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Franz Peter Fischer

Technische Universität München, Germany, fpf@cipl.zoo.chemie.tu-muenchen.de

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GENERAL PATTERN AND MORPHOLOGICAL SPECIALIZATIONS OF THE AVIAN COCHLEA

Franz Peter Fischer
Institut für Zoologie, Technische Universität München
Lichtenbergstraße 4, D-85747 Garching, Germany

Phone No.: (49) 89-3209 3662; FAX No.: (49) 89 3209 3674; E-Mail: fpf@cip1.zoo.chemie.tu-muenchen.de

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Abstract

In different bird species, there is a common pattern in the hair-cell morphology and innervation of the basilar papilla; the absolute values, however, are species-specific. In the barn-owl papilla, an extreme being case, the basal high-frequency part of the papilla is greatly expanded. In this behaviorally most important frequency range of the barn owl, the number of afferent nerve terminals to neural hair cells is extensively increased. Instead of about 2 afferent terminals as in other species, up to 20 afferents are present. In the bird species studied (chicken, starling, emu, barn owl), the area of the afferent nerve terminals correlates well with the best hearing range. There is a continuous transition from neural to abneural, and from apical to basal in the morphological hair-cell parameters. Thus, the only precise and functionally relevant classification of avian hair-cell types (tall hair cells versus short hair cells) must be based on whether the hair cells have an afferent innervation or not. The differentiation of the evolutionarily-new short-hair-cell type is apparently essential in the high-frequency area of the papilla. This probably functionally supportive type has lost its afferent innervation; its function must therefore be within the papilla itself.

Introduction

Retzius (1884) and later Held (1926) published basic papers on the avian cochlea at the light microscopic level. They described the avian hearing organ as a primitive, compact receptor organ, composed of sensory and supporting cells. Boord (1961) also postulated, in analogy to the situation in mammals, an efferent system in the avian basilar papilla; Cordier (1964) and Vinnikov et al. (1965) described two different types of nerve terminals at the TEM-level. Later Boord (1969) showed the existence of the efferent system to the hair cells in the avian cochlea. Schwarz et al. (1978, 1992) and Strutz and Schmidt (1982) investigated the exact patterns and Fritsch et al. (1993) studied the ontogeny of the efferent system using different nerve cell staining techniques. The function of the efferent system is still unknown in the vertebrate inner ear (Roberts and Meredith 1992). In birds, a functional analysis could be more easily performed than in mammals, since the efferents synapse directly with all hair cells.

Jahnke et al. (1969) and Rosenhall (1971) described gradients in hair-cell morphology over the width of the basilar papilla, and stated that there is no cause for distinguishing hair-cell populations in birds such as in mammals on that basis. Takasaka and Smith (1971) analyzed in detail the ultrastructure of the pigeon basilar papilla. They classified hair-cell types according to a shape factor, the hair-cell length/width ratio: they named hair cells with a ratio > 1 THC ("Tall Hair Cells") and hair cells with a ratio < 1 SHC ("Short Hair Cells"). They also mapped the distribution of these hair-cell types along the length and width of the papilla. Additionally, they observed that THC are mainly innervated by afferents and SHC mainly by efferents. Later TEM studies followed this classification (Tanaka and Smith (1978, chicken); Hirokawa (1978, chicken); Chandler (1984, duck); von Düring et al. (1985, various species); Smith et al. (1985, barn owl); Umemoto et al. (1993, budgerigar). Quantitative data were, however, very rare in these papers.

A number of SEM-studies of the surface morphology of the avian papilla also revealed gradients over the length and width, e.g. in the number and height of stereovilli in the hair-cell bundles: about 200 short stereovilli in the hair-cell bundle in the base and about 50 long stereovilli in the apex (e.g. Tilney and Saunders, 1983, chicken; Tilney et al. 1987, chicken; Counter and Tsao 1986, seagull; Gleich and Manley 1988, starling, pigeon; Fischer et al. 1988, barn owl; Manley et al. 1993, budgerigar). An important finding was the discovery of tip links in the stereovillar bundle of mammalian hair cells in mammals by Pickles et al. (1984). This structural basis of transduction was also demonstrated in

Key words: Bird, chicken, barn owl, emu, starling, hair cell, basilar papilla, innervation, afferent, efferent.

birds and reptiles by Pickles et al. (1989).

To produce a functional-morphological analysis of the avian basilar papilla, it was essential that frequency maps on the basis of single-cell recordings and subsequent staining of primary auditory neurons were available (Manley et al. 1987 (chicken), Gleich 1989 (starling), Köppl et al. 1993 (barn owl), and Klinke and Smolders 1993 (pigeon)).

In the last few years, the emphasis in the investigation of the morphology of the avian inner ear has concerned in the analysis of the regeneration of the basilar papilla after acoustic or other trauma (e.g. Corwin and Cotanche 1988, Corwin 1992, Oesterle and Rubel 1993, Raphael 1992, 1993, Duckert and Rubel 1993).

As a precondition for detailed studies e.g. on hair-cell regeneration, quantitative data on the normal pattern of the hair-cell morphology and innervation in the avian cochlea must be available, as well as their species-specific variation. In this paper, I will first present a survey on the morphology of the avian cochlea. Then I will go into some detail in the chicken basilar papilla, for example, and finally, I will compare the papillae of different bird species in two different ways.

Methods

The basilar papillae of four avian species were studied systematically with the TEM. Complete hair-cell rows (chicken, emu, barn owl) or small hair-cell groups (starling) across and at different positions along the papilla were reconstructed from serial sections and quantitatively analyzed. In this study, 5 chickens (*Gallus domesticus*, breed: selected Leghorn, post-hatching day 7), 7 starlings (*Sturnus vulgaris*, adult), 4 barn owls (*Tyto alba guttata* and *T.a. pratincola*, adult), and 3 emus (*Dromaius novaehollandiae*, 1 adult, 1 post-hatching day 4, 1 post-hatching day 13) were used. In the chicken, barn owl and emu, one ear each was analyzed in great detail. In the starling, the basal half of one individual and the apical half of another one were studied in a similar way. The additional individuals served as control animals to exclude the possibility that the intensively-studied individual of each species was abnormal and also to determine the intraspecific variation. The fixation process in these cases was slightly different, but there were no substantial differences in appearance in TEM. Some aspects of the morphology and innervation in the chicken, starling and barn owl cochleae have been described previously (Fischer 1992, Fischer et al. 1992, Fischer 1994). Most data presented here come from the same set of hair cells as in these papers; a number of new unpublished data are also included, especially for the chicken papilla.

The preparation of the cochleae was slightly different in the four species: The owl was anaesthetized with a combination of Ketamine and Xylazine (Köppl et al. 1993), the round window was opened and the columella gently removed. A total of approximately 4 ml of fixative (5% glutaraldehyde in phosphate buffer, pH = 7.4) was introduced into the ear via a canula at the oval window and left the ear at the round window, where it was absorbed by tissue paper tips. The animal was then sacrificed by a lethal dose of Nembutal. The skull was placed in chilled fixative and the left cochlea carefully dissected free while still in the fixative. The total time in the fixative was 3 hours. The chickens (posthatching day 7) were decapitated, the cochleae rapidly removed and fixed for two hours at 4°C in glutaraldehyde fixative as above. Owls and chickens were postfixed for 2 hours in 2% osmium tetroxide in phosphate buffer (4°C). The starlings were anaesthetized with 0.14 ml 6% nembutal i.m., and

subjected to transcardial perfusion with 500 ml of 5% paraformaldehyde and 1.5% glutaraldehyde. Two postfixations were employed for these specimens: 5% glutaraldehyde in phosphate buffer for 2 hr at 4°C, followed by 1.5% osmium tetroxide in buffer for 3 hours at 4°C. The emu (hatchling) was anaesthetized with Chlorthesin and Nembutal and the fixative applied as for the barn owl. The left ear was then placed into fixative overnight at 4°C, washed with phosphate buffer and postfixed in 2% osmium tetroxide in phosphate buffer at 4°C.

Thereafter the specimens were washed several times with chilled phosphate buffer, dehydrated in ethanol and, after 2 hours in propylene oxide, embedded in Durcupan. Serial semithin and ultrathin sections were cut with a Reichert Ultracut ultramicrotome. The semithin sections were stained with 1% toluidine blue in 1% borax solution, the ultrathin sections with uranyl acetate and lead citrate.

The TEM series were of at least one complete hair-cell "row" across the papilla at 4 positions along the papilla's length in the chicken (4%, 34%, 59%, 82%) and the starling (13%, 40%, 60%, 90%), at 5 positions in the emu (11%, 32%, 59%, 74%, 91%), and at 9 positions in the barn owl (9%, 17%, 27%, 39%, 59%, 73%, 81%, 94%, 97%); the percentages are the relative positions as functions of the distance from the apex of the papillae. In the additional ears of each species, short TEM series were analyzed in similar positions.

Ultrathin sections were studied and photographed in a Jeol SEM-100 electron microscope at a primary magnification of 2600x. An exact calibration of the TEM was performed in every session. The photographs were enlarged to a final magnification of 5000x. It proved sufficient in most cases to photograph every 4th section. Details were studied at higher magnifications and in every consecutive section. All measurements given are for the fixed and embedded specimen, without a correction for the shrinkage due to these procedures.

The hair cells and their nerve endings were drawn on transparent sheets, placed on top of each other and, with the nucleus and the hair-cell surface as landmarks, these sheets were used for the reconstructions. For the chicken, 139 hair cells were reconstructed, for the starling, barn owl and the emu, 36, 196 and 103 hair cells were reconstructed, respectively.

For the maps of the chicken papilla (Figs. 5-6), the values of the intensively-studied papilla were used as well as the values of the additional chicken ears. For the comparison of the different species (Figs. 7-8 and 9-10), only the values of the intensively-studied ears are presented. Here the actual values for the most neural hair cell are shown, as well as the mean values for the hair cells in the neural (excluding the most neural hair cell), medial and abneural third of the basilar papilla.

Distance measurements such as hair-cell length, and also the numbers of afferent and efferent nerve terminals were directly derived from the reconstructions. The contact areas of afferent and efferent nerve fibers on the hair cells were calculated from the length of contact zones in the sections and the thickness of these sections. The data for the characteristic frequencies (Figs 9-10) of the respective regions on the papillae were estimated from the frequency maps given by Manley et al. (1987), Gleich (1989) and Köppl et al. (1993). For the emu, such a frequency map is not yet available.

The techniques used for obtaining and preparing specimens were in conformity with the German law for animal protection.

Results and Discussion

A general scheme of the avian basilar papilla

The avian hearing epithelium, the basilar papilla, and the mammalian organ of Corti have independently evolved from the hearing organ of the stem reptiles. As the papillae of primitive reptiles like turtles and the tuatara suggest, no differentiation of different hair-cell populations was present in the hearing epithelia at the early stage (Manley 1990). On the evolutionary line of the mammals, the formation of two morphologically and functionally distinct hair-cell types over the width of the hearing epithelium has finally led to the Inner and Outer Hair Cells (IHC, OHC). In the evolutionary line of the Archosauria a similar pattern developed in birds (Takasaka and Smith 1971) and in crocodiles (v. Düring et al. 1974): Tall Hair Cells (THC) on the neural side of the papilla grade into Short Hair Cells (SHC) on the abneural side. Thus in both mammals and birds, on the abneural side of the hearing epithelium a derived new hair-cell type with probably a supportive function has evolved from the original hair cells, that are still represented by the IHC and THC, respectively. In some respects, the avian SHC seem even more specialized than the mammalian OHC.

The avian basilar papilla is a long flat band of species-specific length (Fig. 1). In most birds, the papilla is 2-4 mm long; the most spectacular exception are some owls with a papillar length of up to 12 mm. We studied, among other species, the papillae of the chicken, the starling, the barn owl and the emu to get an idea of the variety in the avian hearing epithelium. In the avian terminology, the terms basal and apical, and neural and abneural are standardly used in the description of the papilla. The total number of hair cells is roughly comparable with the number in mammals. However, the hair cells are distributed in a mosaic over the entire surface, i.e. there are no distinct hair-cell rows across the hearing epithelium as in mammals (Fig. 2). The individual hair cells are usually separated from each other by supporting cells. These supporting cells are much less specialized than the supporting cells in mammals, they possess e.g. a pair of centrioles. Avian supporting cells are even capable of differentiating into hair cells, e.g. after acoustic trauma (e.g. Corwin and Cotanche 1988; Raphael 1992; Stone and Cotanche 1994). In the avian cochlea, contacts between neighbouring hair cells are a common feature (Fischer et al. 1991). There are different types of contacts, the most interesting ones being true cell fusions which implicate an electric coupling; this means that some avian hair cells may function as groups. As in the hearing epithelia of the other vertebrates, the hair cells possess a bundle of stereovilli on the endolymphatic space. TEM studies demonstrate that the stereovilli are clearly different from a ciliary ultrastructure; the widely-used term "stereocilia" should therefore be abandoned. The stereovilli insert into the cuticular plate. A kinocilium in front of the tallest stereovillar row may or may not be present, depending on the bird species. In any case, a basal body (with or without the kinocilium) is found, positioned beside the cuticular plate. On the neural side of the papilla, the hair cells extend over the cartilage-like limbus. On the abneural side, they are located on the free basilar membrane. Afferent as well as efferent nerve fibers contact the hair cells. Afferent fibers contact single hair cells or small groups of them in a rather direct way, whereas the efferent system is characterized by extensive branching.

As in mammals, the avian sensory epithelium is tonotopically organized, the high frequencies being represented basally and the low frequencies towards the apex. This goes along with morphological gradients in the

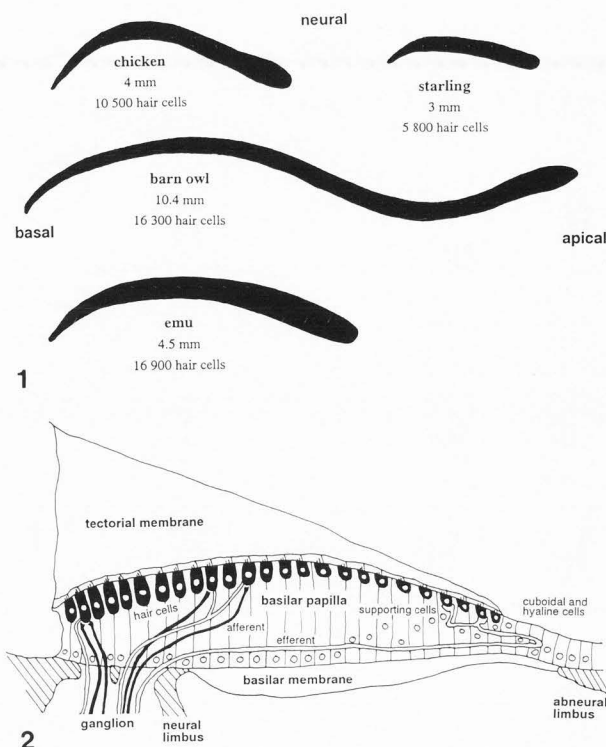
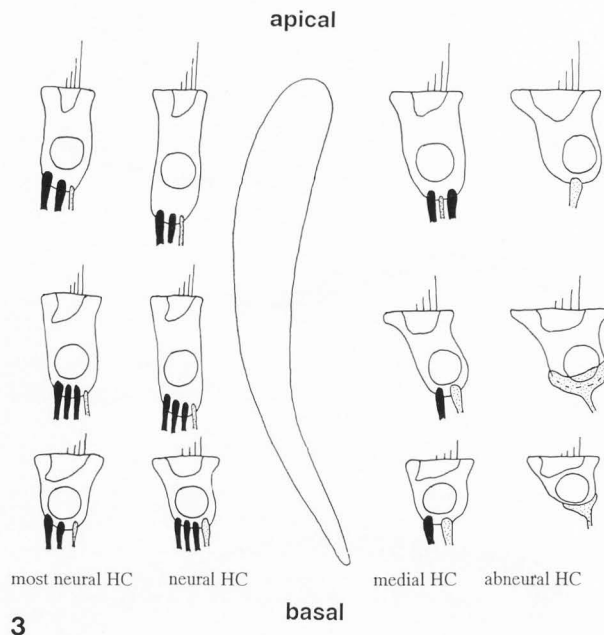


Fig. 1: The general shape of the basilar papillae of the chicken, starling, emu and barn owl. The barn owl is exceptional, in that the basal part of the papilla is greatly expanded. The left side is basal, the right side is apical; the upper side is neural, the lower side is abneural. The length measurements are derived from fixed and embedded specimens. Specimens prepared for SEM are smaller, due to the drying process. The counts for the hair-cell numbers for the starling, chicken and emu were provided by O. Gleich (personal communication).

Fig. 2: Schematic drawing of a transverse section of the starling (*Sturnus vulgaris*) basilar papilla at about 30% from the apex. The left side is neural, the right side is abneural. The innervation pattern (black: afferent; white: efferent) is only shown for a few selected hair cells on the neural side, in the middle of the papilla, and for the abneural side. In contrast to afferents, efferent nerve fibers exhibit extensive branching.

hair cells along the length of the papilla (Fig. 3). Thus, apical and basal hair cells, as well as neural and abneural hair cells, have a very different shape. Neural hair cells, especially in the apical area of the papilla, are generally elongate and show ultrastructural characteristics of high metabolic activity (Fischer et al. 1992). In contrast, abneural hair cells, especially towards the papilla's base, are much shorter and have less active cytoplasm. Abneural hair cells at the extreme base are to a great extent filled by the nucleus and the cuticular plate. Interestingly, the most neural hair cells show less morphological difference along the length of the papilla than do their more abneural neighbours.

A most important functional characteristic of sensory cells is their innervation pattern. Neural hair cells are mainly



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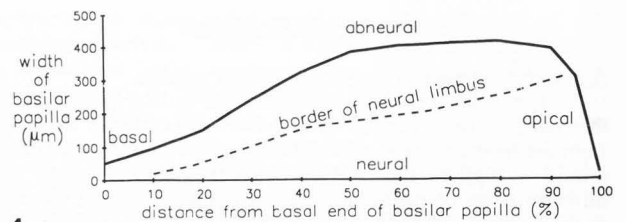
Fig. 3: Schematic drawing of the avian basilar papilla, showing essential features seen in all birds in the hair-cell shape and innervation pattern along the length and across the width of the papilla. For the apical, middle and basal part of the papilla, one hair cell at the extreme neural edge (left), for the neural third (second from left), for the medial third (second from right) and for the abneural third (right) is shown. Black nerve terminals are afferent, dotted terminals are efferent.

innervated by afferent nerve fibers whereas abneural hair cells have only efferent innervation (Fischer 1992, 1994, Fischer et al. 1992). As far as we know, these are the only sensory cells routinely lacking afferent innervation.

Although a great body of morphological and physiological data has been derived for the avian inner ear in recent years, the hearing mechanism is less clear than it is in mammals. In particular, the function of the SHC is completely unknown. One approach to studying structure-function relationships is the quantitative comparison of the inner ear morphology of differently-specialized bird species. In the present study, we compare the basilar papilla of the rather primitive emu, of the chicken, of the starling (a songbird) and of the barn owl (a highly specialized nocturnal predator using auditory cues). The aim is to elucidate which features of the hair-cell morphology and innervation are common to all, and which ones probably represent some specialization.

A map of hair-cell morphology and innervation of the chicken basilar papilla

The pattern of hair-cell morphology and innervation varies along the length and across the width of the avian basilar papilla. In figures 5-6, the pattern of some of these parameters is shown for the chicken, in this example as isoline maps on the basilar papilla. One should bear in mind that only the abneural part of the basilar papilla lies on the free basilar membrane (Fig. 4). The neural hair cells, in



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Fig. 4: Schematic outline of the chicken (post-hatching day 7) basilar papilla showing the limbic borders. The free (abneural) part of the basilar papilla is found over the basilar membrane (upper part), the neural part of the basilar papilla is fixed on the cartilaginous-like limbus (lower part). The neural edge is drawn as if it were straight (x-axis).

contrast, are fixed in the papilla on the cartilaginous-like limbus.

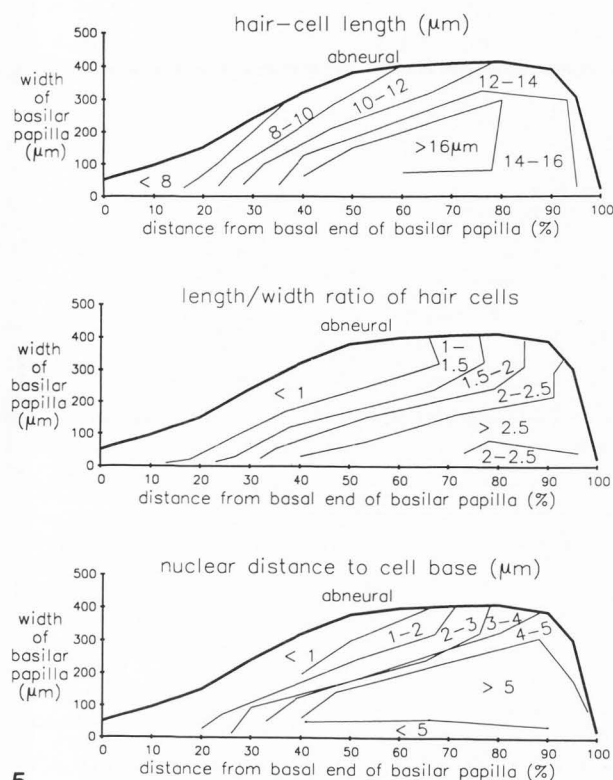
The length of the hair cells increases steadily from the base of the papilla towards the apex (Fig. 5a). The isolines run rather diagonally and not perpendicularly to the papilla's neural and abneural borders. That is, basal hair cells are shorter than apical ones, and abneural hair cells are shorter than neural ones. However, in the chicken, the tallest hair cells are found in the middle of the papilla's width, at about 2/3 of the length from its base. The hair cells along the neural edge and also at the extreme apex are not as tall.

Takasaka and Smith (1971) defined hair cells with a length/width ratio > 1 as THC and hair cells with a ratio < 1 as SHC. The hair-cell shape factor follows a similar pattern as the hair-cell length (Fig. 5b): "classical SHC" with a ratio below 1 are found in a 120 μm -wide zone along the abneural edge, up to 2/3 of the way from the papilla's base. At the extreme base, only SHC are found across the whole papilla. There is a continuous increase in the shape factor towards the medial part of the apex.

The distance of the nuclear membrane from the base of the hair cell is a parameter that characterizes the metabolic activity in the synaptic region (Fischer et al. 1992). This morphological parameter also follows the pattern in the hair-cell length (Fig. 5c): the nuclei of abneural hair cells in the papilla's base nearly touch the cell membrane, thus leaving no space for many organelles in this zone; apical hair cells have much active cytoplasm in this region, and contain numerous organelles such as mitochondria, endoplasmic reticulum, vesicles etc. Again, the most neural hair cells differ from their neighbours in this respect.

Most hair cells on the chicken's basilar papilla have only one to three afferent terminals (Fig. 6a). The exception is the region of the SHC. In a 50-100 μm wide stripe along the abneural edge, no afferent synapses are found in the basal half of the papilla. This means that the hair cells in this area must fulfil a function of a yet unknown nature within the papilla itself.

The synaptic area of afferents per hair cell (Fig. 6b) increases from the base to the apex, the highest afferent innervation being at the neural edge, and, in addition, in a median area at the papilla's apex in the chicken basilar papilla. Here, the afferent contacts are largest. We have shown that at least in the chicken, the size of the thickened membrane areas in the afferent synapses, and thus probably the synaptic sites, are directly correlated with the synaptic area (Fischer 1992). Therefore the diagram also shows the pattern of afferent synaptic activity.

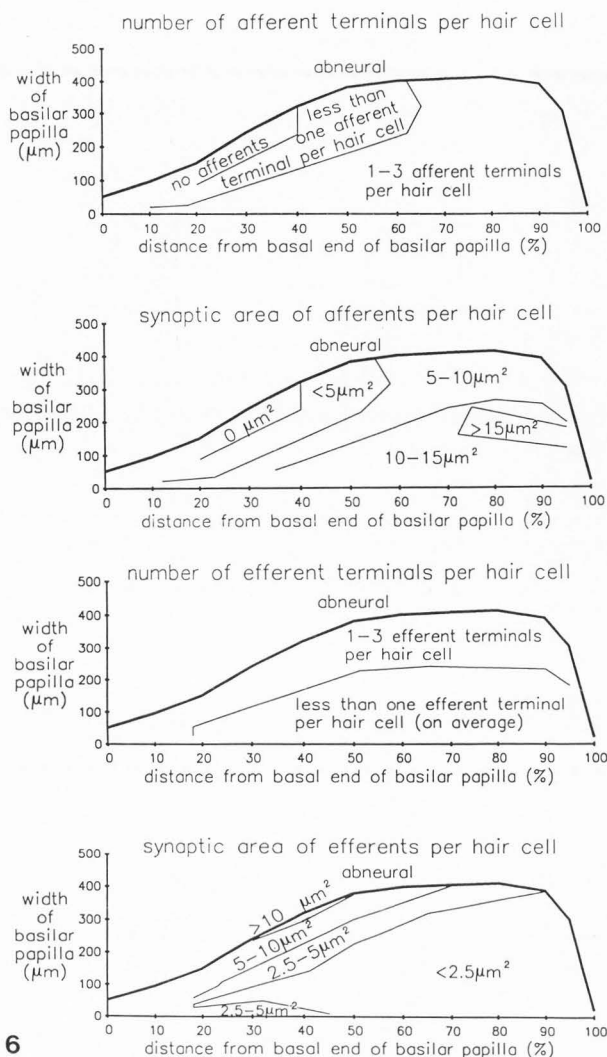


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Fig. 5: Outline diagrams of the chicken basilar papilla showing the patterns in some features of morphology: a) hair-cell length; b) hair-cell length/width ratio; c) distance from the nucleus to the hair-cell base.

In birds, the number of efferent terminals on average is one per hair cell (Fig. 6c). Every hair cell has at least one efferent terminal in the abneural half of the papilla, in a few cases even up to three. In the neural half of most of the papilla's length, scattered hair cells without any efferent terminals can be found. There is no distinct population of hair cells without efferents in the chicken.

The efferent synaptic area per hair cell (Fig. 6c) is small in the apex and increases towards the base. The highest efferent innervation is found abneurally, at about 1/3 to half way from the papilla's base. The form of the efferent terminals varies considerably. In the apex, the terminals are tiny, and finger- or knoblike. The largest terminals at the abneural edge surround as cup-like structures the whole base of their hair cells. As already shown for the membrane thickenings in the afferent nerve terminals, the size of the subsynaptic cisterna (SSC) in the hair cells at efferent synapses is directly correlated with the overall size of the efferent nerve terminal. In contrast to the mammalian situation, the SSC in birds are derived from the rough ER and therefore bear ribosomes on the side towards the hair cell's nucleus. In mammals, the SSC are a derivative of the smooth ER (Fischer 1992).



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Fig. 6: Outlines of the chicken basilar papilla showing the hair-cell innervation patterns: a) number of afferent terminals per hair cell; b) synaptic area of afferents per hair cell; c) number of efferent terminals per hair cell; d) synaptic area of efferents per hair cell. Please note that in basal hair cells on the abneural papillar side, there is no afferent innervation, thus restricting their function to within the basilar papilla itself. Apically, not every hair cell is contacted by an efferent fiber.

A comparison between the chicken, emu, starling and barn owl

The basilar papillae of different birds have common features on one hand, but also some marked species-specific components. Therefore it is not possible to draw a "typical avian papilla" from the analysis of only one species. A comparison of differently-differentiated birds therefore is inevitable. Because of the very different length of the basilar papillae (Fig. 1), a direct comparison of hair-cell morphology and innervation pattern for different species is most easily carried out in two ways:

a) as a function of the position along the basilar

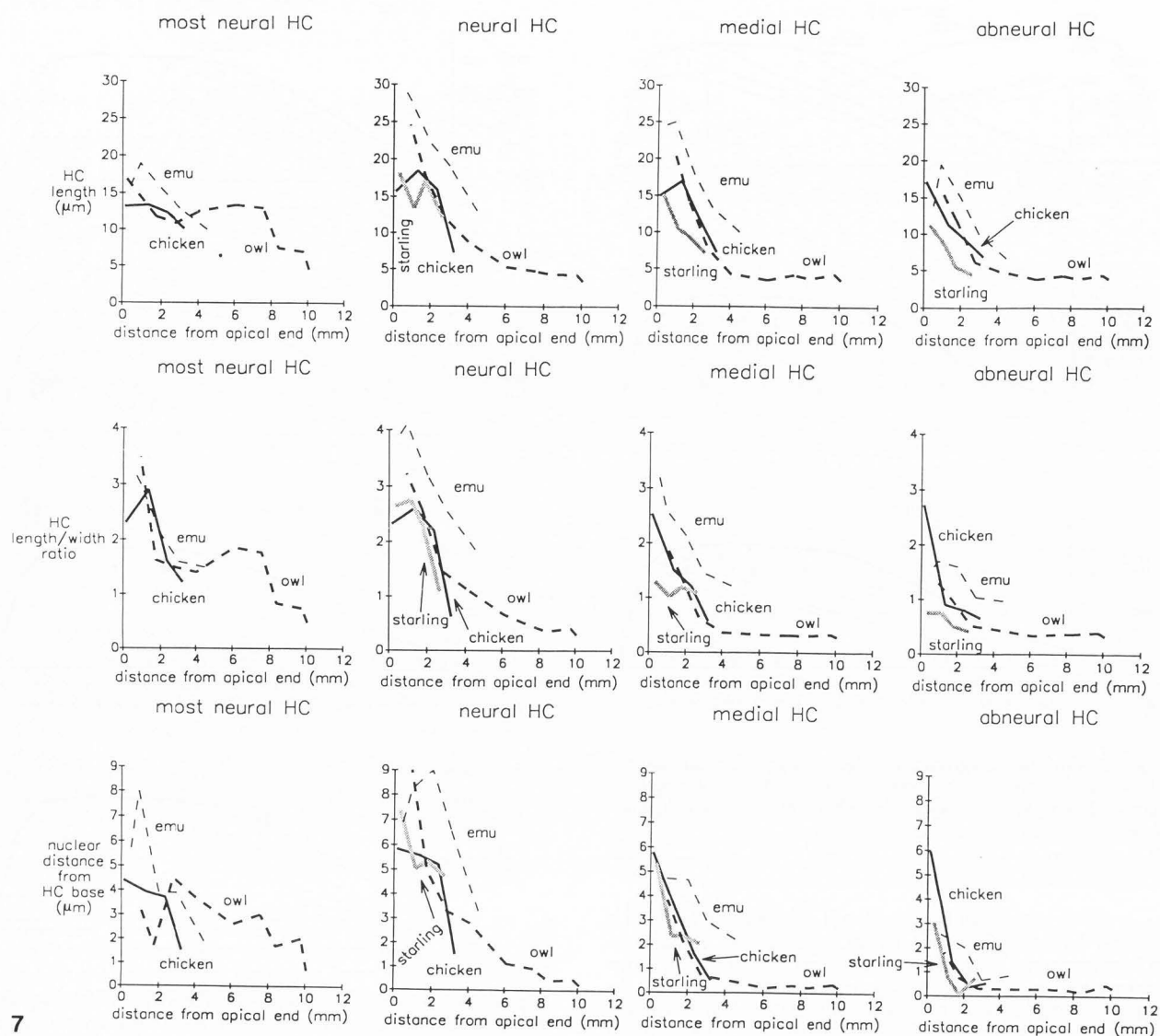


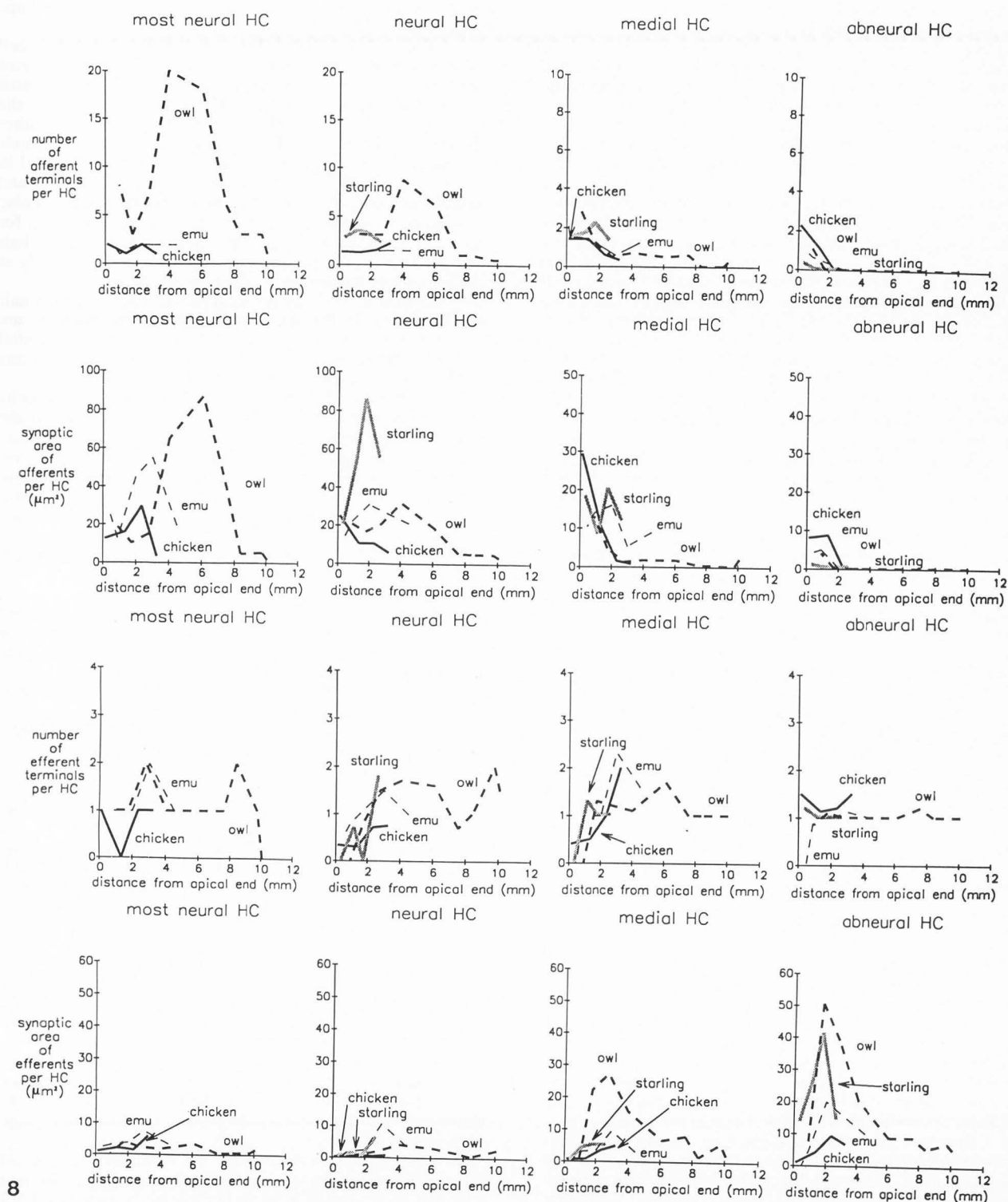
Fig. 7: Parameters of hair-cell morphology along the length of the basilar papilla, shown separately for most neural, neural, medial and abneural hair cells, as a function of the distance from the papilla's apical end (mm). a) hair-cell length (μm); b) hair-cell length/width ratio; c) nuclear distance from hair-cell base (μm).

papilla's length, with reference to the evolutionarily-older apex (Lavigne-Rebillard et al. 1985, Smith 1985, Smith et al. 1985, Fischer et al. 1992).

b) as a function of the characteristic frequency of the afferents to the hair cells at the different positions along the papilla's length. Since the frequency maps are known for the chicken, starling and barn owl, a direct comparison for these three species is possible in this way. For the emu, a similar frequency map is not yet available. There are marked differences in the hearing ability: the starling has a frequency range from about 50 Hz (apical) up to 6 kHz (basal; Konishi 1970, Gleich 1989), whereas the highest frequencies found in the chicken are not much above 4 kHz (Manley et al. 1987). The barn owl, by contrast, has a hearing range up to 12 kHz (Konishi 1973, Köppl et al. 1993).

First, I present a comparison of the parameters in the four species according to the absolute place of the hair cells on the hearing epithelium, i.e. as a function of the distance from the apical end of the papilla. For clarity's sake, I will show the results in the remaining figures separately for the (a) most neural hair cell, (b) for neural hair cells, (c) for medial hair cells and (d) for abneural hair cells (see Fig. 3).

As the first example, the patterns in the hair-cell length are shown (Fig. 7a). Since in the starling no complete hair-cell rows across the papilla were analyzed, no values for the most neural hair cells can be given for this species. The barn owl's papilla has a greatly extended basal region, and this fact is mainly responsible for the unusual length of the papilla in this species. Based on several criteria, the apical third of the barn owl's papilla is equivalent to the whole papilla of the



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Fig. 8: Parameters of hair-cell innervation along the length of the basilar papilla, shown separately for most neural, neural, medial and abneural hair cells, as a function of the distance from the papilla's apical end (mm). a) number of afferent terminals per hair cell; please note the different values for the y-axis; b) synaptic area of afferents per hair cell; please note the different values for the y-axis; c) number of efferent terminals per hair cell; d) synaptic area of efferents per hair cell.

other species (Fischer 1994).

The general pattern in the four species is similar although the absolute values are different. The emu has relatively tall hair cells, the starling has the shortest. In all cases, there is a continuous transition in hair-cell morphology from neural to abneural and from apex to base. The most neural hair cell is shorter than the other neural hair cells (the base of the barn owl's papilla being an exception) and they are more similar to each other along the length of the papilla. Then there is a general decrease in hair-cell height from neural hair cells to medial hair cells and to abneural hair cells. The slope of the curves is rather similar in all species. The basal 2/3 of the barn owl papilla are a marked exception; the hair-cell length is rather constant in this zone, especially in medial and abneural hair cells. If hair-cell morphology reflects functional properties, one must suggest that in the base of the barn owl's papilla physiological changes occur very slowly. In fact, by labelling physiologically characterized primary afferents, Köppl et al. (1993) have demonstrated a spatial overrepresentation of the high frequencies in the basal 2/3. Other parameters such as the stereocillar height also shows a direct correlation with the best frequency.

The hair-cell length/width ratio is, as mentioned before, often used for classifying avian hair-cell types. In all species, there is a similar decrease of this value from apical to basal, and from neural to abneural (Fig. 7b). An exception is the rather unusual most apical position in the chicken, where all hair cells over the entire width are tall. In the expanded basal area of the barn-owl papilla, the ratio is constant, at least in the medial and abneural hair cells. According to the definition derived from hair-cell shape factor, the patterns would mean that the emu has only very few SHC, found at the extreme base. On the other hand, the barn owl would not have THC in the basal 2/3 of the papilla, with the exception of the most neural hair cells in most parts.

The distance of the nucleus to the hair-cell base shows similar patterns (Fig. 7c). In the emu, the distance is larger, and correlates with the greater length of its hair cells. A marked exception in the emu is that the most neural hair cells, although they are not as tall as their more abneural neighbours, are not as uniform in their morphology along the papilla's length as are such cells in the chicken or in the barn owl.

The number of afferent nerve terminals per hair cell varies systematically from the neural to the abneural side of the papilla (Fig. 8a). Neural hair cells along the whole length of the papilla all synapse with afferent fibers. The number of connections very rapidly decreases to zero towards the abneural edge, except in the apical (low frequency) end of the papilla. The most neural hair cells have 2-3 afferent terminals. In this respect, the barn owl is exceptional: In the expanded base of the papilla, up to 20 afferent terminals per hair cell are found in the most neural hair cells and up to 10 in other neural hair cells. This is reminiscent of the situation on IHC of mammals, where 15-30 afferents per IHC are normal in the most sensitive area of the cochlea (Libermann 1980a,b; Spoendlin 1971, Dannhof and Bruns 1993).

Except in the apex (and here to a different extent), abneural hair cells in birds consistently do not synapse with afferent fibers. This provides a functional basis for clearly distinguishing between THC (with afferents) and SHC (without afferents). The distribution of the afferents suggests that the previously-used classification on the basis of the hair-cell shape factor is of less relevance. It should be remembered that there are continuous gradients in the shape factor from apical to basal and from neural to abneural, a fact

which makes a real classification on the basis of cell shape impossible.

The pattern of the afferent synaptic area per hair cell (Fig. 8b) in the barn owl is similar to that of the number of afferents per hair cell; a marked peak is seen for the most neural hair cell between 4 and 8 mm from the apex. In the chicken, the starling and the emu, the pattern is rather different to the pattern of the numbers of afferent terminals per hair cell. A peak in the afferent contact area is found in the emu at about 2 mm from the apex for most neural and neural hair cells. In the starling, at this position, there is also a peak for neural hair cells, and there is a small maximum for the most neural hair cells in the chicken, too. Abneural hair cells have, if at all, small afferent contacts, and these only at the apex. Note the different values for the y-axis.

The number of efferent terminals is usually one per hair cell (Fig. 8c). In the apex, hair cells without efferents are frequent in all species, especially for the neural and medial hair cells. Abneural hair cells have, in nearly every case, one efferent terminal. The pattern is similar in all four species.

The synaptic contact area of efferents on the hair cells (Fig. 8d) increases in all species from small contacts at the neural side to larger areas at abneural hair cells. As for the afferent contact areas on neural hair cells for 3 of the species, a marked peak is found at 2 mm from the apex at abneural hair cells. At this position, there are the largest efferent synapses, cuplike in appearance. This zone is in a middle part of most papillae except for the barn owl, where, correlated with the elongation of the basal part of the papilla, the largest efferent contacts are in the apical 1/3.

Another approach in the comparison of different species is according to the characteristic frequencies of primary afferents to the hair cells. It is not possible to include the emu, as its frequency map is not yet known.

The parameter "hair-cell length" has come into discussion because its absolute value seems to be correlated with the best frequency in mammalian OHC (Pujol 1991, Dannhof et al. 1991). The most neural hair cells have a more constant pattern along the papilla's length than the average of hair cells of the neural third (Fig. 9a). The patterns for the hair-cell length are similar in different species; the absolute value is, however, species-specific. In most parts of the papilla, especially towards the abneural side, there is a direct correlation with the characteristic frequency. The hair-cell shape factor (length/width ratio) shows similar patterns (Fig. 9b). The correlation between the distance of the nucleus to the hair-cell base and the characteristic frequency is very similar in medial and abneural hair cells in the three species (Fig. 9c), in contrast to neural hair cells. Above 1 kHz, abneural hair cells obviously do not have much metabolic activity in the synaptic region. This agrees very well with the finding that above 1 kHz, abneural hair cells lack afferent innervation (Fig. 10a).

The number of afferent terminals per hair cell (Fig. 10a) is fairly constant at about 2-3 for most of the frequency range. The zone of hair cells lacking afferents on the abneural side of the papilla extends over the frequency range above 1 kHz.

Among the species studied, the barn owl is a marked exception, having an unusually high number of afferent terminals at the neural edge in the frequency range of 4.5-9 kHz. This is the behaviourally most important and expanded high-frequency base of the owl's papilla. The high frequencies occupy a large space, the highest octave takes about 1/2 of the whole papilla (6 mm). Correlated with this, the hair-cell morphology is rather constant in these basal 2/3

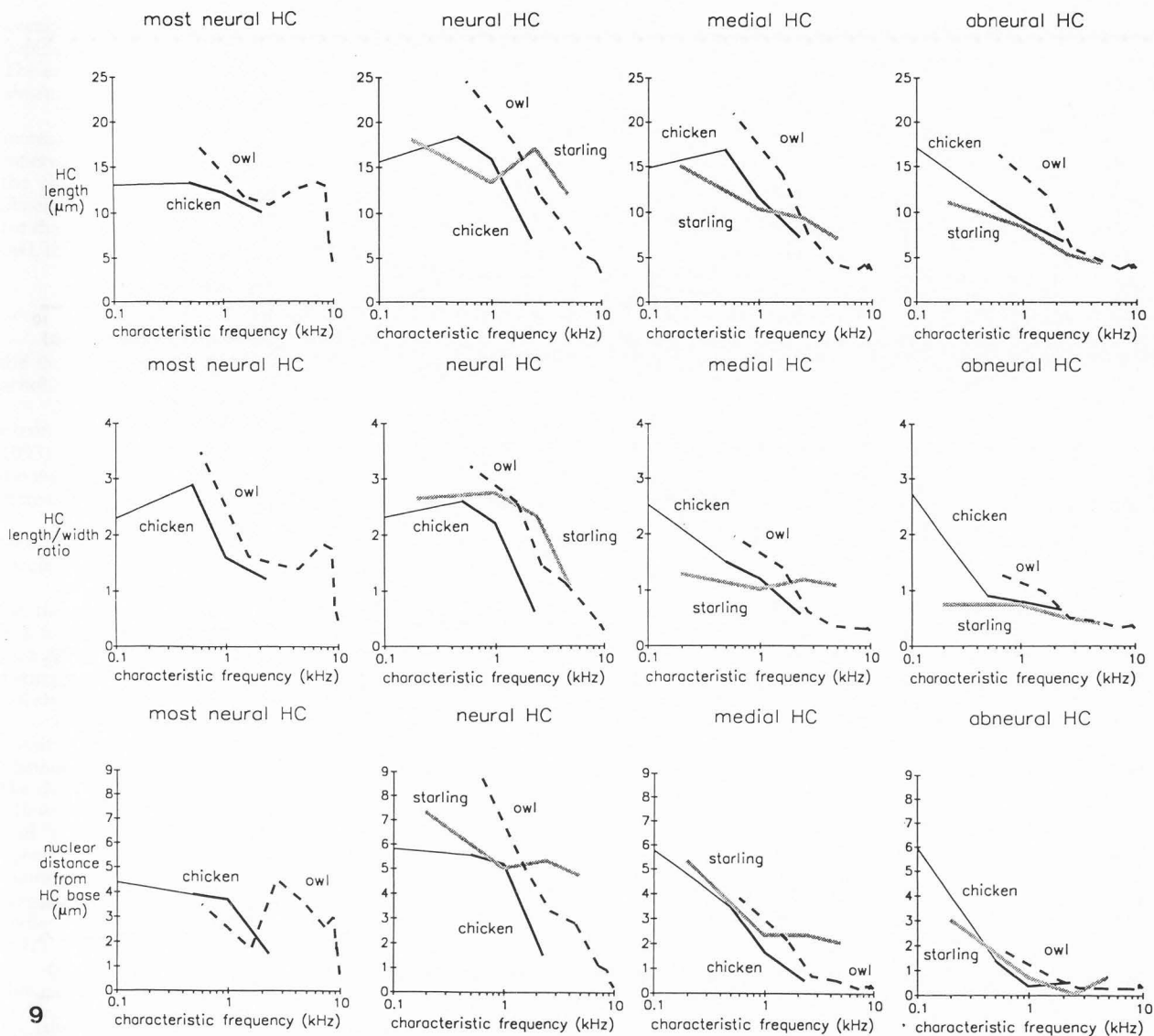


Fig. 9: Parameters of hair-cell morphology along the length of the basilar papilla, shown separately for most neural, neural, medial and abneural hair cells, as a function of the characteristic frequency (kHz). a) hair-cell length (μm); b) hair-cell length/width ratio, c) nuclear distance from hair-cell base (μm).

of the papilla. This spatial overrepresentation of the high frequencies has been called an "auditory fovea" in analogy with the situation in some bats (Köppl et al. 1993). Lower frequencies occupy 0.35-1 mm per octave in the owl, in other bird species this value is 0.1-0.6 octaves per mm.

The contact area of the afferents (Fig. 10b) shows marked peaks for neural or most neural hair cells for all three species. For the barn owl, this range is 4.5-9 kHz, for the starling at about 3 kHz and for the chicken at 1 kHz. For each species, this is the hearing range of great behavioural importance; these are the respective ranges where the three species hear best (Konishi 1970, 1973, Sachs et al. 1978, Dooling 1980, Klump et al. 1986). In the chicken, there is

another zone of large afferent contacts, in the medial hair cells at very low frequencies. It has been shown that, as the pigeon (Schermuly and Klinke 1990a,b), the chicken has the ability to hear infrasound (Warchol and Dallos 1989). The infrasound fibers contact medial hair cells in the apex. In the chicken, the most apical position studied was in the untypical "most apical part" (Lavigne-Rebillard et al. 1985); this part has not been studied with regard to the frequency map; the frequencies in this position are probably very low (Warchol and Dallos 1989). There are also indications that the apex of the barn owl's papilla is specialized in some way; the hair cells are very tall, the efferent innervation is weak, the hair cells are interconnected by numerous non-exclusive afferents

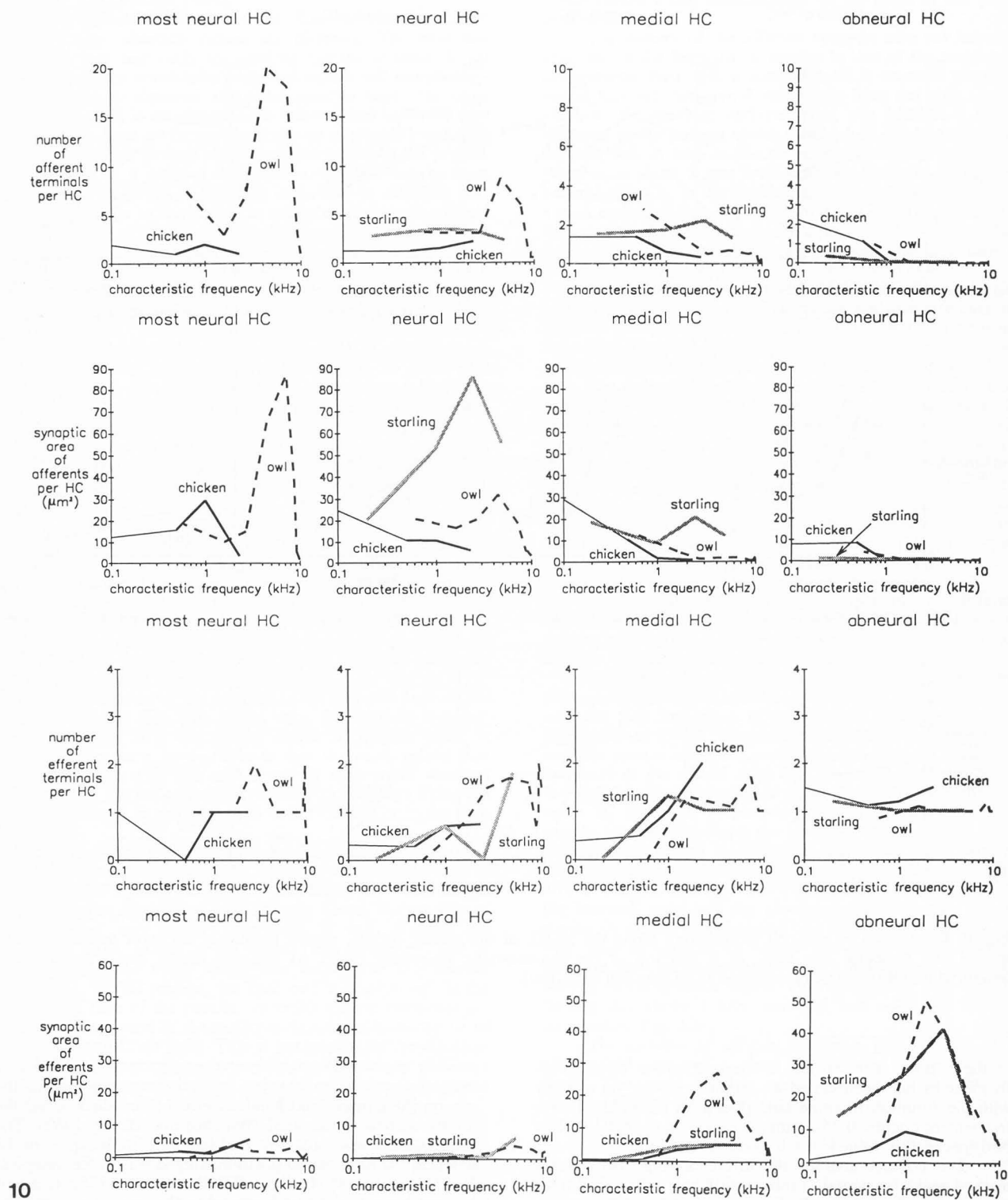


Fig. 10: Parameters of hair-cell innervation along the length of the basilar papilla, shown separately for most neural, neural, medial and abneural hair cells, as a function of the characteristic frequency (kHz) of the hair-cell responses in each position. a) number of afferent terminals per hair cell; please note the different values for the y-axis; b) synaptic area of afferents per hair cell; c) number of efferent terminals per hair cell; d) synaptic area of efferents per hair cell.

and by hair-cell contacts including fusions (Fischer 1994).

The number of efferent nerve terminals (Fig. 10c) is on average one per hair cell. In the low-frequency apex of the papilla, however, hair cells without efferents are frequent. The efferent innervation obviously is of special importance in abneural hair cells, and at higher frequencies.

The efferent innervation area per hair cell (Fig. 10d) increases in the opposite direction to that of the afferent innervation, i.e. from neural to abneural. In the starling and the chicken, the maxima for the efferent contact areas on abneural hair cells lie in the same frequency range as those for the afferent contact areas on neural hair cells. For the barn owl, the two maxima are in different frequency ranges.

Conclusions

1. There are common patterns in the morphology and in the innervation patterns of the avian basilar papilla, but the absolute values are species-specific.

2. In the barn-owl papilla, the high-frequency area is expanded, representing an "auditory fovea" (Köppl et al. 1993). In this behaviorally most important frequency range, the number of afferent nerve terminals to THC is extensively increased.

3. The area of the afferent nerve terminals correlates well correlated with the best hearing range of the different species.

4. In all morphological hair-cell parameters studied so far, there is a continuous transition from neural to abneural, and from apical to basal. Thus morphological parameters, such as the hair-cell length/width ratio, are not suitable for distinguishing between distinct hair-cell types in birds. Hair-cell shape is not directly correlated with innervation pattern.

5. The only straight-forward, and functionally relevant, classification of avian hair-cell types has to be based on whether the hair cells have an afferent innervation or not. The definition of THC and SHC should thus be modified as follows: THC are all those hair cells which have an afferent (and normally also efferent) innervation. SHC are the (specialized) hair cells without afferent innervation; obviously their function is restricted to the papilla itself. This suggestion is in agreement with the results of physiological studies (Manley et al. 1989, Gleich 1989, Smolders et al. 1992).

6. The SHC are an extremely-specialized hair-cell type. This can be seen by the lack of afferent innervation, the low content of active cytoplasm and the lack of subsurface cisternae as compared to the OHC in mammals. SHC are exclusively situated on the free basilar membrane. They also do not show active movements (Zimmermann et al. 1989, Brix and Manley 1994) as mammalian OHC do (e.g. Zenner 1988, Holley and Ashmore 1990). Thus the mechanisms underlying the function of SHC probably differs from that of OHC, although the result may be similar. As the function of the SHC cannot be sensory, they probably are effectors that can be stimulated by their efferents. Possibly they change the mechanics of the tectorial membrane, e.g. by a change in stiffness or bundle movement, and therefore they also modify the stimulus on the THC; in this way, they would be a functionally supportive hair-cell type.

7. The differentiation of the SHC type is apparently essential in the high-frequency area of the papilla. There is, however, no direct correlation between the degree of efferent innervation and the characteristic frequency, for the largest efferent contact areas are not found at the highest frequencies. The process of the differentiation of distinct SHC is well advanced in the high-frequency area of the

papilla, but is found to a different extent towards the apex in the species studied.

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Discussion with reviewers

Reviewer I: You use different fixatives and times to prepare the avian specimens, which you later compare in your study. How do you evaluate the influence of these methodological variations on your results?

Author: The Papilla basilaris is a rather difficult tissue for electron microscopy, and various artefacts such as a swelling of the afferent nerve terminals occur easily (e.g. Picard and Cotanche, 1990; Park and Cohen, 1984; de Groot et al., 1987; Billett et al., 1989). These artefacts must be minimized. The same fixation procedure may cause a different appearance of the papilla in different species. For this reason, different fixation methods were used in every species studied, in order to optimize the procedure, i.e. a minimum of artefacts. The ear with the fewest artefacts was used for the quantitative study, the others were used as "controls" to exclude the possibility that the intensively-analyzed ear was abnormal and also to estimate the intraspecific variation.

Reviewer I: In your TEM series you use different positions throughout the length of the papilla depending on the species (4 in the chicken and starling, 5 in emu, 9 in barn owl) and different relative positions distance from the apex. Could you explain the reason for these methodological variations?

J.O. Pickles: How were the percent distances along the cochlear duct, at which the reconstructions were made, determined?

Author: The number of positions studied in each species mainly depended on the length of the papilla. The aim was to study in detail a number of representative positions in order to assess the morphological gradients. The papillae were cut completely (serial semithin sections), and the ultrathin series were inserted at positions which were apparently undamaged. Due to individual variations of the papillar length, the exact positions in many cases could only be determined when the papilla was completely cut, i.e. after the series had been performed. Since the semithin sections proved that all parameters changed gradually, the method used to compare the different species seems reasonable. In the long and curved papilla of the barn owl, a special method to determine the exact positions was used; this is described in detail in Köppl et al. (1993).

J.O. Pickles: The author is to be congratulated on undertaking what is clearly a great deal of systematic, time-consuming and painstaking work in making his detailed TEM reconstructions. Nevertheless, I have a concern about the small numbers of cells analyzed, and the lack of any indication of the variability of the data. As an example, the author says that a total of 36 hair cells were analyzed in the starling. 4 positions along the cochlear duct were studied, giving an average of 9 cells per position along the duct. Hair cells at each position are divided into 4 groups (most neural, neural, medial, abneural), meaning that there are just over two cells per condition (did each condition have the 2 - or did some only have one?) This is the extreme: the other species have 5 or more cells per condition. However, the numbers are still small to base conclusions on, and we need some indication of how typical the cells are, and the degree of variability expected in the data. Please comment.

Author: In the starling, only a relatively small number of hair cells was completely reconstructed. They were divided in 3 groups (neural, medial, abneural) and each group contained 3 cells. As mentioned in the methods, 7 starlings, 5 chickens, 4 barn owls and 3 emus were used. In these additional ears, small ultrathin series were also cut, but the hair cells were not completely reconstructed. The results were very similar to the closely-studied ears and showed, as did the semithin sections between the closely-studied positions of these ears, that the hair cells presented in this study really were representative. It is, however, not sensible to include the values of the additional ears in the graphs. Although there is, of course, some individual variability, the papilla of each species can be recognized by an expert by the patterns in the morphological and innervation gradients.

J.O. Pickles: How does the author know that the SHC are a "derived hair cell type", while the IHC and THC are the original types? We can only guess what the original hair cells were like. Perhaps they could do a bit of everything (transduce, have motile stereocilia, and motile walls, adapt the offset or gain of mechanotransduction, release neurotransmitter, have electrical tuning), some of which may be now confined to specialized types. Similarly, is it really

definite that no differentiation of the different hair-cell populations were present in the hearing epithelia at the early stage (of evolution)? How does the author know that the apex is "evolutionarily older"?

Author: The comparative anatomy in primitive reptiles like turtles and the tuatara shows that the papillae of these animals are simpler and it is reasonable to assume that these resemble an early stage of evolution. In the papillae of the tuatara and the turtles, there is very little gradient in HC morphology and innervation over the width of the papilla. The ultrastructure of the HC has primitive characteristics (good afferent and poorer efferent innervation, kinocilia are present, no subsurface cisternae or other signs of specialization). In contrast, during the - independent - evolution of the mammalian and avian inner ear, the neural HC in both cases retained many of these original features, whereas the abneural hair cells developed clear specializations, the extreme being the avian SHC. A cell like the SHC, which has lost its afferent innervation, simply cannot "do a bit of everything", but is now a specialized and therefore derived hair cell type. Its function must be confined to the papilla itself. The ultrastructure in mammalian OHC and avian HC strongly suggests that their functional mechanisms must be different, and this agrees with physiological studies on their motility (e.g. Zenner 1988, Holley and Ashmore 1990, Zimmermann et al. 1989, Brix and Manley 1994). Avian SHC have unique features which are certainly derived, such as the lack of afferent innervation, the low content of active cytoplasm (in extrem, in the base of the barn owl most of the cell is occupied by the nucleus and the cuticular plate; Fischer, 1994) and the large cuticular plate.

In the apex, morphological gradients over the width of the papillae are generally much weaker than in the rest of the papilla. The hair cells have more primitive characteristics, such as a good afferent and a poorer efferent innervation, more non-exclusive afferent innervation, more HC with kinocilia. In this sense, the apex has undergone fewer changes during the evolution than the rest of the papilla.

J.O. Pickles: If the definition "without afferents" is to become the definition of the SHC, then only a very small proportion indeed of the hair cells will be candidates (Fig. 6A,B). Would this really be a good idea?

Author: This is only true for the chicken, and here possibly due to a domestication process. In the other, non-domestic species studied, the proportion of HC without afferents nearly reaches the apical end of the papilla. This is even true in the emu which is considered to be rather primitive (Carroll 1988). According to the previously-used definition on the basis of a shape factor, the emu would have nearly no SHC at all. On the other hand, the barn owl would have nearly no THC in 2/3 of its papilla. In fact, there is no indication that the artificial classification of HC using a length/width factor has any functional significance whatever. On the other hand, it is very likely that a functional difference exists between HC with and without afferents.

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