Hearing Research, 24 (1986) 1–15
Elsevier

HRR 00805

Phase-locking in the cochlear nerve of the guinea-pig and its relation to the receptor potential of inner hair-cells

A.R. Palmer* and I.J. Russell

Laboratory of Experimental Psychology and MRC Research Group in Neurobiology, University of Sussex, Falmer, Brighton BN1 9QG, England

(Received 2 December 1985; accepted 21 March 1986)

The high-frequency limit of phase-locking has been measured in fibres of the auditory nerve in the guinea-pig. It is shown that phase-locking begins to decline at about 600 Hz and is no longer detectable above 3.5 kHz which is about 1 octave lower than in the cat, squirrel monkey and some birds. Direct measurements of the cochlear afferent fibre synaptic delay are consistent with indirect estimates from phase-locking, both giving values of 0.7–0.8 ms. Measurements of the receptor potentials of inner hair-cells in the guinea pig cochlea indicate that as the stimulus frequency is increased there is a progressive decrease in the a.c. component compared to the steady depolarization. The cause of this decline is the low-pass filtering of the a.c. component by the hair-cell membrane. The cut-off and slope of the decline in the a.c. component is consistent with the suggestion that this process is the limiting factor in cochlear nerve fibre phase-locking. The implications of these findings for interspecies variation in phase-locking cut-off, for cochlear mechanisms and for the encoding of complex sounds are discussed.

cochlear nerve, phase-locking, hair-cells, receptor potentials

Introduction

In response to low-frequency sinusoidal stimuli the discharges in fibres of the auditory nerve do not occur randomly in time. Because action potentials are elicited by unidirectional movements of the basilar membrane (Davis et al., 1950; Goldstein, 1968; Brugge et al., 1969), discharges occur within a well defined time window relative to a single cycle of the sinusoid. This occurrence of action potentials at preferred times has been termed phase-locking of the discharge (Rose et al., 1967). Phase-locking occurs in auditory neurones of all vertebrate classes; squirrel monkey (Rose et al., 1967; Geisler et al., 1974), cat (Galambos and Davis, 1943; Rupert et al., 1963; Kiang et al., 1965; Pfeiffer and Molnar, 1970; Kim and Molnar, 1979; Evans, 1980; Johnson, 1980), chinchilla (Woolf et al., 1981; Oshima and Strelioff, 1983; Ruggero and Rich, 1983), guinea-pig (Tasaki, 1954; Harrison and Evans, 1979), birds (Sachs et al., 1974, 1980; Sullivan and Konishi, 1984), crocodile (Klinke and Pause, 1980; Smolders and Klinke, 1985), turtle (Crawford and Fettiplace, 1980), frog (Narins and Hillery, 1983) and fish (Fay, 1978).

Phase-locking in response to low-frequencies can be observed in all auditory nerve fibres irrespective of their best frequency. However, the ability of the whole ensemble of auditory nerve fibres to phase-lock decreases with stimulus frequency. Interestingly there are large differences between species in the frequency range over which phase-locking is observed. Thus in cat, squirrel monkey, blackbird and pigeon phase-locking is constant up to about 2 kHz and then declines so that it is no longer detectable at 5–6 kHz (Rose et al., 1967; Kiang et al., 1965; Kim and Molnar, 1979; Johnson, 1980; Sachs et al., 1974, 1980). The owl appears to be rather exceptional in this respect since good phase-locking is observed up to 9 kHz (Sullivan and Konishi, 1984). In poikilotherms the frequency range of phase-lock-

* *Current address*: MRC Institute of Hearing Research, University of Nottingham, University Park, Nottingham NG7 2RD, England.

ing is more restricted than in mammals and birds, being limited to frequencies below 0.9 kHz in the frog (Narins and Hillery, 1983) and 1.5 kHz in the crocodile (Klinke and Pause, 1980), in fish phase-locking is observed up to at least 1 kHz (Fay, 1978). Rodents such as the guinea-pig and chinchilla occupy an intermediate position with the decline beginning at below 1 kHz with no phase-locking detectable at 3.5 kHz (Harrison and Evans, 1979; Woolf et al., 1981).

Our interest in phase-locking is two-fold; first, phase-locking provides an indirect means of studying cochlear function (Anderson et al., 1971; Gibson et al., 1977) and second, phase-locking has been suggested as a means of encoding the frequency position and amplitude of the formant peaks of speech sounds (Young and Sachs, 1979; Palmer et al., 1986). Thus differences in phase-locking between species may affect their ability to utilize temporal coding of complex sounds and may provide us with clues concerning the transduction process.

A number of factors such as temporal jitter (Anderson et al., 1971), temperature (Narins and Hillery, 1983) and filtering by the hair-cell membrane (Sellick and Russell, 1980) have been suggested to affect, or to be responsible for, the high-frequency limit of phase-locking. In the present experiments we have addressed the question of whether the time constant of the hair-cell membrane is indeed the limiting factor in phase-locking.

In order to test this hypothesis we have made extensive detailed measurements of phase-locking in guinea-pig cochlear nerve fibres and have directly compared these data with intracellular receptor potentials from the same species under the same anaesthetic conditions. This has enabled us to confirm the octave difference in the phase-locking limit between cat and guinea-pig and to demonstrate that the limits of phase-locking in guinea-pig are consistent with the suggestion that the limiting factor is the hair-cell time constant. These findings are discusssed in the context of cochlear transduction mechanisms and of the encoding of complex sounds.

Methods

Recordings were made from coloured guinea-pigs (200–450 g) anaesthetized by neuroleptanaesthesia (0.06 mg atropine sulphate s.c.; 30 mg/kg sodium pentobarbitone; 4 mg/kg droperidol; 1 mg/kg phenoperidine (Evans, 1979)). Phase-locking data were also collected from animals anaesthetized with urethane (20% solution 6 ml/kg) and these are described separately. The animals were tracheotomized and core temperature was maintained at 37°C.

Detailed methods for recording from cochlear nerve fibres may be found in Palmer et al. (1986) and details of the hair-cell recordings in Russell and Sellick (1983). The techniques employed to make intracellular recordings from inner hair-cells were also used to make recordings from afferent nerve terminals just before or just after penetrating an inner hair-cell.

The spontaneous rate, the minimum threshold and the most sensitive frequency (characteristic frequency, CF) of each cochlear nerve fibre were routinely determined. Phase-locking was then assessed from the responses to the last 40 ms (to avoid spikes locked to the stimulus onset) of a tone burst of 50 ms duration with 5 ms linear rise and fall presented at 5/s. A computer was used to set the intensity, using a digital attenuator, and the frequency of a free-running waveform synthesizer (Hewlett Packard 3314A). In a single analysis 11 frequencies were presented in 1/10 octave steps spanning a specified frequency. The first analyses were usually centred on the fibre CF with later analyses at higher or lower frequency if the fibre responses to such off-CF tones were sufficient. At each frequency the threshold was first determined automatically using the computer and digital attenuator with a PEST procedure (Taylor and Creelman, 1967). Phase-locking was then determined using 64 presentations of a fixed level tone. The sound system was flat $\pm$ 4 dB over the range from 50–10,000 Hz and thus the sound level was approximately constant at 80 dB SPL irrespective of frequency. For some experiments phase-locking as a function of level at fixed frequency was measured: the threshold was again determined at the outset and tones at a maximum of 7 levels in 10 dB steps were presented from 10

dB below threshold up to 90 dB SPL. The waveform synthesizer synchronization pulse was used to start the computer clock at 1 MHz and times of occurrence of spikes were recorded. Period histograms were constructed from the spike trains by wrapping around at the period of the stimulus waveform and these were saved on disk. Strength of phase-locking was estimated from the period histograms using the synchronization index (calculated by normalizing the vectorial sum of the histogram bins, each bin being assigned a vector angle based on its position within the cycle and an amplitude equal to the number of spikes in the bin; Goldberg and Brown, 1969) or by the coefficient of synchronization (the number of discharges in the most effective half cycle as a proportion of the total number of discharges; Rose et al., 1967).

Results

Phase-locking in cochlear nerve fibres

Fig. 1 shows the phase-locking data collected from two fibres in the guinea-pig auditory nerve. The fibre in Fig. 1A had a CF of 0.6 kHz while that of Fig. 1B had a CF of 3.5 kHz. The spontaneous discharge rates of the fibres were 3 and 25 spikes/s, respectively. Both fibres were from the

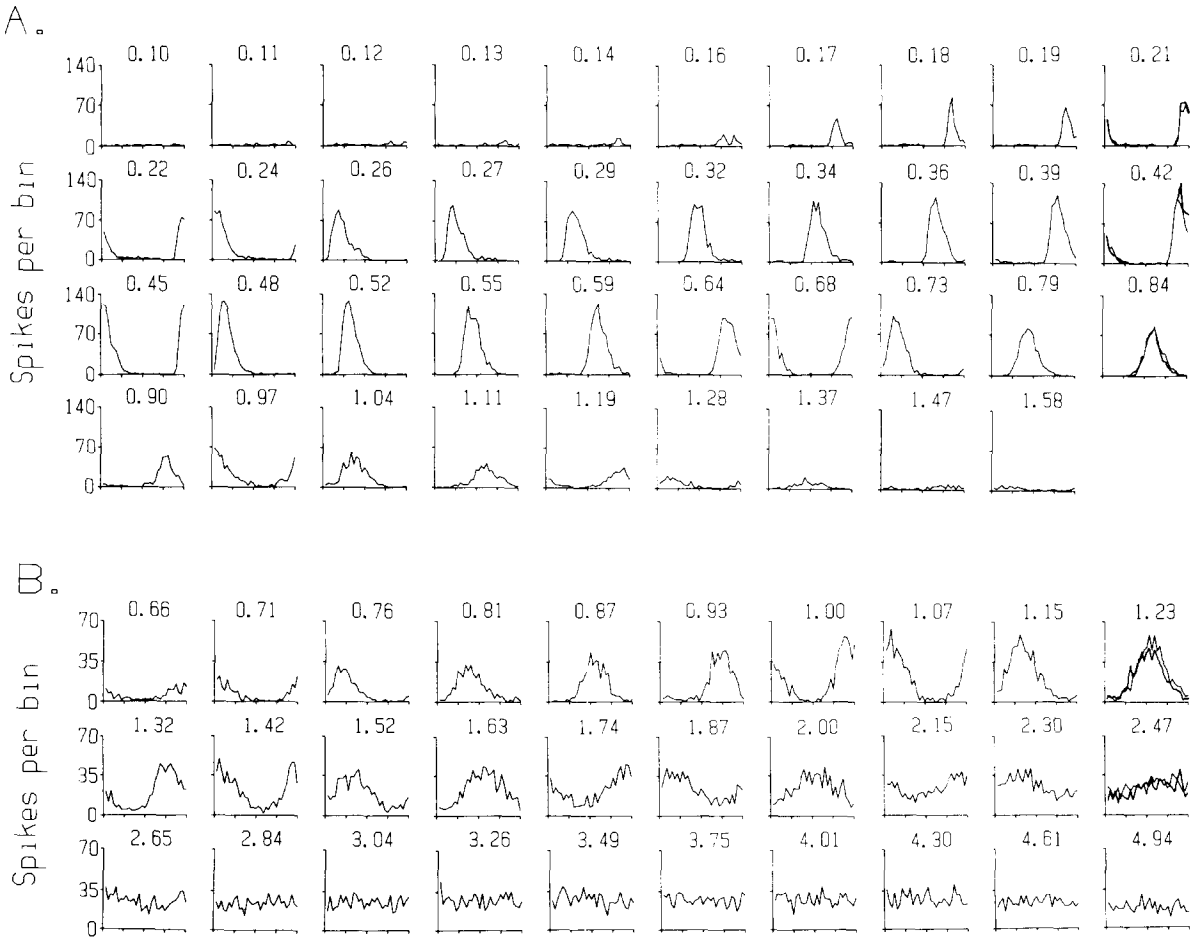

Fig. 1. Period histograms of the responses of two cochlear nerve fibres to sinusoidal stimulation. The numbers above the histograms indicate the stimulus frequency. The ordinate of each histogram is one cycle. (A) Cochlear nerve fibre with CF 0.6 kHz, (B) CF 3.5 kHz.

same electrode track recorded 0.42 mm and 80 min apart. The period histograms in Fig. 1 were collected for a range of tone frequencies at constant attenuation, which over this range of frequency corresponded to 80 dB SPL at the tympanic membrane. As will be seen later, this form of data collection takes no account of the tuning properties of the neurones or of the variation in phase-locking with stimulus level. As a result, the degree of modulation of the period histograms in Fig. 1 reduces, for both fibres, at the extremes of the frequency ranges tested. Thus the period histograms in response to frequencies 0.1–0.18 kHz in Fig. 1A and in response to 0.66–0.81 kHz in Fig. 1B show poor phase-locking which is a result of inadequate effective stimulus level because of the fibres' filtering properties. The data of Fig. 1A were collected in four overlapping series of frequencies and those of Fig. 1B in three series. The first frequency of a series was the same as the last of the previous series and the two histograms resulting from these analyses are plotted together at the 0.21, 0.42 and 0.84 frequencies in Fig. 1A and at 1.23 and 2.47 in Fig. 1B. The repeatability of the responses is in all cases excellent. The decrease in the degree of period histogram modulation at the higher frequencies in Fig. 1 has two causes. The major effect is due to the deterioration in the ability of cochlear nerve fibres in the guinea-pig to phase-lock to high-frequency tones. This is compounded in Fig. 1A, but not in Fig. 1B, by the filtering properties of the 600 Hz CF fibre.

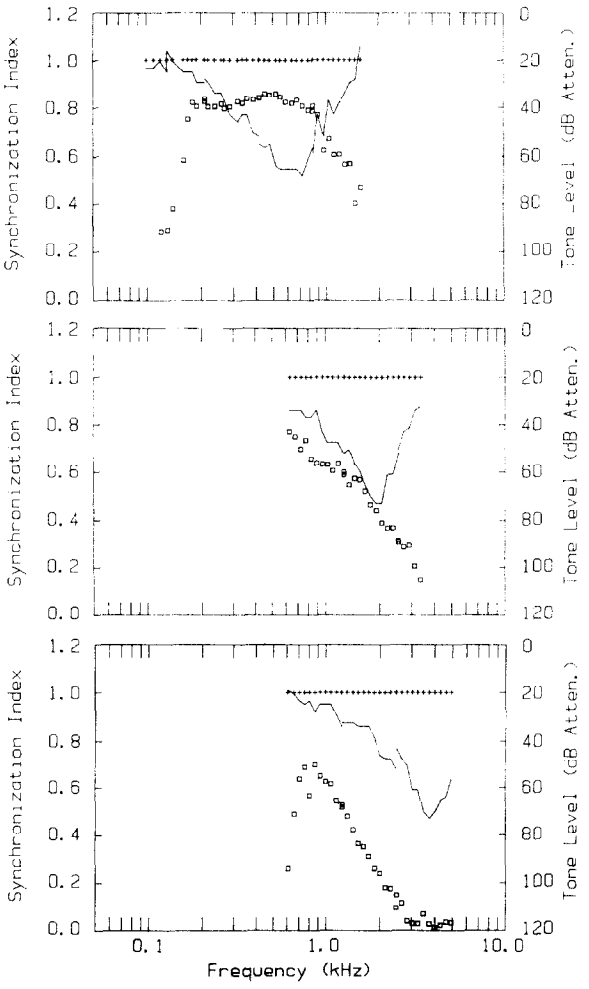

Fig. 2. Phase-locking in single cochlear nerve fibres quantified using the synchronization index (□; see Methods). The thresholds at each of the test frequencies are joined by the continuous lines which therefore represent the frequency threshold curve. The crosses indicate the frequencies and level relative to the fibre response area at which phase-locking was measured. Fibre CFs were 0.6, 2.0 and 3.5 kHz from top to bottom of the figure.

A quantitative measure of the degree of modulation of the period histogram is the synchronization index (see Methods) which we have computed mathematically from data such as those of Fig. 1. The synchronization indices for the fibres shown in Fig. 1 and for a fibre with intermediate CF are shown in Fig. 2. The continuous lines in this figure join the thresholds at each test frequency (see Methods) and therefore constitute the fibre frequency threshold or tuning curve. The crosses show the frequency and attenuation of the test tones used to measure the phase-locking. This figure clearly demonstrates that the falling off of phase-locking in Fig. 1 is the result of the filtering at low frequency. The fall-off of phase-locking at high frequency, however, except perhaps in the top fibre in Figs. 1 and 2 is not due to filtering. The decrease in phase-locking in all three fibres takes place over the same range of frequencies (approximately 0.6–3.0 kHz) and declines smoothly with increase in frequency even for the fibres of CF 2.0 and 3.5 kHz in which the increase in frequency results in the tones becoming more effective in stimulating the fibre, at least up to the CF frequency. The same cut-off was found in

fibres with CFs of 8–9 kHz. Our fixed level of 80 dB SPL was insufficient to make similar measurements in fibres from more basal cochlear positions. Even when the tones are presented at or below the mean rate threshold there is reasonably good phase-locking at least in high spontaneous rate fibres; a result which is well known from previous reports (Rose et al., 1967; Sachs et al., 1974; Johnson, 1980; Evans, 1980; Narins and Hillery, 1983). The PEST criterion used gave threshold as that intensity at which more spikes occurred in the stimulus epoch compared with a similar following epoch on 75% of trials. For fibres of zero spontaneous rate this difference was 1 spike per presentation and for high spontaneous rate fibres it was 2–3 spikes per presentation. The thresholds measured in the phase-locking program were therefore reasonably conservative estimates.

The degree of phase-locking also depends on the level of the stimulus with respect to the threshold of the neurone as has been demonstrated previously in the cat (e.g. Rose et al., 1967; Johnson, 1980; Evans, 1980). As shown in Fig. 3, for the guinea-pig, phase-locking increases with stimulus level from below threshold up to a saturation value, which is attained in every case at levels exceeding 20 dB above mean rate threshold. Fig. 3A shows data collected from a single fibre using tones of different frequency. The vertical lines show the frequencies and levels of the tone bursts used, the lowest of which was 10 dB below the automatically determined mean rate threshold (filled symbol). Fig. 3B shows pooled phase-locking versus level data for 41 fibres at a range of frequencies. Maximum values are obtained within 20 dB of threshold and further increases in level often cause a slight reduction in phase-locking. The vertical line indicates the level of the tone bursts used in the frequency sweeps.

Fig. 4 shows the maximum values obtained from the intensity functions of Fig. 3B, for all functions which extended at least 20 dB suprathreshold and only taking values for stimuli evoking at least 1 spike per two presentations. The

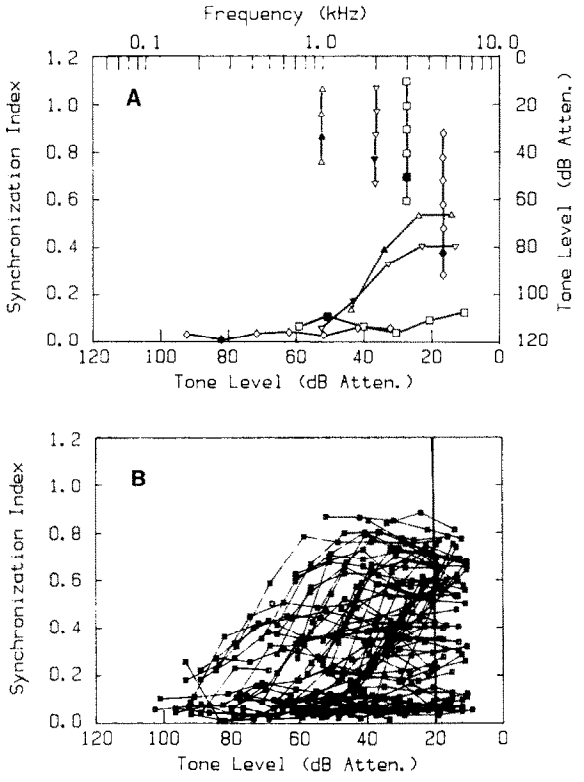

Fig. 3. Variations of phase-locking with stimulus intensity. (A) The vertical lines show the levels at which phase-locking was measured at the frequencies indicated by the top ordinate. The filled symbols show the mean rate thresholds determined by the PEST procedure. The same symbols are used to indicate the synchronization index as a function of the level shown by the bottom ordinate. Phase-locking can be measured below mean rate threshold and reaches a maximum within about 20 dB of threshold. (B) Phase-locking versus intensity for 41 fibres. The continuous vertical line shows the level at which phase-locking was measured as a function of frequency.

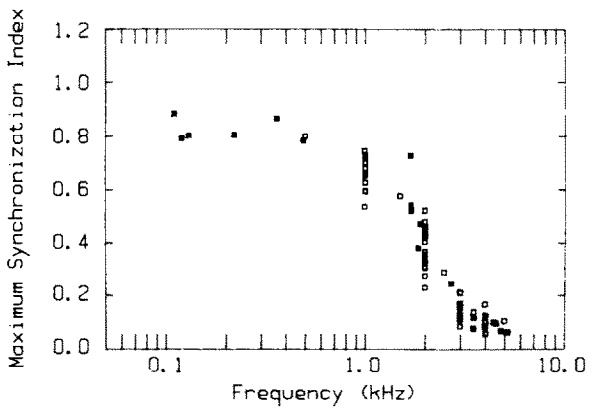

Fig. 4. Maximum synchronization index measured from the phase-locking versus intensity functions of Fig. 3 as a function of stimulus frequency. The filled symbols indicate intensity series at the fibres' CF.

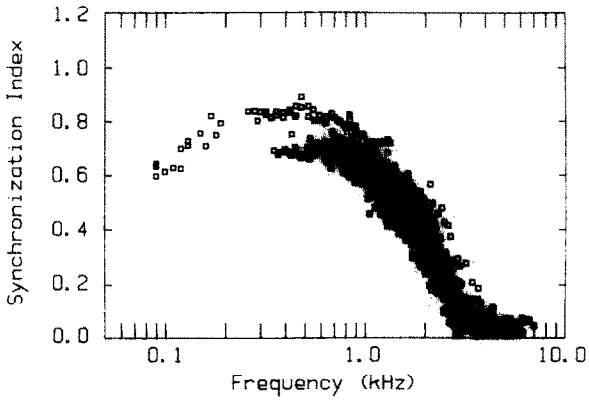

Fig. 5. Phase-locking as a function of frequency for data at least 20 dB above the mean rate threshold. The filled symbols are from animals anaesthetized with urethane and the open circles from animals anaesthetized with the neuroleptic technique.

filled symbols indicate intensity functions at CF. The somewhat isolated value at 1.7 kHz was obtained in this fibre at threshold and 10 dB higher with values at still higher levels only reaching a synchronization index of 0.6 which is more consistent with the other data. Taking the maxima only for those functions at 1, 2 and 3 kHz separately failed to reveal any strong relation of synchronization index with spontaneous rate.

In order to estimate near maximum values from our swept frequency data we have used only histograms obtained with stimuli 20 dB or more above the automatically determined mean rate threshold. As we have demonstrated, such thresholds are conservative estimates and are well above the threshold for detectable phase-locking. Fig. 5 shows the variation in phase-locking as a function of frequency, pooled across fibres and animals for tones more than 20 dB suprathreshold. It is clear from the comparison of Figs. 4 and 5 that this selection procedure does give near maximum values so that Fig. 5 may be used to estimate the phase-locking cut-off. Two different symbols used in Fig. 5 show the data obtained under urethane (filled squares) and neuroleptic anaesthesia (open squares). The urethane data appear to be displaced very slightly downwards at all frequencies. Plotting these guinea-pig data from the 0.6–3.0 kHz region on linear axes produces a remarkably good fit to a straight line (correlation coefficients for the two data sets were -0.948 and -0.934, respectively). Linear regression analyses allowed statistical comparison of the two sets of data and indicated that the slopes of the fall-off with frequency were not significantly different ($P < 0.05$), but that the intercepts were significantly different ($P > 0.05$). An estimate of the difference in cut-off frequency for the two sets of data is the horizontal displacement of the regression lines, which was 240 Hz. There was no difference in the distribution of mean rate thresholds at CF or in the spontaneous discharge rate between the urethane and neuroleptic data samples. The fall-off of phase-locking in the guinea-pig begins at 0.6 kHz and little phase-locking is evident beyond 3.5 kHz. This cut-off is well below that reported for the cat (e.g. Johnson, 1980) and about the same as that reported for the chinchilla (Woolf et al., 1981). In Fig. 6A our data, plotted as synchronization index, are compared directly with the cat data of Johnson (1980) plotted on the same axes. In this and Fig. 6B only our data obtained under the neuroleptic technique are shown for clarity. The cat data points (crosses) lie well above our guinea-pig data points at all frequencies above about 800 Hz. It is interesting to note in passing that the cat data of Johnson (1980), from 1–5 kHz, are also well fitted by a straight line on linear coordinates with a correlation coefficient of -0.944. The frequency at which the synchronization index fell to the arbitrarily chosen value of 0.5 was used to estimate the difference in cut-off of the cat and guinea-pig data. The cat data reached this value at 2.9 kHz compared to 1.53 kHz for the guinea-pig; a difference of 0.92 octaves. The only other quantitative data on phase-locking in the guinea-pig are those of Harrison and Evans (1979) which were presented in terms of the coefficient of synchronization. Our data quantified in this way are shown with those of Harrison and Evans in Fig. 6B. Despite very different methods of measurement it is clear that our data are entirely consistent with these other data from guinea-pig (Fig. 6B), are very similar to those from chinchilla (Woolf et al., 1981) and are very different from those of cat (Fig. 6A).

Johnson (1980) reported that at any frequency the fibres with lower spontaneous rates gave higher values of synchronization index. This was also

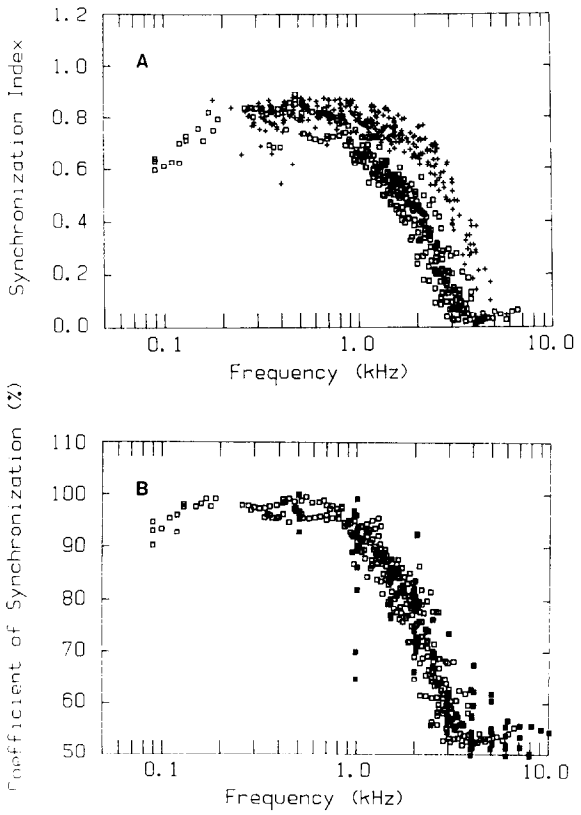

Fig. 6. (A) Phase-locking as a function of frequency for guinea-pig (○) and cat (+; Johnson, 1980). (B) Phase-locking as a function of frequency for guinea-pig quantified by the coefficient of synchronization (□) compared to previous measures for guinea-pig (■; Harrison and Evans, 1979).

observed during data collection, but pooling of data to a large extent swamped this effect. In an attempt to quantify the variation we made use of the linear regression and collapsed data from different frequencies by compensating for the slope. Plotting these corrected synchronization indices against fibre spontaneous rate did indicate a very slight tendency for phase-locking to decrease with increase in spontaneous rate.

Response phase

From Fig. 1 it can be seen that the central tendency of each period histogram (i.e. the response phase) changes progressively with stimulus frequency. The cumulative response phase angle computed from the period histograms is plotted for 9 fibres (pooled from different animals) in Fig.

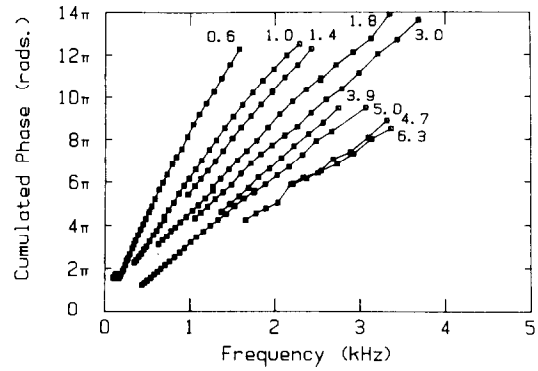

Fig. 7. Cumulative phase angle of the response to sinusoidal stimulation as a function of tone frequency for 9 fibres with CFs indicated by the parameters. The curves have been shifted vertically by multiples of one cycle so that they extrapolate to the origin.

7; each curve has been shifted by an integer number of cycles so that they extrapolate to the origin. The parameters on the curves indicate the fibre CF and a clear dependence of the slope of the phase versus frequency curve with CF is evident, as found previously for the cat and squirrel monkey (Pfeiffer and Molnar, 1970; Anderson et al., 1971; Geisler et al., 1974): the higher CF fibres show a slower accumulation of phase angle than do the low CF fibres. After removal of a phase slope from his cat data Allen (1983) revealed a lot of fine detail in the variation of phase angle with stimulus frequency. There are also detailed variations in the curves of Fig. 7, but in most cases the data are fitted by two (or more) line segments as in the cat data of Pfeiffer and Molnar (1980).

A linear phase frequency relation is indicative of a time delay which is independent of signal frequency. An estimate of this delay may be obtained from the slope of the phase-frequency curve by the relation:

$r = \Delta\theta / 2\pi\Delta f$ (see Anderson et al., 1971),

where r is the delay in seconds, $\Delta\theta$ is the phase difference in radians for a difference in frequency of Δf Hz.

The relationship of the delay to the CF has been used to form an estimate of the travel time of the propagating mechanical disturbance along the basilar membrane (Anderson et al., 1971; Gibson et al., 1977) and even as evidence of the presence

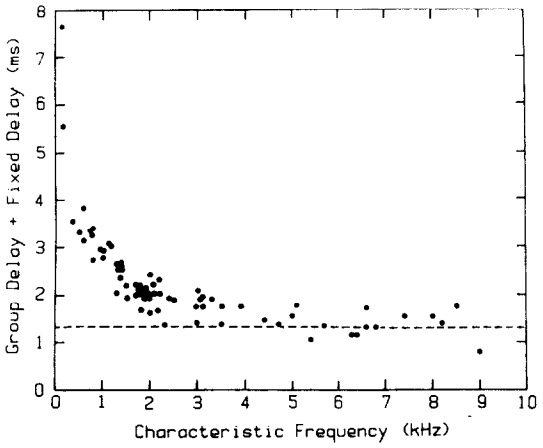

Fig. 8. Delay as a function of the CF of the fibres for the guinea-pig obtained by linear regression of curves such as those in Fig. 8. The dashed line indicates the fixed, frequency independent, delay (see text) estimated from the asymptote.

of a travelling wave motion (Hillery and Narins, 1984). However, as Smolders and Klinke (1986) pointed out and as will be clear from the data below, such conclusions may not be justified. Measurements of delay from our own data using a simple linear regression estimate of the slope were not different from estimates from the asymptote of the instantaneous slope (possibly because the first differentials were often very variable; see Ruggero (1980) for discussion). The delay associated with each fibre as a function of CF is shown in Fig. 8. These data are almost exactly coincident with those of Fig. 3 of Anderson et al. (1971) from squirrel monkey. At high frequency the asymptote gives an estimate of the component of the delay which is common to every fibre. This fixed delay consists of acoustic conduction time from the transducer to the eardrum, middle-ear conduction time, synaptic delay at their hair-cell base and conduction time in the auditory nerve to the recording site. Correction for this frequency independent delay gives an estimate of the group delay which presumably contains at least two components; the cochlear propagation time and the response times of the filters associated with each fibre (see detailed discussion in Smolders and Klinke, 1986; and also in Goldstein et al., 1971; Evans, 1975; Eggermont, 1979; and Ruggero, 1980). The response time of the filter produces a 'virtual delay' (Smolders and Klinke, 1986) which reflects the number of cycles of the stimulus required before the filter output reaches maximum and may be estimated theoretically as 0.5/CF (Smolders and Klinke, 1986; De Boer, 1979). Correction of our group delays by this factor brings them into good agreement with Evans (1972) click latencies (which were to the first spike or at worst the first peak, not the mode of the PSTH which would correspond to the group delay; Goldstein et al., 1971). Smolders and Klinke (1986) indicated that such click latencies are not unaffected by filter response times and thus even after these corrections we cannot get a correct estimate of the residual delay caused by cochlear propagation. Our corrected data plotted on double logarithmic coordinates were not well fitted by a straight line. Thus the straight line fits of Anderson et al. (1971) and Gibson et al. (1977) may give more information about the filter response times than the propagation time. In view of these problems we have not attempted to carry this analysis to its conclusion and present our data as estimates of group delay in guinea-pig cochlear fibres.

Intracellular responses of inner hair cells to tones

Fig. 9 shows intracellular recordings from an inner hair-cell in the basal turn of the guinea-pig cochlea in response to tone bursts. The inner hair-cell receptor potential is sinusoidal and asymmetrical in response to low-frequency tones (e.g. 100 and 300 Hz in Fig. 9). As the stimulus frequency is progressively increased the responses become more asymmetrical in the depolarizing direction (the resting membrane potentials of inner hair-cells are in the range -25 to -45 mV; Russell and Sellick, 1978). Increasing the stimulus frequency beyond about 700 Hz causes a decrease in the size of the sinusoidal component of the receptor potential compared to the steady depolarizing component so that by 2 kHz in Fig. 9 the a.c. component has shrunk to a fraction of the d.c. component (note the doubling of the vertical scales in this figure). At 3 kHz the a.c. component has all but disappeared leaving the unattenuated d.c. component. The reduction in the size of the a.c. component compared to the d.c. component is shown for 9 inner hair-cells (including that shown in Fig. 9) in Fig. 10 where we plot the a.c./d.c.

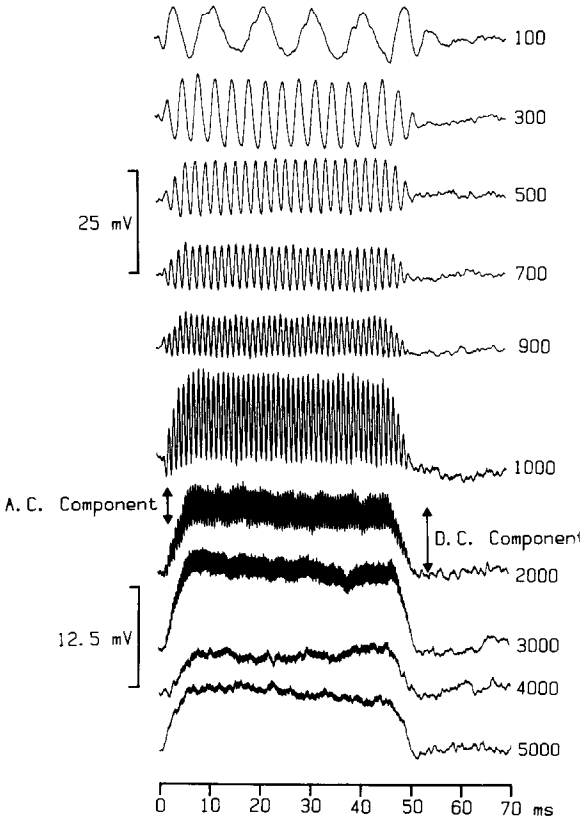

Fig. 9. Intracellular receptor potentials recorded from an inner hair-cell in response to 80 dB SPL tones at frequencies indicated in Hertz by the side of each trace. Notice that relative to the d.c. component, the a.c. component of the receptor potential is reduced as the frequency is increased, and above 1 kHz the response is dominated by the d.c. component. The upper scale bar is for the 100–900 Hz records and the lower for the 1000–5000 Hz records. The resting membrane potential for this inner hair-cell was −37 mV.

ratio as a function of stimulus frequency. Note that for all of the curves the ratio remains fairly constant at low frequency and then drops off smoothly as the stimulus frequency is increased beyond about 300–800 Hz. This fall-off has been ascribed (Sellick and Russell, 1980; Russell and Sellick, 1983) to the low-pass filtering of the hair-cell apical membrane and the direct measurements of the membrane time constant and the a.c./d.c. ratios for the same cells are consistent with this hypothesis. The data in this figure appear to fall into two groups with very different cut-offs and we emphasize this by plotting the two sets in open and closed symbols. We shall return

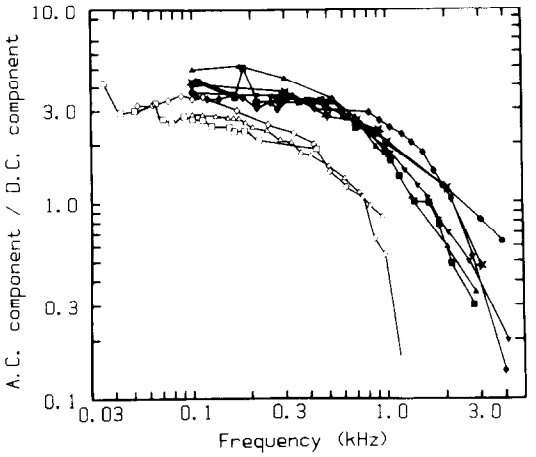

Fig. 10. The ratio of the a.c. and d.c. components of the inner hair-cell receptor potential in 9 hair-cells. The d.c. component was either calculated from the difference between the half-peak a.c. component and the resting potential or by the integral of the waveform.

to this difference in the Discussion.

In Fig. 11 we compare the limits of the phase-locking which we have measured in guinea-pig cochlear nerve fibres with the decline in the a.c./d.c. ratio of inner hair-cells also from guinea-pig and under the same anaesthetic. The a.c./d.c. ratio is a direct equivalent of the synchronization index measure which is the ratio of the phase-locked discharge of a neurone (the a.c. component of the discharge) of the mean discharge rate (the d.c. component of the discharge). In making this comparison we have normalized the hair-cell data

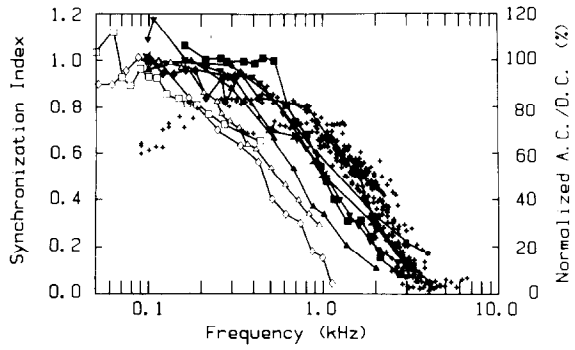

Fig. 11. Comparison of the limits of phase-locking in the guinea-pig auditory nerve and the decrease in the a.c. component of the intracellular receptor potential of 9 inner hair-cells. The hair-cell data have been normalized by plotting as a percentage of the low frequency a.c./d.c. ratio.

by expressing the ratios as a percentage of the constant value at low frequency. There are a number of points of note in this figure; first there appears to be considerably more variability in the measured a.c./d.c. ratios for different hair-cells than there is in the cut-off of phase-locking measured in different cochlear nerve fibres. The difference in cut-off frequency of the hair-cell a.c. receptor potential varies over about two octaves. The frequency at which the open diamond hair-cell a.c./d.c. ratio falls to the arbitrary value of 50% is 450 Hz whereas this occurs for the filled diamonds at 1 586 Hz. The second point to note is that the normalization process has somewhat increased the scatter in that the filled triangles now exhibit an intermediate cut-off frequency and the delineation of filled and unfilled data into separate groups is less clear. Third, the slopes of the cut-offs are quite similar for all of the hair-cells and for the single fibre data. A good match could be achieved by sliding the functions along the frequency axis. Finally, the frequency at which the a.c./d.c. ratio begins to fall from its maximum value occurs for the functions described by the filled symbols (except the triangles) at about the same frequency as the fall in phase-locking i.e. 500–600 Hz.

In the course of experiments recording the intracellular receptor potentials of inner hair-cells we have also made intracellular recordings from afferent nerve fibres. These were identified from their electrophysiological properties and location near the neural membranes of the inner hair-cells and the habenula perforata of the osseous spiral lamina. Invariably it was possible to record intracellularly or extracellularly from the adjacent inner hair-cells when the micropipette was advanced a further 10 μm or so. The resting potentials of the presumed afferent nerve terminals (-40 to -60 mV) were more negative than those of the inner hair-cells (-25 to -45 mV; Russell and Sellick, 1978), but not as negative as the resting membrane potentials of supporting cells (-80 to -90 mV; Russell and Sellick, 1978). Spontaneous and acoustically evoked nerve impulses were recorded extracellularly in the vicinity of the habenula perforata. However, we have not seen impulse activity in our recordings from the presumed afferent terminals. In these recordings we found spontaneous and acoustically evoked fluctuations in membrane potential which were similar in appearance to the excitatory post-synaptic potentials which have been recorded in the afferent nerve terminals of the goldfish sacculus (Furukawa and Ishii, 1967; Furukawa and Matsuura, 1978) and in the lateral line system of Lota (Flock and Russell, 1976). The fluctuations in membrane potential are quite unlike anything we have recorded in the hair-cells and supporting cells of the organ of Corti. Because we have not recorded impulses from the afferent nerve terminals we presume, therefore, that the spike initiation site of the afferent fibres is close to the habenula, and impulses do not invade the terminals (or alternatively that the impulse generating mechanism has been disrupted by the intracellular recording electrode). The receptor potentials from an inner hair-cell and the post-synaptic potentials from an adjacent afferent nerve terminal, recorded in close succession, to 16 kHz tone bursts at 75 dB SPL are shown in the top part of Fig. 12. In the lower part of Fig. 12, on an expanded time scale, the average of four successive hair-cell receptor potentials is compared to the average of four afferent terminal responses. Note that the afferent terminal responses occur later than the receptor potentials. The difference between the onsets of hair-cell and terminal responses is an estimate of the synaptic delay which in the case illustrated is 0.83 ms. There is a difference of about 0.4–0.5 ms between our measurement of the afferent synaptic delay (about 0.85 ms) and our estimate of the fixed delay (about 1.3 ms) which is associated with each nerve fibre (from the asymptote of the phase-locking data of Fig. 8). This discrepancy can be accounted for if it is remembered that the fixed delay is a composite time interval comprising the acoustic delay (0.1 ms), the synaptic delay (0.85 ms) and the time taken for the impulses to propagate to the recording site. In round figures, the distance to the recording site is about 3–4 mm for basal fibres and the conduction velocity for myelinated fibres of diameter 2–5 μm (in guinea-pig; Gacek and Rasmussen, 1961) is of the order of 10–20 m/s (see Rushton, 1951). This gives a value for the propagation time in the range of 0.15 to 0.4 ms. Thus our measured fixed delay associated with each fibre is entirely consistent with our measured synaptic delay. A very low value for the

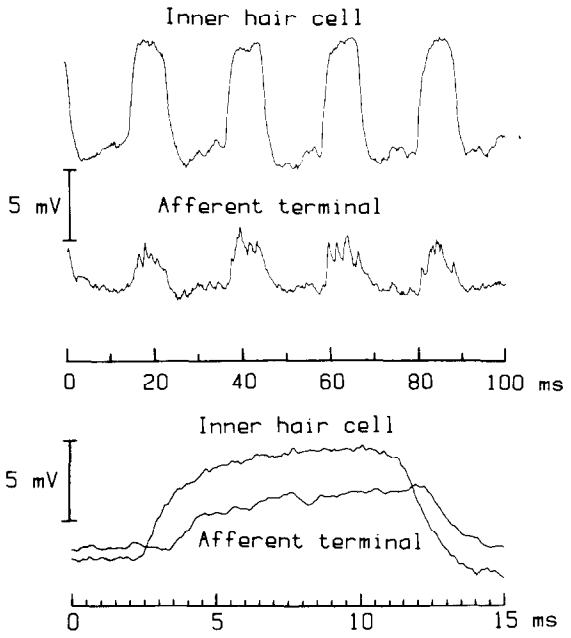

Fig. 12. Direct estimation of the afferent synaptic delay in the guinea-pig cochlea. Top: Single receptor potential and post synaptic potentials from inner hair-cell and afferent terminal in response to 16 kHz tone bursts at 75 dB SPL. Bottom: Averages of four successive responses from hair-cell and afferent terminal shown on expanded time scale. Note that the averaging removes the randomly occurring excitatory post synaptic potentials from the afferent terminal response. The resting membrane potential of the inner hair-cell was −40 mV and of the afferent terminal was −52 mV.

fibre fixed delay of 0.7 ms may be obtained from Evans (1972) click latencies (see Smolders and Klinke, 1986). We are unable to account for the discrepancy between this and our own estimate of the fixed delay (1.3 ms).

Discussion

Our results may be briefly summarized as follows: (i) phase-locking is found in all guinea-pig auditory nerve fibres when stimulated by low frequencies and is maximal for stimuli more than 20 dB above mean rate threshold; (ii) in spontaneously active fibres phase-locking is found well below mean rate threshold; (iii) the maximum degree of phase-locking is approximately constant up to 600 Hz, decreases linearly with further increase in frequency and is no longer evident above 3.5 kHz; (iv) the synaptic delay in the guinea-pig cochlea, obtained by direct measurement of the receptor potential and post-synaptic response is of the order of 0.8 ms; (v) the cumulative phase slope for phase-locking shows a CF dependent delay associated with each fibre; (vi) phase-locking in the guinea-pig falls off an octave below that in cat, squirrel monkey and birds (except owl which is much higher), but above that in frog and crocodile; (vii) the ratio of the a.c. component to the d.c. component of the intracellularly recorded inner hair-cell receptor potential decreases with frequency beyond 500–600 Hz; (viii) the cut-off and the slope of the decrease in the a.c. receptor potential in some inner hair-cells is consistent with the suggestion that the hair-cell membrane time constant is the limiting factor in phase-locking in guinea-pigs.

Johnson (1978) discusses the factors which may affect the degree of phase-locking which is measured. Chief among these factors are wideband baseline noise and baseline noise which mimic the stimulus such as remote microphonic, frequency following responses or signal interference. As in nearly all single unit studies we have used an amplitude discriminator: times of discharges are measured to the point at which the voltage crosses a fixed threshold level. Johnson (1978) demonstrates that the effect of wideband baseline noise is to introduce temporal jitter which acts as a low-pass filter and thus potentially an artificially low cut-off frequency could be measured. Johnson's calculations showed that these effects could not have affected the cut-off frequency which he measured in the cat. We also believe that our measurements were similarly unaffected for the following reasons: (i) our recording conditions were similar to Johnson's in the cat; (ii) direct measurements from the oscilloscope of the temporal jitter allowed us to calculate the filtering effect of such jitter (by the method of Anderson, 1973) again suggesting that our data were free from these effects and (iii) we have made measurements in cochlear nucleus phase-locking cells under conditions of signal-to-noise ratio such that we could exclude baseline noise effects and found exactly the same cut-off frequency.

The net effect of stimulus related baseline noise is to erroneously produce good phase-locking when

the discharge is in fact random. During data collection baseline noise at the stimulus frequency was only very occasionally seen and then at very low frequencies at which our fibres phase-locked extremely well anyway. Since the phase-locking cut-off we observe in the guinea-pig is worse than some other homeotherms and birds, this would appear not to be a problem.

An alternative cause for the lower cut-off frequency in the guinea-pig than cat was proposed by Smolders and Klinke (pers. commun.). They suggested that just as the phase-locking differences between poikilotherms and homeotherms may be attributable to differences in temperature so, if the guinea-pig cochlea had cooled sufficiently during the preparation and experiment, a lower cut-off frequency might result. This is of course difficult to rebut since we did not attempt to directly measure cochlear or intrabulla temperature, merely maintaining core temperature at 37°C. However, a number of observations are relevant: we record under closed bulla conditions; our data match those from other rodents; we do not observe a scatter with a systematic lowering of cut-off frequencies with time as presumably more cooling would have occurred; and finally, an octave shift in phase-locking cut-off, over what must be a relatively small temperature change, seems to be a rather larger shift in a physiological measure than has been found for other measures in mammals (see Gummer and Klinke, 1983, for a review of temperature effects in hearing). We cannot exclude possible temperature effects from our measurements and certainly since hair-cell recordings are performed open-bulla a larger cooling effect would be expected for the receptor potential data.

For the remainder of this discussion we shall take our present data at face value and assume that the cut-off frequencies which we have measured do represent the true physiological conditions.

There is clearly an extremely wide variation of the upper frequency limit at which phase-locking is observed in cochlear nerve fibres in different animals. The lowest reported cut-off (350 Hz) is in frog measured at 15°C (Eggermont, 1983) and the highest in owl (9 kHz; Sullivan and Konishi, 1984) which is a range of 4.68 octaves. Some of this range may be accounted for by the differing body temperatures, but as we have shown in the present study (and indeed as was clear from comparison of data in the literature) a difference of an octave is found between rodents and other mammals, and owls (also homeotherms) phase-lock up to two octaves higher in frequency than rodents.

The limiting factor for phase-locking must reside peripheral to the auditory nerve recording site which gives us a number of candidates. There is, for example, very different cochlear mechanics in the frog compared to the mammal. There is not, however, a qualitative difference in the cochlear mechanics between cats, squirrel monkey and guinea-pig (Khanna and Leonard, 1982; Rhode, 1971; Sellick et al., 1982) and the cochleas of bird and caiman appear to be structurally similar and both support a travelling wave (Wilson et al., 1985; Gummer et al., 1984). It seems unlikely, therefore, that the differences in phase-locking can be directly attributed to differing cochlear mechanics per se.

A second candidate is, as has been suggested previously, the filtering out by the hair-cell membrane time constant of the a.c. component of the receptor potential. This possibility is very attractive since it is intuitively obvious that, if the receptor potential directly leads to release of neurotransmitter and afferent fibre activation, a steady potential cannot produce periodic discharges. The present data were intended as a direct test of this hypothesis. Given the fact that the slope of the a.c./d.c. function is similar to that of the phase-locking and the beginning and end points of the functions match reasonably well (at least for the best of the hair-cells) it seems justifiable to consider the data shown in Fig. 11 to be a good match. Thus the limitation in phase-locking may well be attributable to the decreased a.c. receptor potential, if so, then all but one (the filled diamonds in Fig. 11) of the estimates of the fall-off of the a.c. component seem to be low, with clear discrepancy between the curves and the phase-locking data in the 0.8–1.5 kHz region. For a single cell plotting a.c./d.c. ratios versus frequency for a wide range of sound levels (see for example Fig. 4, Russell and Sellick, 1978) and plotting a.c. amplitude versus frequency at constant d.c. amplitude (5 or 10 mV) yielded the same

functions. We do not, therefore, feel that the mismatch of some of the hair-cell data with the phase-locking data is a result of using a constant 80 dB SPL stimulus as opposed to lower constant sound levels or iso-d.c. amplitudes. Although the hair-cell data are from the basal turn, whilst the highest CF fibre in which we measured phase-locking was 9 kHz, it does not seem likely that the cut-off in fibres from the cochlea base would be significantly different from that in the 9 kHz region. It is possible that both the scatter in the hair-cell data and the lower cut-off might reflect a temperature effect since the recordings are necessarily open bulla (see earlier discussion), this we feel is not the main cause. A difficult problem in making hair-cell recordings is the filtering effect of the electrode impedance and stray capacitance. This electrode filter has been compensated in the data shown in Fig. 11, but any changes in the electrode time constant during penetration of the hair-cell would have resulted in incorrect compensation and a correspondingly lower cut-off frequency.

Accepting, for the time being, that the guinea pig phase-locking cut-off is a result of the hair-cell membrane time constant, what are the implications of this conclusion? Presumably similar constraints will operate in the other homeotherms. Thus the time constants of the hair-cells at a particular frequency position in the cochlea would appear to be longer in rodents than those of cats, squirrel monkeys, pigeons and blackbirds, which in turn are longer than those of owls. Given the two octave spread in phase-locking cut-off amongst these species the R or C components in the hair-cell membrane would have to change by about 4 times which would be an extremely large variation in the properties of the membrane. One further observation from our phase-locking data is consistent with a variation in hair-cell properties. As a consequence of the increasing asymmetry of the inner hair-cell receptor potential with increasing frequency, phase-locking should result in minimum instantaneous discharge rates below spontaneous rate for low frequency (below 1 kHz; Russell and Sellick, 1983), but only down to spontaneous rate for high frequency (above 1 kHz, see Fig. 9). We have observed this phenomenon in our guinea-pig data and it is clearly present in the cat data of Johnson (1980). If the differing phase-locking cut-offs are a result of the hair-cell membrane properties we would expect the transition between minima below or above spontaneous rate to be species dependent. In our data the transition is near 1.5 kHz whereas in a single fibre from cat (Johnson, 1980; Fig. 2) it appears to be above 2 kHz; data from other species are not available.

An alternative to variations in hair-cell properties is that what we observe in the cochlear nerve fibre responses is a result of two transfer functions (i) the mechanical to electrical transfer which results in the declining a.c./d.c. ratio and (ii) the transfer function of the hair-cell synapse. If the same sized a.c. component is applied to transfer characteristics with differing slopes a greater or lesser modulation of the discharge would result. Thus biassing the synaptic transfer function might well produce different phase-locking cut-offs. We might expect that if this were the case that the biassing would be evident in some other features of the discharge. While there are no apparent differences between rodents and cats, the bird cochlear nerve is characterized by the fact that all fibres are spontaneously active, a result suggestive of a different biassing! One way to produce a different biassing would be to alter the standing current passing through the hair-cell. If this were achieved by tonic displacement of the stereocilia another likely outcome would be a reduced d.c. component and therefore an increased a.c./d.c. ratio which would further increase the transfer of modulation to the discharge.

It has recently been suggested that the temporal pattern of discharge across the whole ensemble of auditory nerve fibres may be the means of signalling the spectra of complex sounds such as speech (e.g. Young and Sachs, 1979). Vowel sounds may be distinguished by the relative frequency position and amplitude of the first two formants and some vowels have second formants as high as 3.5 kHz (Peterson and Barney, 1952). Thus the upper frequency limit of phase-locking will affect the internal representation of such speech sounds. While we do not know the upper frequency limit of phase-locking in humans, recent data suggest that phase-locking in guinea-pig occurs at sufficiently high frequency to produce a good internal

representation of vowels with formant peaks up to 3 kHz (Palmer et al., 1986). Use of the guinea-pig as an animal model for the temporal representation of speech sounds is more likely to underestimate rather than overestimate the temporal encoding abilities of the human auditory nerve.

Acknowledgements

We thank Dr. D.A. Lowe and Mr. I.M. Winter for helpful criticism of the manuscript, Ms. E. Cowley for technical assistance and Mr. C. Atherton for photographic assistance. A.R.P. was supported by the Royal Society and both A.R.P. and I.J.R. by grants from the Medical Research Council.

References

Allen, J.B. (1983): Magnitude and phase-frequency response to single tones in the auditory nerve. J. Acoust. Soc. Am. 73, 2071–2092.

Anderson, D.J. (1973): Quantitative model for the effects of stimulus frequency upon synchronization of auditory nerve discharges. J. Acoust. Soc. Am. 54, 361–364.

Anderson, D.J., Rose, J.E., Hind, J.E. and Brugge, J.F. (1971): Temporal position of discharges in single auditory nerve fibers within the cycle of a sine-wave stimulus: frequency and intensity effects. J. Acoust. Soc. Am. 49, 1131–1139.

Brugge, J.F., Anderson, D.J., Hind, J.E. and Rose, J.E. (1969): Time structure of discharges in single auditory nerve fibers in the squirrel monkey in response to complex periodic sounds. J. Neurophysiol. 32, 386–401.

Crawford, A.C. and Fettiplace, R. (1980): The frequency selectivity of auditory nerve fibres and hair cells in the cochlea of the turtle. J. Physiol. 306, 79–125.

Davis, H., Fernandez, C. and McAuliffe, D.R. (1950): The excitatory process in the cochlea. Proc. Natl. Acad. Sci. USA 30, 580–587.

De Boer, E. (1979): Travelling waves and cochlear resonance. Scand. Audiol. Suppl. 9, 17–33.

Eggermont, J.J. (1979): Narrow-band AP latencies in normal and recruiting human ears. J. Acoust. Soc. Am. 65, 463–470.

Eggermont, J.J. (1983): Comment to Narins and Hillary (1983). In: Hearing – Physiological Bases and Psychophysics, pp. 75. Editors: R. Klinke and R. Hartmann. Springer, Heidelberg.

Evans, E.F. (1972): The frequency response and other properties of single fibres in the guinea-pig cochlear nerve. J. Physiol. 226, 263–287.

Evans, E.F. (1975): Cochlear nerve and cochlear nucleus. In: Handbook of Sensory Physiology, vol. V/2, pp. 1–109. Editors: W.D. Keidel and W.D. Neff. Springer, Heidelberg.

Evans, E.F. (1979): Neuroleptanaesthesia for the guinea-pig: an ideal anaesthetic procedure for long term physiological studies of the cochlea. Arch. Otolaryngol. 105, 186–196.

Evans, E.F. (1980): 'Phase-locking' of cochlear fibres and the problem of dynamic range. In: Psychophysical, Physiological and Behavioural Studies in Hearing, pp. 300–309. Editors: G. van den Brink and F.A. Bilsen. Delft University Press, The Netherlands.

Fay, R.R. (1978): The coding of information in single auditory-nerve fibers of the goldfish. J. Acoust. Soc. Am. 63, 136–146.

Flock, Å. and Russell, I.J. (1976): Inhibition by efferent nerve fibres: action on hair cells and afferent synaptic transmission in the lateral line organ of the Burbot, *Lota lota*. J. Physiol. 257, 45–62.

Furukawa, T. and Ishii, Y. (1967): Neurophysiological studies of hearing in the goldfish. J. Neurophysiol. 30, 1377–1403.

Furukawa, T. and Matsuura, S. (1978): Adaptive run down of excitatory post synaptic potentials at synapses between hair cells and eighth nerve fibres in goldfish. J. Physiol. 276, 193–209.

Gacek, R.R. and Rasmussen, G.L. (1961): Fiber analysis of the statoacoustic nerve of the guinea-pig, cat and monkey. Anat. Record 139, 455–463.

Galambos, R. and Davis, H. (1943): Responses of single auditory nerve fibres to acoustic stimulation. J. Neurophysiol. 6, 39–57.

Geisler, C.D., Rhode, W.S. and Kennedy, D.T. (1974): Responses to tonal stimuli of single auditory nerve fibers and their relationship to basilar membrane motion in the squirrel monkey. J. Neurophysiol. 37, 1156–1172.

Gibson, M.M., Hind, J.E., Kitzes, L.M. and Rose, J.E. (1977): Estimation of travelling wave parameters from the response properties of cat AVCN neurons. In: Psychophysics and Physiology of Hearing, pp. 57–68. Editors: E.F. Evans and J.P. Wilson. Academic Press, London.

Goldberg, J.M. and Brown, P.B. (1969): Response of binaural neurons of dog superior olivary complex to dichotic stimuli: some physiological mechanisms of sound localization. J. Neurophysiol. 32, 613–636.

Goldstein, M.H. (1968): The auditory periphery. In: Medical Physiology, vol. 2, pp. 1165–1198. Editor: V.B. Mountcastle. Mosby, St. Louis.

Goldstein, J.L., Baer, T. and Kiang, N.Y.S. (1971): A theoretical treatment of latency, group delay, and tuning characteristics for auditory nerve responses to clicks and tones. In: Physiology of the Auditory System, pp. 133–156. Editor: M.B. Sachs. National Educational Consultants, Baltimore.

Gummer, A.W. and Klinke, R. (1983): Influence of temperature on tuning of primary like units in the guinea-pig cochlear nucleus. Hearing Res. 12, 367–380.

Gummer, A.W., Smolders, J.W.T. and Klinke, R. (1984): Mechanical vibration in the pigeon ear. Pflügers Arch. Suppl. 400, R35.

Harrison, R.V. and Evans, E.F. (1979): Some aspects of temporal coding by single cochlear fibres from regions of cochlear hair cell degeneration in the guinea-pig. Arch. Otol. Rhinol. Laryngol. 224, 71–78.

Hillery, C.M. and Narins, P.M. (1984): Neurophysiological evidence for a travelling wave in the amphibian inner ear. Science 255, 1037–1039.

Johnson, D.H. (1978): The relationship of post-stimulus time and interval histograms to the timing characteristics of spike trains. J. Biophys. 22, 413–430.

Johnson, D.H. (1980): The relationship between spike rate and synchrony in responses of auditory-nerve fibers to single tones. J. Acoust. Soc. Am. 68, 1115–1122.

Khanna, S.M. and Leonard, D.G.B. (1982): Basilar membrane tuning in the cat cochlea. Science 215, 305–306.

Kiang, N.Y.S., Watanabe, T., Thomas, E.C. and Clark, L.F. (1965): Discharge patterns of single fibers in the cat's auditory nerve. MIT Press, Cambridge, Mass.

Kim, D.O. and Molnar, C.E. (1979): A population study of cochlear nerve fibers: comparison of spatial distributions of average rate and phase-locking of responses to single tones. J. Neurophysiol. 42, 16–30.

Klinke, R. and Pause, M. (1980): Discharge properties of primary auditory fibres in *Caiman crocodilus*. Comparisons and contrasts to the mammalian auditory nerve. Exp. Brain Res. 38, 137–150.

Narins, P.M. and Hillery, C.M. (1983): Frequency coding in the inner ear of anuran amphibians. In: Hearing – Physiological Bases and Psychophysics, pp. 70–76. Editors: R. Klinke and R. Hartmann. Springer, Heidelberg.

Oshima, W. and Strelioff, D. (1983): Responses of gerbil and guinea-pig auditory nerve fibers to low-frequency sinusoids. Hearing Res. 12, 167–184.

Palmer, A.R., Winter, I.M. and Darwin, C.J. (1986): The representation of steady-state vowels in the temporal discharge patterns of guinea-pig cochlear nerve and primary like cochlear nucleus neurones. J. Acoust. Soc. Am. 79, 100–113.

Peterson, G.E. and Barney, H.L. (1952): Control methods used in a study of the vowels. J. Acoust. Soc. Am. 24, 175–184.

Pfeiffer, R.R. and Molnar, C.E. (1970): Cochlear nerve fiber discharge patterns: relationship to the cochlear microphonic. Science 167, 1614–1616.

Rhode, W.S. (1971): Observations of the vibration of the basilar membrane in squirrel monkeys using the Mössbauer technique. J. Acoust. Soc. Am. 49, 1218–1231.

Rose, J.E., Brugge, J.F., Anderson, D.J. and Hind, J.E. (1967): Phase locked response to low-frequency tones in single auditory nerve fibers of the squirrel monkey. J. Neurophysiol. 30, 769–793.

Ruggero, M.A. (1980): Systematic errors in indirect estimates of basilar membrane travel times. J. Acoust. Soc. Am. 67, 707–710.

Ruggero, M.A. and Rich, N.C. (1983): Chinchilla auditory-nerve responses to low-frequency tones. J. Acoust. Soc. Am. 73, 2096–2108.

Rupert, A., Moushegian, G. and Galambos, R. (1963): Unit responses to sound from auditory nerve of the cat. J. Neurophysiol. 26, 449–465.

Rushton, W.A.H. (1951): A theory of the effects of fibre size in medullated nerve. J. Physiol. 115, 101–122.

Russell, I.J. and Sellick, P.M. (1978): Intracellular studies of hair cells in the mammalian cochlea. J. Physiol. 284, 261–290.

Russell, I.J. and Sellick, P.M. (1983): Low-frequency characteristics of intracellularly recorded receptor potentials in the guinea-pig cochlear hair cells. J. Physiol. 338, 179–206.

Sachs, M.B., Young, E.D. and Lewis, R.H. (1974): Discharge patterns of single fibers in the pigeon auditory nerve. Brain Res. 70, 431–447.

Sachs, M.B., Woolf, N.K. and Sinnott, J.M. (1980): Response properties of neurons in the avian auditory system: comparison with mammalian homologues and consideration of the neural encoding of complex stimuli. In: Comparative Studies of Hearing in Vertebrates, pp. 323–354. Editors: A.N. Popper and R.R. Fay. Springer, Heidelberg.

Sellick, P.M. and Russell, I.J. (1980): The responses of inner hair cells to basilar membrane velocity during low frequency auditory stimulation in the guinea pig cochlea. Hearing Res. 2, 439–446.

Sellick, P.M., Patuzzi, R. and Johnstone, B.M. (1982): Measurements of basilar membrane motion in the guinea-pig using Mössbauer technique. J. Acoust. Soc. Am., 72, 131–141.

Smolders, J.W.T. and Klinke, R. (1986): Synchronized responses of primary auditory fibre populations in *Caiman crocodilus* (L.) to single tones and clicks. Hearing Res. 24, 89–103.

Sullivan, W.E. and Konishi, M. (1984): Segregation of stimulus phase and intensity coding in the cochlear nucleus of the barn owl. J. Neurosci. 4, 1781–1786.

Tasaki, I. (1954): Nerve impulses in individual auditory nerve fibres of guinea pig. J. Neurophysiol. 17, 97–122.

Taylor, M.M. and Creelman, C.D. (1967): PEST: Efficient estimates on probability functions. J. Acoust. Soc. Am. 41, 782–787.

Wilson, J.P., Smolders, J.W.T. and Klinke, R. (1985): Mechanics of the basilar membrane in *Caiman crocodilus*. Hearing Res. 18, 1–14.

Woolf, N.K., Ryan, A.F. and Bone, R.C. (1981): Neural phase-locking properties in the absence of cochlear outer hair cells. Hearing Res. 4, 335–346.

Young, E.D. and Sachs, M.B. (1979): Representation of steady-state vowels in the temporal aspects of the discharge patterns of populations of auditory nerve fibers. J. Acoust. Soc. Am. 66, 1381–1403.