JOURNAL OF MORPHOLOGY 199:103–118 (1989)

Ear Morphology of the Frog-Eating Bat (*Trachops cirrhosus*, Family: Phyllostomidae): Apparent Specializations for Low-Frequency Hearing

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ABSTRACT The frog-eating bat (*Trachops cirrhosus*) is unusual among bats studied because of its reliance on low-frequency (<5 kHz) sounds emitted by frogs for prey localization. We investigated the ear of this bat in order to identify anatomical features that might serve as adaptations for low-frequency hearing. *Trachops cirrhosus* has a variety of anatomical features that might enhance low-frequency hearing, either by increasing sensitivity to low-frequency sounds or expanding the total frequency range to include lower frequencies. These bats have long pinnae, and a long and wide basilar membrane. The basal portion of the basilar membrane is much stiffer than the apical portion, and the basal portion of the tectorial membrane is more massive than the apical portion. There is also a concentration of mass in the apical portion of the cochlea. *T. cirrhosus* possesses the largest number of cochlear neurons reported for any mammal, the second highest density of cochlear neuron innervation known among mammals, and three peaks of cochlear neuron density. Other bats have two peaks of cochlear neuron density, lacking the apical concentration, while other mammals usually have only one. *T. cirrhosus* differs from most other small mammals and bats in characteristics of the apical portion of the cochlea, i.e., that area where the place theory of hearing predicts that low frequencies are detected.

Microchiropteran bats possess an exquisite acoustical-imaging system in which the returning echoes of primarily ultrasonic signals (>20 kHz) emitted by the bats provide information about the surrounding environment; this information is used for navigation and foraging. The detection and processing of high-frequency sounds is facilitated by a suite of auditory specializations reviewed by Neuweiler ('84) and Pollak et al. ('86). Given the extensive reliance of these bats on ultrasonic sounds and their specializations for processing such sounds, the discovery by Tuttle and Ryan ('81; see also Ryan et al., '81, '82; Tuttle and Ryan, '82; Ryan and Tuttle, '83; Ryan et al., '83) that the frog-eating bat *Trachops cirrhosus* relies on relatively low-frequency (<5 kHz) frog calls for prey localization was astonishing. This low-frequency hearing is not at the sacrifice of ultrasonic sensitivity; these bats also utilize FM echolocation signals (ca. 50–90 kHz) for hunting and navigation (Barclay et al., '81). Although other bats use passive localization of ultrasonics produced by their prey and perceive sonic frequencies, as yet no other species of bat is known to rely extensively on passive localization of low-frequency sounds for localization of its prey (see review in Ryan and Tuttle, '87). If *T. cirrhosus* is not unique in this ability, it certainly is unusual.

The unusual foraging behavior of *T. cirrhosus* suggests specializations for detection of low-frequency sounds. The purpose of this study is to investigate the ear and associated auditory structures of *T. cirrhosus* and compare these structures to those of other bats and to mammals in general. Because the place theory of pitch discrimination (von Békésy, '60) predicts that low-frequency sounds are detected in the apical portion of the cochlea, we were interested especially in differences between *T. cirrhosus* and other mammals with respect to this region of the auditory system. Based on structure-function inter-

pretations from studies of microhiropteran and mammalian audition, we interpret differences between *T. cirrhosus* and other bats in the context of possible specializations for low-frequency hearing.

MATERIAL AND METHODS

Six ears of three adult specimens of *T. cirrhosus* were examined. Animals were collected in the Republic of Panama, near the Panama Canal. Immediately after capture, animals were decapitated under narcosis, and a 10% solution of neutral formaldehyde was injected into the mouth, masticatory muscles, and braincase via the occiptal foramen. The head were then submerged in formaldehyde.

The structures of the outer and middle ears were examined and measured with an ocular micrometer under a stereomicroscope. The middle ear and cochlea were photographed with a home-made device using ZEISS Luminar object glasses.

Serial sections were made by standard histological procedures. Specimens were embedded in celloidin-paraffin and sectioned (15 μm) in three different planes. A computer was used for 3-D reconstruction of the basilar membrane and quantitative analysis of the following structures: length of the basilar membrane, width of basilar membrane, thickness of the basilar membrane, cross-section areas of the fluid spaces and the tectorial membrane, and the distribution of cochlear neurons. For detailed description of the method see Kraus et al. ('81). The location of points within the cochlea are indicated by their distance from the base, either in turns and half turns, or in mm. In describing the entire cochlea and in comparing cochlea among species, we usually refer to the number of turns. When discussing the location of microscopic sections we indicate the number of half turns from the base. Finally, after computing the entire length of the basilar membrane we refer to absolute distances in mm.

The right cochlea of three ears was processed by means of total surface specimen technique, the cochlear partition being stained in toto by toluidine blue and Ehrlich's hematoxylin. The specimens were mounted in glycerine for light-microscopic examination.

The density of hair cells was estimated on the basis of measurements of the area occupied by the hair cells taken at a magnification of 1,000 × in each successive visual field along the total organ of Corti. Seven of ten measurements of each structure or dimension were taken per 1 mm of the organ of Corti in each ear. The data from three cochlea were averaged within the respective 1 mm segments, and the means plotted as a function of the middle of each segment.

RESULTS

Outer ear

T. cirrhosus has long, prominent pinnae ($\bar{x}$ = 31.2 mm, SD = 0.4, N = 6, measured along the longest axis from top to bottom), which are longer than the head. Pinnae are provided with well-developed ear muscles, and muscles of all the usual topographical groups are present [cf. descriptions of *Aselia* and *Myotis* by Schneider ('61)].

Middle ear

The eardrum has an approximate area of 3.76 mm^2 (N = 3). The auditory ossicles are of a common "microchiropteran type" (Henson, '61). However, there is a rather conspicuous and relatively long crus breve incudis, which is almost as long as the crus longum (Fig. 1). The area of the stapedial footplate is estimated to be 0.21 mm^2 (N = 3).

Cochlea

The cochlea in general

The bony cochlea is coiled in 3.25 turns (or more than 6 half turns) (Figs. 2–5). The basal region is characterized by a steep rise and a conspicuous inward bending hook (Fig. 6). The remainder of the cochlea is characterized by a more or less constant rise and a continuous decrease of the radius toward the apex. The oval window has a kidney-like shape, and its area is estimated to be about 0.45 mm^2 (N = 3).

Figures 3 and 4 demonstrate the peculiar shape of the apex. The cochlea of most mammals has the shape of a cone with a pointed apex. In *T. cirrhosus* it has a flat apex with an upper turn of relatively large diameter. The scala tympani is very large in the region of the cochlear aqueduct. From the middle of the first turn (4 mm from the base) toward the apex, the fluidspace below the basilar membrane (scala tympani) is always smaller than the fluidspace above the basilar membrane. The cross-sectional area of the lower fluidspace in the fourth half turn is greatly reduced.

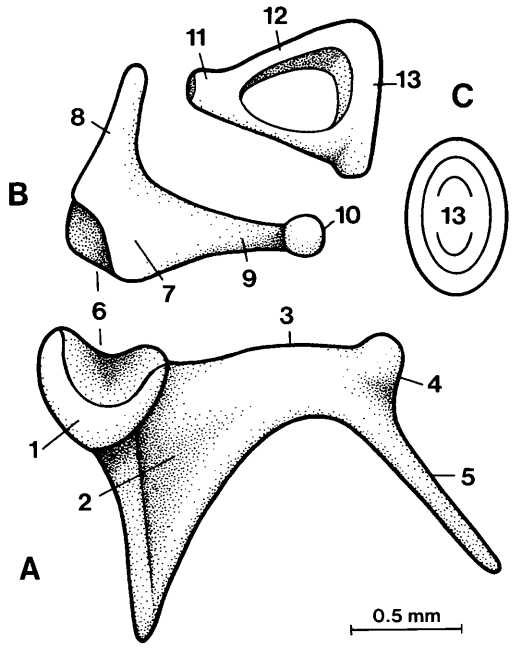

Fig. 1. Auditory ossicles of *Trachops cirrhosus* in dorsal view. **A:** Malleus. **B:** Incus. **C:** Stapes. 1, caput mallei, lamina transversalis; 3, collum mallei; 4, apophysis orbicularis; 5, manubrium mallei; 6, articulatio incudomallearis; 7, corpus incudis; 8, crus breve incudis; 9, crus longum incudis; 10, proc. lenticularis; 11, caput stapedis; 12, crus stapedis; 13, basis stapedis.

Basilar membrane and anchoring system

The length of the basilar membrane measured along the organ of Corti was 14.5 mm in all specimens, whether measured on surface specimens or on the computer reconstructions of the cochlea. The first turn is from the base to 7.5 mm, the second turn is from 7.5 mm to 11.5 mm, and the third turn is from 11.5 mm to 14.5 mm. The width of the basilar membrane increases abruptly along the first two basal millimeters from 55–90 μm (i.e., 17.5 μm/mm). Further increase in width from the basal end is less conspicuous, about 2.5 μm/mm reaching a maximum width of 110 μm at 9–10 mm from the basal end. From this point toward its maximum of 165 μm at 13 mm, the width increases substantially (18.3 μm/mm). Toward the apex, the width decreases slightly (Fig. 7).

The thickness of the pectinate zone of the basilar membrane (the outer segment between outer pillar and spiral ligament) decreases from about 20 μm at the base to about 1.5 μm at the apex (Fig. 8). This overall decrease occurs in three regions. From the base to 3 mm there is a conspicuous thickening to at least 16 μm. From 3–7 mm there is a substantial decrease to less than 4 μm, and in the remaining length, from 7–14.5 mm, there is a slow decrease in thickness of the pectinate zone.

The inner anchorage of the basilar membrane consists of the primary osseous spiral lamina, which is a rigid inner anchor for the basilar membrane and a strong support for the feet of the inner pillars. The outer anchorage of the basilar membrane consists of the spiral ligament and the secondary osseous spiral lamina. In the three basal half turns (0–9.5 mm), the secondary spiral lamina consists of a massive ledge of the outer bony wall, whereas in the three apical half turns the secondary spiral lamina is reduced to an inconspicuous eminence of the outer bony wall (Fig. 4).

Tectorial membrane

The size and shape of the tectorial membrane change conspicuously along the cochlear duct (Figs. 9, 10). The cross-sectional area of the tectorial membrane increases from 102 μm^2 at the very base to 447 μm^2 at 6.5 mm. Beyond this point, the increase is much greater and leads to a maximum of 3,136 μm^2 at 13.5 mm. From this point toward the apex, there is a decrease to 964 μm^2 (mean values of three cochlea).

Hair cells

The geometrical surface pattern of the reticular lamina is arranged basically in the same regular way as in all mammals studied to date. The surface pattern of the reticular lamina in *T. cirrhosus* has a qualitatively different pattern between the basal half (i.e., along the 1st turn) and the apical half (i.e., along the 2nd and 3rd turns) of the organ of Corti (Fig. 11). In the basal portion of the organ of Corti, the "V-like" formations of rows of stereocilia on cuticular plates of outer hair cells (OHC) are opened widely, the rows of hair cells (especially those of OHC) are compressed, and the rows of cells are packed together. The cuticular plates of the hair cells are elongated in a baso-apical direction and are longer in the first than in the second and third cochlear coils. However, in the apical half of the organ of Corti, the "V formations"

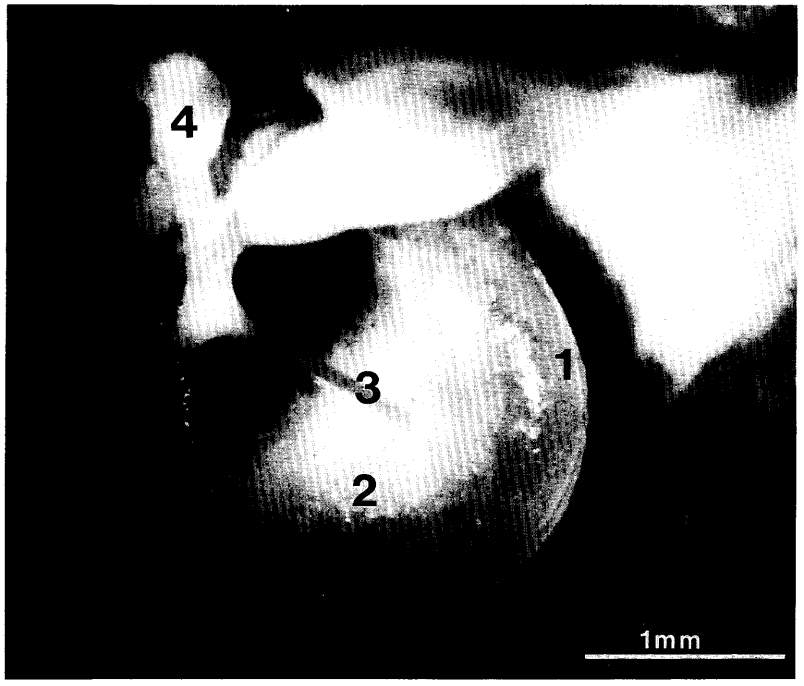

Fig. 2. Tympanic membrane and the ossicles of the middle ear of *Trachops cirrhosus*. 1, tympanicum; 2, tympanic membrane; 3, manubrium mallei; 4, incus.

of hairs on OHC are more closed, cuticular plates are shorter, the rows of hair cells are farther apart from one another, and the surface pattern gives the impression of being slightly less regular.

The greater compression of the rows of OHC in the basal relative to the apical half of the cochlear duct may be quantified by measuring the radial width of the three rows of cuticular plates of OHC (Burda, '85). These values fluctuate only slightly in the apical half of the organ of Corti ($\bar{x}$ = 22.3 μm, SD = 1.2), whereas along the same length in the course of the basal turn they decrease linearly to 11.5 μm at the very base (Fig. 11).

The average density of hair cells per 1 mm was found to be almost the same in all ears under study: 394.6 (SD = 0.5) and 109.9 (SD = 0.4) for outer and inner hair cells, respectively. Thus the total average number of OHC is 5,721; that of inner hair cells (IHC) is 1,594.

As discussed above, the size and packing of hair cells are not constant along the organ of Corti, and hence the density of hair cells also changes. These intracochlear changes may be expressed by averaging the values of mean densities estimated for each 1-mm segment along the organ of Corti: 395.1 ± 34.1 and 110.1 ± 12.5 for OHC and IHC, respectively. The highest density of both OHC and IHC are found in the apical half of the organ of Corti (Fig. 11). There are 84.8 IHC/mm at 0.5 mm from the basal tip, and this increases to 121.5 IHC/mm at 8.5 mm. Generally, this rise in IHC density is linear and statistically significant ($P < 0.005$). The changes in IHC density between 7.5 and 13.5 mm are not statistically significant; the mean value in this region is 119.9 (SD = 1.5). Toward the terminus of the apical tip, the density decreases slightly; there is an average of 116.2 IHC/mm at the apex. The density of OHC/mm, which is 326.8 at the basal tip, is subject to similar changes. In the course

of the basal turn, there is again a marked increase in density. The density of OHC is the highest in the apical half to the organ of Corti, the area in which there are two plateaus of density maxima. A lower OHC density maximum of 412.0 (SD = 4.0) lies between 6.5 and 9.5 mm; the second maximum of 429.0 (SD = 5.4) occurs between 10.5 and 13.5 mm. Within these maxima the current density changes are not statistically significant. There are on average 411.3 OHC/mm at the apical tip of the organ of Corti.

Although the densities of both OHC and IHC are subject to similar changes along the organ of Corti, their relative abundances change (Fig. 11). Thus, there are 386 OHC to 100 IHC at the base, whereas there are only 341 OHC/100 IHC 8.5 mm from the basal tip; the latter is the minimum value of the ratio. The mean ratio is 360 (± 12.8) OHC/100 IHC.

Cochlear neurons (spiral ganglion)

The total number of neurons in the spiral ganglion is 40,500. The average density is 2,793 neurons per millimeter of basilar membrane length. Reconstruction of the density along the cochlear duct reveals considerable regional differences. There are three peaks of innervation. The greatest (5,500 neurons/mm) is between 5 and 6 mm from the base. The two others (ca. 4,800 neurons/mm) are found in the first millimeter and between 9.5–11.0 mm, respectively.

Between these areas of maximal density are two areas of minimal density: 3,000 neurons/mm between 2–3 mm, and 2,600 neurons/mm between 8.5 and 9.5 mm. The apical concentration requires elaboration. Here, the spiral ganglion does not reach the helicotrema; thus, the course of the fibers between the spiral ganglion and the organ of Corti must diverge from the radial direction.

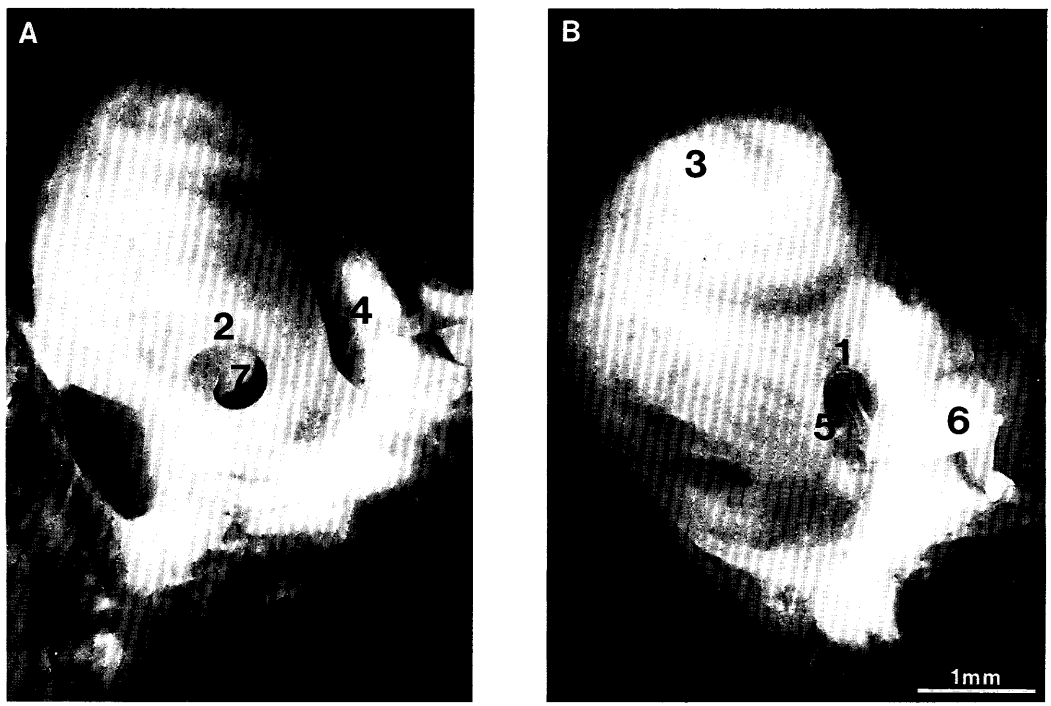

Fig. 3. The cochlea of *Trachops cirrhosus* **A:** View onto the round window. **B:** View onto the oval window. 1, oval window; 2, round window; 3, apex; 4, stapes; 5, arteria stapedia; 6, incus; 7, basal part of basilar membrane.

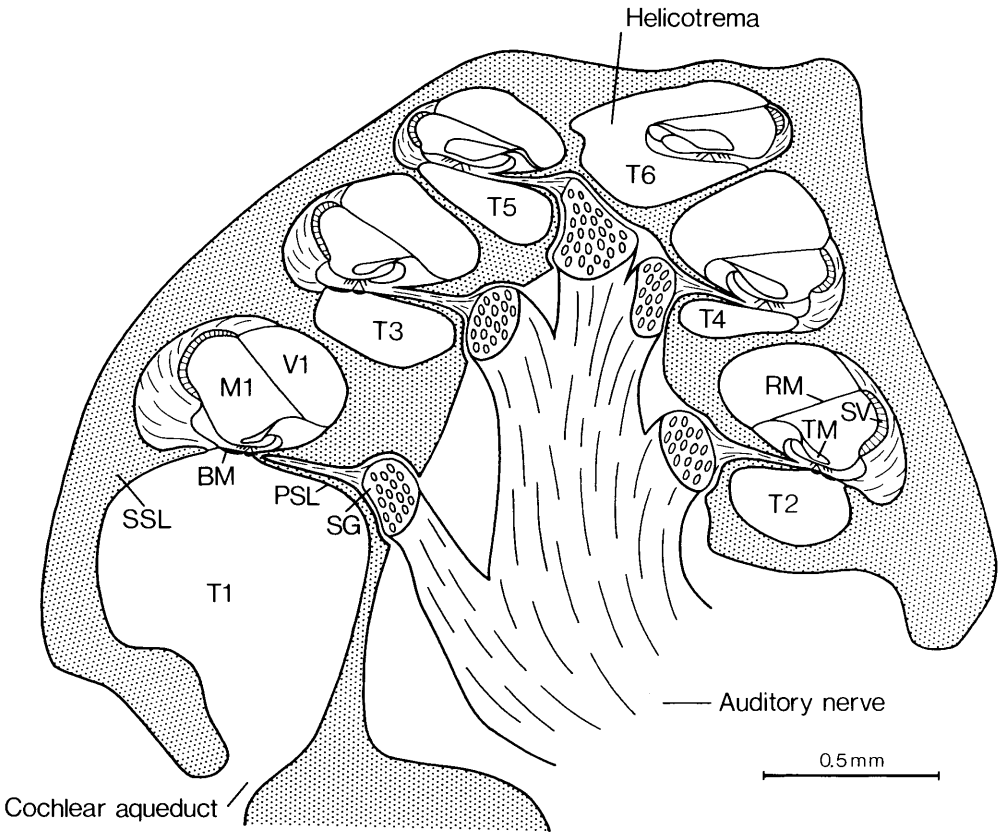

Fig. 4. Midmodiolar section of the cochlea of *Trachops cirrhosus* demonstrating six half turns, the cochlear aqueduct (below left), and the helicotrema (above). The half turns from base to apex can be followed by the number of the cross sections of the scala tympani (T1, first half turn; T2, second half turn, etc.; T1 + T2, 1st turn; T3 + T4, second turn; T5 + T6, third turn). The fluidspaces above the basilar membrane (BM) are marked only in the first half turn (M 1, scala media; V 1, scala vestibuli). Remaining abbreviations: PSL, primary spiral lamina; RM, Reissner's membrane; SG, spiral ganglion; SL, spiral ligament, SSL, secondary spiral lamina; SV, stria vascularis; TM, tectorial membrane.

Because we do not know the exact relationship, we did not classify each apical 0.5 mm basilar membrane section with a definite section of the spiral ganglion. Instead we pooled the neurons from 11–14.5 mm and calculated the average density for this region.

DISCUSSION

In this section we compare the ear and associated structures in *T. cirrhosus* to homologous structures in other bats and to mammals in general. We especially stress those differences that would appear either to enhance low-frequency sensitivity or expand the frequency range of hearing in *T. cirrhosus*.

Outer ear

Compared with measurements of 18 other species in the family Phyllostomidae, as summarized by Firbas ('72) or with illustrations and data given by Walker ('75), the absolute and relative size of pinnae in *T. cirrhosus* seems to be rather exceptional both among the Phyllostomidae and among other Microchiroptera in general.

According to Firbas ('72) there is a close correlation between the size of pinnae, characteristics of echolocation calls, and capabilities of hearing; larger pinnae enable collection of sounds of lower frequencies and intensities more effectively. Because of its large pinnae, *T. cirrhosis* probably is capa-

ble of enhancing sounds of lower frequencies and/or of lower intensities better than most other bats.

Middle ear

The area of the eardrum (ca. 3.8 mm^2) places *T. cirrhosus* among the so-called intermediate ultrasonic forms of echo-locating bats (according to a division proposed by Henson, '61). *Eptesicus fuscus* is considered typical of this group by Henson. This species has an audible range from 0.2–100 kHz (Poussin and Simmons, '82). Correspondingly, we suggest that the area of the eardrum of *T. cirrhosus* should enable it to perceive well both relatively higher as well as lower frequencies. With the exception of the crus breve incudis, the auditory ossicles are of a common "microchiropteran" type (Stanek, '33; Henson, '61). The functional role of the relatively long crus breve incudis is not clear. From comparison with European bats described by Stanek, a similarly long crus breve is also found in horseshoe bats (*Rhinolophus* sp.).

The ratio between the area of the eardrum and stapedial footplate (which defines the amplification of sound across the middle ear) is about 18:1 in *T. cirrhosus*. This value falls in the range of values given for this ratio in bats by Henson ('61).

Cochlea

The cochlea in general

The number of turns in the cochlea of *T. cirrhosus* (3.25) exceeds that in the shrew, *Sorex araneus,* (1.5 turns) and house mouse, *Mus musculus* (2 turns) (Bruns and Burda, personal communication). The number of cochlear turns in bats ranges from 2.5 (*Myotis lucifugus*) to 3.5 (*Rhinolophus ferrumequinum*) (Henson, '70; Bruns et al., '84). The 3.25 cochlear turns in *T. cirrhosis* is a common feature in the Phyllostomidae (Pye, '67, '80). The cochlea seems to be relatively large, but comparative data on the length of the cochlear duct in other phyllostomids are lacking.

In terrestrial mammals, West ('85) found a correlation between the frequency range and the number of turns in the cochlea, but this correlation does not hold for some bats. For example, the horseshoe bats, *Rhinolophus ferrumequinum* and *R. rouxii,* with 3.5 turns exhibit the maximum for microchiropteran bats. However, these bats do not exhibit an extraordinary frequency range but an acoustic fovea; a small frequency range is spread over about half the length of the cochlea. Regarding *T. cirrhosus,* we suggest that in bats with small skulls architectural constraints might limit cochlear expansion. Specifically, the area for expansion of the first turn is restricted, thus elongation results in an increase of spiralization.

Scala tympani and helicotrema

The scala tympani is very large in the region of the cochlear aqueduct. This is also true in other bats (Fiedler, '83; Kraus, '83). From the second turn toward the apex, the fluidspace below the basilar membrane (scala tympani) is always smaller than the fluidspace above the basilar membrane. In the family of Muridae (Old World mice and rats) such an asymmetry is found in species with good low-frequency hearing (rats), whereas mice with poorer low-frequency hearing have more symmetric fluidspaces (Bruns and Burda, personal communication). There is a reduced cross-sectional area of the lower fluidspace in the fourth half turn. The region of very low-frequency hearing in *T. cirrhosus* is to be expected between that minimum and the apex, since, in general, higher frequencies are represented more basally while lower frequencies are represented more apically (von Békésy, '60).

Basilar membrane

Compared with the basilar membrane of eutherian insectivores and rodents of similar body size, the basilar membrane of *T. cirrhosus* is remarkably longer. For example, it is 3.6 times longer than in the shrew and 2.1 times longer than in the house mouse (Bruns and Burda, personal communication). Even among bats, which generally have longer basilar membranes than most mammals, most species have a shorter basilar membrane (e.g., *Myotis lucifugus,* 6.9 mm; *Megaderma lyra,* 9.9 mm; *Rhinopoma hardwickei,* 11.8 mm; and *Pteronotus p. parnellii,* 13.2 mm). Only *Molossus ater* (14.6 mm) and *Rhinolophus ferrumequinum* (16.1 mm) have longer basilar membranes (Bruns et al., '84).

The diameter of the largest turn (the basal turn) of the basilar membrane of *T. cirrhosus* is similar to that in the shrew, house mouse and many bats. Only *Rhinolophus* and *Pteronotus* have clearly greater diameters in their basal turns. Therefore, the elongation of the basilar membrane in *T. cirrhosus* compared with that in other mammals is due to an increase in the number of turns.

In terrestrial mammals, West ('85) found a correlation between the length of the bas-

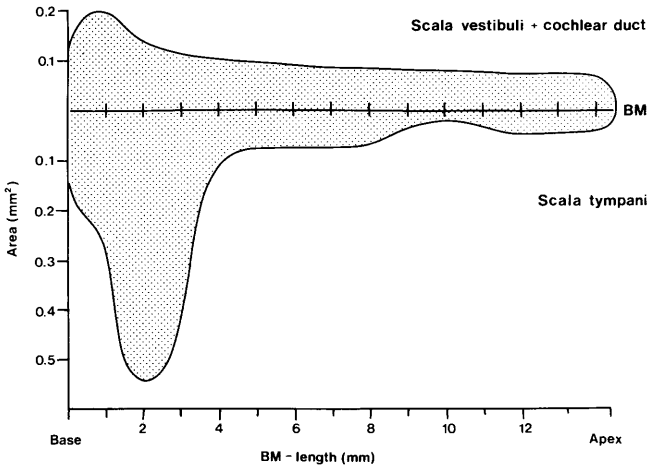

Fig. 5. Cross-sectional area of the cochlear fluid spaces above and below the basilar membrane (BM-length, length of the basilar membrane), which is decoiled and shown as a horizontal line.

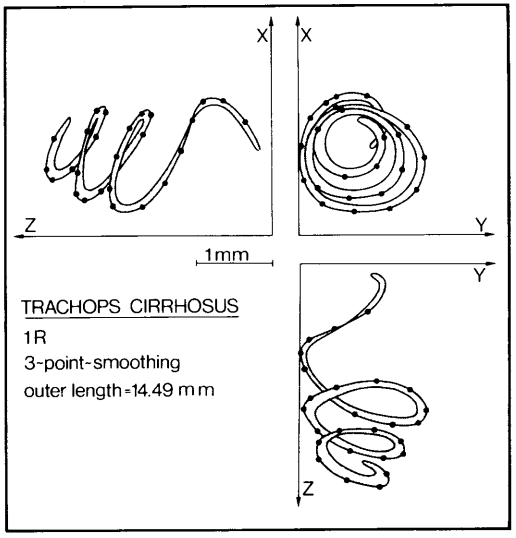

Fig. 6. Computer reconstruction of the basilar membrane in three perpendicular planes. Points on the outer margin of the basilar membrane indicate every 0.5-mm distance.

ilar membrane and low-frequency hearing; the longer the basilar membrane, the lower the frequencies that can be heard. Comparative studies show that the elongation of the basilar membrane may have two different functional implications: 1) an overall extension of the frequency range, or 2) an expansion of biologically relevant frequency bands. The latter is exhibited in *Rhinolophus ferrumequinum, R. rouxii,* and *Pteronotus p. parnellii* in which the small frequency band around the pure tone echolocation signal is expanded in an "acoustic fovea" (Bruns, '76a,b; Vater et al., '85; Kössl and Vater, '85). In *T. cirrhosus,* which does not have a pure tone echolocation signal (Barclay et al., '81), we suspect an overall frequency expansion.

Basilar membrane width

In a comparative study of nine species of New World bats, Pye ('80) described the basilar membrane width of *T. cirrhosus* in the following way: "In *T. cirrhosus* a strange pattern occurs: the basilar membrane is exceptionally wide (150 μm) at the first half turn, decreases rapidly to 106 μm at the second half turn, and then increases again towards the apex to a value of 156 μm at the sixth half turn. This, therefore, shows only a slight difference in width between the base and the apex of the cochlea, although an increase of 50 μm takes place from the second half turn to the apex."

This observation is contradicted by our investigation, which shows a narrow basilar membrane at the base (55 μm) and a basoapical increase of 100 μm. This discrepancy is probably due to differences in methodol-

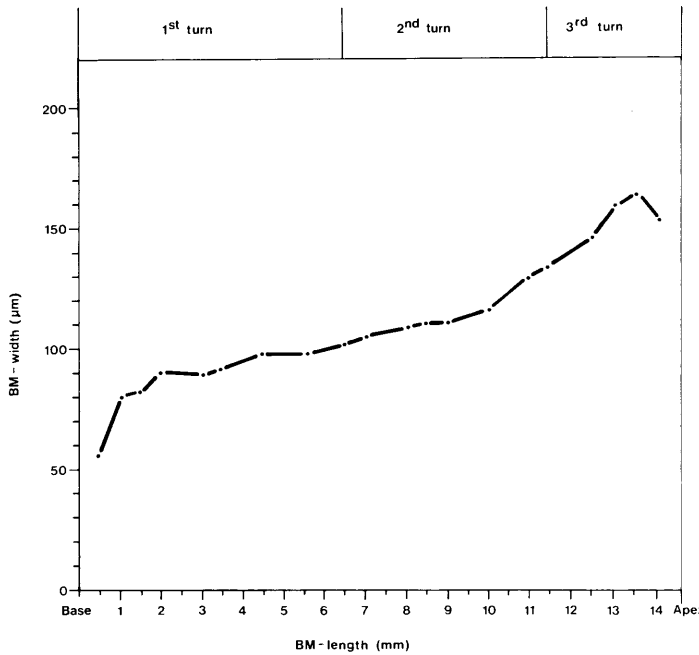

Fig. 7. The width of the basilar membrane (BM).

ogy. Our 3-D reconstruction demonstrated that in the basal cochlear region there is a steep rise and a conspicuous inward bending of the basilar membrane (Fig. 6). In Pye's study, the head of *T. cirrhosus* was serially sectioned in the horizontal plane; that is, more or less parallel to the modiolar axis. In such a plane, virtually no section cuts the basilar membrane in the radial direction. If the section is not in the radial direction, the width of the basilar membrane more or less increases corresponding to the angle of divergence. Without 3-D reconstruction one cannot assume this angle and make the respective correlations. In our method, the evaluation of the basilar membrane width is independent of the plane of sectioning.

In our comparison of the basilar membrane width in shrews, rodents, and bats *T. cirrhosus* shows the greatest baso-apical difference in absolute as well as in relative dimensions (Bruns and Burda, personal communication). According to von Békésy ('60), the basilar membrane width is the main parameter defining the stiffness of the cochlear partition. The stiffness is inversely correlated with the 4th power of the basilar membrane width. The baso-apical change in stiffness of the cochlear partition causes the baso-apical frequency distribution (place theory). Following this argument, *T. cirrhosus* should have the largest frequency range among most species of shrews, rodents, and bats studied to date.

Basilar membrane thickness

Pye ('80) found measurable thickening of the basilar membrane (more than 5 µm) at the base of the cochlea. In her Figure 3, she shows three measurements in *T. cirrhosus:* first half turn, ca. 9 µm; second half turn, ca. 11 µm; and third half turn, ca. 5 µm. Pye's value of the first half turn is only half of ours. From our 3-D reconstruction we can conclude that the first half turn extends from 0–3.5 mm. Here we found that the basilar membrane is between 15 and 20 µm thick. However, Pye's value of the second half turn is clearly higher than ours. In the second half turn, extending from 3.5–7.5 mm, the basilar membrane was between 15 and 4 µm thick.

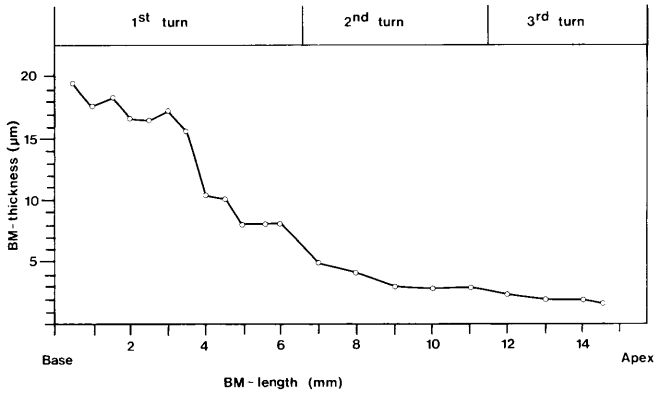

Fig. 8. The thickness of the outer segment (zona pectinata) of the basilar membrane.

We measured the thickness of the basilar membrane at about 290 points along the cochlear duct and defined the exact longitudinal position between base and apex. One possible explanation of the data of Pye is that the values of the first and second half turn were both measured around the 3.5-mm position, i.e., at the extreme apical end of the first half turn and the extreme basal end of the second half turn. In this case one could miss the conspicuous thickening of the basal region and could get a value of the second half turn that is greater than the averaged thickness of this cochlear region.

Thickening of the basilar membrane in the basal region, which ranges between 15 and 20 µm, is found in most bat species; also, obvious but less pronounced thickenings are found in other small mammals. Bat species of the genera *Hipposideros, Rhinolophus,* and *Molossus* have conspicuous thickening between 30 and 40 µm. Yet in these cases the arrangement of filaments and growth substance differs qualitatively from the "normal" mammalian pattern (Kraus, '83). A quantitative and functional analysis is possible only for a qualitatively uniform construction, which is true of most mammals, including *T. cirrhosus.*

The stiffness of a vibrating plate depends not only on its width (b) but also on its thickness (h), such that

$$f \sim \frac{h^3}{b^4}$$ (von Békésy, '60).

This formula can be applied to the properties of the basilar membrane, if the number of radial filaments is proportional to the thickness.

In *T. cirrhosus* the thickness of the filament layer at different points along the cochlea is proportional to the total thickness of the basilar membrane. The great basal thickness suggests representation of high frequencies, and the great baso-apical difference is further evidence for a broad frequency range.

In those species where the basilar membrane structure is of the "normal" type, we calculated the relative baso-apical difference in stiffness with the following formula: f (basal): f (apical). In the shrew, *S. araneus,* the stiffness ratio is 300 × higher at the base than at the apex. In rodents it varies from about 1,000:1 to about 33,600:1 (Fernandez, '52; Bruns and Burda, personal communication). In bat species other than *T. cirrhosus* with a "normal" basilar membrane, we found the baso-apical difference varying between 2,100:1 in *Rhinopoma hardwickei* to 22,800:1 in *Molossus ater.* The highest values computed are in the cat (50,000:1), and in the dolphin *Tursiops truncatus,* (100,000–200,000:1; Wever et al., '71a,b). In *T. cirrhosus* the baso-apical difference in the stiffness ratio is 128,600:1.

The cat and the dolphin are among the mammals with the greatest frequency range. The house mouse and the bats have excel-

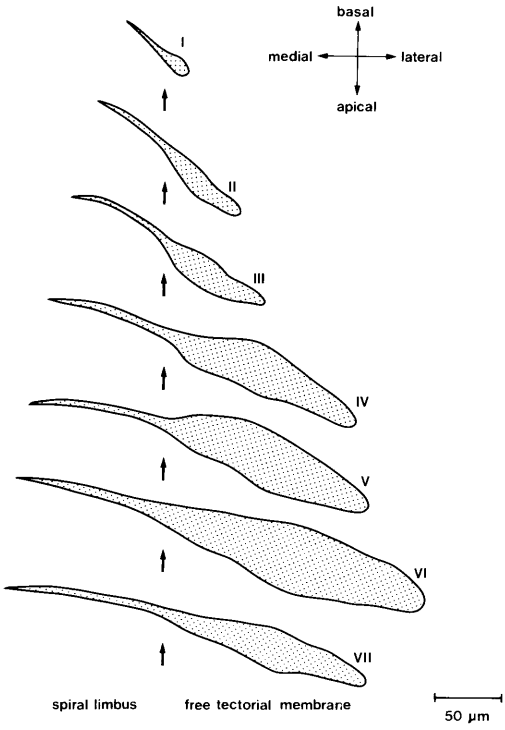

Fig. 9. Radial sections of the tectorial membrane from the base (I) and from every half turn (II, first half turn; III, second half turn; etc.). The exact longitunal position can be taken from Figure 10. The arrows indicate the outer edge of the spiral limbus.

lent high frequency hearing but, with the exception of *T. cirrhosus,* generally do not have good low-frequency hearing. Thus it seems that the baso-apical stiffness difference correlates quite well with the expanded frequency range of hearing suggested for *T. cirrhosus* (Ryan et al., '83).

Anchoring system

Mammals with low-frequency hearing have a well-developed secondary osseous spiral lamina only in the basal cochlear region (guinea pig, and gerbils), or only in the lower part of the basal turn (e.g., man, mole; Firbas, '72; Fleischer, '73). By contrast, mammals with high-frequency hearing, such as shrews and bats, have a secondary spiral lamina along the entire cochlear duct (Firbas, '72; Fleischer, '73). The inconspicuous secondary spiral lamina in the apical region of *T. cirrhosus* indicates low-frequency hearing in this region, and suggests that *T. cirrhosus* differs from other bats that have been studied.

Tectorial membrane comparison

There are few published quantitative data of the cross-sectional area of mammalian tectorial membranes. Bruns and Burda (personal communication) recently analyzed the organ of Corti in a dozen mammalian species by means of semi-thin sections. The baso-apical pattern of the cross-sectional area differs among these species. Nevertheless, there is an overall increase in the area from base to apex. The ratio of the basal area to apical area varies between 1:1.7 in the guinea pig and 1:10 in *T. cirrhosus.* The shrew *S. araneus* (1:7.4), the mouse (1:9.7), and the bat *Hipposideros fulvus* (1:8.3) have only slightly lower ratios than *T. cirrhosus.* Like *T. cirrhosus,* some species have local maxima of their tectorial membrane area. In the bat *H. fulvus,* this maximum is in the basal region at 22% of the cochlear length; in the guinea pig, it is in the middle region at 50% cochlear length, and in the gerbil, *Pachyuromys duprasi,* it is in the upper middle region at 65% cochlear length (Bruns and Burda, personal communication).

In his model of the stereocilia-tectorial membrane system, Zwislocki ('80) assumed that the resonance frequency of this system is inversely proportional to the mass of the tectorial membrane—that is, the greater the mass the lower the resonance frequency. This suggests that most structural elements of the cochlea in *T. cirrhosus* indicate a wide frequency range; thus, one would expect a great baso-apical difference in the mass (measured as cross-sectional area) of the tectorial membrane. As expected, the ratio of basal to apical mass in *T. cirrhosus* is the highest of any species investigated, but it is only slightly higher than that of the house mouse and the bat *Hipposideros fulvus,* two species with only a moderate frequency range. Thus there might be only a weak correlation between this ratio and the frequency range.

Frequency mapping in the gerbil, *Pachyuromys duprasi,* showed that the most sensitive frequency of this species (1.5 kHz) is

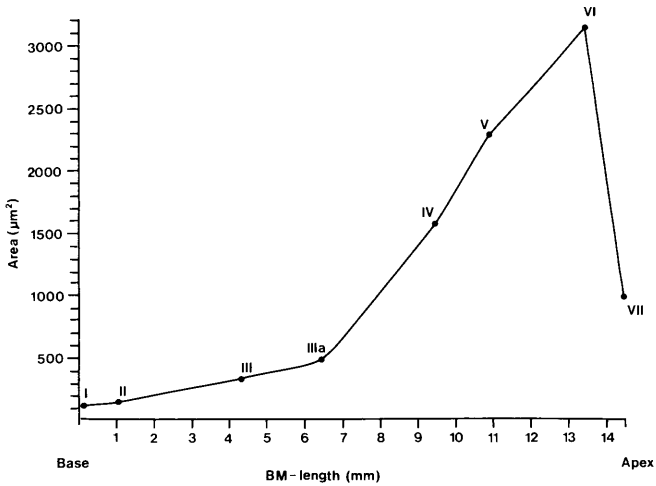

Fig. 10. Radial sectional area of the tectorial membrane.

represented at the place of maximum mass of the tectorial membrane (Ott et al., '86a,b; Dannhof et al., '86). For *Hipposideros fulvus* there is indirect evidence from the frequency map of the related species *Rhinolophus rouxii* (Vater et al., '85) that the maximum mass of the tectorial membrane lies at the point corresponding to the frequencies of the pure tone echolocation signal. In analogy to these species, we assume that the apical maximum of the tectorial membrane mass in *T. cirrhosus* could enhance the sensitivity of the species in the low-frequency region.

Cochlear receptors

The increase of the radial width of the OHC triad is a feature common to all mammalian species studied so far. There is no particular difference in absolute maximum and minimum values of this parameter between the house mouse (Bruns and Burda, personal communication) and *T. cirrhosus*. Nevertheless, whereas in the mouse (and most other mammals studied so far) the slope of increase is linear along the organ of Corti, it is different in the basal and apical cochlear halves in *T. cirrhosus* (Fig. 12). The functional significance is not clear. This parameter has been discussed by Burda ('85) as a quantifiable correlate of postnatal maturation of the reticular lamina of the organ of Corti. We have described it for *T. cirrhosus* in the present paper as a morphometric correlate of changes in packing of rows of OHC, and differences in qualitative appearance of the geometrical pattern of the reticular lamina between the basal and apical cochlear turns (see also Fig. 11).

Comparative data on the total number of cochlear hair cells in other phyllostomids are lacking. The length of the basilar membrane usually is more variable among mammals than the mean receptor density; thus, membrane length is used for determining the total number of receptors. According to a number of studies (cf. survey provided by Burda and Voldrich, '84, personal communication) the mean mammalian receptor density is 380 (±19) OHC/mm and 105 (±10) IHC/mm. (The standard deviations refer to interspecific differences of respective values of mean density estimated for each species.) Thus, intracochlear changes in receptor densities are not reflected. The mean densities of both types of receptors for *T. cirrhosus* (395 and 110 for OHC and IHC, respectively) fall into the range of values commonly found in mammals.

As in most of the mammals studied so far, the density of hair cells along the organ of Corti is not constant. It is obvious that the densities of both IHC and OHC are highest throughout the course of the apical half of the organ of Corti where there is a plateau of maximum density. In contrast, the density drops linearly and steeply in the course of the first coil toward the base. The pattern of hair cell distribution described for *T. cir-*

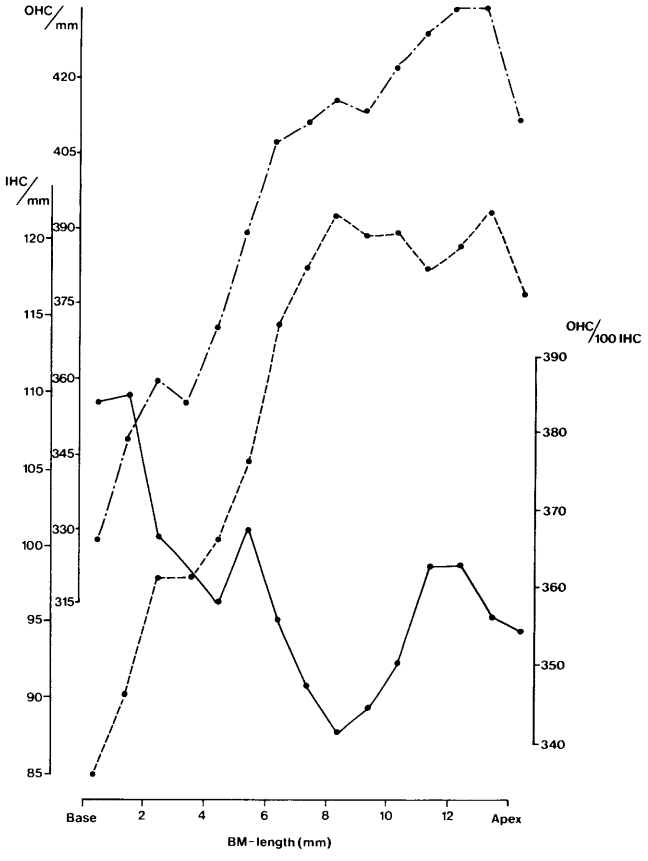

Fig. 11. The density of inner hair cells (IHC) and outer hair cells (OHC) per 1 mm of the length of the organ of Corti, and the ratio between the number of OHC and IHC along the organ of Corti. BM-length, position on the basilar membrane (BM) from the base of the cochlea.

rhosus is quite different from that in any other bat or small mammal studied so far. The variable density has been correlated with sensitivity and/or with hearing discrimination capabilities in several mammalian species (Burda, '84).

If there is a relatively constant resolution capability at the cochlear segments of the same length, there must be more receptors in apical segments where smaller frequency differences are detected than at the base (Burda and Voldrich, '84). Consequently, we suggest that a high density of hair cells in the apical half of the organ of Corti be considered as an adaptation for relatively good hearing sensitivity and/or discrimination in the low-frequency range.

In previous studies of 30 different species of eutherian mammals (Burda and Voldrich, '84, personal communication), the mean mammalian ratio of OHC/100 IHC (i.e., the number of OHC per 100 IHC) was 364 (SD = 6). The mean value estimated for *T. cirrhosus* (360, SD = 13 OHC/100 IHC) is similar. In general, the average cochlear value of the ratio tends to be lower than the above "mammalian" mean in bats and other "high-frequency" mammals, whereas it tends to be higher in "low-frequency" specialists including humans. One possible interpretation of the fact that the OHC/100 IHC ratio in *T. cirrhosus* is close to the mammalian mean is that it should be considered specialized in respects to both high-frequency and low-frequency hearing.

The functional significance of the changes of the OHC/IHC ratio along the organ of Corti is not fully understood. This phenomenon

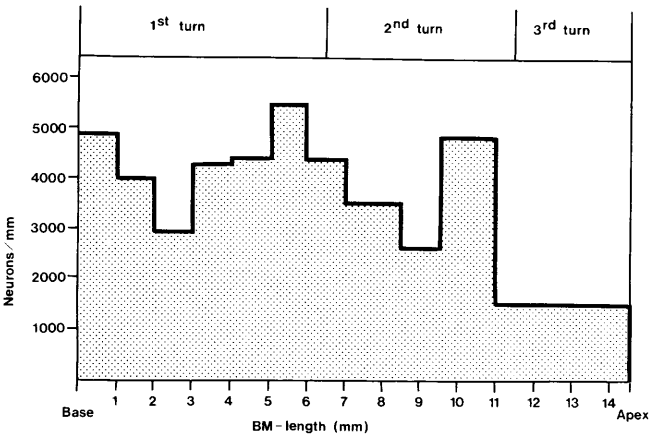

Fig. 12. The density of cochlear neurons in the spiral ganglion. BM-length, position on the basilar membrane (BM) from the base of the cochlea.

was discussed briefly by Burda and Voldrich ('84). It should be noted here that the pattern of changes of this ratio in the organ of Corti of *T. cirrhosus* is opposite for the basal portion than for the apical one.

Cochlear neurons

The 40,500 neurons in the spiral ganglion of *T. cirrhosus* is one of the highest in mammals so far studied. It is about 50% higher than in man and is surpassed only by the little brown bat *Myotis lucifugus* (55,300, Ramprashad '79) and the dolphin (95,000, Wever et al., '71c). Other small mammals of comparative body size have a much smaller number: e.g., the shrew 5,200, and the house mouse 7,200 (Bruns and Burda, personal communication). All other bats, with the exception of *M. lucifugus,* have smaller numbers: *Hipposideros fulvus,* 13,500; *H. speoris,* 15,800; *Rhinolophus ferrumequinum,* 16,000; *Megaderma lyra,* 17,000; *Rhinopoma hardwickei,* 18,000; *Taphozous n. kachhensis,* 23,000; and *Molossus ater,* 31,800 (Bruns and Schmieszek, '80; Fiedler, '83; Kraus, '83).

The average density of neurons per millimeter basilar membrane length is 1,061 in the shrew *S. araneus,* and 1,028 in the house mouse (Bruns and Burda, personal communication). Bats have a density between 993 neurons/mm (*Rhinolophus ferrumequinum,* Bruns and Schmieszek, '80) and 7,971 neurons/mm (*Myotis lucifugus,* Ramprashad, '79). The value for *T. cirrhosus* (2,790 neurons/mm) is the second highest among bats and also the second highest in all mammals, even surpassing the dolphin *Tursiops truncatus* (2,560 neurons/mm, Wever et al., '71c).

Information on the regional distribution of cochlear neurons is available for only a few mammals. Ehret ('79) found a maximum density in the middle of the cochlea of the house mouse and a continuous decrease toward the base and the apex. This is considered the normal mammalian pattern. The results on the house mouse were confirmed in our laboratory (Ballast, unpublished dissertation). We also evaluated the regional distribution of cochlear neurons in other mammals, among them nine bat species (Bruns and Schmieszek, '80; Fiedler, '83; Kraus, '83). In all bats, we found one maximum density in the middle of the cochlea and a second one in the basal region. The latter is thought to be involved in the analysis of echolocation sounds. Only *T. cirrhosus* has three maxima. An apical maximum was found in one other mammalian species, the gerbil, *Pachyuromys duprasi.* By frequency mapping (Ott et al., '86a,b,c) we have demonstrated that this apical maximum is found at 1.5 kHz, the frequency for which this species is most sensitive. In summary, *T. cirrhosus* has 1) a maximum of innervation in the middle of the cochlea as found in all other mammals so far studied, 2) a basal maximum as in all other bats, and 3) an apical maximum as in the low-frequency specialized gerbil.

In the cat (Schuknecht, '60) and in the house mouse (Ehret, '79) the distribution of cochlear neurons correlates with the shape of the audiogram. Demonstration of a causal relationship requires an exact determination of the frequency representation in the cochlea. In the cat, frequency mapping was done by Liberman ('82), who found that 6 kHz is represented at the place of maximum innervation density (Spoendlin, '72), that frequency for which the cat is most sensitive (Heffner and Heffner, '85). Vater et al. ('85) established the frequency map for the horseshoe bat *Rhinolophus rouxii,* compared it with the innervation pattern of closely related species (Bruns and Schmieszek, '80), and found a correlation of the pure tone echolocation signal and a peak of innervation. In the gerbil, Ott et al. ('86a,b) also demonstrated that the most sensitive frequency of 1.5 kHz is represented at that place where an innervation maximum is found. Although we agree with Ehret ('79) that a local increase of the innervation density need not always cause an increase of sensitivity of the auditory system, the data are at least suggestive that *T. cirrhosus* has evolved an increase in low-frequency sensitivity through an increase in the density of innervation at the apical end of the cochlea.

ACKNOWLEDGMENTS

This investigation was supported by the Deutsche Forschungsgemeinschaft (SFB 45/B 21) and the A.V. Humboldt-Foundation. The authors would like to thank G. Elter for histology, graphic work, and typing the manuscript. We thank W. Wilczynski and H. Zakon for comments on the manuscript.

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