Chapter 1

Temporal patterns of the responses of auditory-nerve fibers to low-frequency tones

Introduction

There are still matters regarding the responses of auditory-nerve (AN) fibers to low frequency (LF) tones that are not completely understood. One of these concerns "peak-splitting". In the responses of AN fibers to a LF tone, usually there is only a single peak in a period histogram constructed on the period of the LF tone. However, at higher stimulus intensities, there are sometimes two or more peaks in the period histogram. This phenomenon of peak multiplication is commonly called "peak-splitting" and has been demonstrated in a number of species (e.g., Kiang and Moxon, 1972, Johnson, 1980 and Kiang, 1984 in cat; Ruggero and Rich, 1983, 1989 in chinchilla; Oshima and Strelioff, 1983 and Ronken, 1986 in gerbil; see Kiang, 1990 for summary). It is often related to a "notch" in the fiber's rate-intensity curve and to a rapid phase shift which occurs there (Kiang and Moxon, 1972; Kiang, 1984). The origin of peak-splitting is of much interest. Although it seems there is no disagreement that it is generated by some nonlinear effect somewhere between the basilar membrane (BM) motion (which shows no such peak-splitting) and the AN spike initiation, the exact site attributed differs in each hypothesis. Proposals ranged from the effect of current in the organ of Corti fluid upon the inner hair cell (IHC)

transmembrane potential (Ruggero and Rich, 1983) and harmonics summed at the IHC level (Liberman and Kiang, 1984; Kiang et al., 1986), to cochlear mechanics (Cody and Mountain, 1989), or more specifically, the outer hair cell (OHC) (Ruggero and Rich, 1989). To decide between these hypotheses will require more data, because there have been few detailed studies of peak-splitting on the AN-fiber responses so far (Ruggero and Rich, 1989, in chinchilla; Liberman and Kiang, 1984, in cat). In this report, we provide a more detailed study of this phenomenon in cat and discuss possible origins.

A second issue not well understood concerns the timing of spike initiation in relation to BM motion. Earlier studies from different animal species indicate that the response phases, normalized to BM motion, seem to be in all possible phases of BM motion (see Ruggero and Rich, 1983 and 1987 for summary). During the past decade, Ruggero and colleagues have explored this issue intensively in one animal species, the chinchilla (Ruggero and Rich, 1983, 1987, 1988; Ruggero et al., 1986a, 1986b). They have shown that the phases of an AN fiber's responses were strongly dependent both upon the characteristic frequency (CF) of the fiber and upon the stimulus intensity. For very low frequency stimuli (30-100 Hz), there was a near-180 degrees phase change in the cochlear region which corresponds to CFs ranging between 2 to 6 kHz (Ruggero and Rich, 1983, 1987). In the basal region, the near-threshold response phase (corrected for synaptic delay and neural travel time) led the BM displacement towards scala tympani (ST) by about 40-60 degrees (Ruggero and Rich, 1987). For fibers from both apical and basal regions, there were abrupt phase changes in the AN responses with increases of intensity (Ruggero and Rich, 1988). However, studies in other animal species produce results which do not agree with the chinchilla results. For example, Oshima and Strelioff (1983) showed a clear difference between the responses of gerbil and guinea pig to 50 Hz tones: there was a near 180-degrees phase change at CFs between 2-4 kHz in gerbil

but not in guinea pig, and there was no sign of an intensity dependence in either species. This possible species difference makes it difficult and even risky to interpret experimental results in one species based on findings in other species. In cat, one of the major animal species used in auditory research, there have been several studies of the phases of AN responses to LF tones (e.g., Pfeiffer and Kim, 1975; Kim et al., 1980; Liberman and Kiang, 1984), but these are limited in terms of stimulus frequencies, intensities and CF range. In this report, we provide results that complement and extend the findings of previous cat studies. We also explore the intensity effects on the response phase and relate the response phase of the auditory-nerve fibers to BM phase (as inferred from cochlear microphonics (CM) data).

Methods

1. Animal preparation

Adult cats of either sex, weighing 2 to 5 Kg, and with no sign of middle ear infections were used in this study. Pentobarbital sodium (40 mg/kg, i.p.) was used to anesthetize the animal and supplemental doses were given whenever necessary to maintain areflexia. A tracheal tube was inserted, and a heating pad was used to maintain the rectal temperature of the cat at about 37.5 °C. The pinna was removed to allow the delivery of sound stimuli directly to the external meatus through a metal ear piece. A small hole was opened on the bulla and a silver wire electrode was placed near the round window (RW) to monitor the condition of the cochlea. A plastic tube of about 40 cm length and with an inner diameter of about 0.6 mm was placed through the opening to provide static-pressure release (Guinan and Peake, 1967). The bulla was then re-sealed with glue and dental cement. The skull was opened in the

posterior fossa area with a rongeur. The use of an electrical drill was avoided since it might cause some damage to the cochlea. The dura was cut and cerebellar tissue was gently pulled toward the midline with a small aluminum retractor, using a manual microcontroller, to expose the auditory nerve. Once in place, the aluminum retractor was glued onto the skull, to keep the nerve exposed during the experiment, which usually lasted for 3 days. A plastic chamber was then cemented over the exposure and filled with warm mineral oil. A Trent-Wells hydraulic microdrive was attached to the chamber to form a hydraulic seal and ensure the stability of the electrode during the experiment. A 30-70 mΩ glass electrode (filled with 3 M KCl) was aimed at and inserted into the nerve under visual guidance. During the whole process, special attention was paid to minimize any possible damage to the cochlea.

2. Sound delivery and recording system.

The sound delivery and recording system was standard in this laboratory (e.g., Deng & Geisler, 1985). Briefly, the acoustic stimuli were generated by a digital stimulus system (Rhode, 1976), mixed electronically, presented by a condenser earphone (Stax) and delivered to the truncated external meatus through the ear piece. All of the parameters of the stimuli (frequency, intensity, delay, rise/fall time, etc.) are controlled by the computer through macro-instructions. For calibration, a probe tube was inserted into the ear piece with its tip seated within several mm of the ear drum. The ear piece was sealed to the auditory meatus with Audalin to prevent acoustic leakage at low frequencies. In each experiment, the system was calibrated after the surgery procedure and before the data collection with a condenser microphone (B & K, type 4134, 1/2 inch) . The neural signals went through a spike detector, the output of which was sent to the computer. The exact time (resolution of 1 μsec) of each neural spike with respect to the stimulus onset was then stored. The rate-intensity curve and

post-stimulus time (PST) histograms were displayed during the experiment. Additional displays were produced during off-line analysis.

3. Stimulus

This study was part of a larger study to investigate the temporal response patterns of auditory-nerve fibers to two-tone stimuli. The stimuli used were two partially overlapping pure tones (Fig. I-1). The low frequency tone (which also served as suppressor) was chosen so that the CF tone and the LF tone were harmonically related. When a fiber was located by a frequency-modulated sweep tone, a threshold frequency-tuning curve was taken. Information about spontaneous rate (SR) and click latency were also usually collected. Then the stimulus paradigm illustrated in Fig. I-1 (with a repetition period of 1 second) was used. Each tone was 500 ms in duration. As the LF tone was delayed relative to the CF tone, there were three successive time windows during each stimulus presentation which corresponded to CF tone alone, two-tone and LF (suppressor) tone alone (cf. Fig. I-1). The intensity of the CF tone was fixed at 10 to 30 dB above threshold. The intensity of the LF tone was then varied over an intensity range (e.g. 70 to 95 dB SPL), usually in 5 dB steps. To minimize possible overstimulation to the cochlea, the highest stimulus intensity was limited to 95 dB SPL. The frequency of the LF tone usually had one of the following values: 50, 100, 200, 500, 1000 or 2000 Hz. For each frequency and intensity combination, the paradigm was repeated 75 times and the responses were combined.

To monitor the physiological condition of the cochlea, the compound action potential (CAP) was recorded through an electrode near the round window before and during the single-fiber data collection. The head holder formed the ground electrode, as in the case of single-fiber

recordings. Acoustic clicks (electrical signal was a 10-μs pulse, repeated 10 times per second) were used to generate the CAP responses, which were averaged 100 times. The electrode configuration for CM recording was the same as for CAP. Thus the same basic acoustic and recording system was used to collect CM, CAP and the single-fiber data, except in the cases of CAP and CM, where the analog signal from the amplifier was sent to the computer's A/D converter directly. For recording CM, the stimulus was a single LF tone (50, 100 or 200 Hz) which lasted 300 ms and was repeated 75 times with a repetition period of 500 ms. The high-side cutoff frequency of the amplifier's filter was 3 kHz, but the low-side cutoff frequency was varied between 10 to 300 Hz in different experiments. The phases of the CM responses were later corrected for the filter setting to an equivalent low-side cutoff frequency of 1 Hz (the hardware does not allow correction to DC).

4. Data analysis

Only data from "normal" preparations are included in this report. When the condition of a cochlea had deteriorated (as judged by a rise in the threshold of the click-evoked CAP response of 10 dB or more, or by fiber thresholds which were more than 15 dB higher than those reported by Liberman, 1978), any data taken were not included in the analysis. Eight cats contributed to the data: C92101, C92106, C92116, C93030, C93036, C93146, C94009 and C94011. For convenience, they are also referred to as cats A, B, D, E, F, H, I and J, respectively. Cat H's cochlea had a traumatic injury but seemed to recover. That injury was caused by an accidental pulling out of the RW electrode when the head holder was turned. Although there was no visible damage to the RW membrane under the surgical microscope, the voice-evoked CM responses were much smaller when the RW electrode was re-implanted, and the CAP threshold increased by about 20 dB, compared to comparable

responses in other animals and the value observed later in the same animal. The initial AN fiber thresholds were also elevated by about 20 dB. However, after about 24 hours, both the CAP threshold and the fiber thresholds returned to "normal". Thus the data from the first 24 hours of experiment H were excluded from the data analysis. The rest of that animal's data points were included (and are identified with special symbols in the pooled data).

In Fig. I-2 we show the thresholds of all fibers (344) from the 8 animals we studied. 195 of the 344 contributed to the data we present in this and other reports. The solid line in the figure is the "best threshold curve" reported by Liberman (1978). The threshold data show a considerable distribution, and fibers with low (<= 1/sec) and medium (> 1/sec and < 20/sec) spontaneous rates (SR) generally have higher thresholds than those with high (>= 20/sec) SRs. There is a general rise of threshold in the 3-4 kHz region, similar to the superimposed best threshold curve. All these features are similar to those reported by Liberman (1978). Based on these threshold data and the CAP data, we judge all 8 cochleae to have been in nearly normal condition at the time the data were collected.

Fig. I-3 shows the click latencies of those fibers. One interesting thing in this figure is that at lower CFs (less than about 4 kHz) the distribution bifurcates: one group has longer latencies while the other group has short latencies. Careful examination reveals that the time difference in the latency of these two groups at one particular CF is the inverse of the CF, suggesting that the longer latency was due to a lower effective neural drive. As is well known, the click response of a LF fiber "rings" at the CF of the fiber. Thus, the lower group, which is close to the latency data of cat reported by Kiang et al. (1965), would be a better representation of the true latencies of those fibers.

Using the period of the LF tone as base, period histograms (64 bins) were constructed for the LF-tone window (with the initial 75 ms excluded, Fig. I-1). In determining the phase of maximum response to LF tone, the vector phase was used (Goldberg and Brown, 1969). Each histogram was statistically tested for departure from uniform distribution using a Rayleigh test (Deng & Geisler, 1985), which is a maximum likelihood ratio test for circular data. A brief description of this test is given in Appendix A. Usually a Rayleigh coefficient of less than 0.05 is considered to be significant enough to reject the assumption of uniform distribution. However, due to the way the test is formulated and to our own experience that a phase value obtained at the 0.05 level may not be reliably repeatable, we only included in our phase analysis those period histograms that had a Rayleigh coefficient less than or equal to 0.001.

The care and use of animals reported in this study were approved by the Animal Care and Use Committee of the University of Wisconsin-Madison.

Results

1. Peak-splitting

Examples of peak-splitting are shown in Fig. I-4 for stimulus frequencies of 50, 100 and 200 Hz. The data were all obtained from one fiber, J42, which had a CF of 1178 Hz. With a stimulus frequency of 50 Hz (column A), the period histogram was basically unimodal at moderate intensity (75 dB SPL). As the intensity was increased, the peak gradually became higher and broader, and eventually two peaks, about half a cycle apart, emerged (90 dB

SPL). The data from column B were taken with a stimulus frequency of 100 Hz. The peak-splitting is quite obvious both at 85 dB SPL and at 90 dB SPL. However, the peaks are not half-cycle apart, as in 50 Hz case, but only about 0.2 cycle apart. In column C, where the stimulus frequency was 200 Hz, the split happened at 75 dB SPL, which is about 10 dB lower than in the 50 and 100 Hz cases, and the two peaks are about 0.3 cycle apart. Although there were exceptions, the pattern shown in columns A and B of Fig. I-4 are typical in our data for peak-splitting at stimulus frequencies of 50 Hz and 100 Hz, respectively. The frequency dependence of the distance between the peaks and of the intensity at which peak-splitting begins as illustrated in Fig. I-4 was a general phenomenon. In columns A and B of Fig. I-5 we show examples from another fiber from the same experiment (J28). The 50 Hz case (column A) was quite similar to that shown in Fig. I-4, with peaks about half a cycle apart. Column B (100 Hz stimulation) also resembles its counterpart in Fig. I-4 in its general shape and inter-peak distances, except that a third peak began to emerge near the phase of 0.8T (T is the period of the LF tone) at 85 dB SPL. Column C of Fig. I-5 shows another example from this same animal using 50 Hz stimulation. The general shape of the histograms and their evolution are similar to those shown in column A of this figure and of Fig. I-4, except that the taller peak further split into two peaks. In column D, a three-peaked example from another experiment is shown for 100 Hz stimulation. The peak produced by 85 dB stimulation (at a phase of 0.25T, marked by arrow) was the first which emerged, but it never grew very large. Two other peaks, located at either side of this peak, became dominant at the higher intensities.

In Fig. I-6, we show some comparable responses from a high- and low-SR fiber pair (each with a CF of about 12 kHz), which were taken consecutively from the same electrode penetration in animal I, and were studied with the same stimulus parameters. We believe

Fig. I-5 Further examples of peak-splitting. Data in columns A and B are from the same fiber (C94011, J28, CF = 700 Hz; SR = 63.0/s, TH = 12 dB SPL). The data in column C are from another fiber in the same animal (J44, CF = 400 Hz, SR = 47.0/s, TH = 8 dB SPL) while the data in column D are from a fiber in another animal (C93030, E56, CF = 700 Hz, SR = 39.5/s, TH = 20 dB SPL).

that, since they had similar CFs and very close proximity in the penetration pathway, they at least innervated neighboring IHCs, if not the same IHC. Their response patterns to single-tone and two-tone stimuli will be reported in more detail in Chapter 3. In response to 500 Hz tone (Fig. I-6), their temporal response patterns are remarkably similar, with both fibers showed very similar peak-splitting. The single-tone responses of another fiber pair are shown in Fig. I-7. This pair of fibers was taken from experiment J. Both fibers had similar CFs (~4.8 kHz), but since their locations in the AN were much farther separated than the fiber pair in Fig. I-6, it is more likely that the fiber pair in Fig. I-7 innervated different IHCs. In response to both LF tones (suppressor, 200 Hz) and CF tones, both fibers showed very similar suppression phases (Appendix B). Yet in response to 200-Hz tones, they showed different response patterns (Fig. I-7). At 85 dB SPL, fiber J75 showed basically a single peak (Fig. I-7A, arrow), at phase 0.12T. At the same intensity, fiber J82 showed two response peaks (arrows), which are slightly less than half a cycle apart (Fig. I-7B). The major peak is at phase 0.67T, and the minor peak is at about 0.1T, approximately the same phase as the single response peak of fiber J75.

Similar to prior studies in cat (Kiang and Moxon, 1972; Johnson, 1980; Kiang, 1984, 1990), we saw peak-splitting mostly for lower CF fibers (Fig. I-8) and with low stimulus frequencies (Table I-1). In fact, among the 45 fibers that showed peak-splitting, 37 of them had CFs below 2 kHz. These 37 fibers constitute about 47% of the fiber population in our sample of that CF region. This CF-dependence is in sharp contrast to the data from chinchilla (Ruggero and Rich, 1983, 1989), where peak-splitting was, for the intensities which we used, also present in higher CF fibers. Although the multiple-peak examples we showed in Fig. I-5 have not been previously shown for cat, they are present in chinchilla (Ruggero and Rich, 1983, 1989) and gerbil (Ronken, 1986) data. However, most fibers in our split-peak data

display only two peaks. In two cases only did we see 4 peaks. A given fiber did not necessarily show peak-splitting at all frequencies. For example, some fibers showed peak-splitting at 100 Hz but not at 50 or 200 Hz at the intensities we used. This indicates a frequency dependence of the intensity at which peak-splitting occurs.

Table I-1 Number of fibers showing 2, 3 and 4 peaks

Number of peaks	Stimulus frequency (Hz)				
	50	100	200	500	1000
2	15	15	12	3	1
3	5	7	1		
4	1		1		
total cases	21	22	14	3	1

2. Phases of responses to LF tones

In determining the phases of the responses to LF tones, the histograms showing peak-splitting were of course excluded. Figure I-9 shows the pooled data for the phases of the threshold-level responses of AN fibers, for frequencies of 50, 100, 200, 500, 1000 and 2000 Hz. As can be seen, the phases of responses are functions of fiber CF. For all frequencies except 50 Hz, the change of phase with respect to CF was monotonic, and showed less phase lag with increasing CF. For 50 Hz, it varied a little with CF, showing a slightly non-monotonic behavior. With 100 Hz stimulation, the phase decreased at about 0.6 cycle per

Fig. I-9 Vector phase of the threshold-level responses to LF tones (50, 100, 200, 500, 1000 and 2000 Hz), in terms of the period of the LF tone (i.e., phase *delay* is plotted on the ordinate). For 100 and 200 Hz, the ordinate was offset by 0.5 cycle to allow better visualization of the distribution. The solid diamonds indicate data points contributed by experiment H (C93146). The solid curves were obtained by averaging over one octave ranges in half-octave steps. The points that are about 180 degrees away from the main groupings as well as the points from experiment H were excluded from the averaging. The small bars on the right ordinate of the plots for 50, 100 and 200 Hz represent the phases of BM motion as inferred from the CM data (see Discussion). See text for details about the labeled fibers.

decade up to a CF of about 1 kHz, and there was basically no change in the higher CF region. For other frequencies, the slope increased with stimulus frequency (from about 0.35 cycle per decade at 200 Hz to about 0.7 cycle per decade at 1000 Hz).

It is interesting to note that some points are clearly outside the usual range formed by majority of the data points (Figs. I-9A, B and C). Some of these points are about half a cycle away from the usual range. Such points also exist in the phase data for cat (Pfeiffer and Kim, 1975), chinchilla (Ruggero and Rich, 1983) and guinea pig (Oshima and Strelioff, 1983). These extraordinary phase responses seem to be dependent upon the stimulus frequency (i.e., they need not occur in the fiber's responses to all stimulus frequencies). For example, for fiber B6, the phase of response to 50 Hz tone (labeled arrow) was about half a cycle away from the usual range, but the same fiber showed response phases at 100 and 200 Hz (labeled arrows) which were inside the usual range for their respective stimulus frequencies. The same is true for fiber E80, which showed a phase about half a cycle away from the usual range with a 200-Hz stimulus (labeled arrow) but phases in the usual range for stimulus frequencies of 50 Hz and 100 Hz (labeled arrows). Of special interest are the points contributed by experiment H (denoted by filled diamonds in Fig. I-9). Most of the fibers from that experiment (which mostly came from the 8-14 kHz CF region) showed unusual response phases nearly half a cycle away from the rest at 100 Hz and about a 1/4 cycle away from the rest for higher frequencies. The fibers with unusual phase values (including those from experiment H) were not any different from the rest in terms of SR and threshold.

The data shown in Fig. I-9 are the threshold-level responses. At the basilar membrane level, the response is known to be a function of stimulus intensity. Since the threshold of each fiber differs, the near-threshold phase data of Fig. I-9 were taken at different stimulus conditions.

For comparison, we show in Fig. I-10 two examples of phase data that were taken at the same stimulus intensity, each from one animal. For data in the upper panel, the stimulus was a 50-Hz tone, presented at 80 dB SPL. Although there are only a limited number of points, they span a relatively wide CF range with a distribution which is very similar to that shown in Fig. I-9A. In the lower panel, the data are from another animal for which the stimulus was a 100-Hz tone, presented at 85 dB SPL. This distribution is also similar to its counterpart in Fig. I-9B. We conclude that near-threshold phase data shown in Fig. I-9 are representative and are not biased due to different stimulus conditions and animal origins.

In order to display the effects of stimulus intensity on the phase of the AN-fiber responses to LF tones, we plotted the relative phase change with respect to intensity of the LF tone for each fiber. A positive value indicates the phase at that intensity lags the phase obtained at the threshold-level intensity, while a negative value indicates it leads that threshold-level phase. Fig. I-11 shows such intensity effects. As can be seen, the amounts of phase change were small for lower frequencies (< 0.15 of a cycle, or 54 degrees), and the phase shifts are dependent upon both the CF of the fiber and the frequency of the stimulus. For 50 Hz, the phase usually shifted up (lag) for most fibers having low CFs (< 1 kHz, Fig. I-11A), but generally shifted down (lead) for those having higher CFs (>= 1 kHz, Fig. I-11B). For 100 Hz, there existed a similar CF dependence, although the division of fiber CF seemed to be at 2 kHz (Fig. I-11C & D). Such CF dependence of the direction of phase change with intensity was also seen in chinchilla (Ruggero and Rich, 1988, stimulus frequency 100 Hz, CF region <1600 Hz and > 6400 Hz). A similar trend also existed at 200 Hz, where the phase shifted up (lag) for fibers with CF less than about 4 kHz (Fig. I-11E). Due to lack of data, the trend for fibers with CFs greater than 4 kHz is not clear, but there are some fibers that showed large phase shifts (Fig. I-11F). For higher stimulus frequencies, there is no clear CF division

Fig. I-11 Normalized phase-intensity plot for several stimulus frequencies. The data are contributed by the same fibers that contributed to Fig. I-9. The phase at the lowest intensity that showed a significant phase-locked response was set to zero. The phase values shown at subsequent intensities were measured relative to the phase produced at that first synchronizing intensity. A positive value indicates a phase lag, while a negative value indicates a phase lead. See text for details about the labeled curves.

in the phase lead/lag effects (e.g., Fig. I-11G).

Although the general trends for lower stimulus frequencies are quite clear, there are some exceptions. In Fig. I-11A, the fiber labeled J44 initially shifted down, then at 80 dB SPL, it changed direction and shifted up, joining the rest of the population. Two other fibers, labeled I7 and J29, shifted down. At 100 Hz, fiber I7 also displayed an unusual direction of shift (Fig. I-11C), together with another fiber from the same experiment (I6). Three of the fibers showing different directions of phase change (I6, I7 and J29) had unusual threshold-level response phases at one or more frequencies.

Some fibers showed large phase shifts (e.g., 1000 Hz, Fig. I-11G). Fig. I-12 shows examples of large phase shifts, where with both 500 Hz and 1000 Hz tone stimulation, the fiber (J4) showed roughly 180 degrees phase shift within 15 dB (vector phase moved from 0.825T to 0.325T at 500 Hz, and from 0.61T to 0.153T at 1000 Hz). However, the same fiber seemed to show no phase shift with 2000 Hz tone stimulation (Fig. I-12C). We have only a few examples of this near-180° phase-shift behavior, probably due to the relatively low stimulus intensities which we used (see Discussion).

In Fig. I-13, the phase data were plotted as a function of fiber SR. Instead of just using the threshold phases, we use the phases at all intensities. It is clear that there is no obvious SR-dependence in the data, for all stimulus frequencies. Low-SRs fibers rarely showed responses to the three lower frequencies (Fig. I-13A-C) at the intensities which we used, but for fibers that did show responses, there was no particular SR-dependence.

Fig. I-13 Vector phases of responses to LF tones as a function of fiber SR. The phase values measured at all intensities were entered so that each fiber may contribute several points. For 100 and 200 Hz, the ordinate was offset by 0.5 cycle as in Fig. I-9. No effort was made to distingush the data points from experiment H due to the lack of SR-dependence for the data points from experiment H.

3. Cochlear microphonics

In order to estimate the phase of the AN responses relative to BM motion, we recorded the cochlear microphonics near the round window in several experiments. The CM data were analyzed in a way similar to that used to generate the period histograms for the responses on the AN fibers (i.e. fold the original responses according to the period of the LF tone), using the same temporal reference points. The data for different frequencies from one experiment (J) are shown in Fig. I-14. The different curves in each plot represent the CM responses at different stimulus intensities. One feature in the responses is the shift of response peaks with intensity. The total shift of the negative peak was 0.23 cycle for 50 Hz, 0.19 cycle for 100 Hz and 0.2 cycle for 200 Hz. Similar phase shifts have also been observed in guinea pig (Klis and Smoorenburg, 1985) and in chinchilla (Ruggero et al., 1986a). At a constant amplitude of about 100 μV, the phases of maximum negativity for the CM responses, after correction for filter setting (10-3000 Hz), were 0.86 (50 Hz), 0.16 (100 Hz) and 0.34 (200 Hz) respectively. The CM responses from another animal (E), which were recorded using a different filter setting (300-3000 Hz), yielded, when corrected for filter setting, about the same phase of maximum negativity at 100 Hz (0.22) as in experiment J. However, the phases of maximum negativity in the CM data from animal H, of which the phases of the threshold-level responses of AN fibers lead those in other animals (Fig. I-9), also slightly lead the CM phases from the other animals (as compared at the same intensities, the CM phase in experiment H lags that in experiment J by 0.02T at 50 Hz, and leads by 0.17T and 0.16T at 100 and 200 Hz, respectively).

Fig. I-14 Cochlear microphonics responses for three stimulus frequencies (50, 100 and 200 Hz) from the same experiment (C94011). The data were collected in a similar manner as that for the neural data, but analyzed as waveforms. They were averaged 75 times and the averaged responses were then folded according to the periods of the stimuli (see Methods). The folded responses were then divided by the number of stimulus cycles in the stimulus and the average amplitude (in mV) calculated. Each curve represents the CM response at one intensity, which ranged from 60 to 95 dB SPL in 5 dB steps. The amplifier's filter settings were 10-3000 Hz. After correction for the lowpass filtering, the phases of maximum negativity at intensity levels that yielded peak amplitude of about 100 μV are, in terms of stimulus cycle, 0.86T (50 Hz), 0.16T (100 Hz) and 0.34T (200 Hz), respectively. These phase values were then corrected for a synaptic delay and neural travel time of 1 ms to represent the maximum BM displacement toward ST in Fig. I-9 (see Discussion).

Discussion

1. Peak-splitting and its origins

Although peak-splitting was first observed in cat (Kiang and Moxon, 1972), ours is the first population study of this phenomenon in this species. It had been observed previously that peak-splitting seemed to be restricted to responses to tones with frequencies below 1 kHz (Ruggero and Rich, 1989; Kiang, 1990). We made a similar observation, finding further that, in cat, it was mainly restricted to stimulus frequencies below 500 Hz (Table I-1). This value agrees well with the finding that, in guinea pig, the counterpart of peak-splitting in the IHC receptor potential was restricted to stimulus frequencies less than about 200 Hz (Cody and Mountain, 1989). We also found that the majority of the fibers that showed peak-splitting had CFs below 2 kHz (Fig. I-8). Although this result seems to be consistent with previous studies in cat (Kiang and Moxon, 1972; Johnson, 1980; Kiang, 1984, 1990), it is in sharp contrast to the findings in chinchilla, for which fibers with higher CFs were more likely to show peak-splitting than those with lower CFs (Ruggero and Rich, 1983), and fibers with CFs as high as 26 kHz commonly showed peak-splitting (Ruggero and Rich, 1989). These differences between our study and those chinchilla studies cannot be accounted for by the relatively lower stimulus intensities which we used (<= 95 dB SPL), since peak-splitting was obvious at those intensities in the high CF fibers in chinchilla (Ruggero and Rich, 1989).

We have shown that more complex response patterns such as the 3-peaked patterns reported for chinchilla (Ruggero and Rich, 1983) and gerbil (Ronken, 1986), also exist in cat (Fig. I-5). In the two-peaked cases, the distance between peaks may be much less than half a cycle (Figs I-4 and I-5), which was also observed in chinchilla (Ruggero and Rich, 1983; although

the half-a-cycle-away case was emphasized), but for which there are no counterparts in previous cat studies. The number of peaks in one cycle of the LF tone, the distance between the peaks and the occurrence of peak-splitting are dependent on the stimulus frequency (Figs. I-4 and I-5). Such frequency dependence, although observed in some previous studies, has not been systematically studied before. We also showed that, in response to the same stimulus, both fibers of a high-SR and low-SR fiber pair with possibly the same IHC innervation showed similar peak-splitting (Fig. I-6). By contrast two other pairs of fibers, each from the same cochlea region, showed similar two-tone suppression patterns (with two suppression phases half a cycle apart), but in response to the LF-tone alone, in each pair, one fiber showed peak-splitting and the other showed basically a single response peak (Fig. I-7).

It has been demonstrated that some form of peak-splitting, a near-180 degrees abrupt change in the phase-level function, and a notch in the rate-level function are all related phenomena, which are thought to have the same origin (Kiang and Moxon, 1972; Kiang, 1984, 1990). This conclusion is based on the observation that during an abrupt transition of an AN fiber response from one phase to its opposite, the response rate sometimes dropped sharply and thus produced the notch in the rate-intensity curve. Moreover, for those same intensities, more than one peak was observed in the period histograms. When a very rapid phase change occurs, peak-splitting sometimes becomes difficult to observe, since it happens over a narrow intensity range. The early cat studies of peak-splitting generally emphasized the small intensity range over which peak-splitting occurs, and the near-180 degrees distance between two peaks. In our study, although such peak-splitting was also observed, the usual observation was that peak-splitting happens over an intensity range of 10 dB or more, and in some cases the distances between peaks (if only two peaks) are much less than half a cycle (e.g., 100 Hz, Figs. I-4 and I-5). These different features might suggest that there are (at

least) two different types of peak-splitting present in the cat: the usual form observed by most early investigators and the somewhat different form we mainly report here.

All the hypotheses to account for peak-splitting and related phenomena seem to have a common feature: that is, they are generated by some nonlinear interaction in the cochlea (see Introduction). A simple scheme was proposed (Liberman and Kiang, 1984; Kiang et al., 1986; see Kiang, 1990 for summary), in which different harmonics were generated at the BM stage and summed at the IHC stage. The stereocilia of the hair cells were assigned rather specific roles in summing the response components. This scheme relies on harmonic cancellation to eliminate the component that gives rise to the response at the lower intensities. However, so far such harmonic cancellation has not been experimentally demonstrated. With this scheme, it is also more difficult to explain more complex peak-splitting phenomenon, such as the 3-peaked cases in this study. Ruggero and Rich (1989), based on the "cochlear microphonic counterpart of 'peak-splitting', which was presumably of OHC origin", proposed that it might be in the BM macromechanics. Their evidence is the 180-degree phase changes which occur with stimulus intensity in the CM response (Zwislocki, 1989). However, there is no direct evidence from BM studies to support this argument. Further support might be the "phase reversal" exhibited by individual OHC responses (Russell et al., 1986; Zwislocki and Smith, 1989), but this phenomenon was observed only in a small number of OHCs. Changes of phase in CM responses as intensity was changed were also observed by other investigators (Klis and Smoorenburg, 1985; Ruggero et al., 1986a), and were present in our CM data (Fig. I-14), although in our and other studies the changes were usually not as large as in Zwislocki's (1989). We think that these smaller phase shifts are more likely to be the result of the spatial summation of the CM responses at different cochlear locations (see part 4 of this section), rather than due to similar

changes with intensity in the OHC responses.

So far there has been no report of any direct observation of the peak-splitting on BM. Although this does not exclude the possibility of its existance on BM, indirect evidence suggests that it is not likely. It has been demonstrated by two-tone suppression studies on both BM (e.g., Rhode and Cooper, 1993) and AN fibers (e.g., Sachs and Hubbard, 1981) that with a LF suppressor, the response to the CF tone is suppressed at one phase of the suppressor tone at lower suppressor intensities, but as the suppressor intensity is increased, there is often a second phase of suppression; the distance between the two suppression phases is always about half a cycle apart. In our two-tone recordings, obtained nearly at the same time as the LF responses, whenever there were two suppression phases, the distance between them was also about half a cycle apart. By contrast, the distance between the split peaks was dependent on the stimulus frequency and was not necessarily half a cycle apart. We have shown that fibers with similar BM origin can have quite different single-tone response patterns but similar two-tone suppression patterns (Fig. I-7). Since two-tone suppression was shown to be related to the saturation of OHC receptor potentials (Geisler et al., 1990), and presumably the OHC function is related to the "active process" of the BM, the difference between the patterns of peak-splitting and suppression argue against the direct involvement of OHC function in the generation of peak-splitting, and thus its BM mechanical origin. The different single-tone response patterns from some fibers with similar BM origins but different IHC innervations (cf. Fig. I-7) also do not support the BM mechanical origin of peak-splitting.

A possible counterpart of peak-splitting was found in IHC receptor potentials (Dallos et al., 1986; Cody and Mountain, 1989) and in the conductance changes of the IHCs (Cody and

Mountain, 1989), suggesting that peak-splitting originates at, or before, the IHC stage. Our finding that a high-and low-SR fiber pair, which are believed to have innervated the same IHC, showed similar peak-splitting (Fig. I-6), is also in favor of this conclusion. Since any direct consequence of BM mechanics was also not likely from the arguments in the previous paragraph, peak-splitting must originate somewhere between the BM and the IHC. Thus, the tectorial membrane becomes an obvious candidate for the generation of peak-splitting, possibly through nonlinear coupling between the tectorial membrane and the organ of Corti (Zwislocki, 1986; Cody and Mountain, 1989). For this mechanism to work, one must assume the mechanical influence of tectorial membrane upon the OHC function to be relatively small. This could be the case, since the strongest evidence of the involvement of OHC function in peak-splitting (and thus of the mechanical feedback) was the phase reversal, which was observed only in a small number of OHCs.

However, there is also a not-so-obvious choice, that is, the transduction-related process, which is also between the BM and the IHC. It has been shown in chick cochlear hair cells that the angular displacement of the shortest stereocilia was much larger than that of the tallest (Duncan et al., 1995), thus it is possible that there are relative movements between stereocilia. The acoustically induced relative movement of the stereocilia has been observed in the alligator lizard cochlea in vitro (Davis and Freeman, 1995). If the tip-links between neighboring stereocilia mediate the mechanoelectric transduction, the stretch of the tip-link is then not simply proportional to the hair bundle displacement due to the relative movement, suggesting a nonlinear mechanical signal-processing stage. The nonlinear nature of this stage might also be a source of peak-splitting.

2. Phase of LF tone response: comparison with previous studies

There are relatively few studies on the phases of the AN-fiber discharge to LF tones in cat (Allen, 1980; Pfeiffer and Kim, 1975; Kim et al., 1980; Liberman and Kiang, 1984; Joris et al., 1994). The Allen study was presented in a different format and thus most of the data are not comparable to the others or to the present study. The paper that is most comparable to this study is that of Pfeiffer and Kim (1975). In their study, they used tones with frequencies from 50 Hz to 2600 Hz, at intensities of 20 dB SPL (except for 50 Hz, which was 60 dB SPL). These intensities are much lower than those which we used. As a result, they only recorded from fibers with CFs in the neighborhood of the stimulus frequencies. In our study, the CF of the fiber was always at least 3 times the stimulus frequency, so the stimulus frequency was always low compared with the CF of the fiber. Thus, there is not much overlap between the CF regions in their represented data and our data. At two frequencies (600 and 1200 Hz), they had a few points in the higher CF region which overlap with our ranges. In the region of overlap, their phase trajectories are comparable to those in our data (500 and 1000 Hz). With 50-Hz stimuli, both studies showed small non-monotonic phase changes.

We showed in our 100 Hz data (Fig. I-9B) that the phase change with CF was relatively larger at lower CF region, and smaller at higher CF region. This trend agrees generally with the findings in other cat studies at other stimulus frequencies (Pfeiffer and Kim, 1975, 600 and 1200 Hz; Kim et al., 1980, ~600 Hz; Liberman and Kiang, 1984, 1000 Hz). Kim et al. (1980, Fig. 2) had shown an abrupt phase change with CF (~130 degrees) in the CF region of 2-3 kHz with 620 Hz stimulation in one cat. However, by shifting some points in their graph by one cycle, that phase change can be eliminated. No similar change was seen in the other

two cats in the same study (with 600 Hz stimulation). We did not see any large phase change with CF in the similar CF region at a comparable stimulus frequency (500 Hz), or at any other frequencies in our data. Although there are not a lot of data points in the high-CF region for the lowest frequency stimuli in our data (due to our use of limited intensities), we see no hint of such changes in those data (cf. Fig. I-9). Neither did Liberman and Kiang (1984) in their 1000-Hz data and Joris et al. (1994) in their 500-Hz data. With higher frequency (> ~ 400 Hz) stimulation, Liberman and Kiang (1984) observed a near-180 degrees phase change with intensity. Due to our use of lower stimulus intensities, we only saw a few examples of such large phase change with intensity at similar stimulus frequencies. We showed that for lower frequency stimuli (< 200 Hz), the phase-intensity changes were small and their directions were dependent upon the CF of the fiber. The small phase-intensity changes agree with similar observations in cat for similar stimulus frequencies (Allen, 1980). However, there were no comparable data available in other cat studies for the CF-dependence of the direction of phase-intensity change.

Although the phase results from this study basically agree with comparable data in previous cat studies, they differ from those of the chinchilla studies (see Introduction) in two main aspects. First, there was no near-180 degrees phase change with *CF* in the mid-CF region in our low-frequency (50 and 100 Hz) data, as was evident in the comparable chinchilla data (30, 60, and 100 Hz). The second aspect in which our data differ from that collected in the chinchilla are the phase changes with *intensity*. Although both studies have showed a CF-dependence of the *direction* of phase change with intensity (in our case: at least 50 and 100 Hz, and probably 200 Hz too, Fig. I-10; in chinchilla: 100 Hz, Ruggero and Rich, 1988), the amount of phase change with intensity was generally small (< 0.15 cycle, or 54 degrees) with lower stimulus frequencies (< 200 Hz) in our study (Fig. I-11). However, large phase

changes with intensity were seen in chinchilla for similar stimulus frequencies over the intensity range which we used (Ruggero and Rich, 1988).

There are at least two possibilities that might account for these differences. The first is the condition of the cochlea. As suggested by Ruggero and Rich (1987), both high stimulus intensity and OHC damage can cause a near 180 degrees phase change. Since we used relatively lower intensities (<= 95 dB SPL, compared to 100+ dB in other studies), and we monitored the cochlea condition with the CAP throughout our experiments to insure minimal damage, we feel that our cochleae were in normal conditions throughout the experiment. On the contrary, the fact that the data points, which are away from the usual range (denoted by diamonds in Fig. I-9), were from an experiment with a slight "damage" history, would suggest otherwise. The second possibility is species difference. The above mentioned differences and the differences in peak-splitting behavior (see part 1 of this section) between our data and those from chinchilla, suggest that there may be real differences in the behavior of the cochleae of these two species.

3. Dependence on stimulus frequency

An important finding in this study was the dependence on the stimulus frequency of the temporal patterns of auditory nerve fibers in response to low frequency tones. That is, many features such as peak-splitting, the number and position of the peaks in one cycle of LF tone (Figs. I-4 and I-5), the points that are half-a-cycle away from the usual range (Fig. I-9), and the phase-intensity effect (Figs. I-11 and I-12) all seem to depend in a complex way upon stimulus frequency. There is no readily available explanation for these phenomena. Among the various aspects of frequency dependence are the points that are half-a-cycle away from

usual range; such points are also seen in other studies (cat, Pfeiffer and Kim, 1975; chinchilla, Ruggero and Rich, 1983; guinea pig, Oshima and Strelioff, 1983). The fact that both fibers in the fiber pair in Fig. I-7, which were taken from the same cat under the same stimulus conditions, showed major response phases nearly half-a-cycle apart (similar cases also exist in the data of Pfeiffer and Kim, 1975 and of Oshima and Strelioff, 1983), suggests that the difference must be due to factors after the OHC/BM stage.

Do such unusual phases exist in the transduction currents of the IHCs? This question is of interest since, if they already exist in the transduction currents of the IHCs, there must be a stage between the BM and the IHC that is responsible for it. After the transduction process there is no known mechanism that might account for it, except through electrical interaction between the organ of Corti and the IHC, which is thought to be weak (Dallos, 1985). Besides, the fact that each of the labeled fibers (B6 and E80) in Fig. I-9 showed an unusual response phase at one of the three frequencies (50, 100 and 200 Hz) but not at the other two also does not support the electrical interaction hypothesis, since the electrical properties of IHC and other structures are similar at these frequencies.

Thus it is more likely that the phase change already existed in the transduction currents of the IHCs. The tectorial membrane hypothesis discussed earlier might be able to account for some of the frequency dependence (such as those related to peak-splitting and the phase-intensity effect), through a nonlinear coupling between the tectorial membrane and the organ of Corti. But to account for the frequency dependence of the points that were half a cycle away, additional assumptions are needed, since, unlike peak-splitting, most of them were not dependent on stimulus intensity (i.e., the half-a-cycle-away points stayed at half a cycle away from usual range when the stimulus intensity was changed, cf. Figs. I-9 and I-11). Those

points might reflect some “normal pathology”, which jeopardizes the structural and functional integrity needed for the coupling between the tectorial membrane and the hair cells (Liberman and Kiang, 1984). The fact that some of the points in Fig. I-9 (which had unusual phase values) were from one experiment with slight damage history, but that the threshold and tuning curve shape of those fibers at the time of recording were not different from the rest, supports this idea.

Another alternative is a strong frequency dependence of the IHC transfer characteristics (since those half-a-cycle away points are stimulus-frequency dependent, Fig. I-9), presumably of the transduction process. This implies that the IHC depolarizes when the stereocilia deflect towards the shortest stereocilia, instead of towards the tallest ones in the usual case (Hudspeth and Corey, 1977), for which there is no evidence so far.

4. Phase of LF tone response re BM motion

Tasaki et al. (1952) showed that at low stimulus frequencies there was little phase difference between the CM responses recorded from the RW or from inside basal-turn ST. Later it was shown that the phase of maximum negativity in CM responses at these low frequencies corresponds to maximum displacement of the BM towards ST (Ruggero et al., 1986a, in chinchilla, with RW electrode; Patuzzi and Sellick, 1984, in guinea pig, with ST electrode), and this relationship has been used to estimate the phase of BM motion corresponding to phases of responses recorded on the auditory-nerve fibers or ganglion cells. We assume that this relationship between the maximum negativity of the CM response recorded with RW electrode and the maximum deflection of the basal BM towards ST also holds for cat.

Since the phase of the negative peak in CM data changes with intensity (Fig. I-14), a question arises naturally as to which response should be used to estimate the phase of BM motion in the basal region. There are two issues which need to be considered. First, the CM responses recorded with the RW electrode have contributions from both the basal and the apical regions. Dallos (1973) compared the CM responses recorded with either a ST or scala vestibuli (SV) electrode with the responses obtained with differential electrodes in the basal turn, and concluded that there were remote contributions in both the ST or SV cases. However, the contributions of the apical region should be relatively smaller at higher intensities than at lower intensities, since at low intensities, the responses in the basal region are very small. Support for this idea comes from the study of Patuzzi et al. (1989), in which they showed that, after ablation of the apical parts of the cochlea, the magnitude of CM at 100 and 200 Hz was reduced in amplitude at low intensities, but suffered very little change at high intensities. A second issue of concern is that, at higher intensities, the CM response becomes nonlinear. Wever (1949) has shown that at low frequencies the CM response in guinea pig began to saturate when the peak amplitude was above 200 to 350 μV. At higher intensities, the CM response might also be contaminated by CAP responses. Considering the above issues, we have chosen an intermediate level for the CM data (peak amplitude of about 100 μV) from which to estimate our BM phases. A similar value was chosen by Ruggero et al. (1986a). However, it should be pointed out that this choice is still somewhat arbitrary, and a different criterion could easily change the phase by some 30-50 degrees.

In order to use the CM data to estimate the phases of the AN responses to LF tone relative to the basilar membrane motion, we need to correct for the synaptic delays and neural travel times, which presumably affected the AN fiber discharge data. This delay is estimated to be 0.8-1.2 ms in cat (Stypulkowski and Honert, 1983) and 0.7-0.8 ms in guinea-pig (Palmer and

Russell, 1986). Thus it seemed reasonable to use a value of 1 ms in this study. At the peak amplitude of about 100 μV, the phases of maximum negativity in the CM responses from experiment J (Fig. I-14) were 0.86, 0.16 and 0.34 cycles for stimulus frequencies 50, 100 and 200 Hz respectively. We then correct for the synaptic delay and neural travel time of 1 ms, which gives us the phases of maximum displacement of BM towards ST as 0.91, 0.26 and 0.54 cycles respectively. For comparison with the AN data, these values are entered on the right-hand ordinates in Fig. I-9 (A-C) as horizontal bars. In comparing the CM phases and the phases of response of AN fibers, we only considered the data points from the higher CF region (where the CM data were collected) and excluded the data points from animal H (diamonds in Fig. I-9, see Methods). Thus, for 50 Hz, the vector phase of the response of the high-frequency AN fibers corresponds to a BM phase that slight lags the maximum velocity of BM towards ST. For 100 Hz, it slightly leads the maximum velocity of BM towards ST. For 200 Hz, it is roughly centered at the maximum BM displacement towards SV, but there is considerable scatter in the data, with variation on either side of up to 0.25 cycle.

The 50 Hz and 100 Hz results agree in general with the findings that, at lower stimulus frequencies, the IHC is responding to velocity of BM motion (Sellick and Russell, 1980; Nuttall et al., 1981). Our result at 50 Hz is also close to those obtained at low frequencies (30-100 Hz) in chinchilla (e.g., Ruggero and Rich, 1987), in which they showed that the phase of the threshold-level responses of AN fibers at higher CF region leads ST displacement by 40-60 degrees. But at 100 Hz, there is roughly 50-60 degrees difference between the average phases recorded in these two studies. Our 50-Hz result is also very close to that obtained in gerbil at similar CF region (Oshima and Strelioff, 1983), but there is a half-a-cycle difference between our data and that obtained in guinea pig by the same

authors. Comparison of our results to other studies (cf. Ruggero and Rich, 1983, 1987) becomes difficult since they either had no direct CM references or used different stimulus conditions. For example, Sellick et al. (1982) showed that in guinea pig the responses in ganglion cells at 40-100 Hz roughly correspond to ST displacement. The nominal difference between our results at 50 and 100 Hz and their results is between 50 to 90 degrees. But since their data were collected at high intensities (> 95 dB), if we were to include the increasingly phase-lead with intensity which we observed for higher CF fibers (Fig. I-11B and D), the difference between the two studies increases by 30-50 degrees. Considering the uncertainties in inferring BM displacement from CM phase and the unknown adequacies of the 1-ms correction for synaptic delay and neural travel time in other studies and ours, resolving the differences between the different studies must wait for better indicators of BM phase.