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Functional parallels between hair-cell populations of birds and mammals

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Introduction

The evolution of land vertebrates has produced an extremely interesting variety of structure and function of the hearing organ. We find not only large differences in the overall dimensions of the basilar papilla, but also in the structure, arrangement and innervation of the hair cells. Large, specialized hearing organs developed during the mesozoic adaptive radiation of the reptiles within two evolutionary lines: the mammals and the archosaurs (crocodilians and birds). Since a number of reptilian groups which also originated during the same adaptive radiation still show simple, hearing organs, it is reasonable to assume from the paleontological evidence that the common, early mesozoic, ancestor of these groups had a rather simple, unspecialized hearing organ. The complexities we see today in the inner ears of mammals and crocodilians are thus independent developments from a common stock. The similarities we discuss below are the result of parallel evolution and show convergence in some important features. In this report, we discuss two points. Firstly, we summarize the evidence concerning both the structural differences but also the similarities between the hearing organs of birds and mammals. Secondly, we point out that our recent physiological studies provide the first evidence for the supposition that functional parallels exist between the hearing organs of birds and mammals.

Structural differences between the anatomy of the avian and of the mammalian hearing organ

The structure of the bird basilar papilla (see below, Fig. 1) is different to that of mammals (Organ of Corti). Although the total number of hair cells can be similar, the sensory epithelium is not so stretched out and coiled. Rather, although also showing the familiar base-to-apex width gradient, it is generally shorter than 5 mm (some owls being a remarkable exception) and, at the most, somewhat bent and twisted along its length (Schwarzkopff and Winter, 1960). The hair cells of the avian papilla are not so clearly divided into two

populations as in the mammalian hearing organ, where there is one single row of inner hair cells (IHC) and mostly three rows of outer hair cells (OHC). Avian papillae do show structural variety of the hair cells, the tall hair cells (THC) looking very different to the short hair cells (SHC). However, the extremes grade into one another, so that a strict division of hair cells into different types should perhaps be regarded as merely convenient for descriptive purposes.

The combination of a relatively large number of hair cells and a short papilla lead to the fact that in a transverse section of the avian papilla at the apical end, there can be up to about 50 hair cells; at the basal end there are about 10 hair cells. The cells on the neural side are columnar in shape (Tall hair cells, THC), those on the abneural edge are bowl-shaped (short hair cells, SHC; Takasaka and Smith, 1971). Unlike in the crocodilians, however, these cell types in birds are not clearly-definable separate classes, but form a morphological continuum. Hair cells with intermediate shape have been termed intermediate hair cells. In some papillae, a fourth type similar to SHC has been recognized (lenticular hair cells; Smith, 1985). Unlike in the mammalian hearing organ, not all hair-cell types are necessarily found throughout the avian papilla. In addition, unlike in mammals, all avian hair cells are firmly connected to the tectorial membrane.

A further difference, which has only come to light through recent investigations, concerns the hair-cell orientation. In mammals, both IHC and OHC have their axis of stimulation (perpendicular to the stereovillar bundle) oriented at or close to a right angle to the edge of Corti's organ. Although early reports suggested that the situation in birds is the same (e.g. Takasaka and Smith, 1971), this was an error. In the chick (Tilney *et al.*, 1987), pigeon and starling (Gleich and Manley, 1988) and the barn owl (Fischer *et al.*, 1988) papillae, hair-cell orientation in the center of the apical part is rotated up to 90° towards the apex. The hair cell orientation changes back to 0° (i.e., abneural) towards both edges of the papilla.

Structural similarities between the two types of papillae

In spite of these considerable differences in morphology, there are striking parallels, both in the arrangement and structure of hair cells and in the patterns of afferent and efferent innervation. These convergences suggest that the phylogenetic development and organization of avian and mammalian hearing organs were strongly influenced by certain common features. We suggest that these features are some of the fundamental mechanisms of stimulus processing in vertebrate hair cells and in hair-cell mosaics.

The structural similarities between IHC and OHC on the one hand and THC and SHC on the other can be summarized as follows:

1) The relative placement of the different cell types in the respective papillae is almost the same. Both IHC and, in general, THC are not found over the free basilar membrane. Except for apical THC, they are situated within the neural side of the papilla, which overlies the superior cartilaginous plate in birds and

the spiral lamina in mammals (Smith, 1985).
2) The hair cells lying on the neural side of the papilla are regarded as being the less specialized in both vertebrate classes (Chandler, 1984, Takasaka and Smith, 1971).
3) Like IHC, THC are usually exclusively innervated, that is, their afferents synapse only with one single hair cell (Liberman, 1982; also see data below). Although Whitehead and Morest (1985) found many afferents which penetrated between two THC and innervated both, this pattern is very seldom in our data. In addition, both IHC and THC are innervated by the bulk of the afferent fibers. In contrast, SHC, like OHC, are innervated non-exclusively by a relatively small percentage of the afferent fibers (mammal 5 to 10%, starling 14%), which have small synaptic endings (von Düring *et al.*, 1985; Spoendlin, 1979).
4) The efferent innervation of both OHC and SHC is markedly stronger than that to THC or that to the afferent fibers of IHC, and the synaptic endings are much larger (Firbas and Müller, 1983; Hirokawa, 1978; Spoendlin, 1979; Takasaka and Smith, 1971). Also, in both cases, the innervation density of efferents is higher in the basal than in the apical half of the papilla.
5) The ontogenetic development of the afferent and efferent innervation follows very similar patterns in birds and mammals (Whitehead and Morest, 1985; Pujol *et al.*, 1978).
6) With regard to their sensitivity to noise damage, both SHC and OHC tend to be the first to show morphological changes (Cotanche *et al.*, 1987; Liberman and Kiang, 1978; Robertson, 1982).

A functional parallel in the two types of cochleae

The morphological parallels outlined above have, to date, not been matched by equivalent findings of similarities in the physiological responses of, for example, primary nerve fibers. Although the response activity of bird primary afferents certainly does resemble that of mammalian afferents in many respects, these similarities are, in general, features of all vertebrate auditory organs (tonotopicity, frequency selectivity, etc.). There are both quantitative and qualitative differences in the activity patterns of primary auditory neurons of birds and mammals (Manley *et al.*, 1985; Schermuly *et al.*, 1983; Schermuly and Klinke, 1985). Even though otoacoustic emissions with features similar to those of mammals have been reported from a bird species (Manley *et al.*, 1987b) and the related Caiman (Klinke and Smolders, 1984), they have also been found in a frog (Palmer and Wilson, 1981). Thus, such phenomena should perhaps be attributed more to general properties of hair cells than to unique properties of hearing organs with specialized hair-cell populations. We report here an investigation of the innervation patterns of active afferent fibers in two avian species, where the results indicate an unexpected and remarkable parallel to the situation in mammals.

We have recently mapped the tonotopic arrangement of the basilar papilla of two bird species, using HRP in the chicken (Manley *et al.*, 1987a) and the

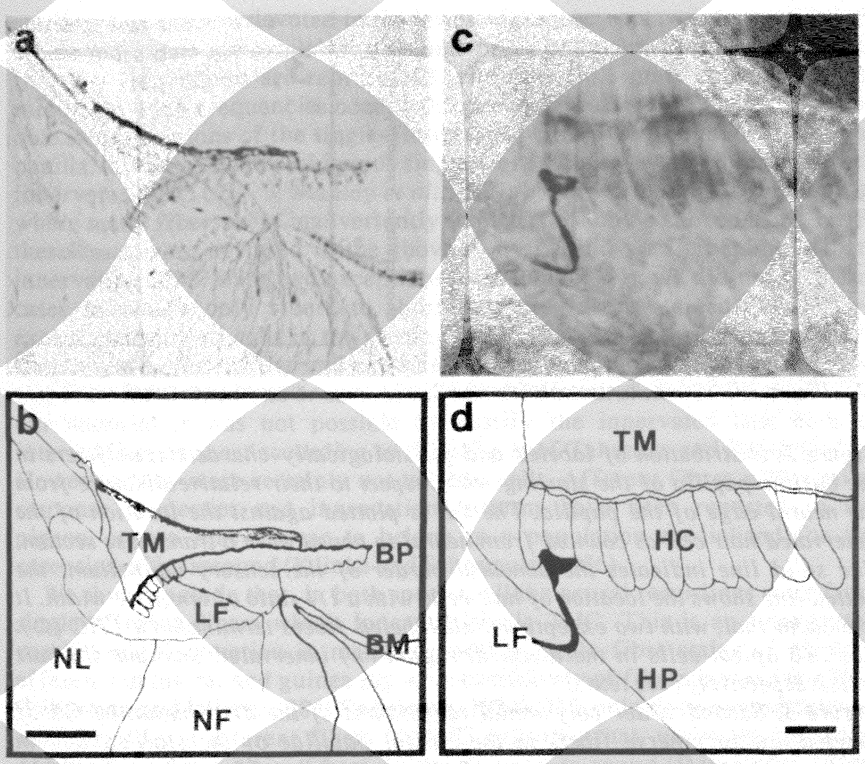
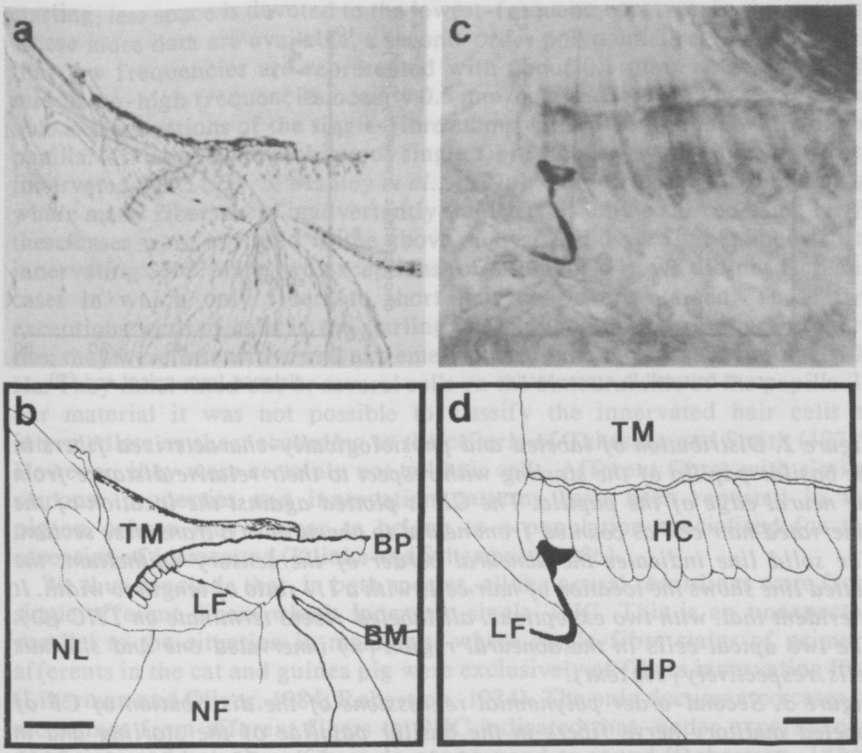


Figure 1 **a**,**b**: *Light micrograph* (**a**) *and corresponding schematic drawing* (**b**) *of a 15 µm transverse section through the starling basilar papilla (bar=50 µm). A single, cobalt-stained afferent auditory nerve fiber (CF=0.4 kHz: threshold=45 dB SPL) runs to the seventh THC from the neural edge (left). The fiber can be followed from the synapse through the habenula perforata and a short distance towards the cochlear ganglion. The receptor epithelium ruptured from the basilar membrane during the histological procedure. For the same reason, the abneural part of the basilar papilla is missing.*
c,**d**: *Higher-magnification micrograph* (**c**) *and schematic drawing* (**d**) *of a HRP-stained afferent auditory fiber in the same frequency range from a chick's basilar papilla synapsing with the second THC (bar=10 µm).*
Abbreviations: BM basilar membrane, BP basilar papilla, HP habenula perforata, HC hair cells, LF labeled fiber, NF nerve fibers, NL neural limbus, TM tectorial membrane.

cobalt technique in the starling (Gleich, in preparation; Köppl and Gleich, 1988). In each case, we stained single auditory-nerve cells or fibers in the cochlear ganglion. Following the investigation of the frequency response characteristics of individual fibers, either HRP or cobalt hexamminechloride



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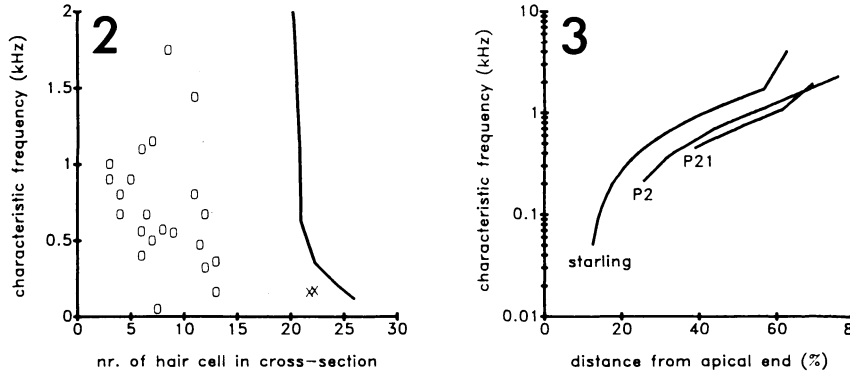

Figure 2. Distribution of labeled and physiologically-characterized fibers in the basilar papilla of the starling with respect to their relative distance from the neural edge of the papilla. The CF is plotted against the location of the innervated hair cell as counted from neural to abneural in a transverse section. The solid line indicates the abneural border of the sensory epithelium; the dotted line shows the location of hair cells with a 1:1 ratio of length to width. It is evident that, with two exceptions, all labeled fibers terminate on THC (○). The two apical cells in the abneural region (×) innervated one and six hair cells, respectively (see text).

Figure 3. Second-order polynomial regressions of the distribution of CF of labeled auditory nerve fibers in the basilar papillae of the starling and in chicks of two different age groups (2nd postnatal day P2 and 21st day P21). Starling: n=34; r^2=0.79; chick, P2: n=13; r^2=0.98; P21: n=8; r^2=0.93.

was injected iontophoretically through the electrode. After a survival time of at least two hours, the animals were perfused through the heart and the cochlear ducts processed to develop the stain. The cochleae were embedded in Spurr, examined and measured both as whole-mount preparations and as serial sections. The position of the fibre terminations were measured from the apical end, as the strong twisting of the basal end makes an accurate correction for the curvature more difficult. As different cochleae differ in length (partly as a result of different ages in the chicks), the locations of the stained terminals (Fig. 1) are given in the figure as a percentage of the distance from the apical end. Further details on techniques are given in the papers cited.

The tonotopic arrangement in both the starling and the chick is unremarkable; the frequency distribution in the starling can be represented as 0.33 mm/oct, in the chicken as about 0.6 mm/oct. Both of these values are obtained from linear regressions over the available range of data. As we have noted elsewhere, however, the distribution of octaves is not uniform in vertebrate hearing organs (Manley *et al.*, 1988). In both the chick and the

starling, less space is devoted to the lowest-frequency octaves. In the starling, where more data are available, a second-order polynomial regression reveals that low frequencies are represented with about 0.1 mm/ octave, whereas middle-to-high frequencies occupy 0.5 mm/octave (Fig. 2). What is remarkable is the locations of the single-fibre stains with respect to the width of the papilla. All cases of unambiguous single fibre staining were of fibers which innervated THC (Fig. 3; Manley *et al.*, 1987a). Only in cases of overstaining, where many fibers were inadvertently stained (this only occurred using HRP, these cases were not used in the above analysis), did we find stained fibers innervating SHC. With two exceptions (of a total of 54), we did not find any cases in which only fibers to short hair cells were stained. These two exceptions were of cells in the starling, which had unusual response properties; they were insensitive and extremely poorly tuned to frequencies near 100 Hz. They innervated one or several cells on the abneural side of the papilla. In our material it was not possible to classify the innervated hair cells as intermediate or short according to the criteria of Takasaka and Smith (1971). However, they were certainly not tall hair cells. Afferent fibres with similar response properties and innervation patterns have been reported in the pigeon, where they appear to belong to a population specialized for the reception of infrasound (Klinke and Schermuly, 1986).

We thus conclude that, in both species, all the neural recordings were from single afferent fibers which innervate single THC. This is an unexpected parallel to the situation in mammals, where single-fibre stains of primary afferents in the cat and guinea pig were exclusively of fibers innervating IHC (Liberman and Oliver, 1984; Robertson, 1984). The only documented cases of recordings from afferent fibers to OHC indicated that, under experimental conditions at least, these fibers do not respond to sound (Robertson, 1984). Taken together, the anatomical and physiological data indicate that it is reasonable to expect that some of the mechanisms underlying the function of the hair-cell mosaics of birds and mammals will be very similar, if not identical. This expectation increases the usefulness of investigations in avian species with respect to the understanding of the function of complex hearing organs and, more specifically, to the elucidation of function in the mammalian cochlea.

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Comments

Evans:

How far can you push this intriguing analogy between mammalian and bird cochleas? Fuchs has recently shown electrical tuning of tall hair cells in the avian cochlea. Does this mean we should expect to find, eventually, electrical tuning in mammalian inner hair cells (for which at present there is contrary evidence) or are there real differences between mammalian and avian mechanisms of frequency selectivity?

Fuchs, P.A., & Mann, A.C. (1986). "Voltage oscillations and ionic currents in hair cells isolated from the apex of the chick's cochlea." J. Physiol. 371, 31P.

Reply by Manley et al.:

Our paper describes data which do not directly address the question of the mechanisms of frequency selectivity in birds and mammals, unless we imagine that the outer hair cells in mammals and the short hair cells in birds are necessary for creating appropriate mechanical conditions for the tuning of inner and tall hair cells, respectively. At present, we know too little to seriously discuss this possibility. We (Manley *et al.*, 1985) emphasized that, although many characteristics of the activity of primary auditory nerve fibres of birds strongly resemble equivalent measures in mammals, there are also consistent differences in both the tuning-curve symmetry and the presence of preferred intervals in spontaneous activity. In the red-eared turtle, such preferred intervals are correlated with membrane-potential oscillations in the hair cells and can be regarded as a neural indicator of the presence of electrical tuning in the hair cell. In the starling, such preferred intervals are characteristic of low-frequency (< 1,5 kHz) fibres. Preferred intervals have also been reported in some reptile preparations at low frequencies. Although

there are few data on low-CF fibres in mammals in the literature, Geisler (pers. comm.) carefully investigated many low-CF fibres of a mammal and found no evidence for preferred intervals in the spontaneous activity. On the other hand, our data from reptiles suggests that the presence of preferred intervals is correlated with fibres only innervating one single hair cell. The avian tall-hair-cell fibres only innervate one hair cell. In mammals, there is evidence (Pujol, pers. comm.) that the innervation pattern of the middle- and basal- turn hair-cell regions is not necessarily continued in the low-frequency apical -turn regions. If apical-turn radial fibres in mammals do not simply innervate one hair cell, then the absence of preferred intervals in primary fibres does not necessarily mean that the hair cells are not electrically tuned. Thus, while we should not necessarily **expect** to find electrical tuning in mammalian hair cells, the present data do not completely rule it out. Nevertheless, there are common patterns across many groups of terrestrial vertebrates, which would suggest that certain fundamental features of frequency selectivity remained unchanged in the evolution along the various lines. One such common pattern is the consistent tendency for the amount of space in the hearing organ devoted to low-frequency octaves to be substantially less than the space devoted to high-frequency octaves (see Manley *et al.*, 1988). In lizards, the low-CF area (below approx. 0.8 to 1.0 kHz) is anatomically separated from the one or two high-CF areas. In at least some of these cases, the presence or absence of strong anatomical gradients suggest a micromechanically-based frequency analysis only in the high-CF area.

Wilson:

Do you think that the graded sensitivity of hair cells across the basilar papilla could be due to differing mechanical input levels to the stereocilia?

Reply by Manley:

The graded sensitivity may well be due to a change in the mechanical input to the hair cells, although this is somewhat counter-intuitive. Almost all of the tall hair cells are not found on the free basilar membrane, so that one might expect their mechanical input to be *reduced* compared to the short hair cells. However, our recent anatomical data indicate strong changes in orientation of hair cells across the apical half of the papilla of the starling (Gleich and Manley, in press). Clearly, we simply know too little about hair-cell stimulation at present.