JOURNAL OF MORPHOLOGY 218:153–165 (1993)

Morphology of the Basilar Papilla of the Budgerigar, *Melopsittacus undulatus*

GEOFFREY A. MANLEY, GABRIELE SCHWABEDISSEN, AND OTTO GLEICH
Institut für Zoologie der Technischen Universität München, 85747 Garching, Germany

ABSTRACT The budgerigar is a representative of the parrot-like birds that, like song birds, have developed complex communication signals. This species is interesting in a psychoacoustic sense, in that it shows unusually good frequency discriminative abilities above about 1 kHz. To begin to understand whether the peripheral hearing organ plays a role in such specializations, we have carried out a quantitative study of the fine anatomy of the basilar papilla and compared it to data from other avian species.

The budgerigar basilar papilla is about 2.5 mm long in the living animal and contains about 5,400 hair cells. The hair cells of the papilla show regional specializations similar to those found in other birds and are described from scanning electron microscopic and light microscopic studies. Regional changes in the basilar papilla, and in the basilar and tectorial membranes are described from light microscopic data. As noted for other avian species, the constellation of morphologic features found in the budgerigar is unique. In general, the hair cell patterns of the budgerigar papilla showed fewer specializations than found in, e.g., a songbird, the starling, but more than seen in a primitive land bird, e.g., the pigeon. There were no features that were obviously related to the unusual psychoacoustic performance of this species.

The comparative approach to studying the inner ear of vertebrates has brought important new insights into its function and its evolution. As the hearing organ of birds shows the greatest structural similarity to that of mammals (Manley, '90), the investigation of hearing and communication in birds is of special interest. Within the birds, certain species such as the canary, starling, and budgerigar have been investigated for years using behavioral techniques. Psychoacoustic techniques have shown that the hearing abilities of the species studied are remarkably similar (Dooling, '80), although there is good evidence for specific differences perhaps related to morphological specializations (Dooling, '92).

Unfortunately, the starling is the only avian species studied in detail with regard to its communication ability from the behavioral, physiological, and anatomical standpoints (e.g., Gleich and Manley, '88; Klump and Gleich, '91; Klump and Langemann, '92; Manley et al., '85). For the other species with a well-known behavioral profile (canary, zebra finch, budgerigar), there is virtually no physiological or anatomical information available. To begin to overcome this deficit, we have undertaken an anatomical study of these species. A second paper (Gleich et al., personal communication) deals with the two song birds, the canary and zebra finch. The present paper describes a quantitative morphological investigation of the auditory papilla of the budgerigar, also known as the parakeet (Psittaciformes). Birds of this order are highly vocal, but have apparently evolved their communication signals independently of those of the song birds, being more closely related to the family of the pigeons (Feduccia, '80).

Although the last 20 years have brought great progress in our understanding of the anatomy and physiology of the avian ear, a number of important questions remain unresolved. As every species investigated to date

Otto Gleich is now at HNO Klinik der Universität Regensburg, Postfach, 93042 Regensburg, Germany.

has a unique papillar anatomy, a comparative study offers the promise of helping to elucidate the correlations between structure and function in this highly complex sense organ. More recent quantitative anatomical studies (for a review, see Manley and Gleich, '92) have made it clear that the well-known differentiation of avian hair cells into tall and short types provides an interesting challenge for the physiologist. Whereas these two hair cell types are systematically distributed into different regions of the hearing organ, they in fact grade continuously into one another both across and along the hearing organ. In the species studied in detail to date, they receive a profoundly specialized innervation, the most striking feature of which is the highly reduced and mostly absent afferent innervation of short hair cells (Fischer, '92; Fischer et al., '92). Many fundamental questions related to this specialized anatomy and innervation are unresolved, for example, the function of the short hair cells that receive only an efferent innervation (Manley et al., '89).

From the species studied so far, it is possible to say that some of the obvious structural specializations found can clearly be related to specific functions. In the barn owl, for example, the cochlea is highly elongated with obvious specializations in the basal half (Fischer et al., '88); here, the high frequencies are analyzed that the barn owl is known to use in its acoustic hunting behavior, the octave between 5 and 10 kHz being highly expanded into an auditory fovea (Köppl et al., '93). This kind of correlation encouraged us to investigate other species with prominent functional features, to try to relate them in some way to anatomical specializations of the hearing organ.

The budgerigar has been intensively studied with regard to its ability to perceive complex sounds, including its own vocalizations, song bird vocalizations, and human speech (for refs. see Dooling, '80, '92). This species is unusual, in that its pattern of frequency selectivity as determined behaviorally (Dooling and Saunders, '75; Saunders et al., '79) shows a very strong improvement in the middle of its frequency range. The budgerigar shows a highly unusual critical ratio function, with a minimal critical ratio at 3 kHz (measuring the critical ratio by studying the bird's response to tones in noise is a method for estimating the frequency selectivity: small critical ratios indicate a high selectivity). In other respects, however, the hearing of budgerigars is quite similar to that of passerine birds (Dooling, '80, '92). If the unusual features of this species' hearing are realized through peripheral specializations and not through specializations in the auditory pathway of the brain, then morphological studies might reveal such differences in the hearing organ. Precisely this reasoning lay behind the motivation for the present study. We have examined the basilar papilla of the budgerigar in the same way as in our studies of other avian species (Fischer et al., '88; Gleich and Manley, '88), so that through standardized methodologies we can be sure that a comparison of species will reveal true differences.

MATERIALS AND METHODS

Four young budgerigars (*Melopsittacus undulatus*, younger than 1 year old, two males, two females from an animal supplier) were deeply anaesthetized and decapitated. The skull was exposed and the inner ear opened widely to provide access for the fixative solution (0.1 M phosphate buffer, 15% of a concentrated solution of picric acid and 2.5% glutaraldehyde, pH 7.4). After 20–40 minutes in this solution at room temperature, the inner ears were post-fixed for a week in the refrigerated fixative solution. Thereafter, the basilar papillae were carefully dissected out of the skulls and the tectorial membranes removed mechanically with great care using fine forceps. Following an overnight wash in 70% alcohol, the five papillae intended for study under the scanning electron microscope (SEM) were transferred via a rinse in 50% alcohol in 0.1 M phosphate buffer to a solution of 1% osmium tetroxide in 0.1 M phosphate buffer for 1 hour. They were then taken through buffer and a series of alcohols for dehydration and, via acetone, into liquid CO_2 in a critical point dryer (Balzers, CPD 020). The preparations were then gold coated (300 Å, Polaron Unit E5000). The three other papillae were dehydrated in an alcohol series and embedded in plastic (Durcupan soft). They were either sectioned transversely at 5 μm for measurement of general hair cell and papillar dimensions, or at right angles to the nerve at 1 μm on an ultramicrotome for counts of the papillar and lagenar nerve fibers. All sections were stained with toluidin blue.

SEM preparations were viewed at an angle perpendicular to the hair cell surfaces and the entire papilla photographed at a magnification of 80×. The resulting micrographs in a montage were used to measure the papillar length along its midline, its width, and the total number of hair cells. The papillar width was defined as the width of the hair cell area and was measured every 10% position along the whole length at an angle perpendicular to the neural papillar edge. Three papillae were preserved well enough to measure details of the hair cell anatomy and were studied further. From these three papillae, strips of hair cells about 60 μm wide were photographed at positions 10% of the papillar length apart at a magnification of 800×. These photographs were used to determine the number of hair cells across the width, for which only cells were counted that were divided almost in half by an imaginary line across the papilla. For each of these strips, a group of three to seven cells at a neural, middle, and abneural position were also photographed at 1,040×. In addition the orientations of the hair cell bundles were measured as the angle by which the long axis of the bundle was rotated with respect to the neural edge (defined as 0°). Positive angles indicate that the tall stereovilli (or, in 20% of cases in which the kinocilium was still present, the kinocilium) were rotated toward the apex, negative angles the reverse. These measurements were carried out within the 60 μm-wide strips, which were again divided by lines oriented *along* the papilla into groups of cells 10 μm wide. The angular rotations of all bundles within each such block of cells were averaged.

The other data were derived from the high-magnification photos (final magnification 7,070×), which were placed on a graphics tablet for tracing and measuring surface areas with the help of a computer program. In this way, the hair cell surface area and the area covered by the bundle was measured for the small groups of hair cells in each strip for the neural, middle, and abneural positions. From the photographs, the number of stereovilli per bundle was also counted. The length of the long axis of the stereovillar bundle and its width (along the axis from the short to the tall stereovilli) were measured as was the distance of the edge of the bundle from the neural and abneural edge of the cell in each case. From these data, the proportion of the cell surface occupied by the bundle and the number of stereovilli per μm^2 were calculated. The stereovillar thickness was assumed to be the same for all stereovilli in a bundle and the diameter was estimated roughly from the known bundle area and number of villi per bundle.

The light microscopical preparations were analyzed using a compound microscope fitted with a video analysis system (Soft Imaging Software GmbH. "Analysis" program) and hard copies were made using a video printer (Mitsubishi P68E). In this way, some nerve sections were reconstructed in montages to count the number of fibers to the basilar papilla and to the lagena macula. In addition, measurements of the height and width of the hair cells, the height of the stereovillar bundles, and the distance of the cells from the neural edge were made, again using positions at 10% distances along the papilla and averaging data in each case from three neighboring sections. At lower magnification, the areas of the papilla, the tectorial, and the basilar membranes were measured in two preparations.

The parameters measured will be described as a function of percentage of the total papillar length for the respective position in terms of the distance of that position from the basal end of the papilla.

RESULTS

Papillar dimensions and the number of hair cells

The length and width of the basilar papilla and the total number of hair cells were measured from four specimens and averaged, but for all other features, only three specimens were used. The fixed, dried papillar length is 2.148 mm (±0.201 mm). Assuming that the tissues shrink about 20% during fixing and drying, the length of the papilla in the living budgerigar would be a little over 2.5 mm. The width of the papilla varies along its length, being 57 μm at a distance 10% of the length from the basal end. From this position, the papilla widens rapidly to 50% of the length, then more slowly to its maximum width of 197 μm at 90% of the length. The width falls rapidly at the very apical end of the papilla, being still 144 μm at 97% of the length from the basal end (Fig. 1a).

The papillae contain an average of 5,372 (±234) hair cells. The number of hair cells across the width increases proportionately to the papillar width and thus continuously from

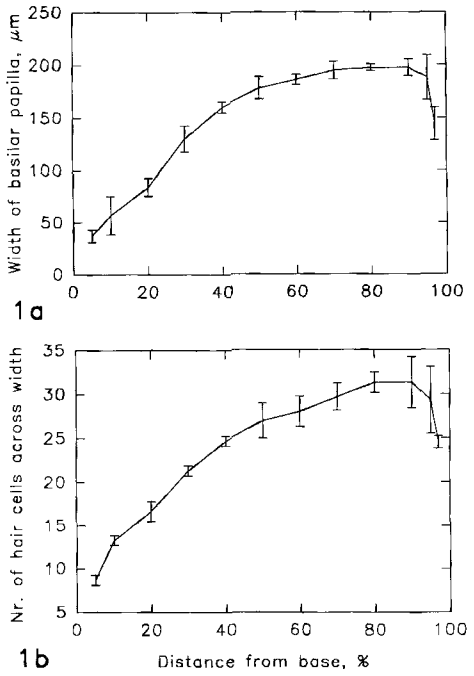

Fig. 1. *Melopsittatus undulatus.* The width of the hair cell area of the basilar papilla (**a**) and the number of hair cells in one cross section (**b**) of the papilla as a function of the distance from the basal end. The data are normalized for the papillar length (to 100%). The continuous lines show the mean values of three preparations and the standard deviations for each position.

the base up to 90% from the basal end. At 10% from the base, there are 13 cells in a transect, at 90%, there are 31 cells (Fig. 1b).

Hair cell bundle orientation

The hair cell bundle orientation sometimes varies greatly between neighboring cells. However, the small values of the standard deviations of the averages for the hair cell blocks measured in the three specimens (S.D. between 7.6 and 8.8°) indicate that, in general, neighboring cells are similarly oriented. The pattern of the distribution of average rotations (as isolines) for two papillae is shown in Figure 2a,b (see also Fig. 3a,b). The rotational angles are small ($< 10°$) along both edges of the papilla, and remain small over the whole basal half (Fig. 3a). In the apical half of the papilla, however, the rotational angle increases across the papilla to a maximum in the middle, and this maximum angle also increases toward the apex, where it reaches average values of $> 65°$ in the medial area (Fig. 2a,b, 3b).

Hair cell surface areas and stereovillar bundle parameters

In the basal 20% of the papilla, the neural, middle, and abneural hair cells all have a similar surface area near 35–40 μm^2 (Figs. 3c, 4a). Further toward the apex, the average areas of these three cell populations diverge strongly. The surface area of neural cells decreases fairly steadily to about 20 μm^2 at 95% from the base. Abneural cells, by contrast, increase dramatically in surface area up to 87 μm^2 at 50% from the base (Fig. 3d), decreasing beyond that (Fig. 4a). Medially lying hair cells show an intermediate behavior. Only the abneural cells are larger at the apex of the papilla than they are at the base. The above data show that, except in the basal

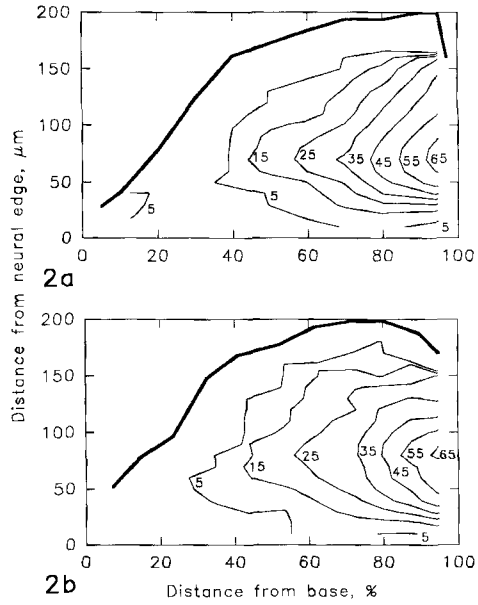

Fig. 2. *Melopsittatus undulatus.* The hair cell bundle orientation patterns over the surface of the papilla in two specimens, **a** and **b.** The graphs are drawn in each case so that the neural edge of the papilla is considered to be a straight line forming the lower axis, with the basal end to the left. The outermost contour (drawn as a thick line) shows the width of the hair cell area as a function of the papillar length. Within this contour, the thin continuous lines show the iso-orientation contours for different values of bundle rotation (the number next to each contour gives the angular rotation toward the apex). In both specimens, there is an area at the apical, medial position that shows the greatest bundle rotations ($> 65°$). From this position, the bundle rotation reduces gradually in all directions.

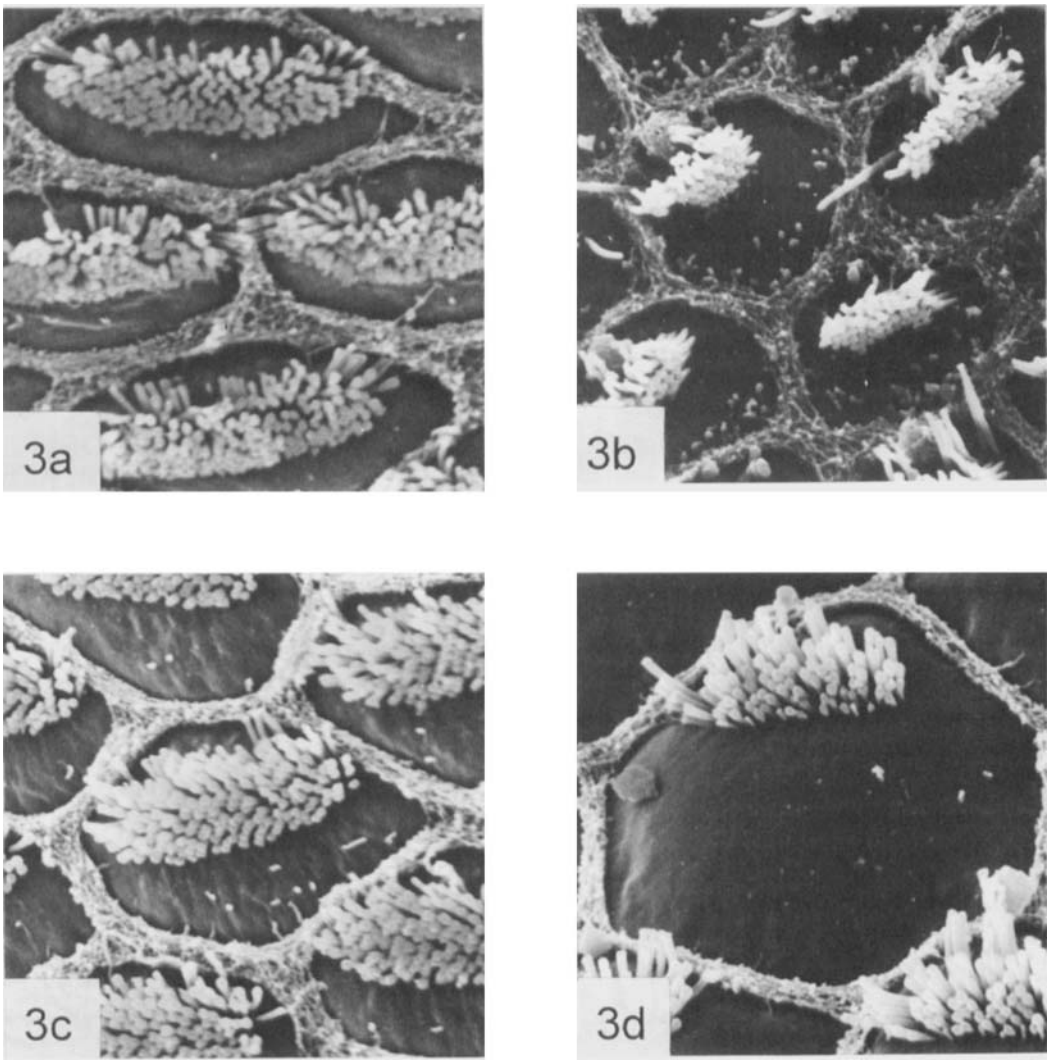

Fig. 3. *Melopsittatus undulatus*. SEM photographs of hair cells from different regions of the auditory papilla to illustrate various aspects of their surface morphology (see text). All photographs are to the same scale, each print has a width of 14 μm, and is oriented such that neural is downward and apical is to the left. Each hair cell is typically surrounded by a hexagon of the microvilli of supporting cells, which still bear remnants of tectorial membrane material. **a:** Neural half of the papilla, 10% from the base; here, the bundles have many stereovilli, occupy a large percentage of the hair cell surface, and are oriented parallel to the neural edge of the papilla. **b:** Neural half of the papilla, 90% from the base; here, the bundles have many fewer stereovilli, occupy a smaller percentage of the cell's surface, and are rotated toward the apex. **c:** Hair cells from a position in the middle of the papilla, 20% from the basal end, where on average the largest number of stereovilli were encountered. **d:** An abneural position, 50% from the basal end. Here, the hair cells have the largest surface area, and the bundle is strongly displaced toward the abneural side of the cell.

quarter, the hair cell surface area increases in any given transverse section across the papilla from neural to abneural sides.

In contrast to the differences in the hair cell surface areas, the areas occupied by the stereovillar bundles are very similar at all three positions across the papillar width. The cells at the papillar base have bundles covering an area of about 14 μm^2, and all cells show a steady decrease in bundle area between papillar positions of about 40% and 90% from the base down to between about 4

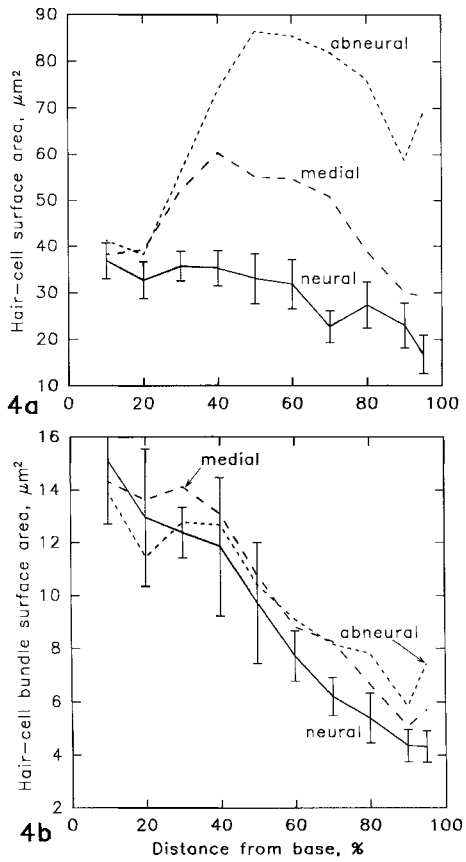

Fig. 4. *Melopsittatus undulatus.* Dimensions of the area parameters for hair cell apical surfaces. In **a,** the average surface area of the hair cells is shown for different positions along the length of the papilla from the basal end for three specimens. Data are shown separately for neurally lying hair cells (continuous line), medial hair cells (dashed line), and abneurally lying hair cells (dotted line). In **b,** the data are presented as in a, except that here, the area of the stereovillar bundle is shown. In these and later figures, two standard deviations are shown only for the neural data, in order to avoid confusion.

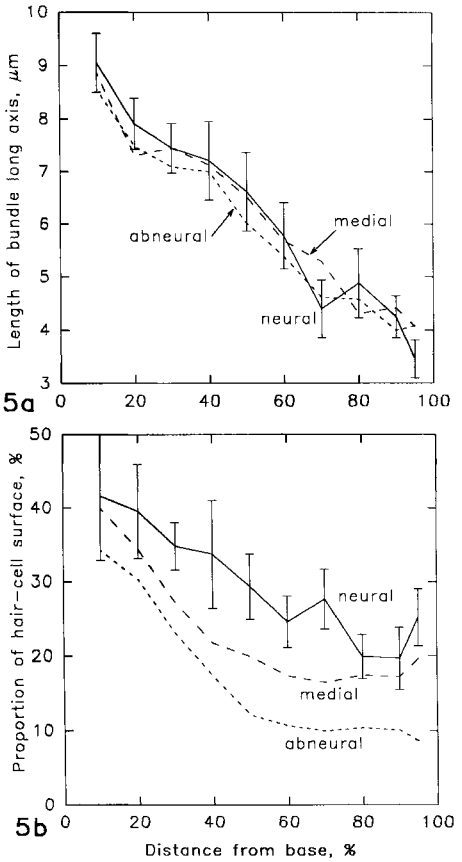

Fig. 5. *Melopsittatus undulatus.* Parameters of the stereovillar bundles. In **a** is shown the length of the long axis of the stereovillar bundles of neural (continuous line), medial (dashed line), and abneural hair cells (dotted line) as a function of their distance from the basal end of the papilla in three specimens. In **b** the percentages of the hair cell apical surfaces occupied by the stereovillar bundle are shown for the three cell groups as a function of their distance from the basal end. The line codes are as in a. In both a and b, the standard deviations are only shown for the neural data, in order to avoid confusion.

and 8 μm^2 (Fig. 4b). The change in area occupied by the bundle is determined mainly by the reduction in the length of the bundles from the base to the apex for all three positions across the papillar width (Figs. 3a,b, 5a). As all the cells analyzed in terms of their surface area and that of their bundles were individually identified, it was possible to calculate the percentage of the cell's surface area occupied by the bundle. This percentage falls for all three cell positions continuously over the basal half of the papilla, becoming then fairly constant over the apical half, whereby it is generally largest in neural cells and smallest in abneural cells (Fig. 5b). The percentages occupied at the apex are roughly one-half (neural cells) to one-third (abneural cells) of those at the base (see also Fig. 3).

The bundle position on the hair cell surface is given by the distance from the neural and abneural cell edges. In all hair cells, the bundle distance from the abneural edge is remarkably constant, at about 1.5 μm (Fig. 6b). In neural hair cells, the bundle distance from the neural edge is also about 1.5 μm, so that in these cells, the bundle is centrally

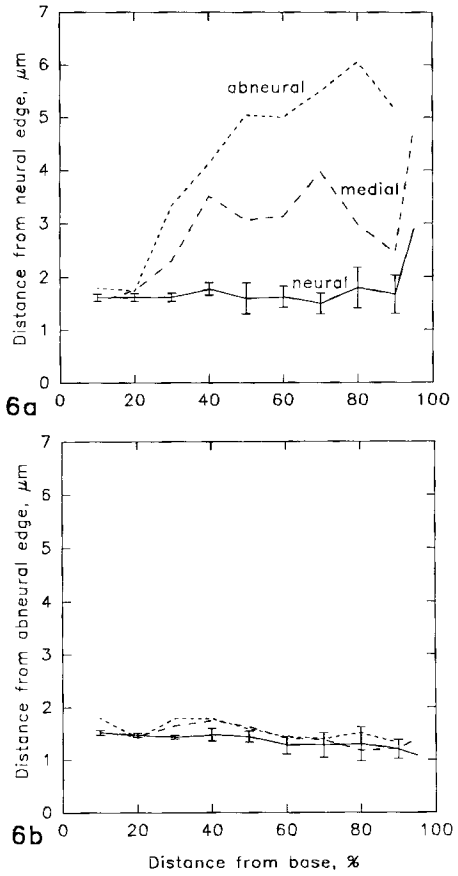

Fig. 6. *Melopsittatus undulatus.* The position of the stereovillar bundle on the hair cell surface expressed as the distance of the bundle's midpoint to the neural (**a**) and abneural (**b**) edge of the hair cell when measured along an imaginary line oriented across the papilla at right angles to the neural edge. The line codes for hair cell position across the papilla are as in previous figures. In both a and b, two standard deviations are only shown for the neural data, in order to avoid confusion.

located (Fig. 3a). As the hair cell area increases in medial and, especially, in abneurally lying cells, there is a resulting large increase in the distance of the bundle from the neural edge in these cells beyond the position about 20% from the basal end of the papilla (Figs. 3d, 6a). In abneural cells, this distance reaches maximally 6 µm at 80% of the papillar length. The bundle is only nearer the neural edge (previously used as one criterion for distinguishing so-called lenticular hair cells) in a very small number of cells.

The number of stereovilli per bundle was counted directly from the high-magnification photomicrographs by two persons independently and the results averaged. There is a clear tendency for the number of stereovilli per hair cell bundle to fall from the largest number at the base to one third or a quarter of that number at the apex (Figs. 3a,b, 7a). Whereas the number falls steadily in the abneural and medial populations, the number on neurally lying hair cells is roughly constant for the basal 40% of the papillar

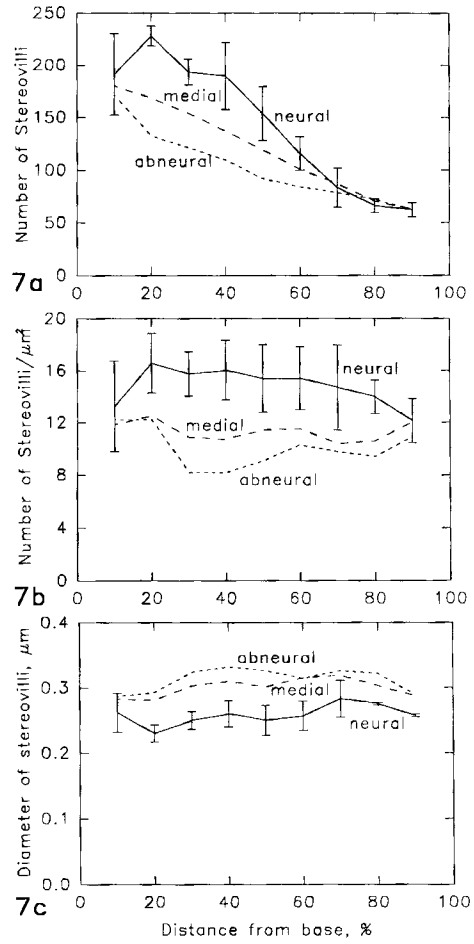

Fig. 7. *Melopsittatus undulatus.* Numbers and sizes of stereovilli as a function of hair cell position on the papilla. The line codes for hair cell position across the papilla are as in previous figures. **a:** The number of stereovilli per hair cell bundle as a function of the distance from the base of the papilla. **b:** The number of stereovilli per square micron, as calculated from the number of stereovilli (a) and the bundle area (Fig. 4b). **c:** The mean diameter of the stereovilli, calculated from the data in b. In a–c, the standard deviations are only shown for the neural data, in order to avoid confusion.

length but then falls rapidly. Over the basal quarter of the papilla, hair cells at the three transverse positions have the same number of stereovilli (Fig. 7a). The largest number of stereovilli is found at the 20% position in neural cells (228 stereovilli/bundle, Fig. 3a), the smallest number at the abneural 90% position (55 stereovilli/bundle, Fig. 3b).

Using the data collected on the area of the bundle and the number of stereovilli per bundle for individual cells, the area available for one stereovillus was calculated (Fig. 7b,c). From this "stereovillus area," the square root was assumed to give a good indication of the diameter of the stereovillus. This calculation, of course, ignores the known fact that the stereovillar diameter within one bundle is not uniform and also assumes that there is no free space between stereovilli, but as an approximation is realistic enough to permit a comparison of bundles in different locations. These calculations indicate that neurally lying hair cells tend to have a higher number of stereovilli within the same area of hair cell surface (Fig. 7b), indicating that their stereovilli are on average thinner (Fig. 7c). Thus the stereovillar diameters of neural hair cells lie between 0.23 μm at 20% from the base and about 0.27 μm in the apical quarter of the papilla. Over most of the papilla, both medial and abneural hair cells have stereovilli with a larger diameter, between 0.28 and 0.33 μm.

Light microscopical analysis

The number of nerve fibers in the auditory nerve of one specimen was counted twice in a section taken at a position just before the proximal axons entered the ganglion. Similarly, the number of fibers to the lagenar macula were counted four times in a transverse section just distal of the hair cell area of the basilar papilla. The counts in each case were averaged and it was assumed that there was no fiber branching in the ganglion area. In total, the nerve entering the cochlea contains 10,893 fibers, of which 1,127 continue through into the lagenar macula. Thus we estimate that nearly 9,800 fibers innervate the basilar papilla. Given that the papillae on average contain 5,372 hair cells, this means that almost two fibers enter the papilla for each hair cell.

The transverse sectional areas of the basilar and tectorial membranes and of the basilar papilla were measured in two specimens, which gave very consistent results (Fig. 8). The cross sectional area of the basilar membrane remains fairly constant over the length of the papilla, which means of course that it is thicker at the (narrower) base. The tectorial membrane is most voluminous at the basal end (about 5,500 μm^2) and its area reduces fairly steadily toward the apex. In contrast, the cross sectional area of the papilla basilaris increases strongly from near 7,000 μm^2 at the base to near 12,000 μm^2 at the apex.

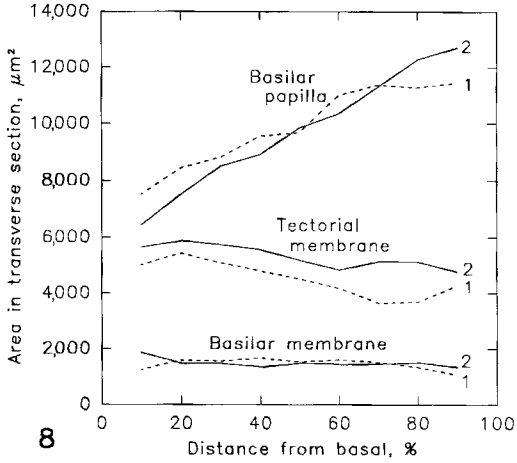

Fig. 8. *Melopsittatus undulatus.* Hearing organ parameters investigated using transverse sections viewed under a video enhanced light microscope. A frame grabber was used, with software to calculate the areas of the basilar papilla (hair cell and supporting cell tissue), the tectorial membrane and the basilar membrane in fixed, embedded specimens. Data sets are shown in each case for two papillae (numbers 1 and 2).

The hair cell dimensions and stereovillar bundle height were measured from the 5 μm sections. The hair cell height is greatest in the apical neural area (maximally 14 to 15 μm) and smallest basally-abneurally (5–7 μm). The variation of this parameter over the papillar surface is shown for two specimens in Figure 9a,c. In contrast, the cell width, as measured in transverse section, varies in an almost contrary fashion. The widest cell apices are found in the apical abneural area, with values from 11 to 15 μm; the narrowest cell apices (5 μm) are found along almost the entire neural edge of the papilla (Fig. 9b,d). As hair cell dimensions traditionally play the most important role in separating "tall" from "short" hair cells (cell height/width ratio above and below the value of 1, respectively), we calculated this ratio for all positions (Fig. 10a,b). The isolines in Figure 10 show that short hair cells (abneural to

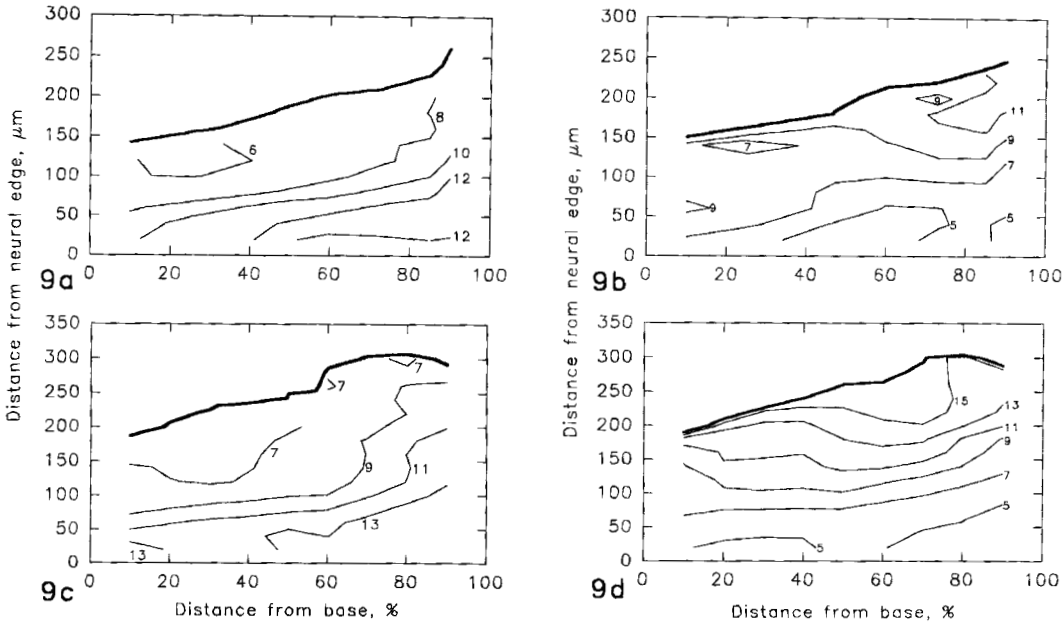

Fig. 9. *Melopsittatus undulatus*. Hair cell dimensions measured from transverse papillar sections for two specimens (left and right columns of graphs, respectively) viewed under a video enhanced light microscope, using a frame grabber and appropriate software. Shown are isolines for the hair cell height (**a** and **c** are two specimens, measured for each cell perpendicular to the papillar surface) and the width at the cell's apex (**b** and **d**, same two specimens). The thick line represents the abneural edge of each papilla. The data are presented as a function of the position of the hair cells along and across the papilla, in the same way as the bundle iso-orientation contours of Figure 2.

the 1:1 isoline) occupy the abneural area of the papilla along its entire length and cover on average 43% of the papillar surface. Cells on the neural edge of the papilla are about 2.5 to 3 times taller than wide. The isolines show that the hair cell shape ratio changes fairly gradually across the papilla, the greatest changes being seen across the apical area. The smallest hair cell shape ratios are about 0.6 on the abneural papillar edge.

The height of the stereovillar bundles is very difficult to measure because they are very short indeed (Fig. 11a,b). The bundle height variation across and along the papilla is not very large (ratio on average about 2:1). The tallest bundles, almost 4 μm in height, were found in the apical abneural papillar area of one specimen (not visible in the averaged data of Fig. 11). The height falls toward the base, where some bundle heights below even 1.5 μm are found. There is also a tendency in the apical half of the papilla for the bundles to be shorter in the medial hair cell area.

DISCUSSION

The quantitative data described in this paper for the budgerigar resemble in many respects those known for other avian species (e.g., Manley and Gleich, '92). It is obvious, however, that every species so far studied is unique with respect to the constellation of features presented by its papilla. Although there is always variation between the individual papillae, they are consistent enough to reveal systematic differences between the hearing organs of different species.

The papillar length in the budgerigar (2.148 mm in the fixed, dried state) resembles the values given by Gleich and Manley ('88) under the same preparation conditions for one budgerigar (2.02 mm) and by Smith ('81, 2.3 mm measured from sections) and Schwartzkopff and Winter ('60, 2.45 mm in whole mounts). Among other species investigated, the papillar length is most similar to that of the starling, as is the number of hair cells (5,830 in the starling; Gleich and Manley, '88).

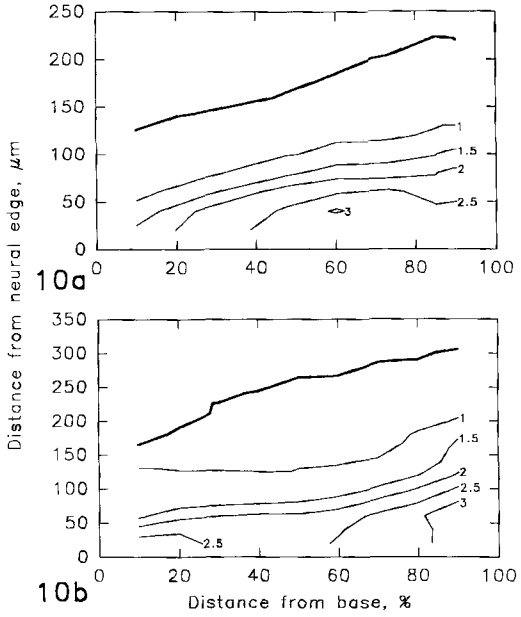

Fig. 10. *Melopsittatus undulatus.* The ratio of the hair cell height-width values calculated from Figure 9 and shown for two specimens in **a** and **b,** respectively. Classically, a height/width ratio greater than 1 is taken to denote "tall" hair cells, below 1, "short" hair cells. The latter occupy on average the abneural 43% of the papilla.

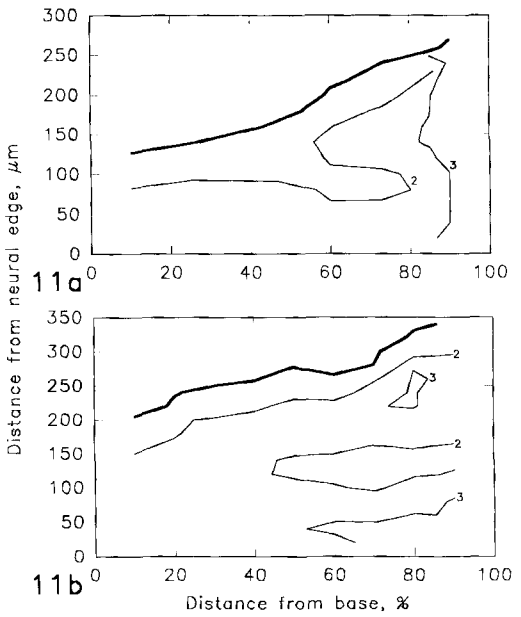

Fig. 11. *Melopsittatus undulatus.* The pattern of the distribution of the height of the tallest stereovilli in each bundle measured in two specimens, **a** and **b** as a function of the position of the hair cells along and across the papilla and shown as isolines. The data are presented in the same way as the contours of Figures 2, 9, and 10. The contours are labeled for the bundle height in µm.

In general, the distribution of hair cell dimensions and stereovillar bundle parameters in the budgerigar is similar to that of the other bird species described in detail so far. Thus in all species, the neurally lying and apical hair cells are taller than the abneural, basal cells. Nevertheless, as noted earlier (Smith, '85; Manley and Gleich, '92), the distribution of hair cell forms is species specific. In the starling, the hair cell shape ratio changes more rapidly across the neural half of the papilla than in the budgerigar. Thus in the starling, a much greater proportion of the papilla (65%, unpublished data) is occupied by short hair cells than in the budgerigar (43%, Fig. 10). As short hair cells are regarded as the more specialized form, in this respect the budgerigar is less specialized than the song bird.

As in other avian papillae, there is a place specific orientation pattern of the hair cell bundles. In general, this pattern is such that all hair cells on the neural and abneural papillar edge are oriented more or less at right angles to the neural edge of the papilla. The more medial apical areas, in contrast, contain hair cells with bundles rotated toward the apex. The largest rotations are shown by medial, apical hair cells (up to about 80° in some cells in the budgerigar at the extreme apex). In comparison to the starling and the pigeon, the isoline pattern describing the stereovillar bundle rotational angles appears shifted toward a more apical position in the budgerigar. The budgerigar orientation pattern appears to be intermediate between those of the starling and pigeon on the one hand, where the largest rotations are not at the extreme apical end (Gleich and Manley, '88) and that shown by the canary and zebra finch on the other hand, where the largest rotations are at the extreme apex, but show smaller angles of rotation (Gleich et al., personal communication).

Although as in the pigeon and starling, the abneural cells in the budgerigar are the largest and the neural cells are the smallest, this difference is maintained in the budgerigar right up to the apical end, where in the other species the cell surface area is more uniform across the width (Gleich and Manley, '88).

Other features, such as the number of stereovilli, also change only gradually along and across the budgerigar papilla. Also, the large change in the surface area of the hair cell bundles from base to apex (14 to 6 μm) resembles that of the pigeon, but is a much greater change than seen in the starling (Gleich and Manley, '88). A similar difference, correlated with this, is seen between these species with regard to the length of the long axis of the stereovillar bundles, which changes much more from base to apex in the pigeon and budgerigar than in the starling. In all species, however, the length of the short axis of the bundle is quite stable from base to apex (at about 2 μm). This means that the large increase in the numbers of stereovilli per hair cell in all species from apex to base is achieved by lengthening, but not widening, the bundle through adding more columns of stereovilli at the ends of the bundle. It has been demonstrated that the stereovilli of each such column are joined by tip links to each other (Pickles et al., '89). The hair cell bundle in budgerigars is placed about 1.5 μm from the hair cell's abneural edge, similar to the starling (about 2 μm) and the pigeon (about 2.5 μm). Changes in the surface area of the hair cell along the papilla result in an increase of the distance of the bundle from the neural edge.

As in other species studied, the number of stereovilli per bundle is a function of the hair cell's position in the papilla. In both the starling and the pigeon, neurally lying hair cells in the basal papillar half have more stereovilli than abneurally lying hair cells. This is also the situation in the budgerigar, except that the difference in the number of stereovilli is greater in the budgerigar and extends further toward the apex. The stereovilli are, on average, about 20% thinner on neural hair cells along the whole length of the papilla. As in the starling and pigeon, the height of the tallest stereovilli decreases from apex to base, but the absolute values in the budgerigar are smaller (less than half as large). Compared to the apical to basal change of about 4 to 1.5 μm in the budgerigar, the starling data show a change from 9.4 to 2.7 μm and the pigeon 12.7 to 4.0 μm. Indeed, the change in bundle height seen in the budgerigar more resembles that seen in the apical half of the barn owl papilla (about 5 to 1.3 μm, Fischer et al., '88). In another respect, however, the bundle heights resemble more the patterns seen in the pigeon and starling, in that medially lying cells, at least in the apical half of the papilla, have shorter bundles than cells on the neural and abneural edges (Gleich and Manley, '88).

The gradual change in the cross sectional areas of the basilar membrane, basilar papilla, and tectorial membrane seen in the budgerigar resembles the situation in the starling (Gleich and Manley, '88). It differs, however, from the situation in the pigeon, which shows a specialization in the apical quarter to third of its papilla, where all three parameters show a disproportionate enlargement.

In attempting to relate quantitatively determined morphological parameters to functional gradients on the avian papilla, we are faced with a very large number of unknowns. The main reasons for this deficit are, first, that so far the techniques used have been far from standardized, and, second, that only four bird species, the chicken (Manley et al., '87, '89), the pigeon (Smolders et al., '92), the starling (Gleich, '89; Manley et al., '89), and the barn owl (Köppl et al., '93), have been studied physiologically in enough detail to even begin to think about such correlations. In these species, gradients in response characteristics along the length of the basilar papilla can, for example, be interpreted in terms of the characteristic frequency of afferent nerve fibers that connect to hair cells in different regions. Thus we know that in these four species, the basilar papillae show a clear and systematic tonotopic organization along their length, from low frequencies apically to high frequencies basally. This correlates with known dimensional changes in accessory structures (e.g., area of the tectorial membrane) and with gradients in hair cells (height of stereovillar bundles, etc.). As the micromechanical response properties of the hair cells at any given location are presumably influenced by many features, it is difficult to assess the influence of differences in individual morphological gradients between species.

Delineating differences in physiological responses *across* the papilla is, however, a much more difficult matter. As seen in Figures 9 and 10, the changes in the hair cell dimensions across the papilla are gradual, suggesting that there would be no sudden changes in function. The problem of understanding function in hair cells at different positions across the papilla is exacerbated by the finding that in the starling and the chicken, at least, basal, abneurally lying hair cells mostly have no

afferent innervation (Fischer, '92; Fischer et al., '92). Most of these hair cells correspond to the "short" category. Thus the function of such cells cannot be studied directly via recordings from afferent fibers.

By recording from the eighth nerve and staining single afferent fibers of known physiological response characteristics, Gleich ('89, in the starling) showed that no fibers could be found that innervated abneural hair cells. Smolders et al. ('92) confirmed this in the pigeon, although the innervation border in the pigeon lies more abneurally than it does in the starling. This is not unexpected, as the morphological gradients across the papilla are more gradual in the pigeon. As avian afferents generally only innervate one hair cell, it was possible to examine the data for correlates of physiological responses with hair cell position across the papilla. In both species, there were systematic differences in response threshold correlated with the position of the innervated hair cells. Unfortunately, the morphological substrate(s), if any, of these changes in threshold are not yet known, making it difficult to use these data to predict the physiology of other species, such as the budgerigar.

Are there any features of the morphology of the budgerigar basilar papilla that are possible candidates for explaining the somewhat unusual psychophysical responses in this species, especially its unusually high ability to discriminate between frequencies? In order to answer this question, we have to know what features determine this ability. Not having a physiologically determined frequency map of the budgerigar papilla and not having detailed information on the innervation patterns or innervation density of the hair cells of different regions as compared to other species makes this task at the moment impossible. The only information available on the frequency distribution in the periphery of this species was obtained using loud bands of noise and looking for changes in hair cell structure correlated with threshold shifts in the audiogram (Dooling, '80). According to these data, and based on the changes induced by the lowest sound pressure used (76 dB SPL), 2 kHz would be analyzed at a position about 40% from the base of the papilla, a frequency value very similar to that for the starling at this position (Manley and Gleich, '92). However, the morphological features of the budgerigar papilla show no hair cell areas that are obviously specialized in any way, as compared to other species. Indeed, compared to the starling, the morphological features of the budgerigar seem to change particularly gradually along the length and across the width, suggesting that a peripheral origin of the known psychophysical specializations is unlikely and certainly not visible at the hair cell level.

In this regard it would of course be very interesting to compare the frequency tuning characteristics of primary auditory nerve fibers in this species to those known in other birds. As yet, however, such data are not available. The small number of neural frequency tuning curves available for the budgerigar (Schwartzkopff, '57) are from unspecified locations in the medulla and show a pattern similar to that seen in primary auditory nerve fibers from other bird species (Manley, '90). If budgerigar auditory nerve fibers show similar tuning, then there is no evidence in peripheral responses for a particularly sharply tuned region near 3 kHz.

ACKNOWLEDGMENTS

This work was supported by the Deutsche Forschungsgemeinschaft within the program of the SFB 204 "Gehör." We thank Prof. R.J. Dooling for assistance in making independent counts of hair cell numbers in these papillae and for making suggestions for improving an earlier version of the manuscript.

LITERATURE CITED

Dooling, R.J. (1980) Behavior and psychophysics of hearing in birds. In A.N. Popper and R.R. Fay (eds): Comparative Studies of Hearing in Vertebrates. New York: Springer Verlag, pp. 261–288.

Dooling, R.J. (1992) Hearing in birds. In D.B. Webster, R.R. Fay, and A.N. Popper (eds): The Evolutionary Biology of Hearing. New York: Springer Verlag, pp. 545–559.

Dooling, R.J., and J.C. Saunders (1975) Hearing in the parakeet (*Melopsittacus undulatus*): Absolute thresholds, critical ratios, frequency difference limens and vocalisations. J. Comp. Physiol. Psychol. *88:*1–20.

Feduccia, A. (1980) The Age of Birds. Cambridge, MA: Harvard Univ. Press.

Fischer, F.P. (1992) Quantitative analysis of the innervation of the chicken basilar papilla. Hear. Res. *61:*167–178.

Fischer, F.P., C. Köppl, and G.A. Manley (1988) The basilar papilla of the barn owl: a quantitative morphological SEM analysis. Hear. Res. *34:*87–101.

Fischer, F.P., C. Miltz, I. Singer, and G.A. Manley (1992) Morphological gradients in the starling basilar papilla. J. Morphol. *213:*225–240.

Gleich, O. (1989) Auditory primary afferents in the starling: correlation of function and morphology. Hear. Res. *37:*255–268.

Gleich, O., and G.A. Manley (1988) Quantitative morphological analysis of the sensory epithelium of the starling and pigeon basilar papilla. Hear. Res. *34*:69–85.

Klump, G.M., and O. Gleich (1991) Gap detection in the starling (*Sturnus vulgaris*). III. Coding of gaps by the auditory periphery. J. Comp. Physiol. [A] *168*:469–476.

Klump, G.M., and U. Langemann (1992) The detection of frequency and amplitude modulation in the European starling (*Sturnus vulgaris*): psychoacoustics and neurophysiology. In Y. Cazals, L. Demany, and K. Horner (eds): Auditory Physiology and Perception. Oxford: Pergamon Press, pp. 353–359.

Köppl, C., O. Gleich, and G.A. Manley (1993) An auditory fovea in the barn-owl cochlea. J. Comp. Physiol. [A] *171*:695–704.

Manley, G.A. (1990) Peripheral Hearing Mechanisms in Reptiles and Birds. Berlin, Heidelberg: Springer Verlag.

Manley, G.A., and O. Gleich (1992) Evolution and specialization of function in the avian auditory periphery. In D.B. Webster, R.R. Fay, and A.N. Popper (eds): The Evolutionary Biology of Hearing. New York: Springer Verlag, pp. 561–580.

Manley, G.A., H.J. Leppelsack, O. Gleich, and H. Oeckinghaus (1985) Activity patterns of cochlear ganglion neurones in the starling. J. Comp. Physiol. [A] *157*:161–181.

Manley, G.A., J. Brix, and A. Kaiser (1987) Developmental stability of the tonotopic organization of the chick's basilar papilla. Science *237*:655–656.

Manley, G.A., O. Gleich, A. Kaiser, and J. Brix (1989) Functional differentiation of sensory cells in the avian auditory periphery. J. Comp. Physiol. [A] *164*:289–296.

Pickles, J.O., J. Brix, S.D. Comis, O. Gleich, C. Köppl, G.A. Manley, and M.P. Osborne (1989) The organization of tip links and stereocilia on hair cells of bird and lizard basilar papillae. Hear. Res. *41*:31–42.

Saunders, J.C., W.F. Rintelmann, and G.R. Bock (1979) Frequency selectivity in bird and man: a comparison among critical ratios, critical bands, and psychophysical tuning curves. Hear. Res. *1*:303–323.

Schwartzkopff, J. (1957) Untersuchung der akustischen Kerne in der Medulla von Wellensittichen mittels Mikroelektroden. Verh. Dtsch. Zool. Ges., Graz 374–379.

Schwartzkopff, J., and P. Winter (1960) Zur Anatomie der Vogel–Cochlea unter natürlichen Bedingungen. Biol. Zentralbl. *79*:607–625.

Smith, C.A. (1981) Recent advances in structural correlates of auditory receptors. Prog. Sens. Physiol. *2*:135–187.

Smith, C.A. (1985) Inner ear. In A.S. King and J. McLeland (eds): *Form and Function in Birds*, Vol. 3. London: Academic Press, pp. 273–310.

Smolders, J.W.T., D. Ding, and R. Klinke (1992) Normal tuning curves for primary afferent fibres innervating short and intermediate hair cells in the pigeon ear. In Y. Cazals, L. Demany, and K. Horner (eds): Auditory Physiology and Perception. Oxford: Pergamon Press, pp. 197–202.