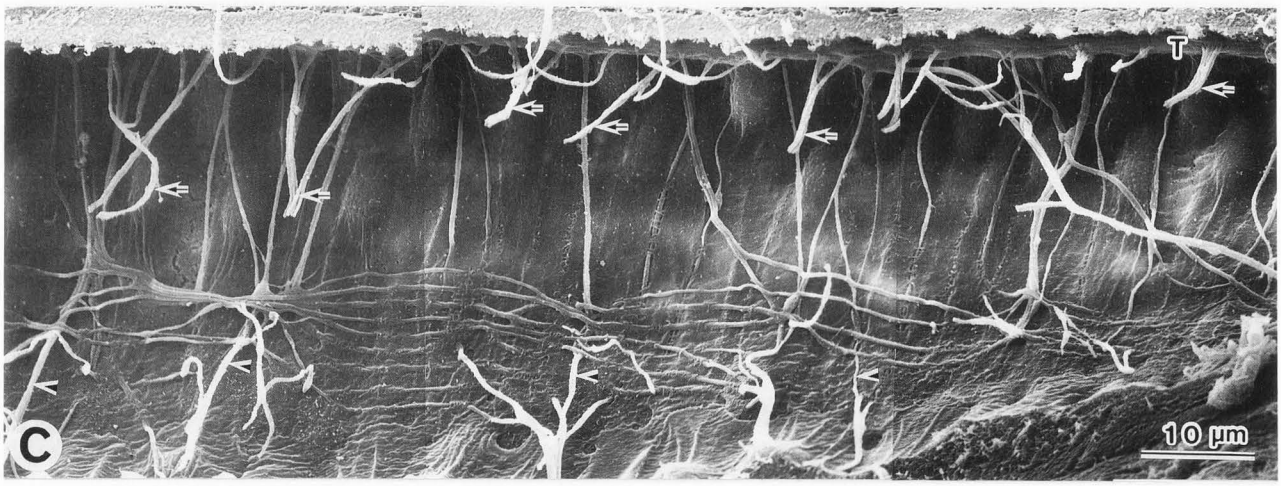
Results

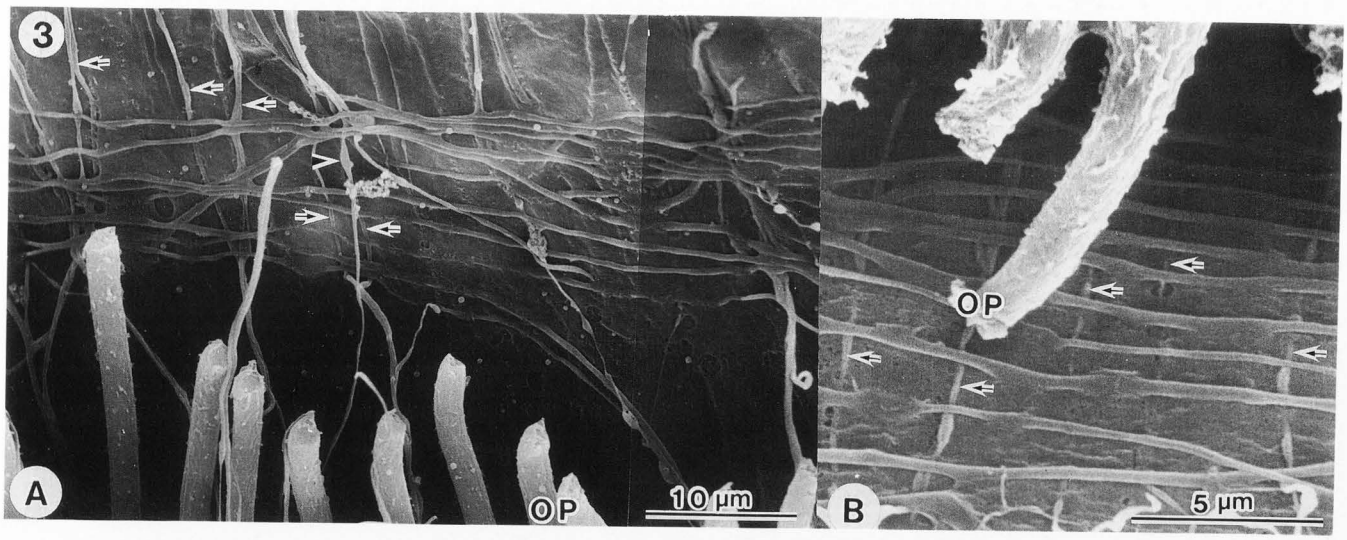
When the cutting plane was parallel to the basilar membrane at the middle of the tunnel of Corti, a considerable portion of the interior of the organ of Corti was exposed in a given section. A length of 100 μm was suitable for observation. Exposures of this length were obtained in the desired cochlear turns in many instances. Nerve fibers inside the fluid spaces, such as the tunnel of Corti and Nuel's space, were clearly seen. As both sides of the cutting plane could be exposed, both the ceiling and the floor of the fluid spaces were observable (Fig. 1). By observing sequential sections, it was possible to study the nerve fiber arrangement throughout most of the cochlea. Although there was a slight shrinkage of outer hair cell bodies, possibly caused by the embedding procedure, few artifacts were present in the nerve fibers.

Four groups of nerve fibers were observed by this method: tunnel basilar fibers, upper tunnel radial fibers, tunnel spiral bundle, and the first outer spiral bundle. Other nerve fibers such as the inner spiral bundle, the 2nd and the 3rd outer spiral bundle were not revealed


2
OP
A
10 µm


OP
B
10 µm


T
C
10 µm


3
A
OP
10 µm
OP
B
5 µm

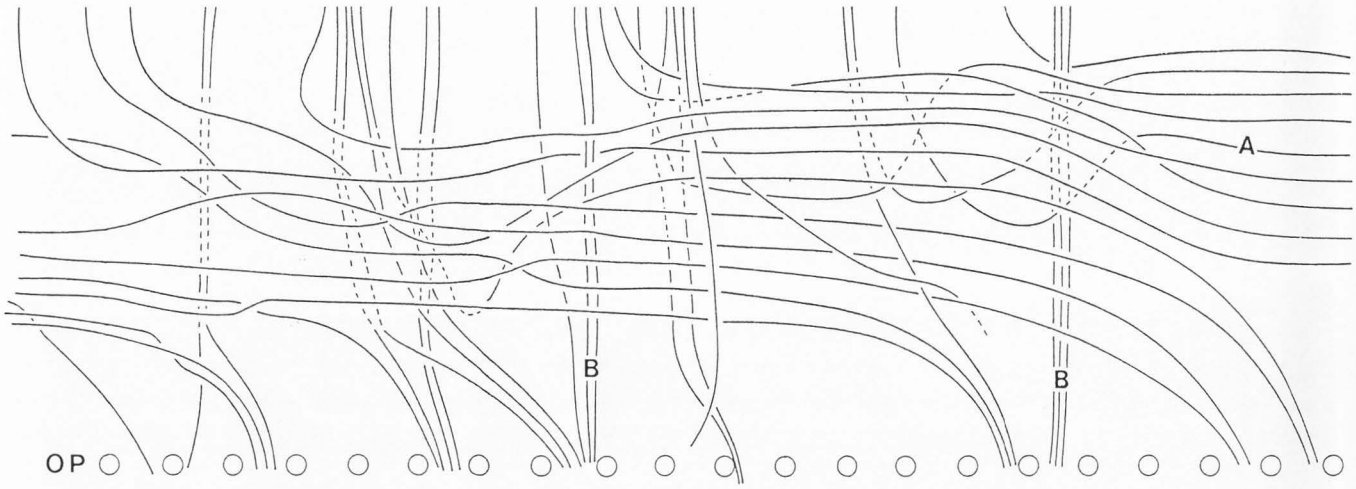


Figure 4 (above). Schematized tracing of nerve fibers on the tunnel floor. The longitudinal bundle of basilar fibers (**A**) and the underpassing radial fibers (**B**) are illustrated. The cochlear base is toward the right. OP: outer pillar cells. (500 g, upper basal turn).

Figure 5. Inner corner of Corti's tunnel. Upper tunnel radial fibers (U) enter the tunnel through the upper part of or just above the tunnel spiral bundle (T), while underpassing radial fibers (arrowheads) enter through the lower part of the tunnel spiral bundle. The underpassing radial fibers ran apart from the floor at this corner, whereas tunnel basilar fibers ran attached to the floor at their entering point (arrows). Some of the underpassing radial fibers are thin with varicosities (*). The cochlear base is toward the right. I: inner pillar cells. (800 g, lower middle turn).

Figure 6. Outer wall of Nuel's space. The first outer spiral bundle (O) is seen along the surfaces of Deiters' cells (D). Most fibers run parallel to each other. Some fibers disappear along the pathway at the junction of neighboring Deiters' cells (arrows in the insert). These fibers may be passing to the second or third outer spiral bundle. Upper tunnel radial fibers (arrowheads) arrive at the level of the bases of outer hair cells (OH). Some vertically running fibers (*) are seen along the Deiters' cells. They are thin with varicosities, and usually climb on the outer spiral bundle, but some of them run under the outer spiral bundle (arrow). The cochlear base is toward the right. (800 g, lower middle turn: insert 500 g, apical turn).

Figure 7. Survey view of Corti's tunnel in an adult rabbit cochlea. Note the radially running tunnel basilar fibers (arrowheads). Upper tunnel radial fibers that originated from the tunnel spiral bundle (T) were cut during preparation (arrows). The cochlear base is toward the right. OP: outer pillar cells, I: inner pillar cells. (lower basal turn).

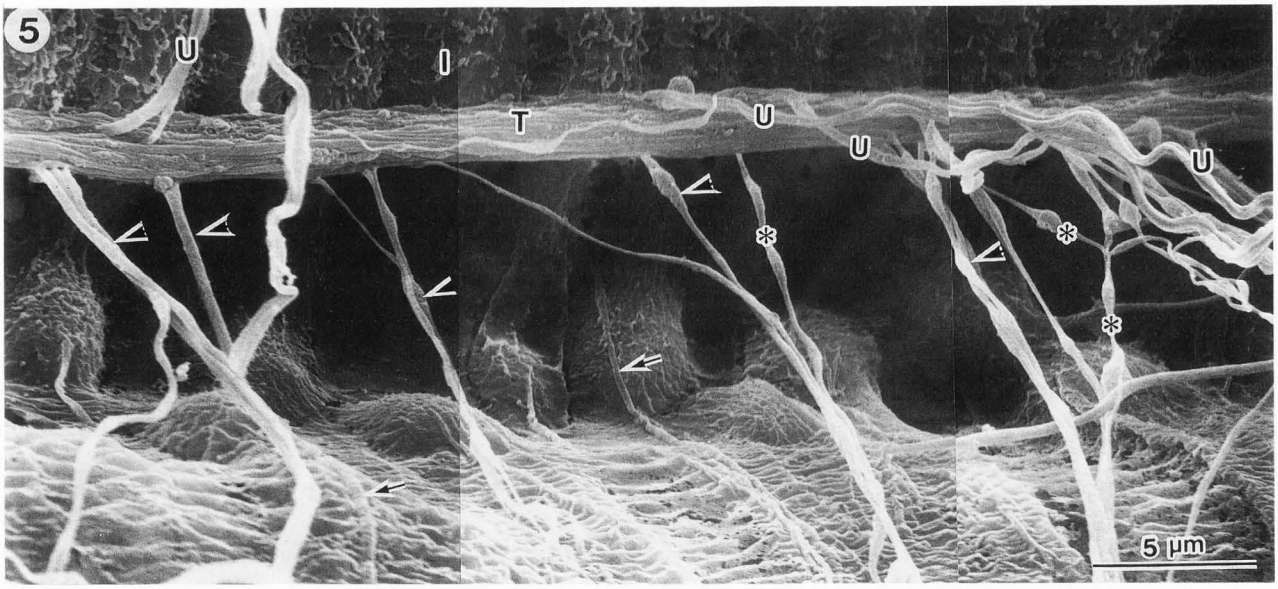
---

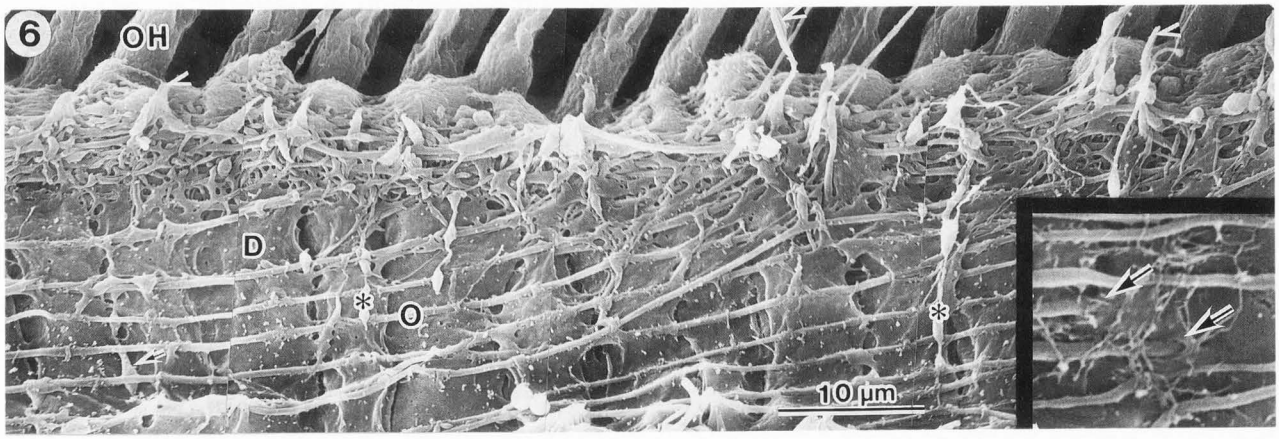
enough for detailed observation because of lack of fluid spaces surrounding them. In the cat, upper tunnel radial fibers and the tunnel spiral bundle are thought to be solely efferent, while the basilar fibers and the outer spiral bundles are afferent (Spoendlin, 1972).

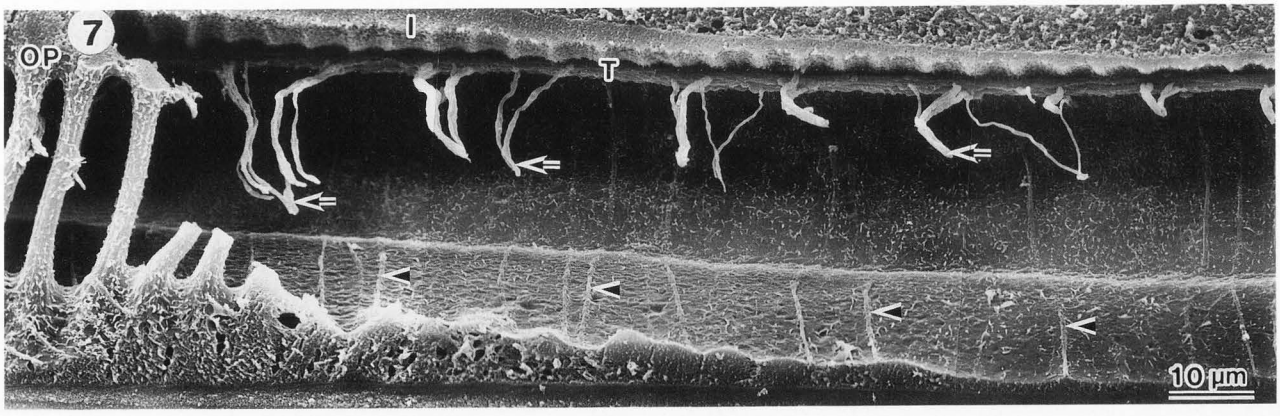
(1) tunnel basilar fibers

The tunnel basilar fibers emerged between inner pillars into Corti's tunnel beneath the tunnel spiral bundle and curved toward the cochlear base, taking a longitudinal course in the center of the tunnel floor; then they ran radially to pass between the bases of outer pillar cells. Because the number of fibers that took a longitudinal course was large, they seemed to compose a broad nerve bundle in the center of the tunnel floor (Figs. 2, 3). The basilar fibers composing the bundle often took parallel courses at about 1-2 μm intervals, but some fibers crossed over one another (Fig. 4). The thickness of the basilar fibers in this bundle seemed uniform, about 0.3 μm, without varicosities. This bundle was well exposed on the tunnel floor in the upper portion of the cochlea, whereas in the lower portion, it was partly buried in the feet of the outer pillar cells, and in the hook portion it was almost completely covered. The number of nerve fibers composing the bundle was less in the apical turn (1-6 fibers) than in the basal and middle turns (8-16 fibers) (Fig. 2), so the width of this bundle was narrower in the upper turns. The longitudinal extent of each basilar fiber in the tunnel could not be measured exactly, because most fibers took a longitudinal course past more than 15 outer pillar cells in the middle and basal turns (Fig. 4). We were unable to expose the tunnel floor along such a long length with this method. In the apical turn, we observed two basilar fibers which traveled past 8 outer pillar cells in the tunnel.

This longitudinal bundle was observed in adult dogs as well as in puppies, and we also found this bundle in a newborn dog cochlea two days after birth. The


5
U
I
T
U
U
U
5 µm


6
OH
D
O
10 µm


7
OP
I
T
10 µm

number of nerve fibers composing the bundle seemed to be almost the same in both adult dogs and puppies. There was some age-related difference in these fibers with respect to their degree of exposure to the fluid spaces, the bundle being more exposed and more easily observed in the puppy than in the adult dog.

(2) tunnel radial fibers

There were 2 distinct types of tunnel radial fibers in the dog cochlea. One type was upper tunnel radial fibers running freely above the tunnel floor and exiting the tunnel at a high level between the outer pillar cells.

The other type of radial fiber ran beneath the longitudinal portion of tunnel basilar fibers and exited the tunnel at a low level between outer pillar cells (Figs. 2, 3). These fibers usually entered the tunnel just below the tunnel spiral bundle or through the lower part of the bundle, whereas most of the upper tunnel radial fibers entered the tunnel through the middle or upper part of the tunnel spiral bundle (Fig. 5). After entering the tunnel, the underpassing fibers ran freely for a short distance, then went under the longitudinal bundle of basilar fibers, while the basilar fibers ran attached to the floor after entering the tunnel. After passing beneath the longitudinal bundle of basilar fibers, the underpassing radial fibers ascended in the tunnel space again, then exited the tunnel at a low level between outer pillar cells. Some of them came together with the basilar fibers when they passed between outer pillar cells (Figs. 3. 4). There were some fibers that passed under only two or three basilar fibers and again ran freely in the space (Fig. 3, arrowhead). Underpassing radial fibers were observed more often in the middle and basal turns than in the apical turn. The upper tunnel radial fibers were also more numerous in the lower turns.

Most of the underpassing radial fibers had the same thickness as upper tunnel radial fibers, but some of them were thin and had varicosities (Fig. 5). This latter type of fiber took an underpassing course similar to the thick type of underpassing radial fibers.

(3) tunnel spiral bundle

The tunnel spiral bundle ran attached to the base of inner pillar cells in the dog, like in the rabbit, guinea pig, and cat. Varicosities on the nerve fibers composing the tunnel spiral bundle were not so evident (Fig. 5) compared to the kitten (Ginzberg and Morest, 1983) and the guinea pig (Bredberg, 1977). The thickness of the tunnel spiral bundle was about 2 μm and seemed not to vary between the middle and basal turns. We could not measure the thickness of the bundle exactly in the apical turn, because in the apical portion of the cochlea there were only a few sections in which the inner portion of the Corti's tunnel was clearly exposed.

(4) nerve fibers on the lateral wall of Nuel's space

Many nerve fibers were observed running on the modiolar side of the first row of Deiters' cells (Fig. 6). These fibers formed the first outer spiral bundle. The view of the outer spiral bundle in the dog was similar to that in the rabbit and the cat. The nerve fibers composing the bundle usually took parallel and longitudinal courses and rarely crossed over. Some fibers in the bundle abruptly disappeared in their course (Fig. 6, insert). They may have joined the second or third outer spiral bundles.

Upper tunnel radial fibers reached the base of the first row of outer hair cells. We could not ascertain that the fibers made contact with outer hair cells because there is a complicated network of nerve fibers at the bases of outer hair cells.

In contrast to the gradually climbing longitudinal fibers and the upper tunnel radial fibers, there were thin fibers that climbed up over the external spiral fibers. They had varicosities and climbed vertically, usually on the outer spiral bundle, and disappeared among Deiters' cells or the complicated network of nerve fibers at the bases of outer hair cells (Fig. 6).

Discussion

Although light microscopic studies of the inner ear of congenitally deaf dogs have been reported by various authors (Igarashi *et al.*, 1972: Branis and Burda, 1985), SEM reports on the dog cochlea have been rare. Mount and Harrison (1987) have published an SEM study on the hair cell stereocilia in the dog, but the nerve fiber network inside the organ of Corti in the normal dog cochlea has not yet been reported.

This study showed that there may be considerable variations in the arrangement of nerve fibers among mammalian species, especially as to tunnel basilar fibers. In the dog, the basilar fibers curved basalward, taking a longitudinal course in the center of the tunnel floor, and formed a spiral bundle both in mature and immature cochleas. In the rabbit, basilar fibers take a straight radial course (Fig. 7). In the cat (Mizuta *et al.*, 1990) the basilar fibers slightly curve basalward but do not form such a bundle as in the dog, except in the immature cat cochlea. In the immature cat cochlea, a spiral nerve bundle, which was thought to be a collection of basilar fibers, is seen on the tunnel floor near the outer pillar cells (Mizuta, unpublished data). In human fetus, Hoshino (1990) reported the curved basilar fibers as 'spiral fibers', which were similar to those of the cat.

This study also showed a variation in the nerve fiber arrangement along the length of the cochlea. The number of basilar fibers composing the bundle was less in the apical portion than in the basal portion. This base-to-apex gradient was thought to be caused either by the difference in the length of the longitudinal course of each basilar fiber, or by the difference in the number of basilar fibers. The length of the tunnel spiral course of the basilar fibers could not be exactly measured in this study. Some fibers ran past 8 outer pillar cells in the apical turn, whereas most fibers in the lower portion passed more than 15 outer pillar cells. This finding suggests that the longitudinal course of each basilar fiber is

longer in the lower turns. Its typical length should be measured by other appropriate methods, such as nerve fiber staining. We also could not determine the variation in the number of basilar fibers in different cochlear turns.

The exposure of the basilar fibers to the fluid spaces varied with the cochlear turn. They were well exposed in the apical portion, and were buried in the feet of the pillar cells in the basal portion. This base-to-apex gradient of exposure was also found in other species, such as, the rabbit and the cat. This exposure also varied with age. The puppy showed more exposed basilar fibers than the adult dog.

The radial fibers running beneath the basilar fibers were predominantly characteristic for the dog. These underpassing radial fibers were also found in the cat (Mizuta *et al.*, 1990), but their number was far less than in the dog. The underpassing radial fibers and the upper tunnel radial fibers enter Corti's tunnel in a similar way, closely related to the tunnel spiral bundle, which is efferent in nature. The underpassing radial fibers were often more frequent in the basal and middle turns than in the apical turn, and this pattern was also recognized in upper tunnel radial fibers, as Ginzberg and Morest (1983) reported in the cat. Varicosities of the nerve fibers, which were found in the efferent nerves in the cat cochlea, were also found in the thin fibers of the underpassing radial fibers. From these findings, we suppose that the underpassing radial fibers in the dog may be outer hair cells (OHCs) efferent branches.

In 1987, Brown reported that roughly 5-10% of the efferent tunnel branches that terminate on the outer hair cells ran at low levels in the outer portion of the tunnel in the guinea pig. These fibers were noted to have small swellings among Deiters' cells and eventually rose to form large boutons on the base OHCs. He suggested that this type of OHC efferent may form axodendritic contacts on afferent outer spiral fibers. In the mouse cochlea, Whilton and Sobkowicz (1989) reported GABA-positive climbing fibers, which are thought to be efferent, in the OHC region. In the cat, Ginzberg and Morest (1984) reported some OHC efferents ran near the tunnel floor. We think that the underpassing radial fibers in the dog are efferent branches that run at a low level in Corti's tunnel and Nuel's space and pass among Deiters' cells, as Brown (1987) showed in the guinea pig cochlea.

The underpassing radial fibers were not so evident in the cat and the human fetus, and were rarely observed in the rabbit cochlea. This difference among species may indicate that the low tunnel-crossing OHC efferents are numerous in the dog. The presence of two distinct populations of tunnel radial fibers warrants further investigation to determine their functional significance. The dog cochlea may be suitable for such study, because of its abundance of underpassing radial fibers.

The thin-type underpassing radial fibers with varicosities, which enter the tunnel similarly to the thick-type underpassing radial fibers, seem like the thin

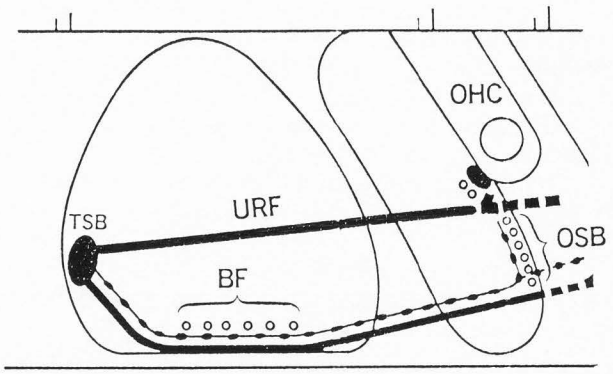


Figure 8. Schema of tunnel radial fibers in the dog. Upper tunnel radial fiber (URF) and two types of underpassing radial fibers (thick fibers and thin fibers with varicosities) are shown. Underpassing radial fibers run beneath the longitudinal bundle of tunnel basilar fibers (BF). Some of the thin type of underpassing fibers climb on Deiters' cells. Most of the thick type of underpassing radial fibers are thought to go to the bases of the 2nd and 3rd rows of outer hair cells (OHC). OSB: outer spiral bundle; TSB: tunnel spiral bundle.

---

irregular tunnel fibers in the human fetus reported by Hoshino (1990). He supposed that these fibers were efferent branches. We also think the thin type of underpassing radial fibers are efferent in nature because of their entering point relative to the efferent tunnel spiral bundle. In Nuel's space, they may connect to the thin climbing fibers, but we could not ascertain the connection in this study. These different types of radial fibers observed in the present study are schematically shown in Fig. 8. The functional difference between the two types of underpassing radial fibers should be determined by another method, such as nerve trans-section. We have tried nerve sections in several animals but so far have not succeeded in obtaining degeneration of efferent nerve fibers.

Acknowledgments

This study was supported in part by the Japanese National Science Research Fund (No.01570950). We are thankful to Y. Muranaka for his help in the preparation of this manuscript.

References

Branis M, Burda H (1985). Inner ear structure in the deaf and normally hearing Dalmacian dog. J Comp Path **95**: 295-299

Bredberg G (1977). The innervation of organ of Corti. A scanning electron microscopic study. Acta Otolaryngol (Stockh) **83**: 71-78.

Bredberg G (1981). SEM studies of Corti's organ with special reference to its innervation. Biomed Res **2** (suppl.): 403-413.

Brown MC (1987). Morphology of labeled efferent fibers in the guinea pig cochlea. J Comp Neurol **260**: 605-618.

Ginzberg RD, Morest DK (1983). A study of cochlear innervation in the young cat with the Golgi method. Hearing Res **10**: 227-246.

Ginzberg RD, Morest DK (1984). Fine structure of cochlear innervation in the cat. Hearing Res **14**: 109-127.

Harada Y, Hirakawa K, Mangape D (1983). Scanning electron microscopy of the nerve endings of Corti's organ. Scanning Electron Microscopy, **1983**; I: 183-188.

Hoshino T, Nakamura K (1985). Nerve fibers in the fetal organ of Corti. Scanning electron microscopic study. Ann Otol Rhinol Laryngol **94**: 304-308.

Hoshino T (1990). Scanning electron microscopy of nerve fibers in human fetal cochlea. J Electron Microsc Tech **15**: 104-114.

Igarashi M, Alford BR, Saito R, Cohn AM, Watanabe T (1972). Inner ear abnormalities in dogs. Ann Otol Rhinol Laryngol **81**: 249-255.

Inoue T, Osatake H (1988). A new drying method of biological specimens for scanning electron microscopy: The t-butyl alcohol freeze-drying method. Arch Histol Cytol **51**: 53-59.

Kiang NYS, Rho JM, Northrop CC, Liberman MC, Ryugo DK (1982). Hair-cell innervation by spiral ganglion cells in adult cat. Science **217**: 175-177.

Lim DJ (1980). Cochlear anatomy related cochlear micromechanics. A review. J Acoust Soc Am **67**: 1686-1695.

Mizuta K, Hoshino T, Morita H (1990). Scanning electron microscopy of the celloidin-embedded inner ear sections. Scanning Microsc **4**: 967-973.

Mount RJ, Harrison RV (1987). Scanning electron microscopic observations of the canine inner ear. Scanning Microsc **1**: 1167-1174.

Simmons DD, Liberman MC (1988a). Afferent innervation of outer hair cells in adult cats: I. Light microscopic analysis of fiber labeled with horse radish peroxidase. J Comp Neurol **270**: 132-144.

Simmons DD, Liberman MC (1988b). Afferent innervation of outer hair cells in adult cats: II. Electron microscopic analysis of fibers labeled with horseradish peroxidase. J Comp Neurol **270**: 145-154.

Smith CA (1975). Innervation of the cochlea of the guinea pig by use of the Golgi stain. Ann Otol Rhinol **84**: 443-558.

Spoendlin H (1969). Innervation pattern in the organ of Corti of the cat. Acta Otolaryngol (Stockh) **67**: 239-254.

Spoendlin H (1972). Innervation densities of the cochlea. Acta Otolaryngol (Stockh) **73**: 235-248.

Takasaka T, Shinkawa H, Hashimoto S, Watanuki K, Kawamoto K (1983). High-voltage electron microscopic study of the inner ear technique and preliminary results. Ann Otol Rhinol Laryngol **92** (Suppl 101): 1-12

Whilton DS, Sobkowicz HM (1989). GABA-like immunoreactivity in the cochlea of the developing mouse. J Neurocytol **18**: 505-518.

Discussion with Reviewers

B.A. Bohne: How and where do the basilar fibers exit the tunnel?
Authors: The basilar fibers passed between the bases of the outer pillar cells, ran freely above the bottom of the Nuel's space, then they reached the medial wall of Deiters' cells and went basal-ward to form the outer spiral bundle.

D.J. Lim: I was impressed by the well-preserved nerve fibers that underwent the acid decalcification. Is it because the nerve fibers are resistant to acid decalcification treatment? What about the stereocilia of the hair cells? Are they also well preserved by this method?
Authors: The nerve fibers inside the organ of Corti were well preserved and the stereocilia were also preserved as well with this method. We used ethylenediaminetetraacetic acid (EDTA) for decalcification in some animals. There was no difference in SEM appearance between acid-treated specimens and EDTA-treated ones. The nerve fibers seem to be resistant to acid decalcification treatment.

D.J. Lim: I am also impressed by the lack of shrinkage artifact which sometimes occurs in this type of preparation causing breaking or cracking of nerve fibers as the tissue shrinks. Have you seen such artifact in your preparation? If not, how do you avoid such artifacts?
Authors: We have seen slight shrinkage of outer hair cell bodies possibly caused by embedding procedure, but the arrangement of nerve fibers seemed to have minimum change. In our experience, the nerve fibers are well preserved with this method if specimens are handled carefully after the freeze-drying procedure.

Y. Harada: Is it possible to observe both light and scanning electron microscopic findings from thin and thick celloidin sections?
Authors: In the horizontal cutting adopted in the present paper, the purpose was to look inside the organ of Corti. The comparative study by light and scanning electron microscope is not suitable for this purpose. When we cut the cochlea vertically, such comparison is easily performed and seems to be meaningful.

Y. Harada: There are a number of beautiful SEM studies about the arrangements of nerve fibers in the organ of Corti by Bredberg and others. What is the difference and merit of the procedure in this paper?
Authors: With this method we can get the interior view of the organ of Corti constantly and easily, whereas the views reported by Bredberg and Hoshino were seemingly obtained after acquiring exceptionally fine skills to break the specimens using needle or scissors at a few spots and after many trials.

R.S. Kimura: The basilar fibers cross the underpassing radial fibers at the floor of Corti's tunnel. Some radial fibers show varicosities at these cross points in the photographs (Figs. 2 and 3). They are most likely to establish synapses as shown in the monkey. How often do you see this type of contact? It is interesting to note that similar contacts are often shown on the lateral wall of Nuel's space (Fig. 6).

Authors: We suppose that there may be contacts between the basilar fibers and the underpassing radial fibers at their cross points. However, the appearance of varicosities at their cross point was rare. Also we could not identify synapses in this SEM study.

R.S. Kimura: Two types of radial fibers on the floor of the tunnel are described. These fibers are both considered to be efferent types. However, one of them is smaller in diameter and shows varicosities at shorter intervals. These small fibers may have a different origin, either unilateral or contra lateral efferent. If the nerve section experiment does not confirm this, they may be sympathetic nerve fibers. Sympathetic nerve fibers are known to come in contact with efferent fibers and cell processes of type II neurons in Rosenthal's canal in the monkey, though they are not identified in the organ of Corti. Because the dog cochlea shows a unique nerve fiber arrangement, it may be worthwhile to study these fibers with immunohistological techniques.

Authors: Thank you for your suggestion; we will plan to perform experiments along the lines you suggest.