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The phase response of primary auditory afferents in a songbird (*Sturnus vulgaris* L.)

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The effects of stimulus frequency and intensity on phase-locking characteristics of cochlear ganglion cells were studied in the starling. All cells showed phase-locking to tone stimuli within their response area. Phase-locking at CF is found on average 9 dB below discharge rate threshold. Phase-locking is best at 0.4 kHz and deteriorates with increasing frequency almost independently of CF. No phase-locking was evident for test frequencies above 3–4 kHz. Phase-locking in cells with CFs above 1.0 kHz is better below CF than at CF. For constant sound pressure, an increase in stimulus frequency always produced an increase in phase lag of the neural response. The phase vs. frequency data obtained at constant sound pressure can be reasonably approximated by straight line functions. The slopes of these functions indicate the latency of the neural response, and are correlated with the CFs of the respective cells; the latency tends to be longer in low-CF cells and shorter in high-CF cells. The latency decreases by 0.04 ms per 1 dB sound pressure increase. The response phase at CF is nearly stimulus level-independent. Increasing stimulus intensity causes increasing phase lag below CF and decreasing phase lag above CF. These results are compared to findings in other vertebrates and demonstrate the similarities of phase-locking characteristics despite the substantial anatomical differences among the vertebrate groups.

Starling; Ear, inner; Cochlear ganglion cell; Phase-locking

Introduction

The comparative investigation of vertebrate groups has proved to be a powerful approach for determining the function of various structures in the inner ear. The avian inner ear exhibits a variety of structural features not found in that of mammals (Takasaka and Smith, 1971; Tanaka and Smith, 1978; Chandler, 1984). The activity patterns of primary auditory fibers of birds and mammals share many common features but also exhibit some distinct differences (Sachs et al., 1974, 1980; Gross and Anderson, 1976; Manley and Leppelsack, 1977; Manley, 1979, 1980; Manley and Gleich, 1984; Manley et al., 1985; Schermuly and Klinke, 1985). In order to provide additional data on the functional aspects of avian primary auditory afferents, we studied the frequency and intensity dependence of the phase-locked response of cochlear ganglion cells in the starling. An increased data for the comparison of structure and function between the mammalian and avian inner ear should help us understand basic principles of inner ear functions in these two groups.

Methods

The results reported here were obtained from anesthetized birds, which were artificially respired, with the body temperature maintained at normal values (39–41°C). Details of the preparation have been previously reported (Manley et al., 1985). After surgery the birds were placed in an electrically-shielded sound-proof chamber.

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Acoustic stimuli were presented via a closed system consisting of an earphone (AKG DKK 32) and a calibrated measuring microphone (Brüel and Kjaer 4133) for monitoring the stimulus at the eardrum. An earpiece was sealed into the outer ear canal and the system was individually calibrated before each experiment. The system output was flat within ±1.5 dB over a frequency range of 0.1–3.6 kHz. Distortion was low, at 90 dB SPL harmonics were at least 60 dB below the fundamental.

Upon encountering an auditory cell in the cochlear ganglion, the frequency threshold curve was determined, generally using an automated procedure controlled by a laboratory computer (MINC 11/23). Tone bursts (100 ms duration, 2.5 ms rise and fall time, 4 per second) were each presented twice over a range of sound pressures (10–90 dB SPL) and frequencies (2–3 octaves). Frequency steps were either 5 per octave or 25, 50, 100, 200 or 400 Hz, for frequencies below 100, 400, 1000, 1600, and 4000 Hz, respectively. The frequency of the tone bursts was randomly varied but presented at a constant sound pressure. After a complete frequency series had been presented, the sound pressure was increased by 4 or 8 dB and the procedure repeated until the highest SPL in the series was reached. Spikes occurring during the stimulus were summed up within each frequency-sound pressure combination and a bar graph was constructed. Isorate curves were derived using rate criteria that resulted in a 'smooth' frequency-threshold curve, typically at about 40% above the spontaneous rate.

Next, the cell's activity was measured in response to continuous pure tones presented in frequency steps of 25, 50 or 100 Hz and in intensity steps of 10 dB over a frequency and sound pressure range (which was as wide as possible, always including the cell's CF, but restricted by the recording time). The responses were recorded on magnetic tape for subsequent off-line analysis. The stimulus tone duration was variable, but adjusted such that a minimum of 200 spikes was obtained. From these, period histograms comprising approximately 100 spikes were constructed. The positive-going zero crossing of the stimulus at the eardrum triggered the input of the computer which then registered the times of spike occurrence with a resolution of 1 μs. In an initial series of experiments histograms constructed from 100 spikes turned out to be a fair compromise between accuracy of the measurements and the required recording time. Although we did not establish any quantitative criteria for confidence limits of the preferred response phase and VS measurements, the 'jitter' in our phase vs. frequency curves is typically quite small.

The whole system was calibrated and corrected for phase shifts introduced by the set up (e.g. sound monitoring system, tape recorder). The vector strength (VS, the length of the normalized sum-vector (Goldberg and Brown, 1969)) and the preferred firing phase (angle of the sum-vector) were then calculated from these histograms. The significance of phase-locking was calculated using a Rayleigh test for circular data with a VS of 0.26 being highly significant ($P < 0.001$) for histograms of 100 spikes (Littlefield, 1973; Buunen and Rhode, 1978).

Results

All primary auditory afferents ($n = 40$) studied in 15 starlings showed phase-locking in response to stimulation with continuous pure tones within their response area. Fig. 1 shows three period histograms demonstrating the fundamental forms that were found. Fig. 1A shows a histogram representing the standard response to a supra-threshold, near CF stimulus showing strong phase-locking with one clear peak. Fig. 1B shows the response of the cell when stimulated with a tone outside its response area. There is no obvious phase-locking, i.e., the histogram is essentially flat. Fig. 1C shows an example of a multiple-peaked histogram. The stimulus frequency was more than one octave below the CF. Note that the peaks do not occur at equal intervals within the histogram.

In seven cells (from a sample of 19 with similar CFs and thresholds) period histograms with two or three peaks were found. In these cases (best thresholds between 10 and 42 dB SPL), multiple-peaked histograms were obtained in response to high intensity (90 dB SPL), low frequency (1–3 octaves below CF) stimuli. The inter-peak interval corresponded to about two or three times the stimulus frequency and was closer to the CF of

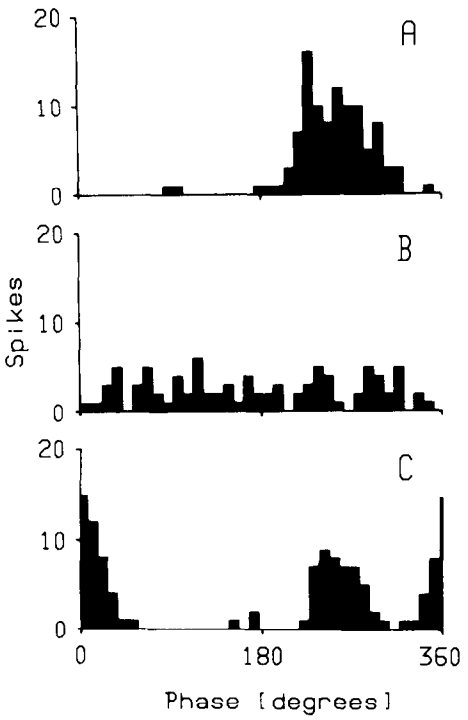

Fig. 1. Three period histograms of a cell with a CF of 0.53 kHz and a CF threshold of 15 dB SPL to three different stimulus frequencies presented at 90 dB SPL. (A) shows a highly phase-locked response to a 0.5 kHz tone. (B) demonstrates the activity during a 1.6 kHz tone, which is not in the response area of this cell. No phase-locking is visible, spikes occur randomly within the stimulus cycle. (C) demonstrates a multiple peaked histogram in response to a 0.2 kHz tone. There are two obvious peaks. The intervals between the modes of the peaks are 1.6 ms and 3.4 ms; the stimulus period is 5 ms.

the cell than to the stimulus frequency. Data from such multiple-peaked phase histograms were not used in assessing preferred response phase.

Effects of intensity and frequency upon VS

Fig. 2 shows intensity functions for three cochlear ganglion cells, obtained at their respective CFs. An increase in sound pressure above the corresponding threshold increases both the vector strength (VS) and the discharge rate of the cells. Both parameters saturate typically within 20–40 dB above threshold and are in some cases reduced again by further increases in sound pressure. In most cases, phase-locking is significant below discharge rate threshold. In 18 cells, we determined both the rate discharge threshold and the threshold for phase-locking (we chose VS = 0.26) at the CF. In 15 cells the latter threshold was 2–26 dB (mean = 11.8 dB) below the discharge rate threshold (Fig. 3). VS threshold was slightly higher (1–2 dB) than discharge rate threshold in two cells and only one cell had a substantially higher threshold (17 dB) for VS compared to that for discharge rate. This difference tends to be larger in cells with low CFs compared to those with high CFs ($n = 18$, $r = 0.47$, $P < 0.05$). Over all 18 cells, significant phase-locking occurs on average 9 dB below the discharge rate threshold.

At constant sound pressure, VS also varies as a function of frequency. For example at 90 dB SPL,

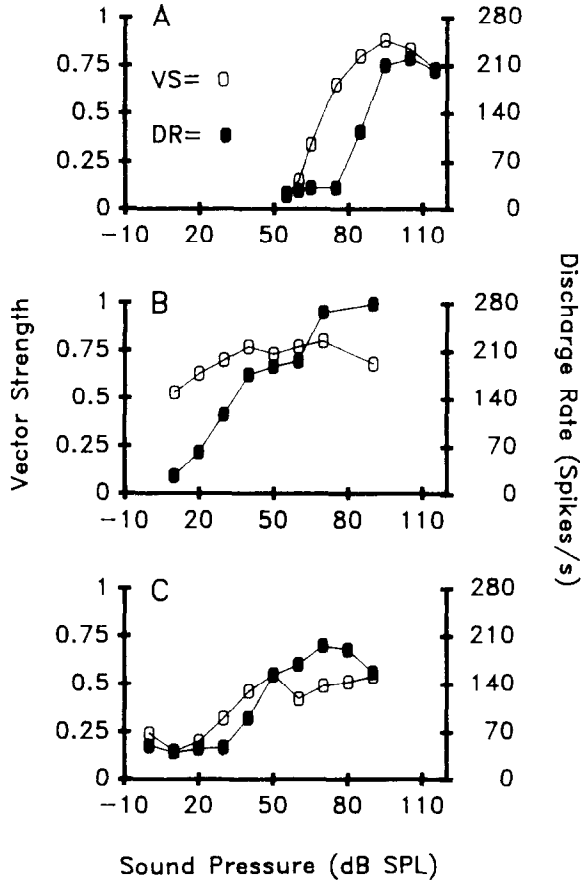

Fig. 2. Intensity functions for vector strength (VS, open symbols) and discharge rate (DR, filled symbols). Both parameters increase with increasing sound pressure, saturate and eventually decrease. In (A)/(B)/(C) CF is 0.3/0.8/1.4 kHz, and the VS maxima are 0.89/0.79/0.55.

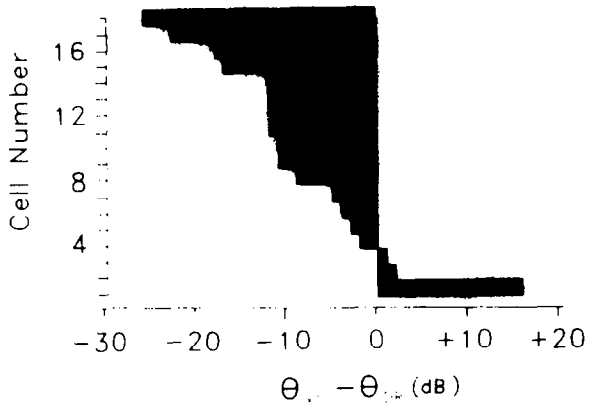

Fig. 3. Threshold for phase-locking (θ_{VS}) relative to discharge rate threshold (θ_{DR}) at CF was determined in 18 cells. The difference $\theta_{VS} - \theta_{DR}$ is plotted in ordered sequence from the bottom (cell one) to the top (cell 18) of the figure.

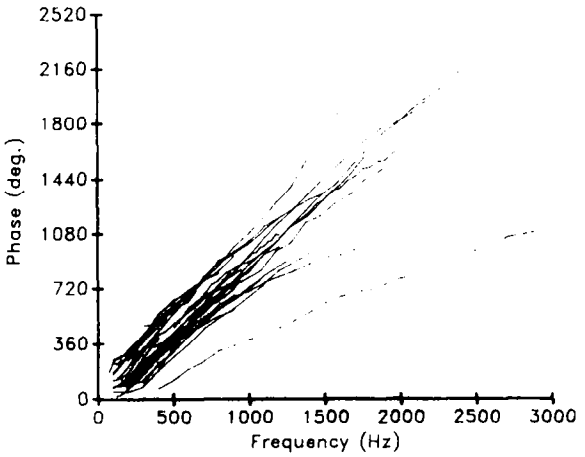

Fig. 5. Preferred response phase versus stimulus frequency for 40 cells tested at 90 dB SPL. Data points for individual cells are connected.

VS is typically higher for low stimulus frequencies than for high frequencies. Fig. 4 demonstrates that phase-locking is best (highest VS) around 0.4 kHz, independent of the CF of the cell under test, and deteriorates at higher and lower frequencies. In this figure, VS was measured at 90 dB SPL over a range of frequencies within each cell's tuning curve ($n = 38$). It is obvious that significant phase-locking (VS > 0.26) is not obtained above 3.0 kHz. Extrapolation of the line delimiting the outer edge of the VS distribution to the frequency axis results in a high frequency limit for phase-locking of about 4.0 kHz.

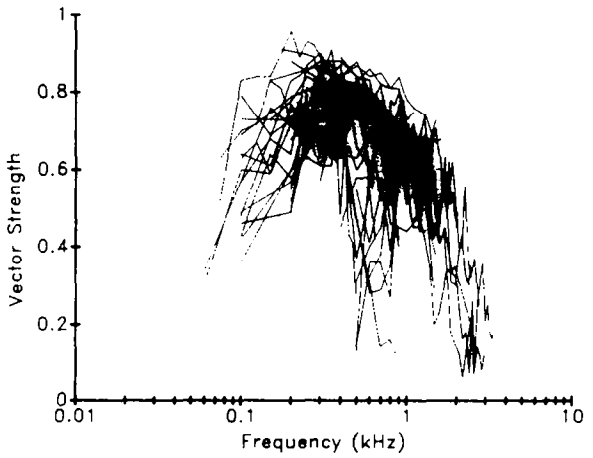

Fig. 4. Vector strength versus stimulus frequency at a constant sound pressure of 90 dB SPL for 38 cells in the starling cochlear ganglion. Only test frequencies within a cell's tuning curve are plotted, always including the CF. Data points from individual cells are connected.

We believe that although the low VS values at frequencies below 0.2 kHz are principally due to the decrease of the stimulus intensity relative to threshold, they are at least in some cases due to multiple peaks in the period histogram.

Preferred response phase: frequency and intensity effects

The preferred response phase depends, in all cells tested, on stimulus frequency. Fig. 5 shows typical plots of the preferred phase versus stimulus frequency. In all 40 cells an increase in stimulus frequency results in an increased phase lag of the neural response. The best-fit linear regression lines through the data points fit the data in all cells remarkably well ($r^2 > 0.9409$).

In seven cells the phase response was also determined following the tuning curve threshold. The phase vs. frequency functions obtained at 90 dB SPL and at threshold are compared in Fig. 6 for two typical cells. The tuning curves and the frequency-sound pressure combinations at which the response phase was determined are shown on the left side of Fig. 6; open symbols indicate measurements near threshold, crosses represent measurements taken at 90 dB SPL. The right side of Fig. 6 shows the corresponding phase vs.

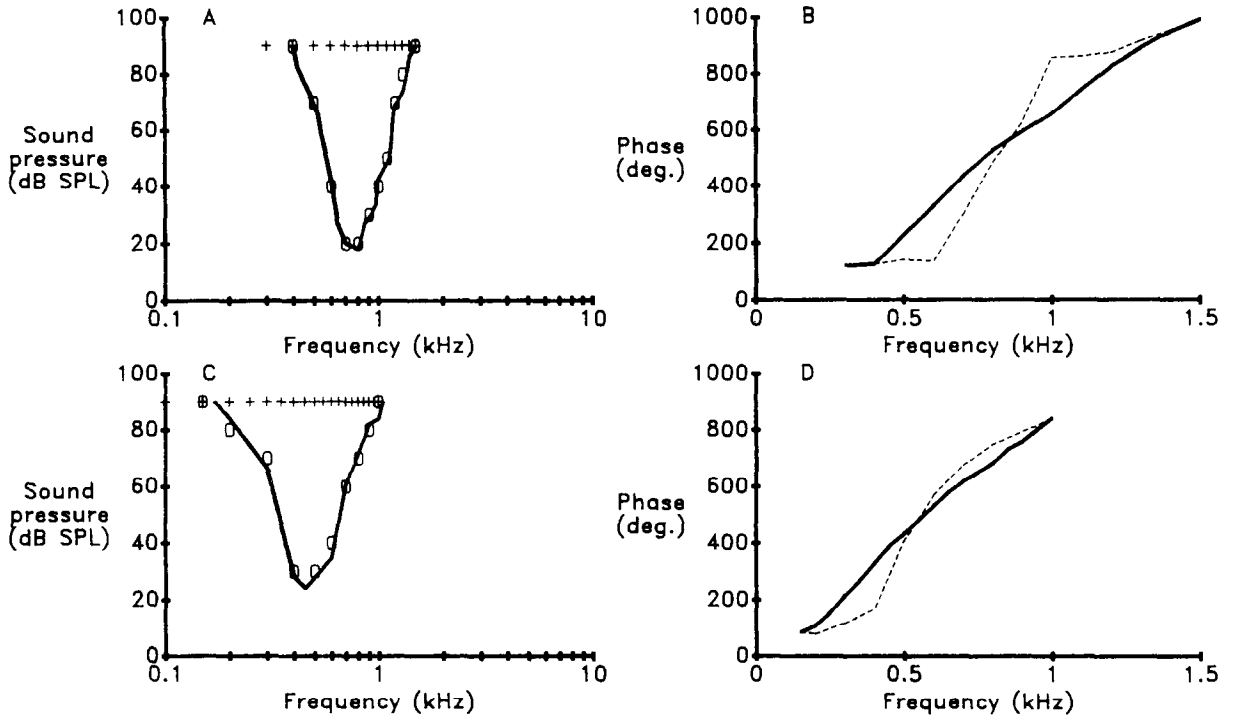

Fig. 6. The preferred response phase of a cell is level dependent. Tuning curves of two representative cells are shown in A and C. The frequency-sound pressure combinations where the response phase was determined are indicated by open symbols (near threshold) and crosses (at 90 dB SPL). B and D show the corresponding phase vs. frequency functions measured at 90 dB SPL (continuous lines), and at threshold (dashed lines). The phase vs. frequency functions obtained at threshold are S-shaped while the 90 dB SPL functions are nearly straight lines. Phase lag is similar in both functions for low and high test frequencies and at the CF. For other frequencies phase lag is decreased below CF and increased above CF in the functions obtained at threshold compared to those determined at 90 dB SPL.

frequency functions; the continuous line represents the 90 dB SPL measurements, the dashed line shows the phase response at threshold. The functions at threshold and those obtained at 90 dB SPL exhibit identical phase lags around CF and at low and high frequencies. Between these extremes, phase lag at threshold is decreased below CF and increased above CF, relative to the 90 dB SPL curve. The curves at threshold do not resemble straight lines as do those obtained at 90 dB SPL; they look much more S-shaped, with the steepest slope of the function around CF.

A constant delay between the input and output of a system results in a linear phase vs. frequency function with the slope of the function being a measure of the delay. Since the phase vs. frequency functions of single cells obtained at 90 dB SPL are very well approximated by their regression lines, it is possible to calculate from their slopes the total delay between the eardrum and the recorded neural response. The total delays were corrected for acoustic and neural transmission times by subtracting 0.8 ms (latency for a 3.0 kHz neuron to a high-level rarefaction click, H. Oeckinghaus, pers. comm.) from the total delay. What remains is regarded as the additional delay produced in the inner ear, which has classically been termed the 'travel time'. This calculated additional delay occurring in the inner ear is plotted versus the CF in Fig. 7; these two variables are significantly correlated according to Spearman's rank correlation procedure ($n = 40$, $r_S = -0.35$, $P < 0.05$) and a linear regression analysis ($r = -0.41$, $P < 0.01$). Despite the scatter this correlation shows a tendency for increasing delay to be associated with decreasing CF. The scatter is at least partly due to

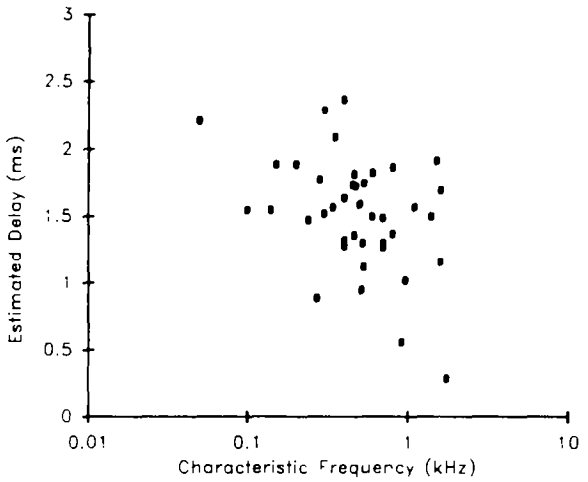

Fig. 7. The total delay occurring between the ear drum and the recording site was calculated from the slopes of phase vs. frequency functions of individual cells. The 'travel time' in the inner ear was then estimated by subtracting 0.8 ms from the total delay to correct for acoustic, synaptic and neural transmission times. This estimated delay is plotted versus the CFs of the respective cells. The delay is significantly correlated with CF ($n = 40$, $r = -0.41$, $P < 0.01$).

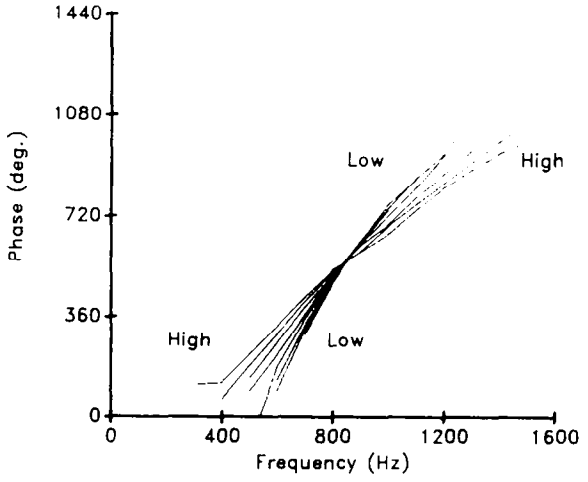

Fig. 8. Phase vs. frequency functions of an individual cell obtained for frequencies spaced by 0.1 kHz and sound pressures between 20 dB SPL and 90 dB SPL in 10 dB steps. The slopes become progressively shallower with increasing stimulus level. The preferred response phase is almost intensity independent around CF (0.8 kHz). Increasing sound pressure causes increasing phase lag below CF and decreasing phase lag above CF.

the fact that data were pooled from different individuals. In 10 out of 12 birds with recordings from at least two cells with different CFs, the delay of the cell with the highest CF was shorter than that of the cell with the lowest CF ($P < 0.05$).

The slopes of the phase vs. frequency functions for starling cochlear ganglion cells are strongly dependent upon the stimulus sound pressure (Narins and Gleich, 1986). Increasing sound pressure reduces the delay, thus resulting in a decreased slope of the phase vs. frequency functions. Fig. 8 shows typical phase vs. frequency functions from a single cell obtained for a series of different sound pressures. Each of the functions, regardless of the stimulus sound pressure, can be fitted very well by a straight line. A decrease of slope with increasing sound pressure is clear. Around the CF of a cell, the preferred response phase is nearly level-independent, resulting in a 'crossover point'. Increasing sound pressure causes a decreasing phase lag (above CF) or an increasing lag (below CF) for a given stimulus frequency.

In Fig. 9, the total delay determined by the slope of the phase vs. frequency function is plotted against the stimulus sound pressure. The decreasing delay with increasing sound pressure is clearly demonstrated, with a mean value of 0.04 ms per dB ($n = 16$ cells).

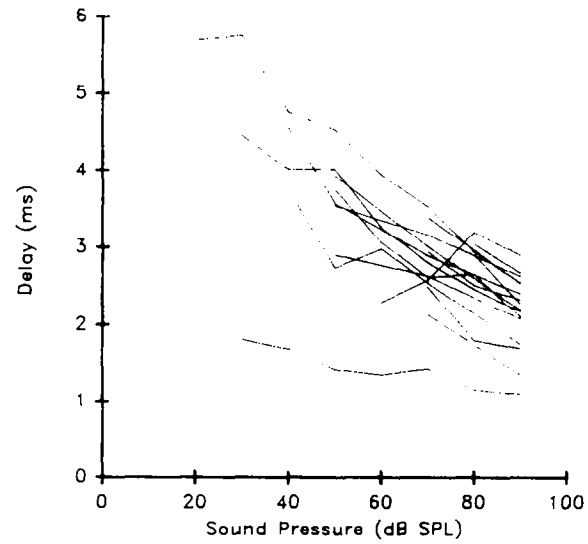

Fig. 9. Total delay, calculated from the slopes of phase vs. frequency functions versus stimulus sound pressure. Data points from individual cells are connected. The mean decrease of the delay is 0.04 ms per 1 dB sound pressure increase.

Discussion

Phase-locking in response to stimulation with effective low-frequency stimuli has been described for primary auditory afferents from amphibians (Narins and Hillery, 1983; Hillery and Narins, 1987), reptiles (Crawford and Fettiplace, 1980b; Klinke and Pause, 1980), birds (Sachs et al., 1974, 1980; Narins and Gleich, 1986) and mammals (Anderson et al., 1971; Palmer and Russell, 1986). Period histograms usually show a single, well-defined peak at the preferred stimulus phase. In seven cells from this study, multiple peaks were found in the period histograms. One explanation for these multiple peaks might be distortion of the stimulus, especially since high stimulus levels are necessary to obtain multi-peaked period histograms. However, two arguments suggest that simple stimulus distortion is probably not responsible for the multiple peaks. First, distortion in our sound system was low and these additional peaks were only found in roughly one-third of the cells, these cells being similar in CF to other low-threshold cells in which no multiple peaks were found. Second, the peaks are not equally spaced within the histograms (Fig. 1C), as might be expected if they were the result of harmonic distortion. Multiple peaks in period histograms of primary afferent activity and corresponding distortion in the hair cell receptor potential in response to low frequency stimuli have been found in mammals (Johnson, 1980; Ruggero and Rich, 1983; Dallos et al., 1986) and the red-eared turtle (Crawford and Fettiplace, 1981b). It was suggested for the turtle that these peaks arose by a nonlinear behavior of the hair cells which, at these low frequencies, exhibited 'ringing' similar to that observed in response to injection of current steps. Thus, Crawford and Fettiplace (1981b) interpreted multiple peaks in terms of an electrical tuning mechanism in the hair cells of the turtle. There is evidence for some form of electrical tuning in the starling inner ear (Manley and Gleich, 1984; Gleich, 1987) which may, as suggested for the turtle, result in multiple peaked histograms for low frequency, high intensity stimuli. In mammals, the mechanism underlying multiple peaked histograms is still uncertain. However, the distortion of the receptor potential (and thus the multiple peaks in period histograms) also seem to be generated within individual inner hair-cells, rather than to originate from a simple transduction of mechanical distortion components of basilar membrane motion (Dallos et al., 1986).

The phase vs. frequency functions at 90 dB SPL show only small deviations from straight lines, compared to functions that are obtained by tracing the phase around the tuning curve threshold of the cell (Fig. 6). In contrast, the pronounced nonlinear component of the phase response measured at threshold, with its steepest slope around the CF, may be the result of complex filter mechanisms. The difference of the response phase between the 90 dB SPL curve and the function obtained at threshold is up to 200° and cannot be explained by a simple non-active filter. In addition, these mechanisms are nonlinear with respect to sound pressure level; their contribution is greatest near threshold and decreases at higher intensity levels. At the moment it is not possible, based on our relatively small data base, to discriminate between all the different processes contributing to frequency selectivity.

Plotting VS versus sound pressure results in input-output functions similar to those for discharge rate (Fig. 2), with, however, a lower threshold for VS relative to discharge rate. This difference is on average about 10 dB (Fig. 3) which is similar to the results from other birds (Sachs et al., 1980), mammals (Palmer and Russell, 1986) and amphibians (Narins and Hillery, 1983). In the starling this difference tends to be larger in cells with low CFs compared to those with high CFs and is in some cases more than 20 dB.

As shown in Fig. 4, above 0.4 kHz, the degree of phase-locking is an inverse function of test frequency at a constant stimulus intensity (90 dB SPL). The figure clearly demonstrates that, for any frequency, there is a maximum VS. The highest VS values are found between 0.3 and 0.4 kHz. Some of the low VS values in our sample are due to low stimulus intensity relative to threshold at the extreme ends of a neuron's tuning curve. The few measurements below 0.3 kHz indicate a decrease of VS with a decrease of stimulus frequency. In at least a few cases, this reduced VS is caused not only by low stimulus intensity relative to

threshold but is also due to the presence of multiple peaks in the period histograms, which has the effect of reducing VS. The maximum VS decreases from about 0.9 at 0.4 kHz to below 0.3 at 3.0 kHz. For stimuli more than 20 dB above threshold, VS is in most cases saturated and nearly independent of the CF of the cell under test. At stimulus frequencies below 1 kHz, VS was typically higher than that obtained for stimulus frequencies above 1 kHz – even for cells with CFs above 1 kHz. This decrease of maximum VS with increasing frequency has also been reported for the red-winged blackbird (Sachs et al., 1980), cat (Johnson, 1980), guinea pig (Russell and Palmer, 1987) and two frog species (Hillery and Narins, 1987). The maximal VS values at a given frequency are species-dependent. Palmer and Russell (1986) arbitrarily selected the frequency where maximum VS had fallen to 0.5 to compare the ability of phase-locking across species. They found this value to be 1.53 kHz in the guinea pig, compared to 2.9 kHz in the cat (Johnson, 1980), and 2.9 kHz in the red-winged blackbird (Woolf and Sachs, 1979). An estimate for this frequency from Fig. 4 would be about 1.7 kHz for the starling.

A comparison of the upper frequency limit of phase-locking also reveals similar species-dependent variations. In the treefrog *Eleutherodactylus coqui* at 22–24° C this limit is 0.9 kHz (Narins and Hillery, 1983). The degree of phase-locking in the frog *Bombina orientalis* is reduced for frequencies above 0.5 kHz compared to cells from *Eleutherodactylus coqui*, both measured at 22–24° C (Hillery and Narins, 1987). In the starling we measured the upper limit of phase-locking at 40° C to be 4 kHz [weighted regression analysis (Cleveland, 1979) of all data points; *x*-axis intersect in Fig. 4], whereas it was reported to be 5–6 kHz in the red-winged blackbird (Woolf and Sachs, 1977) and 9 kHz in the barn owl at 41° C (Sullivan and Konishi, 1984). In mammals, measurements of the upper limit of phase-locking revealed 5–6 kHz in cat (Johnson, 1980) and 3.5 kHz in guinea pig (Palmer and Russell, 1986) and chinchilla (Woolf et al., 1981) at 37° C. There are probably several factors contributing to the variation in the upper limits of phase-locking across taxa. Clearly, core temperature affects the high-frequency cut-off for significant phase-locking (Narins and Hillery, 1983). However, the differences among the endotherms and two frog species measured under identical conditions require an additional explanation. Russell and Palmer (1987) suggest that the decline of the AC component of the receptor potential is responsible for the decline of phase-locking with increasing frequency. From this point of view, one would expect specialized hair cells (e.g. very short membrane time constants) to be found in the inner ear of the barn owl.

The latencies obtained from single cell recordings have been investigated in a variety of species. In the starling, estimates of the total delay, calculated from our phase vs. frequency measurements, are between 1.09 and 3.17 ms. To reveal the classical travel time component this total delay has to be corrected for contributions from the middle ear, the hair cell synapse and the neural conduction time. These delays are assumed to be frequency independent in the range of frequencies studied. The sum of these delays, 0.8 ms, common to all fibers, was estimated from the latency of a high-CF cell to a high intensity rarefaction click. The additional delay (Fig. 7) is significantly correlated with CF such that low frequency cells have, on average, longer delays than high frequency cells. Below about 1.0 kHz the total delay (calculated from the phase vs. frequency functions) is smaller than in mammals (Anderson et al., 1971; Kettner et al., 1985; Palmer and Russell, 1986), caiman (Smolders and Klinke, 1986) and frog (Hillery and Narins, 1984). This difference, especially between caiman and starling, is surprising, as the anatomy of their inner ears is very similar, and as the delays are much more similar within the other vertebrate groups despite significant anatomical differences. The delay in these vertebrate groups is also correlated with CF: low-CF cells having longer delays than high-CF cells. The CF-dependence of the delay in mammals has been interpreted to be the direct result of the travelling wave (Anderson et al., 1971), and in frogs it was used as supporting evidence for the presence of a travelling wave (Hillery and Narins, 1984). The fact that low-CF cells have longer delays than high-CF cells may also be interpreted as the result of a travelling wave in the starling inner ear. The CF-dependence of the delay is small, however, compared to the other vertebrate groups.

This indirect measurement of the travel time tends to overestimate the delay (Ruggero, 1980) and does not consider other possible components introduced by the phase contributions of filter mechanisms. The response time of a tuned filter (the build-up time to maximal output after the onset of a tone, which is inversely related to the bandwidth 3 dB above threshold), does not influence these phase measurements since they are obtained from the steady-state response to continuous tone stimuli. Nevertheless filter mechanisms may contribute to the cells' phase response. The scatter of the delays in cells with comparable CFs (which is at least partly due to pooling data from different individuals) in addition to the inherent methodological complications make a detailed analysis of the travelling wave in the starling with this method impossible.

The existence of a travelling wave has been shown by direct mechanical measurements for the basal part of the pigeon's basilar papilla (Gummer et al., 1985). These measurements were made on the free basilar membrane at locations corresponding to basilar membrane CFs between 1.5 kHz and 4.0 kHz. Estimates of the basilar membrane delays obtained by Gummer et al. (using the straight low frequency portion of the phase vs. frequency functions from their Fig. 2B) are between 0.6 ms and 0.3 ms for CF-locations corresponding to 2.0–3.3 kHz. These short delays agree well with those of the high frequency cells in our sample. Furthermore, the increased delay with decreasing CF of starling ganglion cells is compatible with the idea of a travelling wave propagating from the base to the apex of the avian cochlea.

We have also shown that the preferred firing phase of the ganglion cells is stimulus level dependent (Fig. 8). The slopes of the phase vs. frequency functions become shallower with increasing stimulus levels (Fig. 9), reflecting shorter latencies of the recorded neural response. This latency decrease, on average 0.04 ms per 1 dB increase in stimulus intensity agrees well with the 0.05 ms click-latency decrease reported in the frog (Hillery and Narins, 1987).

In the starling we find that the influence of stimulus intensity on the preferred response phase is smallest around CF and becomes progressively larger for test frequencies further and further from CF. Increasing sound pressure causes increasing phase lag below CF and decreasing phase lag above CF. The intensity dependence of the phase vs. frequency functions is qualitatively similar to that reported by Anderson et al. (1971) in the squirrel monkey, and by Klinke and Pause (1980) in the caiman. Crawford and Fettiplace (1981a) showed no influence of increasing sound pressure at low intensities in turtle hair cells; at higher levels, the phase lag below CF increased with increasing sound pressures. The intensity effects in frogs on the preferred response phase are more complicated, but qualitatively similar (Narins, 1987; Hillery and Narins, 1987; Narins and Wagner, in preparation).

The source of these various intensity-dependent effects in the different vertebrate groups is not completely understood. Measurements of the basilar membrane motion in the squirrel monkey and the guinea pig revealed that increasing stimulus intensity resulted in increasing phase lag for stimulus frequencies below the CF of the recording point, and increasing phase lead for stimulus frequencies above the CF of the recording point (Rhode and Robles, 1974; Sellick et al., 1982). This finding suggests that in mammals the observed neural intensity effects reflect the vibration pattern of the basilar membrane. Evidence is accumulating that in mammals, the outer hair cells act as active modifiers of the basilar membrane vibration pattern (Manley, 1986; Brownell et al., 1985). However, a similar intensity dependence of the phase response is found in animals with rather different inner ear structures (and thus probably differing inner ear mechanics). Thus the question arises as to the mechanisms which cause these phenomena. To what extent do they have their origin in the mechanics of the basilar membrane and to what extent do they originate from intrinsic properties of hair cells, which in turn are reflected in basilar membrane vibration pattern?

We are aware of the problems concerned with the interpretation of the delays calculated from phase vs. frequency functions. Primary auditory afferents of the starling are highly frequency selective with high- and low-frequency slopes of their tuning curves being up to 200 dB per octave (Manley et al., 1985). The mechanical basilar membrane tuning curves measured by Gummer et

al. (1985) in the pigeon were comparatively poorly tuned, with slopes of about 10 dB per octave. While the possibility exists that the gap in frequency selectivity between the basilar membrane and the single cell level may at least partly be caused by a poor cochlear condition in the mechanical measurements, it is necessary to consider that additional filter mechanisms might be required to produce the sharply tuned response of the afferents. There is evidence for electrical tuning from previous studies (Manley and Gleich, 1984; Gleich, 1987) and in addition, the complex phase responses obtained near threshold (Fig. 6) indicate a phase contribution of filter components. However, the delays calculated from our phase vs. frequency functions (obtained at 90 dB SPL, resembling straight lines) coming from cells innervating the apical half of the starling cochlea are consistent with the mechanical measurements from the basal half of the pigeon basilar membrane (Gummer et al., 1985). At the moment it is not possible to discriminate the amount of phase shift introduced by the different sources in the avian inner ear. The investigation of isolated hair cells might contribute to the solution of these problems.

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References

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 sinewave stimulus: frequency and intensity effects. J. Acoust. Soc. Am. 49, 1131–1139.

Brownell, E.W., Bader, C.R., Bertrand, D. and Ribaupierre, Y. (1985) Evoked mechanical responses of isolated cochlear outer hair cells. Science 227, 194–196.

Buunen, T.J.F. and Rhode, W.S. (1978) Responses of fibers in the cat's auditory nerve to the cubic difference tone. J. Acoust. Soc. Am. 64, 772–781.

Chandler, J.P. (1984) Light and electron microscopic studies of the basilar papilla in the duck, *Anas platyrhynchos*. I. The hatchling. J. Comp. Neurol. 222, 506–522.

Cleveland, W.S. (1979) Robust locally weighted regression and smoothing scatterplots. J. Am. Stat. Ass. 74, 829–836.

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.

Crawford, A.C. and Fettiplace, R. (1981a) An electrical tuning mechanism in turtle cochlear hair cells. J. Physiol. 312, 377–412.

Crawford, A.C. and Fettiplace, R. (1981b) Non-linearities in the responses of turtle hair cells. J. Physiol. 315, 317–338.

Dallos, P., Cheatham, M.A. and Oesterle, E. (1986) Harmonic components in hair cell responses. In: B.C.J. Moore and R.D. Patterson (Eds.), Auditory Frequency Selectivity. Plenum, New York and London, pp. 73–80.

Gleich, O. (1987) Electrical tuning in the avian inner ear. Abstr. Assoc. Res. Otolaryngol. 10, 22–23.

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

Gross, N.B. and Anderson, D.J. (1976) Single unit responses recorded from the first order neuron of the pigeon auditory system. Brain Res. 101, 209–222.

Gummer, A.W., Smolders, J.W.Th. and Klinke, R. (1985) The mechanics of the basilar membrane and middle ear in the pigeon. In: J.B. Allen, J.L. Hall, A. Hubbard, S.T. Neely and A. Tubis (Eds.), Peripheral Auditory Mechanisms. Springer-Verlag, Berlin, Heidelberg, New York, pp. 81–88.

Hillery, C.M. and Narins, P.M. (1984) Neurophysiological evidence for a traveling wave in the amphibian inner ear. Science 225, 1037–1039.

Hillery, C.M. and Narins, P.M. (1987) Frequency- and time-domain comparison of low-frequency auditory fiber responses in two anuran amphibians. Hear. Res. 25, 233–248.

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.

Kettner, R.E., Feng, J.-Z. and Brugge, J.F. (1985) Postnatal development of the phase-locked response to low frequency tones of auditory nerve fibers in the cat. J. Neurosci. 5, 275–283.

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

Littlefield, W.M. (1973) Investigation of the linear range of the peripheral auditory system. D.Sc. Dissertation. Washington Univ., St. Louis, MO.

Manley, G.A. (1979) Preferred intervals in the spontaneous activity of primary auditory neurones. Nat. wiss. 66, 582.

Manley, G.A. (1980) Response characteristics of auditory neurones in the cochlear ganglion of the starling. Acta XVII Congr. Int. Ornithol. 697–700.

Manley, G.A. (1986) The evolution of the mechanisms of frequency selectivity in vertebrates. In: B.C.J. Moore and R.D. Patterson (Eds.), Auditory Frequency Selectivity. Plenum, New York and London, pp. 63–72.

Manley, G.A. and Gleich, O. (1984) Avian primary auditory neurones: the relationship between characteristic frequency and preferred intervals. Nat. wiss. 71, 592–594.

Manley, G.A. and Leppelsack, H.-J. (1977) Preliminary data on activity patterns of cochlear ganglion neurones in the starling. In: M. Portman and J.-M. Aran (Eds.), Inner ear Biology-XIVth Workshop, INSERM, Paris, pp. 127–136.

Manley, G.A., Gleich, O., Leppelsack, H.-J. and Oeckinghaus, H. (1985) Activity patterns of cochlear ganglion neurons in the starling. J. Comp. Physiol. 157, 161–181.

Narins, P.M. (1987) Phase-locking characteristics of amphibian auditory nerve fibers in tones and noise. Abstr. Assoc. Res. Otolaryngol, 10, 230–231.

Narins, P.M. and Gleich, O. (1986) Phase response of low-frequency cochlear ganglion cells in the starling. In: B.C.J. Moore and R.D. Patterson (Eds.), Auditory Frequency Selectivity. Plenum, New York and London, pp. 209–216.

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

Palmer, A.R. and Russell, I.J. (1986) Phase-locking in the cochlear nerve of the guinea-pig and its relation to the receptor potential of inner hair-cells. Hear. Res. 24, 1–15.

Rhode, W.S. and Robles, L. (1974) Evidence from Mössbauer experiments for nonlinear vibration in the cochlea. J. Acoust. Soc. Am. 55, 588–596.

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.

Russell, I. and Palmer, A.R. (1986) Filtering due to the inner hair-cell membrane properties and its relation to the phase-locking limit in cochlear nerve fibers. In: B.C.J. Moore and R.D. Patterson (Eds.), Auditory Frequency Selectivity. Plenum, New York and London, pp. 198–207.

Sachs, M.B., Lewis, R.H. and Young, E.D. (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: comparisons with mammalian homologues and consideration of the neural encoding of complex stimuli. In: A.N. Popper and R.R. Fay (Eds.), Comparative Studies of Hearing in Vertebrates. Springer, Berlin, Heidelberg, New York, pp. 323–353.

Schermuly, L. and Klinke, R. (1985) Change of characteristic frequency of pigeon primary auditory afferents with temperature. J. Comp. Physiol. 156, 209–211.

Sellick, P.M., Patuzzi, R. and Johnstone, B.M. (1982) Measurement of basilar membrane motion in the guinea pig using the 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. Hear. 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, 1787–1799.

Takasaka, T. and Smith, C.A. (1971) The structure and innervation of the pigeon's basilar papilla. J. Ultrastruct. Res. 35, 20–65.

Tanaka, K. and Smith, C.A. (1978) Structure of the chicken's inner ear: SEM and TEM study. Am. J. Anat. 153, 251–272.

Woolf, N.K. and Sachs, M.B. (1977) Phase-locking to tones in avian auditory-nerve fibers. J. Acoust. Soc. Am. 62, 46.

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