AUDITORY PATHWAY
Structure and Function

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NEW ASPECTS OF COMPARATIVE PERIPHERAL AUDITORY PHYSIOLOGY

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Although the study of the auditory system of nonmammals has a long
history, it has only been in the last ten years that there has been
a greatly increased interest in comparative studies. There are two main
reasons for this upsurge in interest. Firstly, the hearing organs of
amphibians, reptiles and birds display a structural variety not found in
the cochlea of mammals, offering the chance to investigate structure-
function relationships without interfering with the normal structure.
Secondly, it has been recognized that the sensory papillae of many non-
mammals are mechanically and physiologically relatively robust, which
allows extensive and detailed investigation of hair-cell function in
isolated organs.

These and other advantages have led to a significant increase
in the quantity of data derived from nonmammalian preparations, data
which has made it possible to distinguish between those functional
features which are also found in mammals and those features which are
apparently observable only in nonmammals. Thus, we are increasingly
in a better position to judge which aspects of comparative auditory
physiology are directly comparable to mammalian inner ear function
and provide an alternative means of investigation, if not necessarily
a 'simple' model of the mammalian ear.

A number of features of the organization of the auditory receptors of
terrestrial vertebrates are common to both mammals and nonmammals. Taken
together with a comparison to other hair-cell systems such as the auditory
receptors of fish, and vestibular and lateral-line receptors, this fact
suggests that many of these features are the result of the conservation
throughout evolution of fundamental, primitive hair-cell properties
(Manley, 1986). Thus, the receptor cells themselves and the genetic
programmes which determine their ontogeny into an organized mosaic are the
primary determinants of many basic features of the function of the inner
ear.

There are, on the other hand, several different mechanisms by which
individual hair cells and hair-cell arrays achieve their frequency
selectivity, and there are variations in the expression of different

mechanisms in the various vertebrate groups (Manley, 1986) and perhaps even in hair cells of different frequency ranges within one papilla. It is useful to begin by summarizing some of the published data on important similarities and differences between nerve-fibre activity in the various groups of terrestrial vertebrates.

Similarities in Auditory-Nerve Fibre Activity between Mammals and Nonmammals

1) In the equivalent frequency range (amphibians up to about 3 kHz, reptiles and most birds up to 5-6 kHz), the frequency selectivity of nonmammalian auditory-nerve tuning curves is generally as high as or even higher than that of mammalian primary fibres (Manley, 1981; Manley et al., 1985; Sachs et al., 1974; Turner, 1987).

2) All vertebrate auditory papillae are strongly tonotopically organized, although in certain cases in lizards, the distribution of CFs is not monotonic as in mammals (Manley, 1981).

3) The general patterns in the distribution of intervals in the spontaneous activity of nerve fibres are similar; that is, a modified Poisson distribution underlies the activity in both mammals and nonmammals.

4) Otoacoustic emissions (OAE) have been demonstrated in frogs (Palmer and Wilson, 1981), in the caiman (Klinke and Smolders, 1984) and in the starling (Manley et al., 1987b), which in most respects resemble those reported from mammals.

Aspects in which Auditory-Nerve Fibre Activity Differs between Mammals and Nonmammals

1) Tuning curve symmetry seldom shows the typical form of the asymmetry seen in mammalian fibres of CF >1 kHz. There are often differences between different frequency ranges, however, as in mammals (Manley, 1981; Manley et al., 1985; Turner, 1987). An explanation of these findings assumes an understanding of the mechanisms of frequency selectivity in each case.

2) Spontaneous activity in a number of nonmammals shows systematic deviations from a Poisson distribution (red-eared turtle, Crawford and Fettiplace, 1980; a lizard, Eatock and Manley, 1981; Manley, 1979; the starling, Manley and Gleich, 1984; Manley et al., 1985; the pigeon, Temchin, 1985). Low-CF (characteristic frequency) cells (seldom above CF 1.5 kHz) show preferred intervals in their spontaneous activity, intervals which are roughly the same as or multiples of the CF period. In the turtle, it has been shown that these intervals reflect the presence of oscillations of the hair-cell membrane potential, oscillations whose main energy lies near the cell's CF (Crawford and Fettiplace, 1981). These oscillations seem to be intimately related to an electrical filter mechanism in the properties of the ionic channels of the cell membrane. Such preferred intervals have not been reported in mammalian auditory-nerve fibres.

3) With the exception of basilar-papilla units in the amphibia (Moffat and Capranica, 1976), all nonmammalian primary auditory-nerve fibres investigated show a systematic temperature sensitivity (Gekko, Eatock and Manley, 1976, 1981; Caiman, Smolders and Klinke, 1984; pigeon, Schermuly and Klinke, 1985). Although the most obvious effect is a reversible rise of CF with a rise in temperature (at about 0.06 octaves/ ^{o}C), the spontaneous activity may also be affected. In mammals, there is no temperature sensitivity over a non-lethal range of temperatures (Gummer and Klinke, 1983). The temperature sensitivity of nonmammalian fibres can be explained in terms of an effect on cell membrane channels involved in an electrical tuning mechanism (Eatock and Manley, 1981).

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4) The primary fibre discharge patterns in nonmammals are not always "primary-like" (Manley, 1981, Manley et al., 1985). For example, evidence is accumulating that many reptile and bird primary fibres display primary suppression, that is, the spontaneous activity is depressed by certain single tones (Gross and Anderson, 1976; Manley et al., 1985; Temchin, 1985). This effect is probably related to a firm connection between most hair cells in nonmammals and the tectorial membrane and the apparent absence of such a connection in mammalian inner hair cells.

Differences in activity patterns between mammalian and nonmammalian auditory-nerve fibres are traceable to features at two different levels. The first level consists of cellular and biochemical features (such as membrane ion channels) which are not amenable to normal anatomical analysis. At the second level are features which can be described in normal anatomical terms.

Morphological Substrate as a Potential Origin of Differences in Activity Patterns

Between mammals and nonmammals there are differences in the size of the papilla, which ranges from little over 100 Λm up to a few cm, associated, of course with enormous differences in the total number of hair cells from about 100 to many thousands. Some papillae are even divided into two subpapillae. The hair cells may or may not be overlaid by a tectorial membrane, whose size and form can vary widely between species and even within one papilla. The hair cells in primitive reptilian papillae (e.g., turtles, Tuatara) all resemble each other, but in other groups of reptiles they are divided into two or more types recognizable by their location and their cytological features (Manley, 1981; Miller and Beck, 1988). Lizards show a division of the two hair-cell types between the two different frequency areas. The low-CF area is covered by unidirectional-type hair cells, the high-CF area by bidirectional-type hair cells (Miller and Beck, 1988). In the Archosaurs (Crocodilia and birds) and mammals, two or more hair-cell types are generally found in all frequency areas.

Additionally, the innervational pattern of the hair cells plays an important role. Recent data indicates that there are systematic innervational differences between different hair-cell types, and between species in reptiles, birds and mammals. It is apparent in mammals that only the inner hair-cell population plays a significant role in stimulus transformation and transmission of information to the brain (Liberman, 1982).

In view of these differences, one might well be surprised that superficially, the response patterns in auditory-nerve fibres of different vertebrates are so similar. As outlined above, however, it should be remembered that many similarities are based on common hair-cell properties. In addition, the possibility of convergent or parallel evolution, this is, the independent evolution of a similar solution to the same problem in different groups, should be kept in mind. Some of our recent data from reptiles and birds indicate that there are greater resemblances between activity patterns which can be observed in mammals and nonmammals than some of us had anticipated. The presence of otoacoustic emissions in nonmammals has already been mentioned above. Here, we will briefly discuss three further recent findings from our research groups, which indicate unexpected similarities in (1) the response patterns of different hair-cell populations, in (2) the mechanical resonance properties which contribute to tuning and in (3) the tonotopic distribution on the papillae of mammals and nonmammals.

Functional Differentiation of Hair-Cell Populations in Birds

The basilar papilla of birds contains a continuum of hair-cell forms, from the columnar tall hair cells on the neural edge to the bowl-shaped short hair cells on the abneural edge. The actual distribution of the hair cells (Smith, 1985) and of various aspects of hair-cell morphology (Gleich and Manley, 1988) are species-specific. For descriptive purposes, different hair-cell types (tall, intermediate, short and lenticular) have been defined (Smith, 1985; Takasaka and Smith, 1971). In the related Crocodilia, the tall and short hair-cells are much more sharply differentiated and easily distinguishable from one another (Leake, 1977).

The tall hair cells are the less specialized of the two. This fact, the pattern of innervation and the fact that the tall hair cells are found on the neural (inner) side of the papilla has led to the supposition that the tall and short hair cells are directly comparable to the inner and outer hair cells of mammals, respectively. It should, however, be kept in mind that mammals and birds are derived from different groups of ancestral reptiles and have a very long history of separate evolution.

The ancestors of the different groups of modern reptiles, of birds and of mammals diverged from each other during permiantriassic times (about 200 million years ago). Reptiles regarded as having primitive ears (e.g., Tuatara, turtles) do not show a division into more than one hair-cell population. Assuming this represents the primitive (triassic) condition, it is unlikely that the reptiles ancestral to birds and mammals already possessed two equivalent hair-cell populations. We are thus forced to conclude that the mammals and birds have evolved neural and abneural hair-cell groups independently. We will describe evidence that there are not only anatomical parallels, but also at least one physiological function common to tall hair cells of birds and inner hair cells of mammals. This is a remarkable case of parallel evolution.

In studies of the tonotopic organization of the basilar papilla and its ontogeny in birds, we have used both HRP and cobalt staining techniques to label physiologically characterized primary nerve fibres in the starling and the chick (Gleich, in prep.;Manley et al., 1987a). With only two exceptions, all characterized, unambiguously-labelled fibres made contact with tall hair cells (usually only one cell, Fig. 1a, b). Two broadly-tuned, insensitive cells in the starling with CF near 100 Hz innervated abneurally-lying cells (Gleich, in prep.) in an area described in the pigeon by Klinke and Schermuly (1986) as responding to infrasound. Thus, as in the cat (Liberman, 1982), only the neurally-lying (tall) hair cells of birds seem to transmit information to the brain. These are the first physiological data which support the working hypothesis that a functional equivalence exists between hair-cell populations of birds and mammals.

A Simple Mechanical Resonance Phenomenon as the Basis of Sharp Tuning in the Auditory Nerve of the Bobtail Lizard

An interactive (hair cell - tectorial membrane) mechanical resonance phenomenon has been suggested to underly the sharp tuning of mammalian nerve fibres (e.g., Neely and Kim, 1983; Strelioff and Flock, 1984; Zwislocki, 1979, 1985). Our study of the tuning properties of the basilar membrane and of nerve fibres in the bobtail lizard suggested that a similar resonance could be involved (Manley et al., 1987c).

The basilar papilla of this skink is about 2mm long and contains almost 2000 hair cells. It is divided into a shorter apical area, where the hair cells are covered by a huge, helmet-like tectorial structure
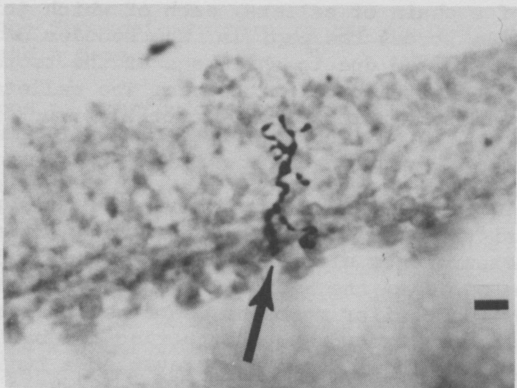
Fig. 1. a) HRP-stained primary nerve fibre in a cross-section of the papilla of a young chick. This fibre appears to make a cup-shaped synapse on a single tall hair cell. b) A primary fibre in the starling papilla seen in surface view. This fibre was stained with cobalt and makes a button-shaped synapse on a hair cell on the neural side of the papilla. c) An auditory-nerve fibre of the bobtail lizard, stained with cobalt during the recording and seen in a wholemount of the papilla. The arrow indicates the fibre's point of entry into the papilla. In sectioned material, this fibre was seen to synapse with at least five different hair cells. The calibration bar is 10 μm in all cases.

and a long basal area, where the hair cells are covered by a delicate
chain of individual "sallets".

These sallets each cover a strip of hair cells running across
the papilla and are connected to each other by a thin strip of tectorial
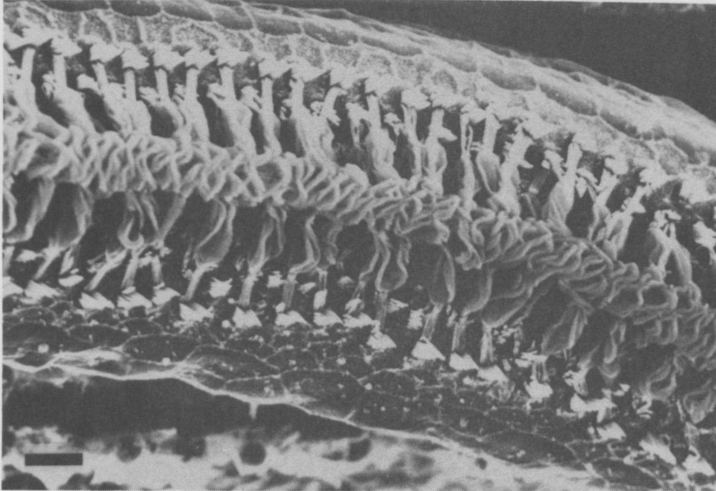material running along the midline of the papilla (Köppl, in prepara-
tion).

Fig. 2.   Scanning-electron-microscope photograph of a small portion of
the upper surface of the basal area of the papilla of the
bobtail skink. It can be seen that the tectorial membrane
consists of a chain of sallets, each of which joins a series
of hair cells across the papilla. The bundles of marginal hair
cells are distorted due to shrinkage of the tectorial structures
caused by the histological procedure. The sallets are themselves
linked together by a rope-like tectorial structure. The calibra-
tion bar is 10 μm.

Unlike the tuning of the basilar membrane, the tuning of primary
nerve fibres was strictly place-specific, revealing a clear tonotopic
organization. Our measurements also showed that at one and the same
location in the same papilla, the neural tuning was substantially
sharper than that of the basilar membrane, each neural curve having a
sensitive tip up to about 40 dB deep. Subtracting the basilar-membrane
tuning from the neural tuning in each case revealed a tuning component
subsequent to the basilar-membrane motion which resembled a sharply-
tuned high-pass filter.

Our model of this tuning assumed coupling between neighbouring,
sharply-tuned elements each represented as a simple, sharply-tuned
high-pass filter. Adding the modelled resonance curves to a typical
basilar-membrane function produced a family of tuning curves for various
CFs which bears a close resemblance to those of the measured neural
elements. It was suggested that the sharp tuning in the basal area of
the bobtail skink papilla is the result of the resonance of local groups
of hair cells (stereovillar bundles) and their associated tectorial
sallet. The shapes of the tuning curves suggest some form of interaction
between adjacent sallet groups, since they are simultaneously relatively
broad and deep. This interaction could be a mechanical one between
adjacent sallets, or it could be due to the parallel connection of
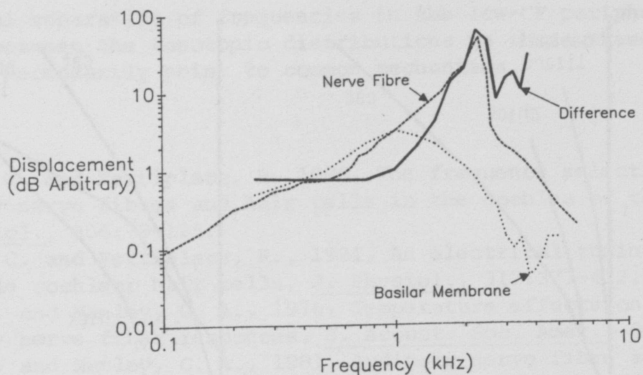several nearby hair cells to one nerve fibre, or be due to both factors

Fig. 3.   A comparison of basilar-membrane (dotted line) and single nerve-
          fibre (dashed line) tuning measured for the same location in one
          papilla of the bobtail lizard. The neural tuning curve has been
          inverted and its placement on the y axis was made using the best
          match for the low-frequency flanks of the basilar-membrane and
          neural curves. The continuous line represents the calculated
          difference curve, which strongly resembles a high-pass filter
          characteristic.

(Manley et al., 1987c). Labelled single auditory fibres in this species
innervated more than five or six neighbouring hair cells. It could be
that the sallets are present as semi-independent tectorial units in
order to increase the isolation of the mechanical resonances of neigh-
bouring elements. In this way, sharp tuning can be achieved over a wider
frequency range in a short papilla.

Similarities in the Tonotopic Pattern in the Auditory Papillae in a Reptile, a Bird and a Mammal

In discussions of tonotopic organization in unspecialized mammals,
it is often assumed that octaves are distributed linearly on the receptor
mosaic. As the amount of space devoted to each octave is roughly
equivalent to the number of receptors responsible for that octave,
variations in the amount of space per octave in specialized mammals has
been taken as an indicator of the relative "importance" of that octave
to the animal. Thus it seems obvious that the "acoustic fovea" of some
bats indicates that a particular frequency region is of special importance
(Vater et al., 1985). Since we can no longer assume that the frequency
analysis mechanisms are the same in all frequency ranges and in all
animal groups, however, the assumption that the amount of space is a
reflection of "importance" may not always be true. If the analysis
mechanisms in different cases are not known, then neither are the
constraints which determine the amount of space devoted to each part
of the frequency range.

A comparison of the frequency distribution on the hearing organs
of the bobtail lizard (Manley and Köppl, in prep.), chick (Manley et al.,
1987a) and cat (Libermann, 1982) revealed that in all three animals,
much less space is devoted to octaves below about 1 kHz than to octaves
above this. The question arises as to whether this means that the lower
frequencies are less "important" to all these animals.

The frequency map in the bobtail was studied both by direct record-
ing of fibres as they emanated from the papilla and by tracing single
fibres labelled with cobalt (Fig. 1c; Köppl and Gleich, 1987). In lizards,
there is a discontinuity between a low-CF hair-cell area (where the hair
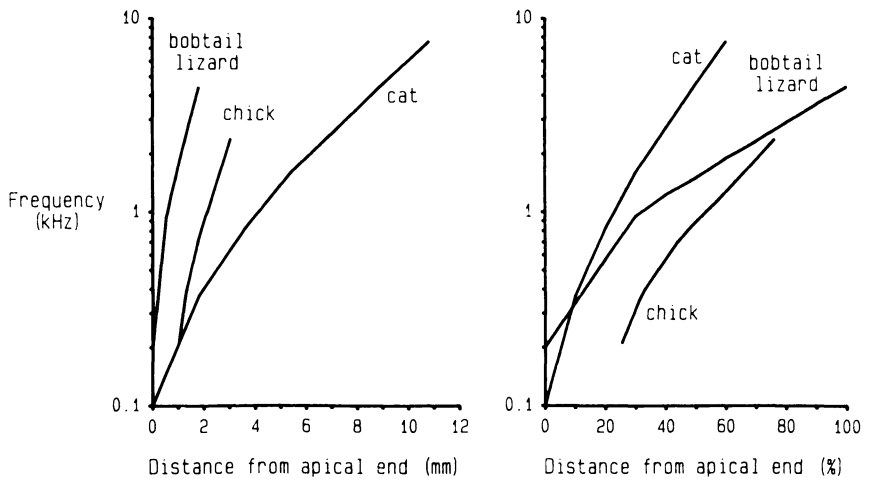
Fig. 4. A comparison of the tonotopic organization of the hearing organs of the cat (after Liberman, 1982), chick and bobtail lizard. The left half of the figure shows the distribution of characteristic frequencies in terms of the absolute distance of the innervated cells from the apical end of the organs. On the right, the values are given as a percentage of each papillar length, in order to make the comparison easier. Less space is devoted to octaves below about 1 kHz in all three cases.

cells are oriented in the same direction) and one or more bidirectionally-oriented areas with CFs above about 1 kHz. More space is devoted to each octave in the higher-CF area than in the low-CF area. The fact that in mammals and in the chick this general pattern is preserved suggests that the division in reptiles is not coincidental, but perhaps correlates with a change in frequency selectivity mechanisms towards higher frequencies.

In nonmammals there are two mechanisms of frequency tuning in which an individual cell is the functional unit and does not greatly influence its neighbours. At lower frequencies, there is evidence for an electrical tuning mechanism. Depending on the required resolution, such a mechanism does not require a large number of receptor cells spread over a large area. At higher CFs, some lizards have a mechanical selectivity mechanism in which individual cells can function independently of their neighbours because they are not attached to them via a tectorial membrane (i.e., the so-called "free-standing" hair cells of iguanid-agamid-anguid lizards, e.g., Weiss et al., 1976).

Where a tectorial membrane is present in an area with CF above 1 kHz, the lateral linking of the hair cells may lead to a reduced selectivity of tuning, unless more space is devoted to each octave. In the latter case, neighbouring cells would have a very similar CF. During the evolution of the some reptile groups, birds and mammals, there was a strong tendency to devote more space to these octaves (Manley, 1986).

What about the low-CF area in mammals? In spite of the apparent absence of electrical tuning in mammals and the fact that a tectorial membrane covers all frequency areas, the cat still devotes less space to lower CF regions than to higher. Perhaps the large brain of mammals (and birds?) is able to use other information in the stimulus at low frequencies (e.g. time information from phase-locked responses). This could function with fewer labelled lines and reduce the necessity for a

large physical separation of frequencies in the low-CF periphery. The resemblance between the tonotopic distributions in these three groups may thus not necessarily point to common mechanisms.

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