Quantitative Analysis of Cochlear Structures in the House Mouse in Relation to Mechanisms of Acoustical Information Processing*

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Summary. 1. Soft surface preparations of the cochlea were used to determine the density of inner and outer hair cells along the basilar membrane. A total number of 2526 outer hair cells (in three rows) and 765 inner hair cells (in one row) were counted. The density of hair cells is significantly higher in the middle part of the cochlea (3.5–4 mm from the apex, total length 6.84 mm) than near apex and base.

2. A special technique was developed for cutting the cochlea on an ultramicrotome radially (2 μm or 3 μm thick sections) throughout the total length. The evaluation of the radial sections showed

a) two significant maxima of density of habenular openings (places where nerve fibres enter the organ of Corti), which can be assumed to represent maxima of innervation density,

b) a significant increase of thickness of the basilar membrane from the apex (1 μm) up to 5.8 mm from the apex (14.5 μm), followed by a plateau up to 6.1 mm and a rapid decrease toward the base (4.5 μm),

c) a significant decrease in width of the basilar membrane from 1.5 mm from the apex (160 μm) to the base (40 μm),

d) a significant reduction in height of the tunnel of Corti from 1 mm from the apex (40 μm) to the base (18 μm).

3. The area of the helicotrema amounts to 0.047 mm^2 .

4. The present data on hair cell and innervation (habenular opening) density are closely related to the frequency region of best hearing (15 kHz) and to features of frequency and intensity discrimination.

5. From the data on width and thickness a function for changing stiffness (z) along the basilar membrane was calculated ($z = 0.0032 \cdot 10^{0.206c}$; c = distance in mm from apex).

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6. The most probable mode of displacement of the basilar membrane and of excitation of hair cells was derived from pictures of radial sections.

I. Introduction

There is a large amount of qualitative data available describing the fine morphology of the cochlea and the possible relation between structure and function. However, only few studies (Wever, 1938; Fernández, 1952; Schuknecht, 1960; Wever et al., 1971 a, b; Spoendlin, 1973; Bruns, 1976 a, b) deal with a quantitative analysis of the variation of structural parameters along the cochlear partition (receptor and innervation density; width, thickness, and stiffness of vibrating membranes, e.g., of the basilar membrane) with a sufficient resolution. A close relation between hearing sensitivity and structure of the basilar membrane was pointed out for the horseshoe bat (Bruns, 1976 a, b), and the innervation density of hair cells in the cochlea of the cat (Schuknecht, 1960; Spoendlin, 1973) also seems to have direct consequences for auditory thresholds.

Hearing in the house mouse (*Mus musculus*) has been psychophysically investigated in detail (see review, Ehret, 1977). In the present study, cochlear structures are quantitatively analysed along their spiral course to give a solid basis for comparative purposes and for correlations with psychophysical and electrophysiological data. A new method is applied for cutting the cochlea radially throughout its total length. By this method, measurements on radial sections yield the wanted information directly, without any reconstruction procedures (e.g., after Guild, 1921).

II. Materials and Methods

1. Subjects

Altogether 47 cochleae from male white laboratory mice (*Mus musculus*, outbred strain NMRI) aged 6–8 weeks were examined. No deficiencies in hearing or structural abnormalities of the acoustic system are known in these mice so far.

2. Cochlea Preparation

Animals were anaesthetized by ether, decapitated, and both cochleae including a portion of the bony labyrinth quickly removed. Under 4% formalin round and oval windows were carefully opened and a small aperture was made at the apex of the cochleae. Then a thin setting pin which was fixed in a snap-fastener of a small glass was stuck through the tissue aside the cochlear capsule and the cochleae were hung into 4% neutral formalin at room temperature for 2 days. Then they were decalcified in EDTA (Titriplex III) for another 2 days.

According to the measurements in question one of two different procedures followed:

- a) Soft surface preparation (e.g. Axelsson et al., 1975). The decalcified bony cochlear capsule was partly removed under a stereomicroscope (Wild, M5). Then the $1\frac{1}{2}$ turns of the basilar membrane were carefully dissected into 4 or 5 portions, which were mounted in glycerin for phase-contrast microscopy. During the whole preparation the cochleae were immersed in a Ringer solution for mammals (8.5 g NaCl+0.25 g KCl+0.3 g CaCl_2 +0.2 g NaHCO_3 +1 g glucose+1000 cm^3 H_2O).

Fig. 1 a-d. Dividing planes (dashed lines in a, b, d) of plastic embedded cochleae. a Division into two halves. b Separation of half turns and division of the large half turns II, III, IV into two parts. c Making sections of part IIIa indicated by the dense parallel lines in three sectors: cutting for about 100 μ m, inclination to the dashed line, cutting, inclination, etc. d Dividing plane for the measurement of the size of the helicotrema and for the evaluation of the basal part (indicated by dots) of the basilar membrane

The hair cell density was determined in such preparations.

b) Block-surface technique (Spoendlin and Brun, 1974). The decalcified cochlea was dehydrated and embedded in Spurr low-viscosity embedding medium (Polysciences, INC., Paul Valley Industrial Park, Warrington, PA 18976, U.S.A.). Special embedding forms (Effa-flat-embedding No. 5209) were used in which the specimens can be handled more easily than in gelatine capsules.

An embedded cochlea was cut two halves using a 0.1 mm thick circular saw, which was driven by a dental drill. The embedded cochlea was mounted on a micromanipulator which could be moved in three directions against the fixed dental drill. Under microscopic control the cochlea was cut either in a mid-modiolar plane (Fig. 1a) or in a less oblique plane (Fig. 1d) preserving the helicotrema and the total basal part. Then the two parts of the cochlea were ready for the separation of half turns (Fig. 1b). Starting with the most apical half turn, small discs were cut down by the circular saw. These discs allow a good surface examination of the organ of Corti. In a further step, half turns II, III, and IV were divided into two parts using a strong razor blade. After determining the length of each cochlear segment and by that the total length of the cochlea (Zeiss microscope with calibrated ocular micrometer), the small segments were again embedded into Spurr to get larger blocks. The blocks were trimmed and radially sectioned on an ultramicrotome (Reichert, OMU 3). A new technique of successive reorientation of the specimens was employed to guarantee radial sections (relative to the modiolus) throughout the whole cochlea. The cochlea segments were cut for about 100 μ m, then inclined by an angle, so that the further cuts were again perpendicular to the bending rows of hair cells (Fig. 1c), then cut for further 100 μ m, inclined, etc. The angle of inclination was determined by optical control through the stereomicroscope of the ultramicrotome and by immediate microscopic control of serial sections, in which three criteria for a good radial orientation were used: the fibres of the basilar membrane should run radially (Figs. 2, 9), and the nuclei and the holes for the lacking kinocilia in the

dense headplates of the three outer hair cells should not be visible in one section at the same time (Fig. 2). The outer hair cells of the second row are normally arranged in a staggered manner against those of the first and the third row. The result is a very orderly pattern in radial direction, so that an ideal radial section hits either the nuclei (respectively holes for the lacking kinocilia) of outer hair cells of the first and third row or of the second row. The kinocilium of the mammalian hair cell is present during development but then degenerates, leaving a hole in the cuticular plate.

Near the base of the cochlea the segments had to be successively reorientated in two spatial planes, which is possible with the OMU 3.

Radial sections were used for the measurement of thickness and width of the basilar membrane, height of the tunnel of Corti, and size of habenular openings.

c) As a control for possible fixating artefacts and (or) shrinking of specimens, unfixated but decalcified cochleae were cut (20 μm sections) vertically (two cochleae) and horizontally (two cochleae) on a freezing microtome (WKf 1150). In mid-modiolar sections width and thickness of the basilar membrane could be measured. Hair cell densities in some regions could be determined from horizontal sections.

3. Measurements

a) *Length of the Basilar Membrane.* The length of a cochlear segment was determined along the pillar cells by measuring tangentially over successive parts of maximally 300 μm . The total length of the basilar membrane is the sum of the length of all segments. In measurements from block-surface preparations additional 200 μm were added for the loss at each sawing place. Although the thickness of the saw blade was only 100 μm , the actual loss was measured to be about 200 μm , somewhat depending on the velocity of revolution of the saw.

b) *Hair Cell Density and Cochlear Structure.* All measurements were carried out under an objective magnification of $100\times$ (phase contrast) with a calibrated ocular micrometer (resolution 1 μm). Hair cell density was determined at known distances from the apex by measuring the longitudinal distance that was occupied by 7 hair cells within a given row. Then the averaged width for a single hair cell was calculated and expressed in $\mu\text{m}/\text{cell}$. The density is the reciprocal of this value. Measurement of outer hair cell density was generally pursued in the middle row of the three rows of outer hair cells. In a control, involving 4 cochleae, the density was determined for all three rows: Measurements were carried out approximately every 350 μm along the basilar membrane, starting at the apex. The locus of measurement depended on the quality of the preparation. Only those parts were used which had no evidence for tissue distortion.

c) The size of the helicotrema was determined using a $16\times$ objective and the calibrated ocular micrometer.

4. Photography

Microphotographs of surface preparations (not included in this paper because of space considerations) and radial sections were made by a Zeiss photomicroscope III. In all cases an Ilford PAN F film, 18 DIN, was used.

III. Results

1. Length of the Basilar Membrane

The length of the basilar membrane of the house mouse was determined from 20 soft-surface preparations (10 right and 10 left side cochleae). Mean and standard deviation for the length of the right side: 6.88 ± 0.17 mm, for the left side: 6.80 ± 0.16 mm. Both values are not significantly different ($p > 0.1$). Thus the averaged length of the basilar membrane of the house mouse is 6.84 ± 0.16 mm.

2. Hair Cell Density along the Basilar Membrane, Total Number of Hair Cells

The hair cell density was measured in 19 soft-surface preparations at successive 500 μm sectors along the basilar membrane. That is, the basilar membrane has a total length of about 7 mm, so that it could be divided into 14 sectors of 500 μm each. An averaged hair cell density within each sector was calculated from a large number of measurements in the total 19 cochleae. Table 1 (see also Fig. 13) presents the mean widths ($\bar{u}$) in μm for single inner and outer hair cells (reciprocal is density $\bar{d}$) in each of the 14 sectors. Standard deviations (s) and numbers of included single measurements (n) are also shown. The measured widths for the outer hair cells are valid for all three rows because they did not differ significantly ($p > 0.1$) between rows at any distance from the apex. It is evident that the smallest values for the widths of outer and inner hair cells occur in sector VIII, thus the density of hair cells is highest between 3.5 mm and 4 mm from the apex and then decreases either toward the apex or toward the base. Values from sector VIII are significantly smaller (the density higher) than those from sector VI ($p < 0.0001$, t -test) and from sector IX ($p < 0.005$). The small decrease in width of hair cells (increase in density) at the apex (sector I and II) is statistically significant only for inner hair cells ($p < 0.025$).

From Table 1 it is possible to determine the total number of hair cells in the cochlea of the mouse by calculating the numbers for each 500 μm sector and summing up. The following values result: 842 outer hair cells per row, making a total of 2526 outer hair cells for the three rows, and 765 inner hair cells in the single row.

3. Size and Density of Habenular Openings along the Basilar Membrane

Two cochleae, embedded in Spurr's were cut radially to the modiolus in 2 μm sections from the apex down to about 1 mm from the absolute base. Thus

Table 1. Means ($\bar{u}$) and standard deviations (s) in μm for the width of one inner and outer hair cell in 14 sectors along the basilar membrane. The values for hair cell density ($\bar{d}$) are the reciprocals of $\bar{u}$. n =number of single measurements included in the means; A=outer hair cells; B=inner hair cells

Apex	0 mm	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	
A	$\bar{u}$	8.07	8.20	8.20	8.00	7.95	7.86	7.64	7.58	7.80	8.03	8.23	8.40	8.74	8.84
	s	0.29	0.35	0.26	0.18	0.21	0.19	0.26	0.18	0.19	0.18	0.15	0.19	0.29	0.29
	$\bar{d}$	0.123	0.122	0.122	0.125	0.126	0.127	0.131	0.132	0.128	0.125	0.122	0.119	0.114	0.113
	n	16	25	25	30	21	27	31	29	25	27	25	29	30	15
B	$\bar{u}$	9.17	9.40	9.00	8.93	8.90	8.75	8.33	8.23	8.46	8.76	8.90	9.10	9.41	9.66
	s	0.40	0.23	0.36	0.29	0.23	0.29	0.24	0.30	0.30	0.33	0.28	0.29	0.32	0.29
	$\bar{d}$	0.109	0.106	0.111	0.112	0.112	0.114	0.120	0.122	0.118	0.114	0.112	0.110	0.106	0.104
	n	18	26	27	24	24	24	26	21	25	19	19	20	16	13

Fig. 5. Distribution of the cross-sectional areas of the habenular openings along the cochlear partition. Each vertical bar indicates the total cross-sectional area (μm^2) of the habenular openings in a cochlear sector of $40 \mu\text{m}$ length

radial (transverse) thin-sections of two cochleae were available except for parts lost by sawing. Further two cochleae were cut in the same way but only in the middle region between 2.5 mm and 4.5 mm from the apex (see Fig. 5).

From the serial sections the opening area of every habenular opening was determined. The radial diameter for an opening was directly measured in a radial section (Figs. 2–4), the longitudinal diameter was calculated from the number of successive sections in which the opening was visible. The habenular openings are elliptic in shape with the radially running axis always being the larger one (Fig. 4). Table 2 presents averaged lengths of radial and longitudinal diameters at every 1 mm along the basilar membrane. Near 3 mm and 4 mm

Fig. 2. Radial section through the cochlear partition about 2.5 mm from the apex (microscope $\times 800$; marker $\pm 25 \mu\text{m}$). Arrow points at the hole in the dense head plate of an outer hair cell of the second row, which is correlated with the lacking kinocilium. *h*, habenular opening; *utr*, upper tunnel radial fibres; *tm*, tectorial membrane; *v*, spiral basilar vessel in pars tecta of the basilar membrane

Fig. 3. Radial section about 3 mm from the apex (mic. $\times 1250$, marker $\pm 10 \mu\text{m}$) showing two habenular openings (arrows) which radially lie one behind the other. *tC*, tunnel of Corti; *sgc*, ganglion cells of spiral ganglion

Fig. 4. Schematic surface view on the distribution of habenular openings in an area where two openings appear. x_1 , radial diameter of an opening; x_2 , longitudinal diameter; *ihc*, inner hair cells

Table 2. Size of habenular openings at 6 positions along the basilar membrane. At 3 mm and 4 mm from the apex the indicated values for radial diameters are the sum from two openings which radially lie one behind each other (compare Figs. 3, 4). s = standard deviation

Apex	1 mm	2 mm	3 mm	4 mm	5 mm	6 mm
Radial diameter μm	4.15 $s=0.72$	4.90 0.88	8.60 1.40	8.00 2.30	7.15 0.99	4.70 0.58
Longitudinal diameter μm	4.0	4.0	3.5	5.0	3.5	2.0

from the apex two rows of habenular openings appear (Fig. 3), mostly in a staggered array (Fig. 4). Figure 5 shows in a diagram the distribution of the area of habenular openings along the basilar membrane, summed over successive 40 μm segments of the membrane. The areas were calculated using the formula for an ellipse. The two peaks, one near 3 mm the other near 4 mm from the apex, are very prominent. The maximal values are significantly larger ($p < 0.005$, t -test) than those 500 μm apart.

4. Width and Thickness of the Basilar Membrane and Height of the Tunnel of Corti

These measures were determined from one of the cochleae used in the habenular measurements and from 4 further cochleae which were serially cut in 3 μm sections. The 4 cochleae were divided into half turns as shown in Figures 1a, b. In such a preparation a part of about 400 μm between half turn IV and V is very difficult to orientate on the ultramicrotome for radial sections and in addition a part of about 200 μm is lost by sawing. Following preliminary examination, however, this region appeared to be of special interest. Therefore 4 additional cochleae were divided as shown in Figure 1d, preserving the transition zone between part IV and V. Only this large most basal portion, indicated by dots in Figure 1d, was evaluated in the additional 4 cochleae.

Figures 6–10 demonstrate clearly the changes in structure of the cochlear partition between apex and base. A thickening of the basilar membrane and a division into two parts (pars tecta or arcuata with the spiral basilar vessel, and pars pectinata, see Figs. 11, 16), a reduction in width of the basilar membrane and of the number of cells below it (tympanic covering layer) is evident. At the absolute base (Fig. 10) the rest of the basilar membrane is incorporated into tissue, the pillar cells are close together leaving only a very small tunnel of Corti, the outer hair cells seem to have disappeared, and the tectorial membrane is connected to the Hensen cells.

Figure 11 shows the change in thickness of the basilar membrane (pars tecta, pars pectinata) along its spiral course. The thickness of the basilar membrane was always measured without the tympanic covering layer. In every section measurements were carried out at the place of maximal thickness of pars tecta

Figs. 6–8. Radial cochlear sections about 1 mm (Fig. 6), 3.2 mm (Fig. 7), and 4.4 mm (Fig. 8) from the apex (mic. $\times 500$, marker $\cong 35 \mu\text{m}$). Arrows in Figures 7, 8 indicate hinge points for the displacement of the basilar membrane, which is in a downward position in Figure 7, and in an upward position in Figure 8. *tm*, tectorial membrane; *tcl*, cells of the tympanic covering layer; *osl*, osseous spiral lamina; *sl*, spiral ligament

Fig. 9. Radial cochlear section about 6.4 mm from the apex (mic. $\times 500$, marker $\approx 35 \mu\text{m}$). Thickening of pars pectinata not only toward the scala tympani (*st*) as in Figures 7 and 8, but also toward the scala media (*sm*)

Fig. 10. Radial cochlear section at the absolute base (mic. $\times 1250$, marker $\approx 10 \mu\text{m}$). Basilar membrane (*bm*) is incorporated into tissue. *pc*, pillar cells; *tm*, tectorial membrane; *v*, spiral basilar vessel

and of pars pectinata. Each point in Figure 11 represents the averaged value of thickness over a distance of $100 \mu\text{m}$. The thickness of pars pectinata increases from the apex up to about 1 mm from the base, stays constant for about $400 \mu\text{m}$, and decreases rapidly toward the base. At the base pars tecta and pectinata coincide to one single part (Fig. 10). The maximum value of thickness

Fig. 11. Mean thickness of the basilar membrane in successive 100 μm segments between apex and base. d_1 , thickness of pars pectinata; d_2 , thickness of pars tecta. In the d_1 curve standard deviations are exemplarily shown between 5 mm and 6.5 mm from the apex

(14.5 μm) between 5.7 mm and 6 mm from the apex is significantly larger than values in a distance 400 μm apart ($p < 0.0005$). The behavior of pars tecta is not as regular. An increase in thickness, however, is evident between 2 mm and 5–6 mm from the apex (Fig. 11). Values between 5–6 mm are significantly larger than those between 3–4 mm ($p < 0.0005$).

Figure 12 presents the variations of width of the basilar membrane and of height of the tunnel of Corti. Near the apex and at the base pars tecta and pars pectinata cannot be distinguished and only the total width is shown. The total width of the basilar membrane, just as the width of pars pectinata, first increases from the apex to about 1.5 mm, then decreases slowly to about 3 mm, then more rapidly to a small plateau around 5.8 mm. A final rapid decrease follows. The width of pars tecta decreases very steadily from the apex to the base. In general, all values of the means which have a difference of more than 10 μm are significantly different ($p < 0.01$). This implies that the small decrease of the height of Corti's tunnel toward the base is significant.

5. Size of the Helicotrema

The size of the helicotrema was determined from 7 Spurr's embedded cochleae which were sawn as shown in Figure 1d. The helicotrema has an elliptic shape

Fig. 12. Mean width of the basilar membrane and mean height of the tunnel of Corti in successive 100 μm segments between apex and base. w , total width with standard deviations; w_1 , width of pars pectinata; w_2 , width of pars tecta; ht , height of the tunnel of Corti (compare Fig. 11)

with axes: $d_1 = 305 \pm 15 \mu\text{m}$; $d_2 = 196 \pm 12 \mu\text{m}$. The averaged area amounts to 0.047 mm^2 .

6. Control Measurements

Width and thickness of the basilar membrane determined from mid-modiolar freezing sections and hair cell densities measured in horizontal freezing sections were identical within 2.3% with respective Spurr's sections or soft surface preparations. This relatively small deviation indicates that the measurements were not influenced systematically by possible effects of fixation and embedding, and that they are reliable.

IV. Discussion

1. Hair Cell and Innervation Density and Auditory Thresholds

It can be assumed that hearing sensitivity improves with increasing receptor, that is hair cell density. Figure 13 shows in one plot the absolute auditory

Fig. 13. Comparison of the absolute threshold curve (*I*, open circles) with the density in $\mu\text{m}/\text{cell}$ of outer (*II*) and inner (*III*) hair cells (closed circles) and the opening density of habenular openings (*IV*, dashed line $\triangleq$ envelope function from Fig. 5). Lower left ordinate: sound pressure level in dB; upper left ordinate: total cross-section of habenular openings within a $40\text{ }\mu\text{m}$ segment in $\mu\text{m}^2/40\text{ }\mu\text{m}$; right ordinate: hair cell density in $\mu\text{m}/\text{cell}$; Abscissa: length of the basilar membrane in mm, given as the distance from the apex, and the respective frequency representation after Ehret (1975b)

threshold curve of the house mouse (Ehret, 1974, 1976) and hair cell densities along its basilar membrane. It is evident that between 3 mm and 4 mm from the apex the absolute auditory threshold is lowest (around 15 kHz) and the width for one hair cell is smallest (the density highest). This fact and the similar shape of absolute threshold and density curves (Fig. 13) may lead to assume a direct relation between hair cell density and absolute auditory sensitivity in this case. However, one has to consider that there is only about 1 more hair cell per $100\text{ }\mu\text{m}$ and row between 3.5 mm and 4 mm from the apex than near the apex and the base, and this small difference, although significant, most probably is not responsible for a threshold decrease of more than 15 dB.

From the distribution of habenular openings (Figs. 5, 13) it can be suggested that another factor may be more important. Habenular openings are the places where all the afferent and efferent nerve fibres, innervating the hair cells, have to run through. In a first approach it is logical to assume that the larger the area of openings is the more nerve fibres can penetrate, in other words, the larger the opening density the larger is the fibre density, provided that

the diameter of fibres remains constant. In the following text density of habenular openings and innervation density will therefore be used as synonyms. Figure 5 demonstrates very clearly the exceptionally large opening areas around 3 mm and 4 mm from the apex.

The functions of hair cell density follow in shape the envelope function of habenular opening areas (Fig. 13) throughout the basilar membrane excluding the two peaks. At the two peaks the number of penetrating nerve fibres (density of hair cell innervation) is, if the preceding assumption is correct, about twice as large as in the neighbouring parts of the cochlea. It will be explained later that the peak near 4 mm from the apex may be related mainly to afferents from the inner hair cells which are situated near 4 mm, and the peak about 3 mm from the apex mainly to afferents from the outer hair cells at the same place. In conclusion, Figure 13 demonstrates that the innervation density (density of habenular openings) follows the hair cell density throughout the cochlea with exception of the region near 4 mm from the apex where the innervation density of sound receptors can be assumed to be much larger than in other parts of the cochlea. Within this special region the frequencies of best hearing (15–20 kHz) are represented. Following this, the rapid threshold decrease from 10 and 30 kHz to 15 kHz might be related to the higher innervation density in the respective region of the cochlea. It has been demonstrated, however, that in cats and guinea pigs the shape of the absolute threshold curve is determined mainly by the frequency response of the external and the middle ear (see Dallos, 1973). This can also be assumed to be true in the mouse. Therefore further experiments have to show whether innervation densities play a role in the determination of absolute auditory thresholds.

The next question is: Can hair cell and innervation densities be related to discrimination thresholds? Ehret (1977) developed a model for frequency and intensity discrimination which is based on the shape of basilar membrane displacement, on receptor (hair cell and innervation) density, and on thresholds of hair cells and afferent nerve fibres to detect a variation of excitation. From this model follows that frequency and intensity discrimination for pure tones improves with an increase of hair cell and afferent innervation density. Considering the present results, differential sensitivity in the mouse should be best at frequencies near 15 kHz. Data from Ehret (1975a) show that neither absolute (Δf , ΔL) nor relative ($\Delta f/f$, $\Delta L/L$) differential sensitivity is best at 15 kHz in the mouse. Following Ehret (1977), however, this can be explained by considering the dependence of difference limens on the shape and sharpness of the displacement of the basilar membrane which is different at different places (frequencies) in the cochlea. If one wants to prove the relation between differential sensitivity and hair cell and innervation density, one has to eliminate effects originating from different displacements at different places along the basilar membrane. This can be done, if one stays at a single frequency and considers the dependence of difference limens on the sound pressure level, i.e., excitation level at this place. According to the model (Ehret, 1977) an increase in sound pressure level (excitation level) leads to a decrease of difference limens which must be the larger the higher the hair cell and afferent innervation density is at a given place (frequency) on the basilar membrane. In fact, the slope

of decrease of frequency difference limens with increasing sound pressure level (excitation level) is largest at 15 kHz in the mouse (Ehret, 1975a; the situation for intensity difference limens is complicated by a nonlinearity and shall not be discussed here). The slope is nearly twice as large at 15 kHz as at 5 and 20 kHz (Ehret, 1975a). This relation also holds for the innervation density of inner and outer hair cells which is about twice as large at 15 kHz than at 5 and 20 kHz (see Figs. 5, 13). This result strongly indicates a direct correlation between hair cell and innervation density and the ability for the detection of changes in excitation along the basilar membrane and supports the model of Ehret (1977).

2. Comparative Hair Cell and Innervation Densities in the Cochlea of Mammals

The hair cell distribution along the basilar membrane is not uniform in the house mouse (Table 1, Fig. 13). Measurements of hair cell density in other mammals were mostly carried out with a smaller resolution as in the present study. In man (Retzius, 1884; Zwicker and Feldtkeller, 1967), cat (Schuknecht, 1960), and guinea pig (Stockwell et al., 1969) hair cell distributions seem to be uniform. In the dolphin, however, the packing of hair cells in the middle part of the cochlea is denser than in the basal and most apical parts (Wever et al., 1971b).

If one takes the total number of inner and outer hair cells (from three rows) and calculates the number per 1 mm length of the basilar membrane the following values result: house mouse, 481; man, 469; cat, 535; guinea pig, 458; dolphin, 446. One can see that man, mouse, guinea pig, and dolphin have rather similar averaged hair cell densities in their ears; cats, however, have more sound receptors per mm length of the basilar membrane than the other mammals for which one has got data.

It has been demonstrated for the cat that the densest innervation appears in the middle part of the cochlea (Spoendlin, 1973, 1975), where frequencies to which the cat is most sensitive are represented (Neff and Hind, 1955; Elliott et al., 1960; Schuknecht, 1960). In the cat the innervation density in this middle region is about twice as large as at the base and at about 5 mm from the apex (Spoendlin, 1973, 1975). The total length of the basilar membrane of the cat is 22 mm (Schuknecht, 1960). Regarding this, the data from cats excellently correspond with the present results from the mouse. Spoendlin (1973) showed for the cat that about 85% of all nerve fibres penetrating the habenular openings innervate the inner hair cells. A similar value (76%) can be assumed from data of the guinea pig (Morrison et al., 1975). The majority of fibres innervating the inner hair cells are afferents, which represent about 95% of all afferents in the cochlea of the cat and 85–90% of all afferents in the cochlea of the guinea pig. This means that an increase in innervation density may be mainly related to an increase of afferent innervation of inner hair cells. It can be assumed that this is the case near 4 mm from the apex in the mouse (Fig. 5). The second innervation maximum (density maximum of habenular

Fig. 14. Radial cochlear section about 2.8 mm from the apex (mic. $\times 2000$, marker $\cong 8 \mu\text{m}$) showing the three Deiter cells (*D*) which are the supporting cells of the outer hair cells. The black dots (some are pointed at by arrows) are transverse sectioned outer spiral fibres which most probably are the afferents from the outer hair cells. *opc*, outer pillar cell; *pt*, pars tecta; *pp*, pars pectinata

openings) near 3 mm from the apex in the mouse can also be interpreted in relation to inner hair cell innervation. There is, however, another possible interpretation: Afferent fibres innervating the outer hair cells (outer spiral fibres) penetrate the habenula, cross the tunnel of Corti, and then run spirally toward the base over a distance of 0.6–0.7 mm in the cat (Spoendlin, 1973, 1975). Similar values were measured in guinea pigs (Smith and Haglan, 1973; Smith, 1975) and rats (Perkins and Morest, 1975). Figure 14 shows in a radial section the Deiter cell region of the cochlea of the mouse. The black dots (arrows) between the Deiter cells and those attached to the Deiter cell next to the tunnel of Corti are transverse sectioned outer spiral fibres which represent the afferents from the outer hair cells. If one assumes that in the mouse the outer spiral fibres also run for about 0.7 mm spirally toward the base, they would innervate outer hair cells about 3.7 mm from the apex, when penetrating the habenula 3 mm from the apex. The distance between the two peaks of the habenula opening density is about 1 mm. That allows that the peak near 3 mm from the apex may be at least partly related to afferents innervating outer hair cells near 4 mm from the apex. Direct measurements of innervation patterns in the mouse will show, whether this interpretation of the present results on a comparative basis is correct.

One more observation concerning nerve fibres crossing the tunnel of Corti should be mentioned. Figure 2 shows that upper tunnel radial fibres can split

into two or more bundles of which one runs directly to the outer hair cells (see also Fig. 7), whereas the others run to the Deiter cells. This splitting of upper tunnel radial fibres was not reported for cat and guinea pig in the above mentioned studies. The significance of this observation in relation to afferent and efferent innervation of outer hair cells has to be clarified.

3. Structure and Displacement Pattern of the Basilar Membrane

Width and thickness of the basilar membrane of the mouse are not constant (Figs. 11, 12). Thickness increases rapidly from 1 μm at the apex to 14.5 μm 5.8 mm from the apex, while the width decreases from about 160 μm 1.5 mm from the apex to about 40 μm at the base; this decrease in width is a factor of 4. A decrease in width (factors are in brackets) from apex to base was also measured for the basilar membranes of man (~ 5), cat (~ 5), guinea pig (~ 3), horseshoe bat (~ 2), and dolphin (~ 14), see Tab. II in Ehret (1977). A small increase in thickness from apex to base was found in guinea pigs (from 1.3 μm to 7.4 μm ; Fernández, 1952), a large one (from 1 μm to 35 μm) in the horseshoe bat (Bruns, 1976a) which, however, is related to a strong discontinuity about 11.5 mm from the apex, where the thickness of pars pectinata drastically increases for more than 20 μm over a distance of only 250 μm . This thickness increase can be measured just at the place on the basilar membrane of the horseshoe bat, where the frequency of echolocation of about 83 kHz is represented to which the bat is especially sensitive (Long and Schnitzler, 1975). A decrease in width (w) and an increase in thickness (d) mean an increase in stiffness (z) of the basilar membrane; this can be expressed as the quotient $d/w=z$. Figure 15 shows the change in stiffness along the basilar membrane of mouse, guinea pig, and horseshoe bat. The curves for mouse and guinea pig can be approximated by a straight line or by the equivalent exponential function:

$$\text{mouse: } \log z = 0.206 c - 2.05 \quad r = 0.997 \quad (1)$$

$$\text{or } z = 0.0032 \cdot 10^{0.206 c} \quad c = \text{distance from the apex in mm}$$

$$\text{guinea pig: } \log z = 0.063 c - 2.38 \quad r = 0.988 \quad (2)$$

$$\text{or } z = 0.0042 \cdot 10^{0.063 c}$$

The stiffness function for the mouse is valid only from the apex up to 6 mm and not for the absolute base. Thickness in the basal turn of guinea pig's cochlea was not measured and the function should not be used in this part. Equations 1 and 2 show that the slope of stiffness increase in the mouse is 3.23 times larger than in the guinea pig. The maximum values differ by a factor of 2 (Fig. 15). This may be the reason why the guinea pig's audible field is limited to about 50 kHz (Heffner et al., 1971), whereas the house mouse hears at least up to 100 kHz (Ehret, 1974, 1976). The rapid stiffness increase 11.5 mm from the apex in the bat seems to be the basis for its high absolute sensitivity at 83 kHz (Long and Schnitzler, 1975). The stiffness increase is followed by a plateau of constant stiffness (Fig. 15). In the mouse the increase is also followed by a plateau which starts at 5.8 mm from the apex. Figure 13

Fig. 15. Stiffness (quotient of thickness and width) of the basilar membrane in dependence on the distance from the apex for house mouse, horseshoe bat, and guinea pig (see Eqs. 1, 2, and text)

shows that 5.8 mm from the apex 50 kHz are represented, and that at this frequency the absolute sensitivity is lower than at neighboring frequencies. The 50 kHz sensitivity maximum may be related to the transition from increasing to constant stiffness, which is present in the mouse like in the horseshoe bat, but only less pronounced.

The thickening of the basilar membrane (pars tecta and pectinata) not only leads to an increase in stiffness with consequences on frequency representation and hearing sensitivity, but also determines the mode of vibration of the basilar membrane. At the apex the basilar membrane is uniformly thin without a difference between pars tecta and pectinata (Fig. 6). Deformation there could generally happen as proposed by v. Békésy (1960), and Steele (1973, 1976, deformation mode I). This is graphically demonstrated in Figure 16. Near the apex the lamina reticularis is parallel to the basilar membrane if the system is not excited (Fig. 6), and the tunnel of Corti is rather high (see Fig. 12). From about 2 mm from the apex the situation changes, because of the thickness increase in pars pectinata and then also in pars tecta (Figs. 7–9). A very thin connection, however, stays between the two parts of the basilar membrane. Three hinge points appear, around which the basilar membrane can rotate: the connection to the osseous spiral lamina, the connection to the spiral ligament, and the connection between both parts. Figure 7 and Figure 8 clearly demonstrate as “standing pictures” a downward displacement and an upward displacement of the basilar membrane. The rotation around the three hinges without any flexing of the basilar membrane is evident. This deformation corresponds to mode II in Steele’s (1973, 1976) model (see Fig. 16).

Fig. 16. Deformation modes of the basilar membrane. Top: two modes as proposed by v. Békésy (1960) and by Steele (1973, 1976). Solid lines: upward deflection of the basilar membrane; dashed lines: downward deflection. Below: deformation of the basilar membrane (upward and downward movement) and the related bending of hairs of the hair cells for the mouse after Figures 7, 8. *ahc*, outer hair cell; *ipc*, inner pillar cell (further abbreviations, see other figures)

It can be recognized (Figs. 2, 7–9) that the foot of the outer pillar cell is situated close to the hinge between pars tecta and pectinata and therefore gets the largest share of the basilar membrane movement. The inner pillar cell, however, is situated on the bone of the osseous spiral lamina, which most probably cannot vibrate. This construction guarantees a very efficient displacement of the hair cells (Figs. 7, 8). The lamina reticularis is parallel to pars tecta in the downward position of the basilar membrane. In the resting position (Fig. 2) and more pronounced in the upward position (Fig. 8) the lamina reticularis forms an angle at the head of the outer pillar cell. In this position the surfaces of inner hair cells lie at one side of the angle, the surfaces of the outer hair cells lie at the other side. This mode of displacement has consequences for the excitation of hair cells by sharing forces, especially for the phase of the response of inner and outer hair cells. Assuming that the hairs of the inner hair cells are, in contrast to the hairs of the outer hair cells, not fixed at the tectorial membrane¹, an excitation of inner hair cells is only possible by fluid motion in the space between lamina reticularis and tectorial membrane. Figure 16 shows the most probable direction of radial sharing (see also Dallos, 1973) of hairs of outer and inner hair cells in an upward and downward position

¹ Kim (1972; see Dallos, 1973) supports this assumption by raster electron microscopy

of the basilar membrane. In the upward position the hairs of the outer hair cells are bent away from the central axis of the cochlea by the movement of the tectorial membrane against the lamina reticularis. The hairs of inner hair cells are bent toward the modiolus by fluid motion. In the downward position the hairs are bent just in the opposite directions. The hairs of the hair cells are arranged in a W-shape (e.g., Spoendlin, 1970; also Ehret, in press). The directional sensitivity is so that both groups of hair cells are excited by a bending of hairs toward the modiolus. It follows that inner and outer hair cells are in phase opposition during the displacement demonstrated in Figures 7, 8 for the mouse. These anatomical results from the mouse support recent electrophysiological findings on phase opposition between inner and outer hair cells (Dallos, 1973; Zwislocki, 1975). Inner and outer hair cells are obviously two very differently working sound receptors concerning the mode of mechanical excitation and the innervation pattern.

4. The Helicotrema

Dallos (1970, 1973) draws the attention to the size of the helicotrema which, as he pointed out, works as a high-pass filter. The larger the size of the helicotrema the better is the pressure equilibration between scala vestibuli and scala tympani and by that traveling waves are fading out earlier when they approach the helicotrema. This means a reduction in low frequency sensitivity. The slope of sensitivity decrease toward the lower frequency limit of hearing becomes steeper the larger the area of the helicotrema is (Dallos, 1970). This is evident from the comparison of absolute threshold curves (see Ehret, 1977) of man, and cat, which are steep at low frequencies, with the one of the guinea pig, which is less steep. The area of the helicotrema amounts to 0.325 mm^2 in man (v. Békésy and Rosenblith, 1951), 0.152 mm^2 in the cat (Dallos, 1970), and 0.022 mm^2 in the guinea pig (Dallos, 1970). The area of the helicotrema in the mouse is also rather small (0.047 mm^2) and a similar slope like in the guinea pig is to be expected at the lowest frequencies in the absolute threshold curve. This agrees with the measured data (Ehret, 1974, 1976) although the lower frequency limit of hearing has not yet fully been explored.

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