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Relationship of the Tectorial Membrane to the Organ of Corti. A Scanning Electron Microscope Study of Cats and Guinea Pigs*

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Summary. The connections between the tectorial membrane and the organ of Corti in the cat and guinea pig were studied by scanning electron microscopy. Postnatal development of the organ of Corti is extensive during the first month after birth. Therefore, young kittens from 1 to 32-day-old as well as adult animals were examined. The outer margin of the tectorial membrane was attached to the 3rd row of Deiters' cells in young animals. This attachment started to deteriorate around the 20th postnatal day. No marginal connections were found in adult animals. In the adult animals, there was a shallow insertion of the tips of the longest hairs of the outer hair cells into the under-surface of the tectorial membrane. With the exception of a 10-day-old cat specimen, the inner hair cell hairs were not inserted into the tectorial membrane and, instead of a direct connection, the inner hair cell region appeared to be loosely connected to the tectorial membrane by fibrous strands. In a 10-day-old cat, the under-surface of the tectorial membrane showed imprints which indicated the insertion and possible attachment of the inner sensory hair tips to the membrane.

The physical relationship between the organ of Corti and the overlying tectorial membrane is important in the perception of sound as it is at this junction where the transduction of fluid vibrations to electrical signals occurs. This relationship has been examined by many investigators using both light and transmission electron microscopy (RETZIUS, 1884; SHAMBAUGH, 1907; HELD, 1926; HILDING, 1952; KIMURA, 1966). Several ambiguous points regarding an actual connection between the organ of Corti and the tectorial membrane have recently been elucidated in studies utilizing the scanning electron microscope (SEM) (LINDEMAN et al., 1971; LIM, 1972).

However, it is the author's contention that the following aspects of the connection between the organ of Corti and the tectorial membrane have not as yet been fully clarified:

1. A possible attachment of the outer margin of the tectorial membrane to the surface of the organ of Corti.
2. The presence of an actual physical connection between the tectorial membrane and the inner and/or outer hair cells on the organ of Corti.

In consideration of these contentions a SEM and a light microscope were used to carefully examine the inner ear of the cat and the guinea pig. An attempt has been made to further clarify, in these species, the relationship between the tectorial membrane and the organ of Corti.

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Fig. 1 A

Fig. 1. Cellular pattern of the reticular membrane of the organ of Corti of 5-day (**a**), 13-day (**b**) and 32-day-old cats (**c**). **A.** A part of the middle cochlear turn (about 15–17mm from the basal end). **B.** A part of the basal turn (about 5–8mm from the basal end). Note marked increase of the size of inner sulcus cells (*S*) and inner pillar cells (*P*) in Figure 1-A. Also, Hensen's cells (*H*) are

Fig. 1 B

arranged in a more regular sequence accompanying this development. Deiters' cells (*D*), especially in the 3rd row, are larger in the middle turn than in the basal turn. *IH* inner hair cell, *OH* first line of outer hair cells.

Materials and Methods

The inner ear specimens were obtained from the cat and the guinea pig. The animals were divided into two groups. Six new-born cats (1 day to 32 days old) and several adult cats and guinea pigs were used for SEM observation. Seven new-born cats (5 days to 32 days old) were utilized for light microscopy. All of the animals were anesthetized by an intraperitoneal injection of pento-barbital and sacrificed by decapitation.

SEM preparations

After decapitation, the inner ears were gently perfused, through the oval and round windows, with a 2% glutaraldehyde solution buffered with 0.1 M phosphate (pH 7.3). The temporal bones were roughly trimmed and further fixed in the glutaraldehyde solution for 5 to 12 hrs. Post-fixation was then applied 1 hr using 1% phosphate buffered osmium tetroxide. Dehydration was accomplished by immersing the temporal bones in an ascending series of ethanol baths. Surface preparations were obtained in 70% alcohol under the operating microscope.

The dissected specimens were placed in a series of baths of amyl acetate for 20 to 30 min and dried by the CO₂ critical point method of ANDERSON (1951) as modified by TANAKA (1972). The tissues were then coated with carbon and gold in a vacuum evaporator.

The inner ear structures were studied under the Hitachi SSM type II and HSM-2A scanning electron microscopes at magnifications ranging from $\times 100$ to $\times 10,000$.

Light microscope preparations

As soon as possible after decapitation the auditory bulla was opened. The stapes was then removed and the round window membrane was ruptured. A third opening was made by removing a part of the bony wall at the middle turn of the cochlea and perforating the scala media. Applying the silver impregnation method of WATANUKI (1969) an aqueous solution of 0.2% AgNO₃ was gently perfused through these openings for a few minutes and then the temporal bones were immersed in a 10% formalin solution and refrigerated. Several hours after perfusion, the cochlear duct was dissected under a dissecting microscope. Staining of the cell borders and the sensory hairs was completed during this phase under a bright light. The dissected cochlear turns were then placed on a slide and covered with a drop glycerin and a cover glass.

Results

The organ of Corti of the cat continues to develop during the first month after birth even though the otic capsule has reached its final dimensions. Developmental differences between several neonatal stages were observed in the silver impregnated preparations (Fig. 1). At each postnatal stage, the organ of Corti in the lower turns of the cochlea showed a more advanced development than in the upper turns. Maturation changes were seen in the surface structure of all of the constituent cells of the organ of Corti. Marked changes of the cell surfaces were seen on the inner pillar and inner sulcus cells and these cells tended to form a more regular pattern of arrangement in the older animals (Fig. 1).

Furthermore, scanning electron micrographs (Fig. 3, 5) demonstrated a single

kinocilium on each sensory and supporting cell in the 1-day and 7-day old cats. However, as an apparent function of development, this kinocilium had disappeared in the lower cochlear turns of the 20-day-old specimen (Fig. 4) but were still present in the upper turn. This cilium was not found in the cochlea of the 32-day-old specimens.

Microvilli, which were found in profusion in younger cats, also diminished both in number and in length in the older animals (Fig. 3-5).

A further development observed during postnatal maturation was found to involve changes in the physical relationship between the tectorial membrane and the organ of Corti. These changes, described below, occurred in the attachment of the outer margin of the tectorial membrane to the organ of Corti, and also in the attachment of the outer and inner hair cells to the tectorial membrane.

A. Attachment of the outer margin of the tectorial membrane to the organ of Corti

In the upper cochlear turn of a 1-day-old kitten, the outer margin of the tectorial membrane was found to be attached along the outside of the third row of outer hair cells of the cochlea (Fig. 2). Because the organ of Corti of the 1-day-old cat is not yet fully developed, it was difficult to ascertain whether or not the tectorial membrane was attached to Hensen's cells or to Deiters' cells. In a more advanced stage

Fig. 2. Upper-surface of the tectorial membrane of a 1-day-old kitten. Note the firm attachment of the outer margin of the tectorial membrane to the organ of Corti. Under the detached portion of the tectorial membrane the hair cells are seen in parallel rows on the right-hand side of the picture. $\times 530$

of postnatal development, evidence that this connection was to the third row of Deiters' cells was distinctly recognized (Fig. 4).

In the lower turns of the cochlea of the 1-day-old cat, the outer edge of the tectorial membrane was purposely pulled away during processing and the remnants of the tectorial membrane were recognized as a line of columnar structures on the supporting cells. The same tectorial membrane remnants were also found in the 7-day-old specimen (Fig. 3).

Examination of a 20-day-old cat specimen showed a structurally more developed

Fig. 3. Surface view of the organ of Corti of a 7-day-old kitten. Remnants of the detached tectorial membrane were found as columns on the third row of Deiters' cells. Sensory and supporting cells distinctly show kinocilia (arrows). The nature of the round globular mass near the kinocilium on each sensory cell is not known.

×2,300

organ of Corti. Remnants of the tectorial membrane were also found as amorphous masses scattered along the periphery of the third row of Deiters' cells (Fig. 4). However, some Deiters' cells of the same specimen did not retain any remnants of the tectorial membrane. Thus, in the 20-day-old specimen, the outer margin of the tectorial membrane seemed to have only an intermittent connection to the outermost row or the third row of Deiters' cells.

In the 32-day-old cat specimen and in adult cats and guinea pigs, remnants of

Fig. 4. The three rows of outer hair cells and nearby supporting cells of a 20-day-old cat. Remnants of the tectorial membrane are seen on the third row of Deiters' cells (black arrows). Some Deiters' cells show none of these remnants (white arrows). Note decreased number of microvilli and smooth cell surfaces. No kinocilia are recognized. $\times 2,700$

Fig. 5. A. Under-surface of the tectorial membrane of a 1-day-old kitten. Outer margin is shown on the left-hand side of the picture. The surface appears rough in the area corresponding to the hair cell region. $\times 2,000$ **B.** Surface of the organ of Corti of the same animal. Fine fibrous structures are seen on the Deiters' cells and hair cells. $\times 3,000$

A

B

Fig. 6. A. Inner hair cells of a 20-day-old kitten. Fine fibrillar substances, strands and some globular structures are seen around the sensory hairs. *P* inner pillar cell $\times 5,600$ **B.** Inner hair cells of an adult guinea pig. Similar fibrillar structures are seen lateral to the sensory hair bundles. $\times 5,000$

the tectorial membrane were not found on Deiters' cells anywhere in the cochlea. Thus, the margin of the tectorial membrane seemed not to have had any attachment to the organ of Corti in this stage of development.

B. Attachment of the outer hair cells to the tectorial membrane

Figure 5-A shows the under-surface (hair cell surface) of the tectorial membrane of a 1-day-old cat. Figure 5-B shows the corresponding surface of the organ of Corti of the same cat after removal of the tectorial membrane. Compared with a similar preparation from a 32-day-old cat (Fig. 7), the under-surface of the 1-day-old specimen was found to be quite rough and irregular. Also, many thick fibrillar structures were found on the surface of the organ of Corti of the 1-day-old cat and these were localized mainly on the supporting cells. These findings suggest the existence of developmentally early diffuse connections between the surface of the supporting cells (Deiters' cells and pillar cells) and the under-surface of the tectorial membrane. During this early postnatal stage the sensory hair bundles of the outer and inner hair cells might have been covered by these fine fibrillar structures.

The relatively smooth under-surface of the tectorial membrane of older animals (32-day-old) were marked with lines of shallow dots arranged in a W-shape (Fig. 7, 8). The sensory hairs of the outer hair cells of these animals are arranged in 3 graded lines of different heights. The single W-shaped imprint therefore indicates that the connection between the hair cells and the tectorial membrane is present only at the

Fig. 7. Under-surface of the tectorial membrane of a 32-day-old cat, clearly showing the imprints of sensory hair tips of the 3 rows of outer hair cells on the smooth surface. $\times 5,300$

tips of the longest hair. The remaining shorter stereocilia of the outer hair cells apparently do not come in contact with the tectorial membrane.

C. Attachment of the inner hair cells to the tectorial membrane

With the exception of the 10-day-old cat, imprints indicating an insertion of the tips of the sensory hairs of the inner hair cells were not found on the under-surface of the tectorial membrane in the specimens examined. In the guinea pig specimens, fine fibrillar structures were occasionally found around the inner sensory hair bundles (Fig. 6-B). The under-surface of the tectorial membrane of the guinea pig bore some fibrous strands which probably corresponded to the position of the inner hair cells (Fig. 8, arrows). Some of these strands seemed to be missing (for example, between the arrows in Fig. 8). In the cat, similar fibrous structures were found lying near the sensory hair bundles of the inner hair cells (Fig. 6-A). However, the corresponding fibrous strands on the tectorial membrane were not as long as they were in the guinea pig.

In the 10-day-old cat, clear imprints, consisting of small dots arranged in a slightly curved line, were recognized on the under-surface of the tectorial membrane corresponding to the position of the inner hair cells (Fig. 9). The short fibrous strands, described above, were also found at both ends of each imprint (Fig. 9, arrows). Most

Fig. 8. Under-surface of the tectorial membrane of an adult guinea pig. Imprints of the sensory hair tips of the first row of outer hair cells are seen. Note short fibrous strands which are disposed intermittently corresponding to the position of the inner hair cells (arrows). $\times 2,100$

Fig. 9. Under-surface of the tectorial membrane of a 10-day-old cat. Irregularly shaped patches, which are distributed from right to left in the photograph, correspond to the inner pillar cell region. Above these patches are seen the W-shaped imprints of two lines of outer sensory hair tips (1, 2). Below the patches are seen the imprints of the inner sensory hair tips. These imprints consist of small dots arranged in slightly curved lines. Short fibrous strands are found at the ends of these curved lines (arrows). $\times 3,700$

of these imprints were arranged arcuate with a notch in the center of the arch. This shape corresponds to the arrangement of the longest line of the sensory hairs of the inner sensory cells.

Discussion

It has been demonstrated that the cat exhibits mature cochlear microphonics and action potentials within 12 to 15 days after birth (PUJOL and HILDING, 1973). Studies by the light and transmission electron microscope have shown that the tunnel of Corti is completely developed and also the hair cells are mature at around 12 days after birth. The present SEM observations of the organ of Corti indicated that the surface structure of the cat's organ of Corti matures around 1 month after birth. Thus, onset of auditory function seems to precede the completion of the surface structure.

Clear imprints in the under-surface of the tectorial membrane, indicating the place of insertion of the hair cell cilia, were demonstrated by the SEM in developed animals. Accordingly, there is little contention relative to the connection between the sensory hair tips of the outer hair cells and the under-surface of the tectorial membrane in the adult cat and guinea pig, as shown in this and other studies. The tips of the outermost row or tallest hairs of each sensory hair bundle are shallowly

inserted into the under-surface of the tectorial membrane. KIMURA (1966) demonstrated this shallow but firm attachment by transmission electron microscope and, according to his results, the hair had a depth of insertion of $0.1\text{--}0.4\mu$ in the tectorial membrane of the squirrel monkey. In younger animals, such as the 1-day-old kitten, the connection between the under-surface of the tectorial membrane and the surface of the organ of Corti did not seem to be limited to the sensory hair tips. In the mammalian fetus, the tectorial membrane has a diffuse connection with the surface of organ of Corti (HELD, 1926). The presence of the connection in the newborn kitten was demonstrated to persist in the upper turn of the cochlea. Disappearance of the fine fibrils connecting the tectorial membrane and the immature organ of Corti may be due to absorption or due to coalescence of the gelatinous substance.

LIM (1972) proposed that the connection between the tectorial membrane and the phalanges of the outermost row of Deiters' cells, or rarely Hensen's cells, persisted even in adult animals. Thus, he supported previous light microscope studies (HILDING, 1952; TONNDORF et al., 1962). On the other hand, LINDEMAN et al. (1971) demonstrated the developmental changes of this connection; namely, a firm connection was present in the newborn kitten between the margin of the tectorial membrane and the outermost row of Deiters' cells but the membrane gradually detached and no connection was found in specimens older than 6 weeks. The present study supports LINDEMAN's findings.

Imprints of the hair tips on the tectorial membrane have not been found from the inner hair cells in previous studies except for a few reports (IURATO, 1967). Many investigators share the opinion that the hairs of the inner hair cells are not attached to the tectorial membrane (HELD, 1926; KIMURA, 1966; LINDEMAN et al., 1971). Instead of a direct attachment at the sensory hair tips, some connections around the inner hair cells have been suggested by other investigators. LIM (1972) postulated the presence of trabeculae-like anchoring devices between the tectorial membrane and the inner phalangeal cells or the border cells, or both.

As proposed by LIM, connections by these strands between the tectorial membrane and the inner hair cell region (probably the inner phalangeal cells) may exist. Furthermore, in the 10-day-old cat specimen, imprints were found on the tectorial membrane which suggest a direct connection between the membrane and the inner sensory hair tips, as was found in the outer sensory hairs. Inasmuch as these imprints were found in only one kitten, further scrutiny of the tectorial membrane is necessary to disclose in what stage this direct connection exists and whether it never is found in the adult cat as many investigators observed.

In SEM specimens prepared by the frozen resin cracking method (TANAKA, 1972), TANAKA et al. (1973) reported that a thick granular substance existed continuously between the tectorial membrane and the lateral aspect of the inner sensory hair bundles of the adult guinea pig. The methodology of the present study involved the use of the critical point drying method. The granular substance reported by TANAKA was not found, though fibrillar strands were observed as mentioned above. Acellular substances, such as the tectorial membrane, may be distorted during specimen preparation, and thus several different preparation procedures seem to be necessary to avoid misinterpretation of the findings.

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コルチ器と蓋膜の接続. ネコとモルモット内耳の 走査電子顕微鏡による観察

星 野 知 之

ネコとモルモット内耳のコルチ器と蓋膜との接続を走査電子顕微鏡で観察した。コルチ器は 生後1ヶ月の間 さらに発育し変化するので、生後1日から32日までのネコと成獣について調べた。蓋膜の外側縁は 生後まもない動物では 3列目のダイテルス細胞に接続しているが、生後20日目頃から次第に離れはじめる。成獣では もはや この接続はみられなかった。成獣では 蓋膜下面に外毛細胞感覚毛の最長の1列の先端が ささっている。内毛細胞の感覚毛の先端は蓋膜にはささらず、蓋膜は 索状の構造物によって 内毛細胞の近くと接続していると考えられたが、生後10日のネコで、蓋膜下面に感覚毛の先端が ささっていたあとが 認められた。

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