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Peripheral auditory processing in the bobtail lizard *Tiliqua rugosa*

IV. Phase locking of auditory-nerve fibres

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Summary. 1. Primary auditory fibres in the bobtail lizard phase-lock up to a maximal frequency of 1.0 to 1.3 kHz at 30 °C body temperature.

2. Phase histograms frequently have two peaks, 180° apart. The frequency of occurrence of this phenomenon in low- and high-CF fibre populations is related to the different tendencies of fibres to innervate both hair-cells' polarities.

3. The vector strength of the phase histograms falls more rapidly with increasing frequency in fibres of a high-CF group than in those of the low-CF group. The corner frequency of the low-CF group is 0.73 kHz, that of the high-CF group 0.51 kHz (at 30 °C). It is suggested that the membrane time constants of high-CF fibres are longer than those of low-CF fibres.

4. The phase delays of the fibres' phase responses below CF vary with CF, from near 3 ms for high-CF cells to 6 ms for low-CF cells. As a travelling wave is not present, these delays must be mainly due to the response times of the hair-cell filters.

Key words: Lizard – Hearing – Auditory nerve – Phase locking

Introduction

The phase-locked responses of auditory-nerve fibres have long been used as a method of investigating the relationships between the mechanical activation of the auditory papilla and the temporal pattern of the stimulus. In the bobtail lizard, we have previously demonstrated that the mechanical responses of the basilar membrane of the high-CF (basal) segment do not show a frequency selectivity or distribution equivalent to those shown by the nerve fibres innervating this region. We also showed that the basilar membrane does not support a travelling wave (Manley et al. 1988a, 1989a). We proposed that the high frequency selectivity of single auditory-nerve fibres from this basal segment is achieved through their innervation of small groups of hair cells which set up a mechanical resonance with an attached portion (sallet) of the tectorial membrane (Manley et al. 1988a, 1989a). In contrast, the basilar membrane of the apical segment was not accessible for measurements. Nerve fibres innervating the apical segment respond to low-frequency sound, appear to be tonotopically organized across the width and have characteristic frequencies (CFs) between about 0.2 and 0.8 kHz ('low-CF' group). As noted in earlier papers (Manley et al. 1990; Köppl and Manley 1990a, b), different lines of evidence strongly suggest that the mechanisms underlying sensory processing in the apical and basal segments are different.

We were interested in seeing whether afferents from these two segments would also differ in their phase-locking characteristics. This was especially interesting, since Rose and Weiss (1988) showed in the alligator lizard that the phase-locking response behaviour of primary auditory-nerve fibres belonging to the low-CF group was better than that of fibres of the high-CF group (CF > 0.9 kHz). These authors suggest that the division of fibres into two groups in the alligator lizard is a peripheral specialization for processing different auditory cues. The low-CF population should, through its better phase locking, be better fitted for processing timing information. Unfortunately, however, their suggestions were based on a set of data derived from nerve fibres whose CFs were not optimally distributed. Of 16 low-CF fibres, 14 had a CF between 0.2 and 0.3 kHz and only 2 had a CF in the frequency range between 0.35 and 1.1 kHz (their Fig. 11). It is thus not clear whether the differences between groups are not simply the extremes of a continuum. As all lizards show a similar division of hair-cell populations (Manley 1990), we have studied this problem in the bobtail lizard, attempting to derive data for an evenly-distributed set of CFs for the entire hearing range. We also attempt to correlate the phase-locking behaviour of bobtail lizard primary fibres to their other physiological response properties and compare them to nerve fibres of species from other classes of vertebrates.

Abbreviations: *CF* characteristic frequency; *PSTH* peri-stimulus time histogram; *VS* vector strength

Materials and methods

The surgical techniques required to expose the auditory nerve in the bobtail lizard have been described in an accompanying paper (Manley et al. 1990). Glass micropipettes were used to record phase-locked responses from 77 single auditory-nerve fibres. Of these, 73 fibres were recorded centrally (see Manley et al. 1990) and only 4 peripherally: the latter did not differ obviously in their response patterns. The sound stimuli used were continuous or pulsed (50 ms duration, 10 ms rise-fall, 6/s) pure tones generated under computer control by a frequency generator (HP 3325A), gated and attenuated (Wilsonics BSIT, PATT). In some fibres which adapted quickly to the tone, it was necessary to make measurements using pulsed tones. Thus in some cases both continuous and pulsed tones were used, in order to test for their equivalence. Both the sound-pressure level and phase of the sound system for the frequencies used were calibrated for each animal.

Action potentials were fed to a computer equipped with a Lab Master board (Scientific solutions, Inc.) and receiving phase information from the frequency generator. The timing of spikes with reference to this phase information was measured using a computer programme which derived a phase histogram for a standard number of 500 spikes. Cycle histograms were collected and analyzed to derive the peak amplitude and phase of the fundamental and second harmonic components of the Fourier transform for as wide a range of frequencies as possible and using SPLs adjusted as close to threshold as possible while still obtaining a substantial increase in discharge rate to the sound. Only at very low frequencies in the case of high-CF cells was it not possible to obtain strong rate increases, due to their high thresholds. We made no systematic attempt to investigate the effects of stimulus level on phase-locking behaviour (measured over a wide range of intensities in 4 cases). Frequencies were changed in 0.05 kHz steps up to the point at which the phase histogram ceased to show phase-locking behaviour, or until contact with the unit was lost. Phase amplitudes in this paper are expressed in terms of the vector strength (Goldberg and Brown 1969).

The phase measurements do, of course, include a 360° phase ambiguity. In plotting the data, we assumed that the phase continued to roll off towards higher frequencies, adding 360° to the relative phase values at each zero-phase crossing. As we used small frequency steps, there was rarely any uncertainty with regard to the degree of phase roll-off.

All experiments were carried out with the animal enclosed in an acoustically – and electrically – shielded chamber and maintained at a (rectal) temperature of 30 ± 1 °C.

Results

We report the phase-frequency response characteristics of 77 primary afferent fibres from 13 animals. In fibres tested with both pulsed and continuous tones, no differences were found (Fig. 1). In the few cells tested for the effect of intensity over a wide range of frequencies (at CF, one octave below and one octave above CF) and sound pressure levels (up to 60 dB), two showed no phase changes greater than 20° over the range tested, two other cases were similarly stable, except that at the highest intensity tested, they showed a large phase jump of about 150°. Thus we assume that, for the SPLs normally used (almost all within 10 and 20 dB above threshold at each frequency), intensity has no important influence on the measured phase angles. In Fig. 2 are shown the mean levels above threshold used at different frequencies for the low- and high-CF cell groups.

Sample phase histograms from 4 cells, 2 from the low-CF and 2 from the high-CF groups are shown in Fig. 3. For each CF group, one fibre shows single, the other double peaks in the histograms. Many cells showed such double peaks (approx. 180° apart, see Fig. 3) in the phase histograms, although the tendency to show such peaks was always stronger at lower stimulation frequencies. Of the 33 fibres analyzed with respect to the number of peaks in the phase histograms and measured over a wide frequency range, 14 were low-CF fibres. Of these low-CF fibres, 13 (93%) showed essentially single-peaked histograms. Of the 19 high-CF fibres analyzed in this way, however, only 4 (21%) had single-peaked histograms, whereas 15 (79%) had double-peaked histograms over the lower part of the frequency range tested.

Single auditory-nerve fibres showed phase locking up to a maximal frequency near 1 kHz. The vector strength (VS) of the phase histograms varied between cells and with frequency (Fig. 4). The quality of phase locking differs for fibres innervating the low-CF (below CF 0.85 kHz; Fig. 4a) and high-CF hair-cell populations (Fig. 4b). Low-CF fibres generally phase-lock well up to 0.3 kHz (Fig. 4a), above which the VS falls slowly. Above about 0.7 kHz, the slope becomes much steeper. High-CF fibres show greater variability in VS, especially below 0.3 kHz (Fig. 4b), where strong double-peaking of the histograms is evident in almost all cases and the

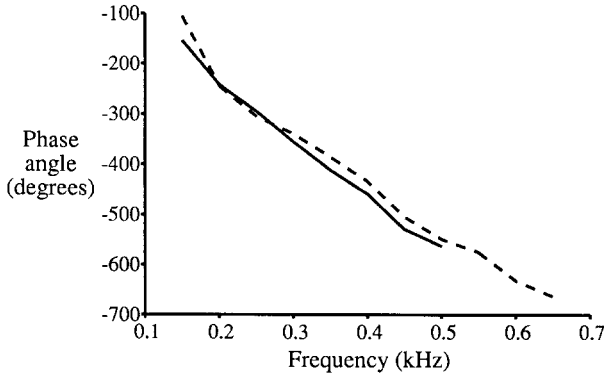

Fig. 1. Phase-frequency data for one fibre tested with both continuous (continuous line) and with pulsed (dashed line) tones

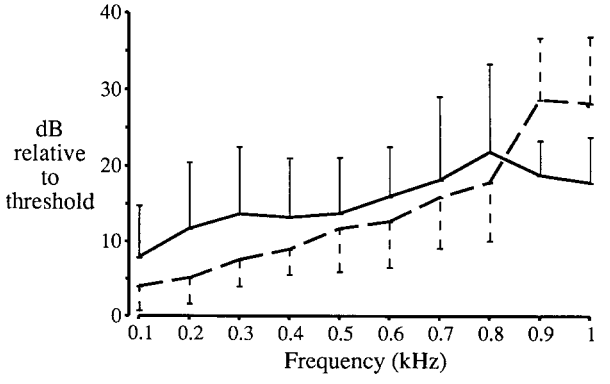

Fig. 2. Mean levels above threshold used for measuring phase data for the low- (continuous line) and high-CF (dashed line) populations of nerve fibres, each shown with one standard deviation

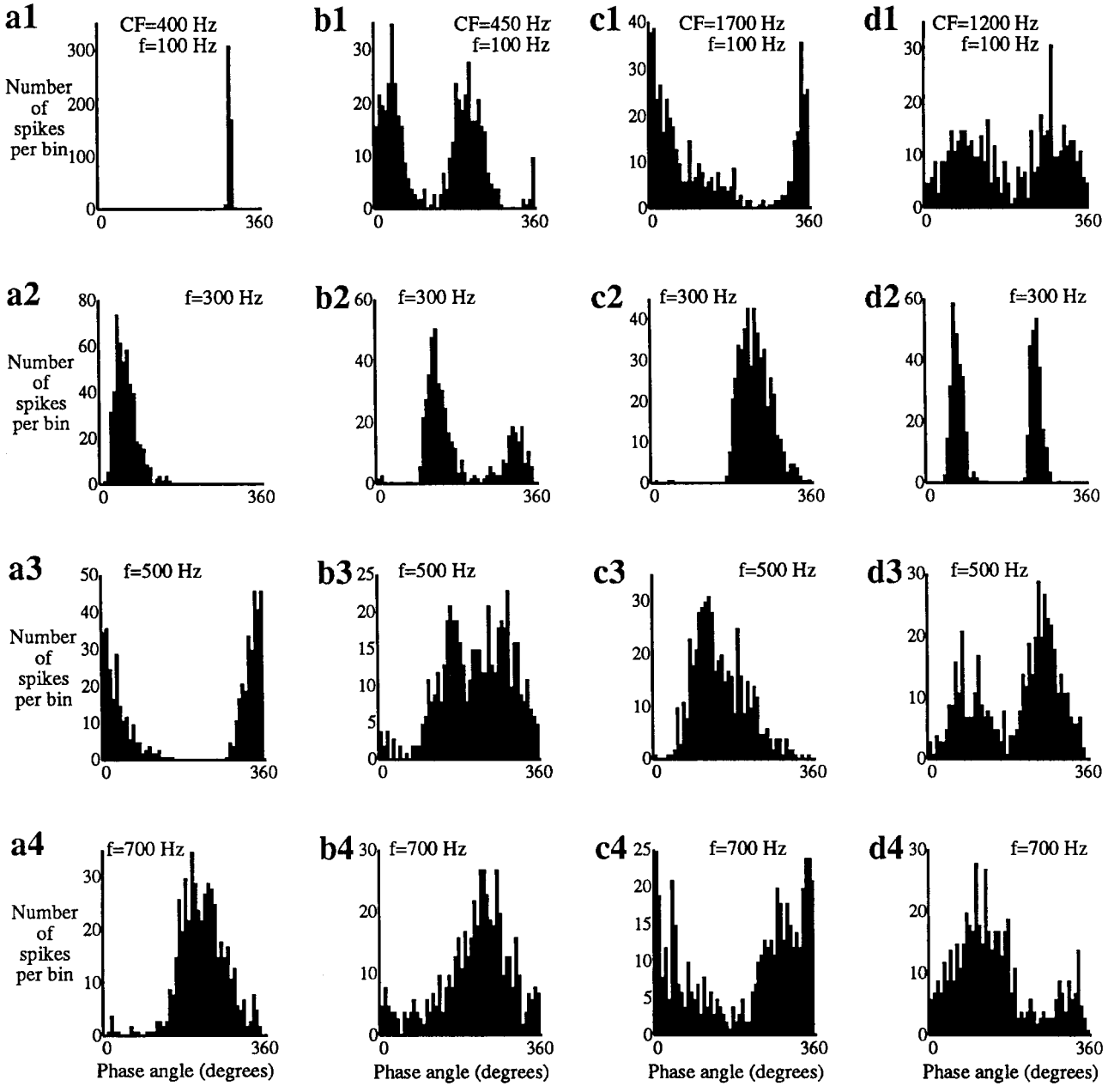

Fig. 3a–d. Cycle histograms of phase-locked responses of four nerve fibres in the bobtail lizard, each showing responses at 4 frequencies (1 = 0.1 kHz, 2 = 0.3 kHz, 3 = 0.5 kHz, 4 = 0.7 kHz). (**a**) a fibre with CF 0.4 kHz and only one peak; (**b**) a fibre of CF 0.45 kHz and showing 2 peaks, at the lower frequencies; (**c**) a fibre of CF 1.7 kHz and a single peak; (**d**) a fibre with CF 1.2 kHz and double peaks. Each histogram represents distribution of 500 action potentials over the 360° (= 50 bins) of a stimulus cycle. The inter-peak distances are: b1, 180°; b2, 173°; b3, 115°; d1, 202°, d2, 184°, d3, 173° and d4, 202°. The histogram trigger was derived from the 'synch' output of the frequency synthesizer

fundamental component of the responses is smaller than the second harmonic. The quality of phase locking for high-CF fibres falls more rapidly above 0.3 kHz than it does over this range for the low-CF fibres, even though the histograms are becoming more single-peaked. Instead, the slope of the VS function for high-CF fibres above 0.3 kHz is more similar to that of low-CF fibres above 0.7 kHz. Thus, in general, the low-CF fibres show somewhat better phase locking, even at frequencies on the high-frequency flank of their tuning curves where they are not more sensitive than high-CF fibres. In Fig. 5, the data for low- and high-CF fibres have been averaged and plotted together to emphasize the differences between them. The corner frequency for each of these averaged curves is that frequency for which each curve reached $1/\sqrt{2}$ of its maximum value (Weiss and Rose 1988b). For low-CF fibres, the corner frequency is 0.73 kHz, for high-CF fibres, it is 0.51 kHz. Differences also exist between low-CF and high-CF cells with regard to the amplitudes of the second-harmonic component of the responses, which, in low-CF cells, was always smaller than the component at the fundamental. In high-

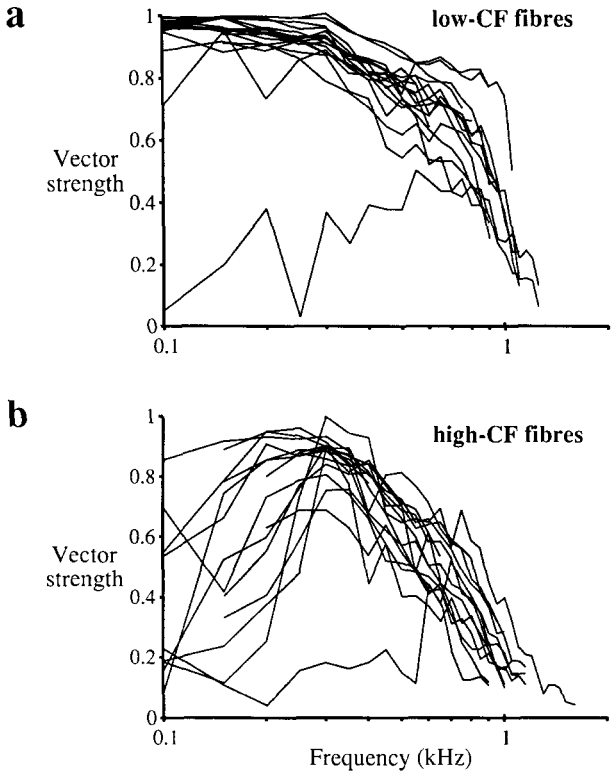

Fig. 4a, b. Vector strength of the cycle histograms over the individually-measureable frequency range for (**a**) low-CF and (**b**) high-CF fibres. The two fibres with very low VS also showed the strongest double-peaking in the phase histograms. They were not included in the cluster analysis (see text)

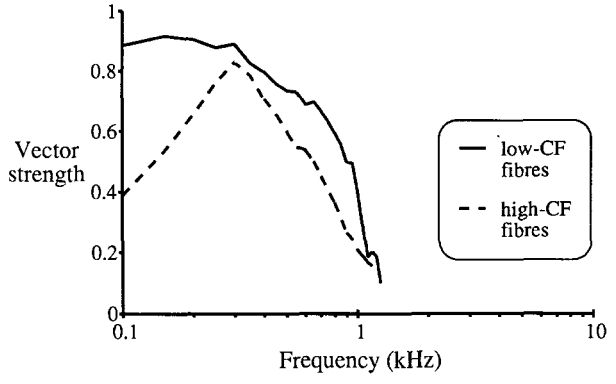

Fig. 5. Averaged data from Fig. 4 of the vector strength of the cycle histograms, to compare the curves as a function of frequency for low-CF (continuous line) and high-CF (dashed line) fibres. Each average value represents the mean of the vector strengths available for all fibres at each frequency

CF cells, in contrast, although the second-harmonic component in the responses was also generally smaller than the fundamental, it was actually larger at frequencies of 200 Hz and below.

The VS data for the two cell populations was compared quantitatively using two measures derived from the VS functions of the phase histograms as shown in Fig. 4. In Fig. 6, the slope of the VS functions is com-

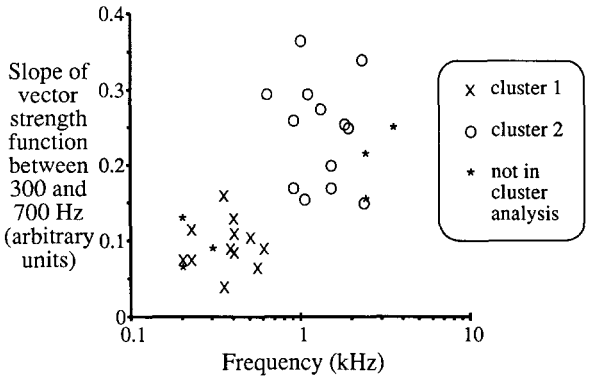

Fig. 6. Slope of the vector strength functions between 0.3 and 0.7 kHz for 31 fibres, as measured from the curves shown in Fig. 4 (using linear approximations to the curve regions in this frequency range), as a function of cell CF. Circle and cross symbols denote the assignment of individual fibres to different clusters (see text). Cells denoted by a star had insufficient data for inclusion in the cluster analysis

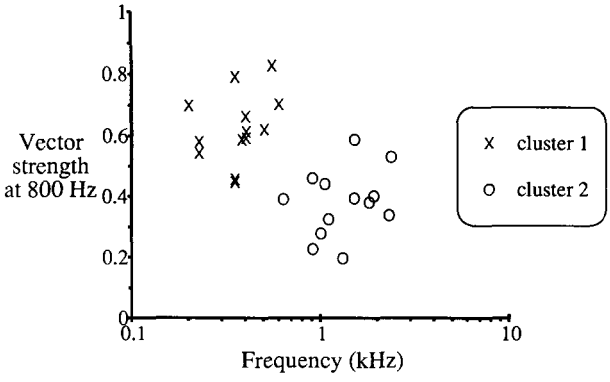

Fig. 7. Value at 0.8 kHz of the vector strength for 26 fibres as measured from the curves of Fig. 4, as a function of cell CF. Circle and cross symbols denote the assignment of individual fibres to different clusters (see text)

pared for the frequency range 0.3 to 0.7 kHz. In Fig. 7, we compare the VS at 0.8 kHz. The data of these two figures were subjected to a statistical cluster analysis (SPSS/PC+; Norusis 1986), using the values for CF, slope of the VS functions between 0.3 and 0.7 kHz and the VS at 0.8 kHz. Two clusters describe the distributions best: the different symbols used in the figures indicate the clusters derived from the statistical analysis. The division into two clusters in Figs. 6 and 7 corresponds closely to the CFs of the fibre groups established on other grounds to originate in the two anatomically-defined papillar regions (Köppl and Manley 1990a).

We investigated the response delays in the phase-locked spikes of units of different characteristic frequency (CF) by measuring the slopes of the individual phase-frequency functions. In the bobtail lizard, the slope of the phase roll-off was not always constant, tending to flatten off slightly at higher frequencies in some cells measured over a wide frequency range (Fig. 8a). In order to analyze the data in a way comparable to

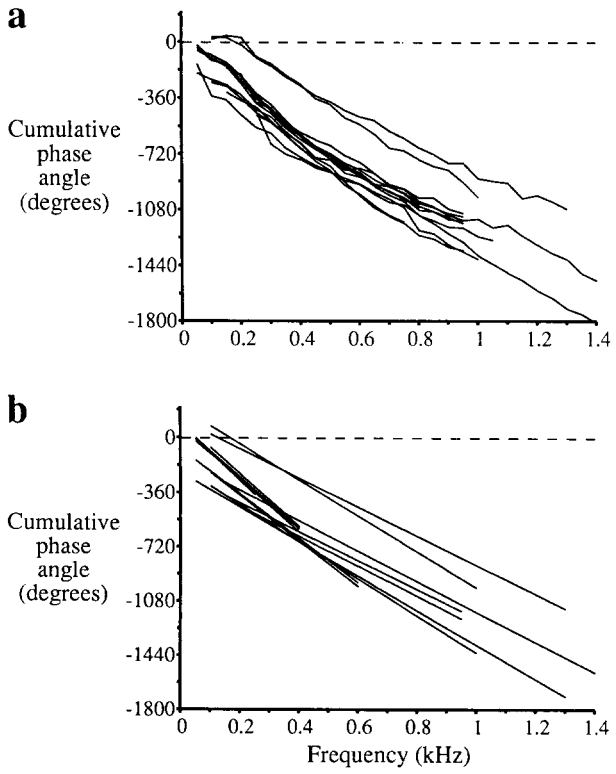

Fig. 8. (**a**) Cumulative phase angle of fibre responses as a function of frequency for cells of different CF in one individual animal. (**b**) Simple regressions for the data in (**a**), using only data up to the individual cell's CF for low-CF cells

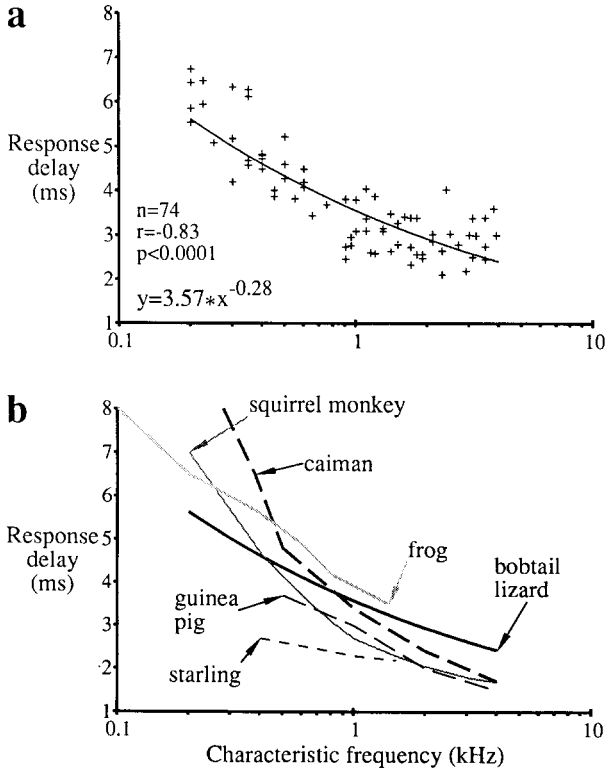

Fig. 9. (**a**) Response delays of fibres calculated from the cumulative phase slopes as a function of CF. The solid line represents the least-squares power fit for the data. The corresponding formula and the statistical values are given. (**b**) Response delays of auditory-nerve fibres for different species of vertebrates. The data for the bobtail lizard are represented by a least-squares power fit as in (**a**): This diagram also shows a simplified representation of data from other vertebrate species, as found for the squirrel monkey (Anderson et al. 1971), for the guinea pig (Palmer and Russell 1986), for the starling (Gleich and Narins 1988), for the caiman (Smolders and Klinke 1986) and for the coqui frog (Hillery and Narins 1984)

publications on other species, we measured the phase-frequency slopes assuming they were adequately approximated by a straight line. The decision on a criterion for the frequency ranges whose slope was to be compared for cells of widely-differing CF was influenced by the fact that it was not possible to derive phase-locked responses for most high-CF fibres near or above their CF. The response delay was thus measured in all cases as the slope of the simple linear regression of the phase-frequency characteristic for frequencies up to the fibre's CF or, in the case of the highest-CF fibres, as far as phase locking was measurable (Fig. 8b).

In Fig. 8, the data have been corrected for the characteristics of the acoustical system, but not to allow for other known constant delays. These are the middle-ear delay (about 0.2 ms in this species; Manley et al. 1988a), synaptic delay (0.8 ms; estimate adopted from measurements of Palmer and Russell (1986) in the guinea pig at 37 °C, without temperature correction) and the travel time of the action potentials to the site of the electrode, estimated to be 0.4–0.2 ms at 10–20 m/s conduction velocity. These estimates are based on the average sizes of high-CF and low-CF fibres in other skinks (Miller 1985) and conduction velocities for myelinated nerve fibres (Rushton 1951; Sugimura et al. 1980). The slopes of Fig. 8b represent constant delays between the stimulus phase at the eardrum and the fibre responses. The response delays derived from these slopes were an inverse function of fibre CF: low-CF fibres had longer delays (near 6 ms) than did high-CF fibres (3 ms; Fig. 9a). The data are well fitted by a power function. A similar constellation of points was found even if phase data for above-CF frequencies for low-CF cells were included. We have included the power-function fit in Fig. 9 in order to compare the data to those of other species. If the actual distribution of data points in Fig. 9a is visually divided into the low- and high-frequency clusters suggested by the cluster analysis, however, it is obvious that the changes in delay within the low-CF cluster are dominant.

Gleich (1987) noted that the phase-frequency curves for many primary auditory fibres of the starling were not simple straight lines, but contained a nonlinear component systematically related to the fibre's CF. He carried out an analysis of the residual phase characteristic derived by subtracting a straight line (representing the constant delays) from the original curve. The residual was analyzed using an iterative procedure to best approximate an LRC filter phase curve with successive approximations of CF and $Q_{3\,dB}$ value. We carried out the same analysis for 37 phase-frequency curves of bob-

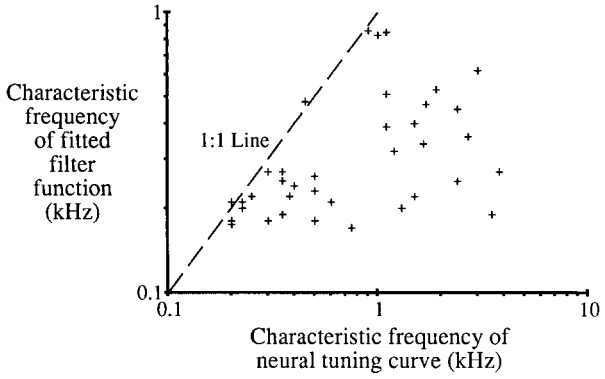

Fig. 10. Variation of the best frequency of the equivalent LRC filter calculated from the residual phase function (see text) as a function of fibre CF

tail afferents. The best frequency calculated for the equivalent LRC filter in each case did *not* vary systematically with the cell's CF in the same way as in the starling (Fig. 10). However, the data do fall into two groups. In a cluster analysis taking CF, slope of the VS function (0.3 to 0.7 kHz), VS at 0.8 kHz and the LRC filter best frequency (15 cases) into consideration, the data were still best described by the two clusters already established for Figs. 6 and 7.

Discussion

Species-specific frequency limits to phase locking

The phase-locking ability of single primary auditory-nerve fibres in the bobtail lizard, with a frequency limit near 1 kHz, strongly resembles that of alligator-lizard fibres (Rose and Weiss 1988). With respect to their phase-locking ability, the two lizard species are intermediate between amphibians on the one hand (Hillery and Narins 1984, 1987) and mammals (Anderson et al. 1971; Palmer and Russell 1986) and birds (Gleich and Narins 1988; Sullivan and Konishi 1984) on the other. Phase-locking data for the caiman are not easily comparable with the present data, as no frequency cut-off information is available (Smolders and Klinke 1986).

As discussed by Weiss and Rose (1988b) and Hillery and Narins (1984), the differences in phase-locking behaviour between auditory-nerve fibres of different vertebrates seems to be real and not, for example, only a function of body temperature. If we can assume that the main difference between the alligator-lizard afferents and the bobtail afferents was the temperature difference of 9 °C, then extrapolating all the data to a temperature of 38 °C based on the observed shift/°C in lizards (Eatock and Manley 1981) would bring the value of the corner frequency of the low-CF fibres near to the corner frequency of guinea-pig afferents (1.1 kHz). If this is the case, the differences in the corner frequencies would indicate that the thermal Q_{10} for the low-CF and high-CF groups (1.45 and 1.57, respectively) are only slightly smaller than calculated by Weiss and Rose (1988b) for the difference between the corner frequencies for guinea-pig and alligator-lizard low-CF fibres (1.1 kHz at 37 °C and 0.48 kHz at 21 °C, respectively, thermal $Q_{10} = 1.68$). It would not, however, produce corner frequencies of phase locking equivalent to those of the cat (2.5 kHz; Weiss and Rose 1988b) or the starling. There is as yet no satisfactory explanation for the great difference between guinea pig and cat in this regard.

A number of factors can lead to a limitation of the frequency range of phase locking, some of which influence the AC response of the hair cells to sound, others of which affect the synaptic transmission of time-locked responses and still others of which result from the electrical properties of the nerve-fibre membrane. Some of these factors have been discussed by Weiss and Rose (1988a). In the guinea pig, Palmer and Russell (1986) proposed that the frequency cut-off of the AC component of the hair-cell responses is sufficient to explain the cut-off in auditory-nerve fibres. Weiss and Rose (1988a), however, showed a difference between the hair-cell and the nerve-fibre data of Palmer and Russell, such that the AC amplitude roll-off is slower in hair-cell AC responses. In the alligator lizard, the slope of the cut-off of hair-cell AC responses (20 dB/octave) is much lower than that of the nerve fibres (>80 dB/octave; Weiss and Rose 1988a). Thus at least in the guinea pig and the alligator lizard, it is necessary to postulate additional filtering stages between the hair-cell electrical activity and the response of the nerve fibres. The fact that the hair-cell AC component falls off more slowly towards higher frequencies may implicate the synapse as the main element limiting normal phase locking of fibres (Weiss and Rose 1988a). A good deal of the reduction in the precision of the time-locked responses can presumably be traced to the stochastic nature of transmitter release.

In cats, direct electrical stimulation of auditory-nerve fibres with sinusoidal stimuli yields better synchronization of action-potential firing than does acoustic stimulation via hair cells. With direct electrical stimulation, the synchronization index is better than the average acoustical fibre synchrony (see Johnson 1980) and decreases only by 6 dB/octave above 4 kHz (Dynes and Delgutte 1989). Thus in the cat, neither the following ability of the nerve fibres nor the 'jitter' of their response latency to constant stimuli can explain the fall-off in phase locking to normal acoustic stimuli. The question in the lizards, of course, is not only why nerve-fibre phase locking falls off more rapidly than that of the hair cells, but why the low- and high-CF populations differ in this respect.

The existence of two groups of nerve fibres

In both the alligator lizard and the bobtail lizard, the averaged responses of low- and high-CF fibre groups differ. Similarly, in the tokay gecko, Eatock et al. (1981) reported that low-CF fibres phase-lock better than higher-CF fibres. In the alligator lizard, the corner frequencies for low- and high-CF fibre groups were 0.48 and 0.34 kHz, respectively. The fact that the corresponding

values of 0.73 and 0.51 kHz in the bobtail-lizard fibres are higher is most probably due entirely to the 9 °C temperature difference between the two experimental series (21 and 30 °C; see above).

The two groups of fibres derived from the cluster analysis of the phase data in the bobtail lizard resemble those suggested for the equivalent populations of fibres in the alligator lizard (Rose and Weiss 1988). Rose and Weiss' suggestion of the existence of two groups of fibres in alligator-lizard afferents was, however, weakened by the fact that their data analysis for CFs in the range from 0.35 to 1.1 kHz was based on only two fibres. Our analysis covers more systematically the available range of CFs in the bobtail lizard, and indicates that indeed the fibre behaviour does not form a continuum. A low-CF population with better phase locking exists and corresponds to the low-CF population defined on other physiological grounds (Köppl and Manley 1990a, b; Manley et al. 1990).

It may, however, be inappropriate to interpret the existence of these two populations in the bobtail and alligator lizard purely in terms of their phase-locking behaviour and thus their ability to encode time information in on-going signals. The behaviour of nerve fibres belonging to these two groups in both species differs in quite a number of respects. In addition to differences in phase-locking behaviour, fibres of the low-CF population in the bobtail lizard are, on average, less well tuned and have differently-shaped tuning curves. They also have different patterns of tone-evoked discharge to those of the high-CF population and show two-tone rate suppression (Köppl and Manley 1990b; Manley et al. 1990). There is thus no a priori reason for supposing that any one of these features would be more important in determining the evolutionary origin of two fibre populations in lizards or their retention over the long period of evolutionary history.

The question as to why there are two fibre populations defined in terms of their phase-response behaviour can also be approached from the point of view of the underlying structural and physiological mechanisms. This question is on a quite different level to the question of the evolutionary origin or 'usefulness' of the response types. The difference between the two fibre populations in their ability to encode the AC component of hair-cell responses may be traceable to properties of the afferent terminals and/or their innervation patterns.

As suggested by Mulroy (1986), the phase-locking ability of fibres may be related to the number of synapses they make on one or many hair cells. In the alligator lizard, single low-CF fibres make, one average, 3 to 4 times as many synapses on a hair cell as do high-CF fibres (Mulroy 1986). In the skink *Mabuya* (same family as the bobtail lizard), low-CF hair cells have 1.2 times as many afferent synapses as high-CF hair cells (Miller and Beck 1988). Indeed, in all 6 lizard families investigated by Miller and Beck (1988), hair cells of the low-CF area formed a larger number of synapses with larger-diameter fibres than hair cells of the high-CF areas. A fibre which maintains a greater number of synaptic endings may be better able to preserve the time-locked information in the transmitter output. This suggestion is in accordance with the idea that the synaptic properties are responsible for the difference between the hair-cell potential and the nerve fibre in terms of their phase-coding abilities. It is also consistent with the finding that, in general, low-CF fibres of lizards tend to have more 'primary-like' discharge patterns in their PSTH (Köppl and Manley 1990b; Manley 1990) except at higher frequencies. The fact that the slope of the VS function in low-CF cells becomes very much steeper at frequencies above about 0.7 kHz is related to their increasingly phasic response pattern at these frequencies (Köppl and Manley 1990b).

This argument concerning the number of synapses is, however, not completely conclusive. While it is true that on average, low-CF hair cells make more synapses with afferent fibres than high-CF hair cells, these numbers say little about the number of synapses per afferent fibre. In skinks no single fibre has been followed to all its hair cells and the total number of its synapses counted (Miller and Beck 1988). Although we have a good estimate of the average number of hair cells contacted by low-CF and high-CF fibres in the bobtail lizard (there is no difference; Köppl and Manley 1990a), we have no information on the number of synapses per fibre. It is of course possible that the synapses of the two hair-cell populations vary in their effectiveness.

There is also the possibility that in lizards, low-CF hair cells utilize an electrical tuning mechanism in addition to any mechanical tuning, whereas high-CF hair cells may have only mechanical tuning (Manley 1990). Thus, all other factors being equal, the potentials produced at the presynaptic cell membrane for a given frequency would differ in these two cell types. Our analysis of residual phase components of the phase-frequency curves of the bobtail lizard did not produce any evidence of tuning such as that found in the starling (Gleich 1987). However, it is very likely that nerve fibre activity can reflect electrical tuning (e.g. in preferred intervals of the spontaneous activity or in the residual phase curves) only if there is exclusive innervation (for discussion see Köppl and Manley 1990b). In birds, we have shown that primary fibres which respond to sound almost all innervate only one tall hair cell, that is, show exclusive innervation (Gleich 1989; Manley et al. 1987, 1989b). As outlined above, the innervation pattern in the bobtail lizard is non-exclusive (Köppl and Manley 1990a). Thus it is not surprising that the residual-phase analysis in the bobtail lizard produced quite different results to those of the starling. The phase data are, however, consistent with the division into two groups based on other physiological response characteristics, whose CFs correspond to the low- and high-CF fibre groups.

When we take both the phase-response data and the patterns of the peri-stimulus-time histograms (PSTH) to pure tones together, however, it becomes obvious that the two groups of fibres differ strongly with respect to the preservation of timing information in their discharge pattern. As we discuss in a companion paper (Köppl and Manley 1990b), there are remarkable parallels between the discharge patterns of low- and high-CF fibres

and the 'primary-like' and 'chopper' types, respectively, of cochlear-nucleus cells of mammals. The behaviour of most higher-CF cells can be well explained in terms of the same neuronal membrane mechanisms as those responsible for the particular activity patterns of mammalian cochlear-nucleus cells. In particular, the chopping behaviour is traced to the presence of a long membrane time constant in the cochlear-nuclear cell (Oertel 1985). If low- and high-CF primary auditory-nerve fibres of lizards not only differ in their anatomy (Miller 1985; Miller and Beck 1988), but also in their membrane properties, then a longer time constant in the membrane of high-CF fibres could explain both the chopper discharge patterns of higher-CF fibres and their poorer phase locking. This is also compatible with the exact match between the VS cut-off slopes towards higher frequencies for the fundamental and the second-harmonic responses in high-CF cells in this study. In contrast, these cut-off slopes for low-CF cells differ more.

It is interesting to note that in all lizards so far investigated, the average diameter of afferent fibres innervating unidirectional-type hair cells (low-CF) is much greater than of those innervating bidirectional-type hair cells (high-CF; Miller 1985; Miller and Beck 1988). Whether fibre size is related to membrane properties in such a way as to explain differing time constants, however, can only be decided on the basis of information on the electrical properties of the dendritic arbors of these fibres.

It might be argued that the fact that most high-CF fibres in the bobtail lizard innervate hair cells of opposite polarity would contribute significantly to a reduced phase-locking ability to the fundamental of the stimulus frequency. Such fibres presumably receive a bombardment of transmitter with a significant component at double the stimulus frequency, resulting in the two-peaked histograms (see below). However, the fact that the high-CF fibres of the alligator lizard show the same reduction of phase-following in spite of their mainly innervating only a single hair cell (Mulroy 1986) would suggest that this factor does not play a major role. At low frequencies in bobtail high-CF cells, the VS of the fundamental is exceptionally small, but that of the second harmonic is larger. At low frequencies in low-CF cells, both the fundamental and the second-harmonic component are large, even though low-CF cells rarely show double-peaking. Although it is not possible to exclude an adaptation phenomenon contributing to the low-frequency slope of high-CF cells, the slope varied greatly between cells in a way unrelated to CF. In addition, the fibres were, on average, only stimulated 4 to 7 dB above threshold, due to their relatively high absolute thresholds in this frequency range.

The ability of lizard nerve fibres to code the timing information of a signal is correlated with the amount of space devoted to that frequency range on the hearing organ: frequencies which are too high to be coded in the time domain (timing of nerve-fibre discharge) must be coded primarily or purely in terms of place (for a discussion see Köppl and Manley 1990a; Manley et al. 1988b; Manley 1990).

Double peaks in phase histograms

The presence of fibre responses showing two peaks in the histogram, roughly 180° apart, can be due to two factors. Firstly, the phenomenon of 'peak splitting' is known from other species (e.g. Johnson 1980). In this case, double peaks have been interpreted as indicating a large second harmonic component in the hair-cell responses. However, in mammals, this is a phenomenon seen at high sound pressures. In the bobtail lizard, in addition, the second harmonic component is in fact larger in low-CF fibres, which very rarely produce double peaks, than it is in high-CF fibres. Perhaps the most important feature is that within any one fibre population, fibres differ very strongly in the presence or absence of double peaks. Some fibres show only a single peak throughout their response range.

The second factor that may give rise to double peaks is that auditory afferents in the bobtail lizard frequently innervate hair cells of opposite polarity. In the basal segment of the bobtail papilla, neurally- and abneurally-located groups of hair cells have their stereovillar bundles oppositely oriented. Across the apical segment of the papilla, the orientation of the hair cells changes twice (Köppl 1988). The innervation patterns of afferent fibres indicates that all fibres innervate several to many hair cells. In the basal segment, they have a strong tendency to innervate hair cells *across* the papilla, which would mean contacting hair cells of opposite orientation. In the apical segment, fibre innervational areas are oriented *along* the papilla (Köppl and Manley 1990a). Here, there should be a much smaller tendency for afferents to innervate hair cells of opposite polarity. These innervational patterns are reflected in the phase-histogram data, in that a much higher percentage of high-CF fibres showed double-peaked histograms (78%) than did low-CF fibres (7%).

Slope of the phase-frequency characteristic

The delay-vs-CF patterns in the bobtail lizard strongly resemble published data for the squirrel monkey (Anderson et al. 1971), guinea pig (Palmer and Russell 1986), caiman (Smolders and Klinke 1986) and frogs (Hillery and Narins 1984, 1987; Fig. 9b). In mammals, these delays have been attributed largely to the delay time of a travelling wave on the basilar membrane (see Smolders and Klinke 1986, for discussion). In the coqui frog, the delays were, in the absence of a basilar membrane both in the amphibian and basilar papilla, attributed to a putative travelling wave in the tectorial membrane of the amphibian papilla (Hillery and Narins 1984, 1987). Pitchford and Ashmore (1987) calculated that, should this be the case, the velocity of a travelling wave in the tectorial membrane would be 10–20 cm/s, about an order of magnitude less than that of the mammalian basilar membrane.

We believe it is more than a coincidence that the delays are so similar in all these highly diverse organs, despite their very different physical dimensions and

structure. We also believe that the presence of a mechanical travelling wave is an unnecessary postulate to explain the equivalence in the behaviour of auditory-nerve fibres in the different species. Firstly, there is no travelling wave on the basilar membrane of the bobtail lizard (Manley et al. 1988a, 1989a). Secondly, the discontinuous structure of the tectorial membrane in this species (Köppl 1988) makes it highly unlikely that it would support a travelling wave. The tectorial structures of the basal and apical segments are not connected to each other and are extremely different in their mass.

Evidence is accumulating that the relatively uniform delay patterns shown in all vertebrate groups are manifestations of delays through similarly-tuned (hair-cell) filter banks. The time delays of electrical resonators of hair cells can explain the phase delays of frog nerve fibres (Pitchford and Ashmore 1987). Similarly, in the caiman, Smolders and Klinke (1986) proposed additional filtering mechanisms subsequent to the basilar-membrane response. In the guinea pig, Gummer and Johnstone (1984) showed that a substantial part of the group delay of high-CF fibres can be accounted for by the sharp tuning of the frequency-threshold curve. The close parallels between the behaviour of the auditory-nerve fibres and the basilar membrane in mammals is presumably the result of the fact that the mechanical activity of hair cells in the organ of Corti also plays an important role in determining the characteristics of the basilar-membrane motion (Johnstone et al. 1986; Patuzzi and Robertson 1988; Yates 1986). Thus, the travelling wave in mammals is at least partly a secondary phenomenon. It is obviously not necessary to assume its existence to explain the phase-delay characteristics of auditory-nerve fibres in species where normal basilar-membrane motion is not strongly dependent on the physiological integrity of the hair cells.

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