Auditory Frequency Selectivity

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PHASE RESPONSE OF LOW-FREQUENCY COCHLEAR GANGLION CELLS IN THE STARLING

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INTRODUCTION

The avian inner ear has recently been the focus of several anatomical studies aimed at characterizing the development and formation of the hair cells along the basilar membrane (Rebillard et al., 1982; Lippe and Rubel, 1983; Chandler, 1984; Ryals and Rubel, 1985; Cotanche and Corwin, 1986), as well as physiological studies of the response properties of the auditory periphery (Sachs et al., 1974, 1980; Gross and Anderson, 1976; Manley and Leppelsack, 1977; Manley, 1980; Manley et al., 1985). It is becoming increasingly clear that the inner ear of the bird is both a convenient and structurally relatively simple model for understanding acoustic processing of sounds by vertebrates.

The purpose of this study is to investigate the preferred phase of firing of starling cochlear ganglion cells as a function of stimulus frequency and intensity. It is hoped that expanding the data base of avian auditory response properties will enable comparisons of similarities and differences to mammalian and other non-mammalian vertebrates to be made, and will yield insights into both the mechanisms and evolution of hearing.

METHODS

Adult starlings (Sturnus vulgaris) (N=11) were anesthetized by intrapectoral injection of pentobarbitol sodium (Nembutal, 90-120 mg/kg body weight). The surgical preparation has been described previously (Manley et al., 1985) and consisted of using a dorso-lateral approach to remove the bone overlying the middle ear cavity in order to expose the basal end of the cochlea. Then, upon opening the inner ear in the region of the recessus scalae tympani, the cochlear ganglion was observable as a white band deep in the scala tympani. Individual cells were penetrated using conventional glass micropipettes filled with 3M KCl with tip diameters of about 0.2 μm and impedances in the range of 15-90 Megohms. The animal was artificially respirated, placed inside a vibration-isolated, anechoic chamber and maintained at a constant temperature of 39°-41°C.

A specially-designed housing contained an earphone for sound presentation (AKG DKK 32) and a calibrated microphone (Brüel and Kjaer 4133) for

monitoring and calibrating the acoustic stimulus. The output port of this housing was sealed around the tympanic membrane of the bird with silicone grease, creating a closed-field acoustic delivery system. The system was routinely calibrated before each experiment; its frequency response was flat (±1 dB) between 0.05 and 4.0 kHz, and the phase response was used to refer all phase measurements to the stimulus waveform at the animal's tympanic membrane.

Acoustic search stimuli were not used, since cochlear ganglion cells in the starling are spontaneously active (Manley et al., 1985). When a single cell was isolated, its CF and best threshold were first determined manually using 50 ms tone bursts presented at 4/s. The FTC was then obtained using 100 ms (2.5 ms rise-fall time) fixed-level tone bursts at 4/s such that each of 24 frequencies was presented twice over the range from 0.025-4.0 kHz. The stimulus level was then incremented in 8 dB steps between 10 and 90 dB SPL. Families of iso-response curves were plotted for different discharge rates; a 40% increase over spontaneous rate was the threshold criterion chosen for the FTC.

In addition, period histograms of 100 response spikes were generated using the positive-going zero-crossing of the stimulus waveform to trigger the digital input of the computer (DEC MINC 11/23) which also logged the real-time of occurrence of the spike events to the nearest 1 μs. Vector strength (Goldberg and Brown, 1969) and preferred firing phase (mode) were determined from each histogram. We calculated the significance of phase-locking using a Rayleigh test of circular data, and a likelihood value $L=2n(VS)^2$, where n=number of spikes, and VS=vector strength (Mardia, 1972; Bunnen and Rhode, 1978). For a histogram with 100 spikes, a $VS \geq 0.26$ is highly significant ($p<0.001$). The phase-vs.-frequency function was then constructed exclusively from those period histograms exhibiting significant phase-locking. Period histograms were obtained in response to pure tones separated by 25, 50 or 100 Hz steps, depending on and always encompassing the cell's CF. All stimulus and response data were stored on an analog tape recorder for subsequent analysis.

The weighted average group delay for a particular cell was estimated by the slope of its phase-vs.-frequency function. Since the weighted average group delays are functions of stimulus level in the mammalian ear (Anderson et al., 1971), it is important to compare these delays at a fixed intensity. We chose 90 dB SPL as our standard since we tested most cells at this level, and it probably results in close to the minimum delay for this method (for a complete description of the technique, see Anderson et al., 1971, or Hillery and Narins, 1984).

RESULTS

We measured the phase response of single low-frequency cochlear ganglion cells as a function of stimulus frequency in the starling (N=28), and the intensity-dependence of this response (N=23). In our preparation only cells with CFs between 0.1 kHz and 1.6 kHz were encountered; over this frequency range all cells exhibited strong phase-locking to sinusoidal stimuli over a wide range of intensities. Fig. 1 shows a typical FTC from the cochlear ganglion of the starling, superimposed on which are the vector strengths of phase-locking tested at the frequency-intensity coordinates of the base of each vertical line. Also shown in fig. 1 are two series of period histograms- one contained at three intensities at the fiber's CF (0.8 kHz), and the other at three intensities above the CF, at 1.0 kHz. Fig. 1 illustrates a result typical for our sample, namely that at or near the neuron's CF, the preferred firing phase changes relatively little with intensity, whereas above CF, there is a progressive phase lead

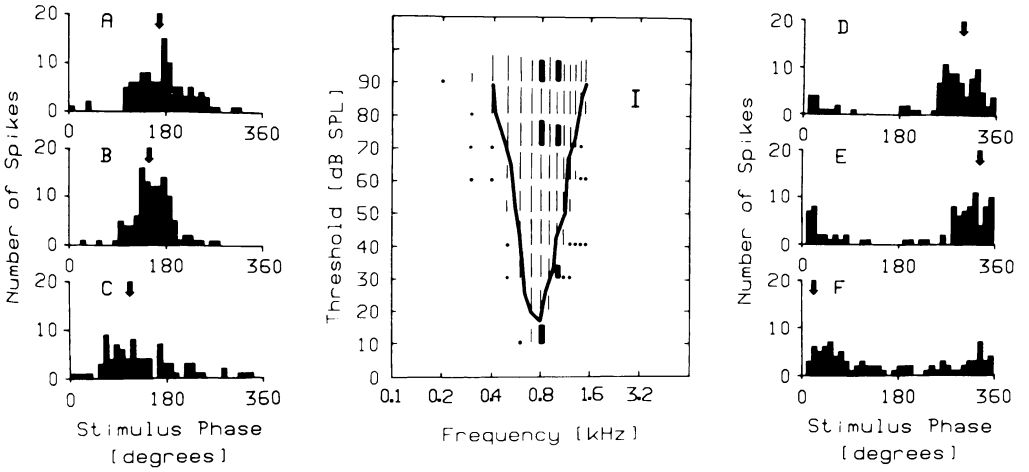

Fig. 1. Typical FTC from starling cochlear ganglion cell (CF=0.8 kHz) with vector strengths indicated by vertical bars (calibration bar: VS=0.5). Heavy bars at CF correspond to period histograms (a)-(c) (VS=0.68, 0.81, and 0.53, respectively), whereas heavy bars at 1 kHz correspond to histograms (d)-(f) (VS=0.66, 0.68 and 0.35, respectively). Phase-locking at indicated points outside FTC is non-significant (see text). Arrows above histograms indicate preferred firing phase. Histogram bin widths: 10°.

with increasing intensity. Likewise, below CF, there is a progressive phase lag with intensity (fig. 4). Similar results have been reported for the auditory nerve of the mammal (Anderson et al., 1971; Allen, 1983;

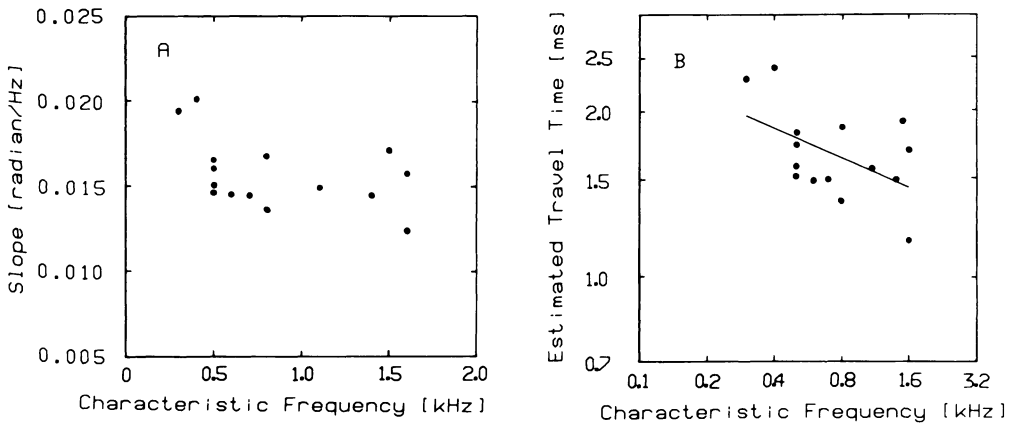

Fig. 2. (a) Slope of phase-vs.-frequency functions obtained at an intensity of 90 dB SPL plotted against the cell's CF. Over the frequency range tested, the slope does not show a significant dependence on CF ($p>0.05$). (b) Travel time estimated from data in (a) by subtracting 0.8 ms from each point, corresponding to the acoustic plus neural delay (see text), multiplying each value by the scale factor $1/2\pi$, and replotting on a log scale. Regression is significant at the 0.05 level.

Ruggero and Rich, 1983), the frog (Narins, 1986; Hillery and Narins, 1986), the caiman (Klinke and Pause, 1980), and the nucleus magnocellularis of the barn owl (Sullivan and Konishi, 1984).

Fig. 2a shows the slopes of the individual best-fit linear regression lines through the phase-vs.-frequency functions for all fibers tested at 90 dB SPL (N=21), as a function of the fiber's CF. Surprisingly, for low-frequency cells in the starling, slope varies little with fiber CF; the best-fit regression line through the data in fig. 2a is essentially flat. All phase-vs.-frequency slopes in our sample for the low-frequency cells are between 0.01 and 0.02 radian/Hz. Transforming the data by subtracting 0.8 ms (obtained from latency measurements for a 3.0 kHz neuron in the starling in response to a high-level rarefaction click, H. Oeckinghaus, pers. comm.), scaling for the appropriate units, and plotting on a log-log scale, one obtains the travel time as a function of CF (fig. 2b). The best-fit linear regression line through these points ($r=0.52$; $p<0.05$) indicates that travel time progressively increases with decreasing CF.

Fig. 3a-c shows typical phase-vs.-frequency functions for all single cells tested at 70, 80 and 90 dB SPL, respectively. We found that the data points for each of these functions could be reasonably well fit by a single linear regression line through them ($0.976<r<0.999$; all $p<0.001$), and that the slopes of the phase-vs.-frequency functions are dependent on the stimulus intensity used to derive them. This may be seen more dramatically in fig. 4a-c which illustrates a series of phase-vs.-frequency functions for three cells, each of which was tested with a range of stimulus levels. Note that for each cell there is a "crossover frequency" at which the phase response is intensity-independent. Unlike the data from the nucleus magnocellularis of the barn owl for which the "crossover frequency" occurred <u>at</u> the cell's CF (Sullivan and Konishi, 1984), in the starling cochlear ganglion it appears to occur consistently <u>above</u> CF for cells with CFs above 0.3 kHz.

From data such as those in fig. 5, one can calculate the slope of the phase-vs.-frequency functions for different stimulus sound pressure levels. Fig. 5a shows such a plot; the functional dependence of the slope on stimulus level is clear: increasing the sound pressure level decreases the slope of the phase-vs.-frequency plot, equivalent to decreasing the weighted average group delay.

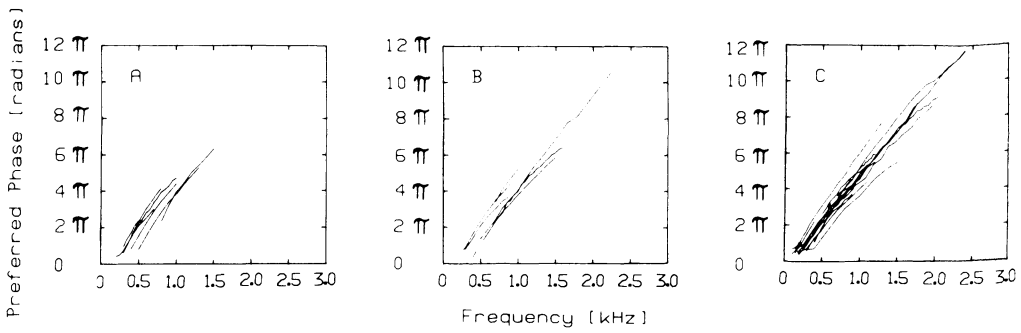

Fig. 3. Phase-vs.-frequency functions for all cochlear ganglion cells tested at (a) 70 dB SPL (N=7), (b) 80 dB SPL (N=6) and (c) 90 dB SPL (N=15). Each function was adjusted along the vertical axis in one cycle increments until it passed as closely as possible to the origin.

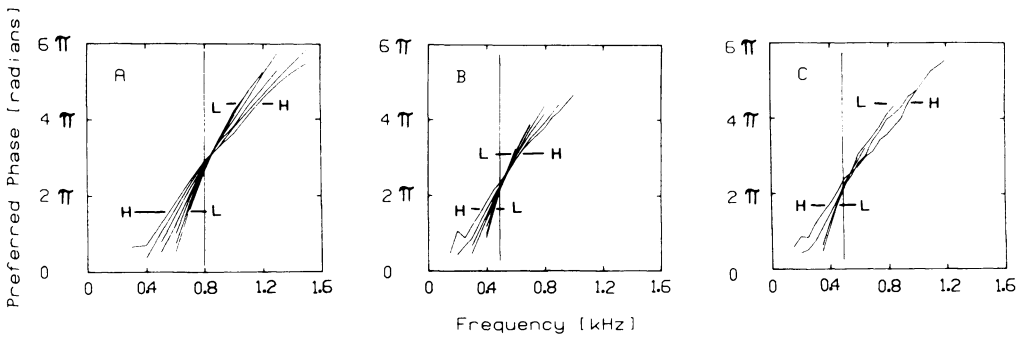

Fig. 4. Phase-vs.-frequency functions obtained at a series of stimulus levels for three cochlear ganglion cells in the starling. Vertical lines in each panel indicate the cell's CF: (a) 0.8 kHz, (b) 0.5 kHz and (c) 0.5 kHz. (a) and (b) show a discrete "crossover frequency", whereas (c) does not. For these three cells, and all cells in our sample with CFs above 0.3 kHz, the "crossover frequency" lies above CF. L and H indicate low and high stimulus levels, respectively.

In fig. 5b we have plotted the slopes of the functions relating the preferred firing phase of a cell vs. stimulus intensity (see inset) at the cell's CF (N=17). We find that the preferred phase of firing for cells with CFs around 0.3 kHz shows the least dependence on stimulus intensity (inset curve 1). Cells with CFs above 0.3 kHz (e.g., cells in fig. 4) showed a progressive phase lag with increasing intensities of Cf tones (inset curve 2), whereas cells with CFs below 0.3 kHz showed a progressive

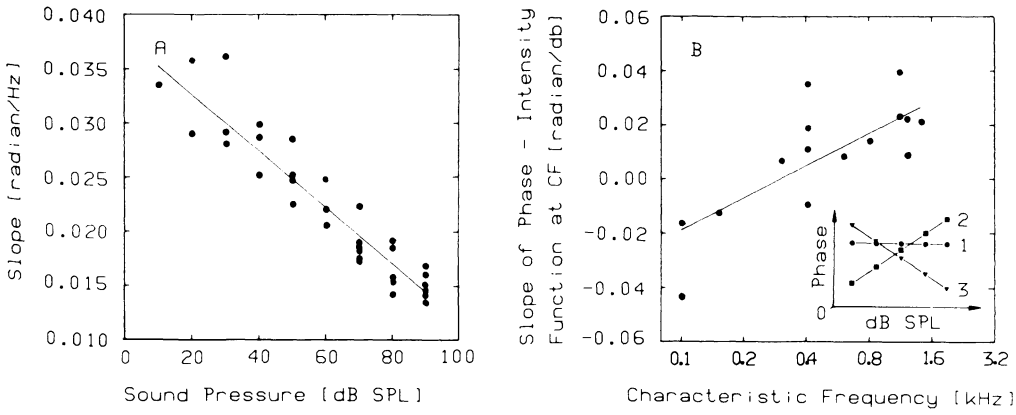

Fig. 5. (a) Phase-vs.-frequency slopes measured for all cochlear ganglion cells for which these slopes were determined at more than one intensity (N=7), as a function of stimulus level. The intensity-dependence of the slopes is clear ($r=-0.94$; $p<0.001$). (b) Slope of phase-intensity function at CF as a function of the CF of the cell. The sign of the slope is zero for cells with CFs near 0.3 kHz (inset, no. 1), positive for cells with CFs > 0.3 kHz (inset, no. 2) and negative for cells with CFs < 0.3 kHz (inset, no. 3).

phase lead with increasing intensities of CF tones (inset curve 3). The best-fit regression line through these data ($r=0.75$; $p<0.001$) crosses the "zero" slope point at about 0.3 kHz.

DISCUSSION

We have shown that low-frequency avian cochlear ganglion cells exhibit remarkably straight phase-vs.-frequency functions over a wide range of intensities. Some exceptions to this goodness-of-fit were indeed found: (1) at the lowest and the highest intensities tested the functions tended to asymptote for frequencies above CF, and (2) at the lowest frequencies tested for a given cell, we occasionally observed a phase plateau (e.g., figs. 3a, 3c, 4). The slopes of the phase-vs.-frequency functions, after subtraction of the appropriate neural and acoustic delays reflect a small range of travel times (1.37-2.40 ms) across the low-frequency (apical) end of the avian basilar papilla. We are aware that in the mammalian ear travel times obtained with this method tend to be systematically overestimated (Ruggero, 1980), but this error should be minimum in the bird since the phase-vs.-frequency functions are extremely straight.

With evidence accumulating for electrical tuning in vertebrate hair cells (Crawford and Fettiplace, 1981; Lewis and Hudspeth, 1983; Ashmore and Pitchford, 1985), it is tempting to look for correlates of our cochlear ganglion cell data in the hair cell response. Crawford and Fettiplace (1981) show one cell in which they measured the phase of the receptor potential as a function of frequency for various sound intensities. This potential shows a progressive phase lag with increasing intensity for test frequencies below CF and a slight phase lead with increasing intensity for test frequencies above CF. This correlates qualitatively with the observed direction of phase shift with intensity for avian cochlear ganglion cells.

In the squirrel monkey, linear phase-vs.-frequency functions for single auditory nerve fibers and a systematic decrease in travel time with increasing CF derived from the slopes of these functions, coupled with the known tonotopy of the basilar membrane have been interpreted as reflecting the travelling wave known to occur on the mammalian basilar membrane (Anderson et al., 1971). Mid-frequency auditory nerve fibers originating in the inner ear of the frog (which contains no basilar membrane), also show linear phase-vs.-frequency functions, with systematic steepening of their slopes for decreasing CFs (Hillery and Narins, 1984). These observations have also been interpreted as being consistent with a low-velocity, propagating mechanical disturbance along the amphibian papilla.

von Békésy (1960) showed that the position of resonance shifts basalward along the cochlear partition with increasing stimulus frequency in the chicken, but to date there has been no convincing demonstration of the existence of a travelling wave in the avian inner ear. The direct mechanical measurements of the pigeon basilar membrane motion (Smolders et al., this volume) and our phase measurements of cochlear ganglion cells in the starling are both consistent with the existence of a travelling wave in the avian inner ear.

If one adopts the simple definition of a travelling wave as proposed by Wilson et al. (1985) based on the observations of von Békésy (1960), then one cannot distinguish, on the basis of neural phase measurements alone, between a wave propagating in a medium, or delay line, and a wave propagating across a bank of resonators. An array of simple resonators will, of course, exhibit a frequency-dependent phase shift in response to a pure tone, with the total phase shift across the array equal to $\pi/2$ times the number of resonator stages (Lewis et al., 1985). It seems

likely to us that active cellular processes coupled to basilar membrane motion give rise to the phase behavior in the avian cochlea.

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