Chapter 3

Responses of auditory-nerve fibers to two-tone stimuli: effect of spontaneous rates

Introduction

One of the main features of mammalian auditory nerve (AN) fibers is their ability to generate spikes (action potentials) in the absence of externally applied sound stimuli. The degree of this spontaneous activity varies greatly, from virtually no spontaneous firing to over 100 spikes per second (Liberman, 1978). Fibers with low spontaneous rates (SRs, <= 1/s) usually have higher thresholds in response to sound stimuli than those with medium (> 1/s and < 20/s) and high SRs (>= 20/s). The threshold intensity at a fiber's characteristic frequency (CF) can be even less than 0 dB SPL in high-SR fibers and more than 60 dB SPL in low-SR fibers. With HRP labeling and other anatomical studies (Liberman, 1980a, 1982; Kiang et al., 1982), it has been demonstrated that the high-SR fibers usually form large synapses with inner hair cells (IHCs), while the low- and medium-SR fibers (they are also referred to as "lower-SR" fibers for convenience) form smaller synapses with the same IHCs. Thus, each IHC makes contact with afferent fibers having different SRs. The lower-SR fiber synapses are usually on the modiolar side, while the high-SR fiber synapses are on the pillar-cell side.

Paralleling the diversity in the physiological characteristics (e.g. SR and threshold) and the

anatomical and structural differences associated with different classes of AN fibers, there are interesting differences in the behavior of these different fiber classes. For example, it has been demonstrated that high- and lower-SR fibers have different adaptation dynamics (e.g., Rhode and Smith, 1985; Relkin and Doucet, 1991). Compared to low-SR fibers, high-SR fibers are easier to adapt in response to a stimulus, they have shorter adaptation time constants, and they also recover from stimulation faster. In response to two-tone stimuli (i.e., a CF tone and a suppressor tone outside CF), these two groups of fibers show different response characteristics. For example, lower-SR fibers are more suppressible once suppression threshold is reached, in the sense that a larger percentage of the driven responses can be suppressed (Fahey and Allen, 1985). In fact, sometimes the responses of low-SR fibers can be suppressed completely (e.g., Fahey and Allen, 1985; Chapter 2). Consistent with this is the finding that greater increase in suppression can be achieved in lower-SR fibers than in high-SR ones by the same increase in suppressor intensity (Delgutte, 1990).

However, our knowledge of the temporal response patterns of high- and lower-SR fibers to both single-tone and two-tone stimuli is quite limited. Since both high- and lower-SR fibers innervate the same IHCs, and their contacts with the IHCs are so close, their synapses should be basically sensing the same receptor potentials, which are thought to be responsible for driving the synapses and giving rise to neural firings. Thus, the basic response patterns of both high- and lower-SR fibers ought to be similar. So far, there is no direct experimental demonstration of this point. In this study, we examine the two-tone responses of cat AN fibers with different SRs, using a CF tone and a low-side suppressor as stimuli. Our results show that the phase response characteristics of high- and lower-SR fibers are similar, but their amplitude response characteristics are different. Some of the data in this report have been presented previously (Cai and Geisler, 1995).

Methods

The study was part of a larger study of the responses of AN fibers to two-tone stimuli. The data were collected from 8 cats (C92101, C92106, C92116, C93030, C93036, C93146, C94009 and C94011) which were sometimes referred to as A, B, D, E, F, H, I and J for convenience. The animal preparation, sound delivery and recording system has been detailed in Chapters 1 and 2. In the following, only points special to this report will be discussed further.

The stimuli were harmonically related CF tone and suppressor (SUP) tone. The frequency of the suppressor was usually 50, 100, 200, 500, 1000 or 2000 Hz and was always below CF. The CF tone and the SUP tone, each 500 ms in duration, were staggered in time (with some overlap). The SUP tone was delayed by 200 ms, so that three stimulus conditions were created: CF-alone, two-tone (CF+SUP), and SUP-alone (Fig. III-2 insert). The intensity of the CF tone was fixed at 10 to 30 dB above threshold. The suppressor intensity was varied over a certain range (e.g., 70 to 95 dB SPL), in 5 dB steps. For each suppressor frequency and intensity combination, the paradigm shown in Fig. III-2 insert was repeated 75 times.

Period histograms (128 bins) were constructed with respect to the period of the suppressor tone for the three time windows: CF-alone (75-200 ms), two-tones (250-500 ms) and SUP-alone (575-700 ms). The initial portions of each stimulus condition were excluded and only the steady-state portions of the responses were analyzed. The rate vs. SUP-intensity curves (hereafter referred to as rate-intensity curves, if there is no ambiguity) were plotted for the three time windows. The phase of maximum suppression and the rate suppression threshold were determined as described previously (Chapter 2).

The rate-intensity curves for a typical AN fiber (D1) are shown in Fig. III-1. As the suppressor intensity is increased, the discharge rate in the two-tone segment gradually decreases. At higher suppressor intensities, the SUP-alone rate increases and the CF-alone rate decreases. The decrease of the CF-alone rate is another phenomenon and is described in more detail in Chapter 5. At 75 dB SPL, the two-tone rate reaches its minimum (arrow) and then increases. This non-monotonic two-tone rate-intensity curve is a general characteristic of an AN fiber for "low-side" suppression (Sachs, 1969; Hill and Palmer, 1991; Hill and Geisler, 1992). Although the two-tone rate at a particular suppressor intensity was lower than the corresponding CF-alone rate, the rate at any phase in a period histogram may be below or above the CF-alone rate, depending on the exact phase (e.g., Figs. III-2 and III-3). To quantify this characteristic, we calculated the ratio of the peak of the two-tone response level to the response level produced by the CF-alone response level (quasi-simultaneously, cf., insert of Fig. III-2). For a particular suppressor intensity, the two-tone response level was defined as the maximum number of spikes (in any one bin) in the two-tone period histogram (cf., Fig. III-2)[1]. To compute a fiber's CF-alone response level, we first determined if the fiber showed responses significantly phase-locked to the CF-tone. If it did, we identified the peaks in the period histogram (binned at the period of suppressor tone), and took the average peak height, plus one standard derivation, as the CF-alone response level at that suppressor intensity. If the fiber did not show significant phase-locking, the average plus one standard derivation of all the bin values in the period histogram was used. The ratio of the two-tone response level and the CF-alone response level at each suppressor intensity was

[1] This simple measure tends to overestimate the two-tone response level and thus the relative-response index. This is especially true for lower-SR fibers since there is generally more variability in the data from lower-SR fibers, cf. Figs. III-2 and III-8.

then computed. This ratio obtained at the suppressor intensity where the two-tone rate was minimum (if a minimum was reached, as in Fig. III-1 at 75 dB SPL) was defined as the *relative-response index* (for the CF-SUP combination) of a fiber. If the suppressor intensity was not high enough to produce a non-monotonic two-tone rate-intensity curve (i.e., the response rate was dropping with intensity, but a minimum had not been reached at the highest suppressor intensity was used), the ratio obtained at the highest suppressor intensity was used as the index.

The method of determining rate suppression threshold was described in Chapter 2. In order to measure the suppression strength, we used the *fractional-response index* (Abbas and Sachs, 1976), which is the ratio of the remaining driven response in the two-tone segment relative to the driven response in the CF-tone alone segment:

$$(R_{tt} - SR) / (R_{cf} - SR),$$

where R_{tt} and R_{cf} are the response rates in the two-tone segment and CF-alone segment, respectively, and SR is the spontaneous discharge rate. A fractional-response index of zero means that the CF driven response is severely suppressed so that the response rate in the two-tone segment equals to the spontaneous firing rate of the fiber, and a value of one means that there is no suppression. In this paper, the "fractional-response index" of a fiber for a particular CF-SUP combination was measured at the same point at the two-tone rate-intensity curve as the "relative-response index" was measured.

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.

Fig. III-2 The response of a lower-SR fiber (C92116, fiber D1-8; CF = 14375 Hz, SR = 2.0/s, TH = 25 dB SPL). The CF tone was presented at 35 dB SPL. The suppressor frequency was 1000 Hz. The vertical scale is that for the two-tone condition (CF+SUP, middle column). In this and other figures, the CF-alone and SUP-alone columns have been scaled so that equal numbers of spikes represent equal firing rates for all three columns. The dashed lines in the CF-alone represent the estimated CF response levels at each suppressor intensity (see Methods). These lines were extended to the two-tone (CF+SUP) column for comparison purposes. The "peak" in the two-tone segment (arrow) is simply the height of the tallest bin. In the display, there are 64 bins in each period histogram and all estimates shown in the succeeding figures are based on 64-bin histograms (except that the population data shown in Fig. III-7 were based on 128 bins). There was basically no difference between the results using 64 bins or 128 bins. At a suppressor intensity of 75 dB SPL, the peak response level in the two-tone segment is below the response level of the CF-alone segment. The rate-level curves for these data are shown in Fig. III-1. The top insert shows the stimulus paradigm.

Results

1. Relative level of responses in two-tone and CF-alone

Figure III-2 shows the period histograms of the responses of a medium-SR fiber (D1). The frequency of the CF tone was 14 kHz and the suppressor frequency was 1000 Hz. The rate-intensity curve for this fiber has been shown in Fig. III-1. For suppressor intensities up to 75 dB SPL, there was no response to the suppressor tone alone. At a suppressor intensity of 60 dB SPL, there was basically no rate suppression in the two-tone segment. As the suppressor intensity was increased to 65 dB SPL, suppression began to show up at a phase of about 0.1T (T is the period of the suppressor tone), but the maximum two-tone response (phase 0.5-0.6T) was still at about the same level as the CF-alone response. Further increases of the suppressor level decreased the responses in the two-tone segment at all phases. At 75 dB SPL, the maximum response level in the two-tone segment is less than half of the CF-alone level (arrows).

In Fig. III-3, the responses of a high-SR fiber (D4) from the same experiment are shown. The CF tone was 18 kHz in this case, slightly higher than that for fiber D1. A low-side suppressor with the same frequency (1000 Hz) was used. Although the phase of suppression in this example is not much different from the one shown in Fig. III-2, the maximum two-tone response level exceeds the corresponding CF-alone level at all intensities, even when there is no appreciable response to the suppressor tone alone (70 dB SPL).

In the next two figures, the responses obtained from two low-CF fibers (~1.1 kHz) are shown. In both cases, a 100-Hz suppressor was used. Figure III-4 shows the responses of

one such fiber (J12-5), one with low spontaneous rate. There was no response to the suppressor tone alone at any intensity by this fiber. At 75 dB SPL, there is significant suppression in the two-tone segment, and the response is completely suppressed at 90 dB SPL. This latter condition (i.e., no response in the two-tone segment), which is called "complete suppression", was seen only in lower-SR fibers (Chapter 2). The responses of another fiber (J42-6) from the same experiment, one with high spontaneous rate, are shown in Fig. III-5. Similar to fiber J12, this fiber showed suppression at a suppressor intensity of 75 dB SPL. But in contrast to fiber J12, this fiber responded to the suppressor tone even at the lowest suppressor intensity (75 dB SPL). Such dual effects of the suppressor (i.e., excitatory and suppressive effects) have been previously documented (e.g., Sachs, 1969; Hill and Geisler, 1992). Since the response is phase-locked to the CF tone as well as to the suppressor tone, we are able to separate the contributions from the CF tone and the suppressor tone in the two-tone responses. At 85 dB SPL, the CF response components were greatly suppressed at all phases except for one peak at a phase near 0.17 (arrow), which coincides with a small peak in the SUP-alone response (arrow). As in Fig. III-3, the maximum two-tone level exceeds the CF response level in this case. At 90 dB SPL, the CF response components were mostly suppressed, but the peak response level in the two-tone segment is still greater that the CF-alone response level.

In Fig. III-6, we show another example of responses from a high-SR fiber (J34-6), which had a lower CF (974 Hz). The suppressor frequency was 50 Hz. At 85 dB SPL, there is no significant response to the suppressor tone alone, but the maximum two-tone level exceeds the CF response level. At 95 dB SPL, the maximum two-tone response is high above the CF response level, and the phase of this peak coincides with a very small peak in the SUP-alone response (arrow). The same fiber did not show similar behavior at other suppressor

frequencies. We have 6 cases that clearly showed such behavior (i.e., a very large peak in the two-tone response which coincides with a very small peak in the CF-alone response). Three of them involve low-CF (~1 kHz) fibers and a 50-Hz suppressor; the others involve higher-CF (4-12 kHz) fibers and higher frequency suppressors.

The examples shown in Figs. III-2 to III-6 are typical in our data for high- and lower-SR fibers, respectively. The latter generally show more overall suppression than do the former. To quantify the data, we used the relative-response index measurement as described in the Methods section. In Fig. III-7, we show the population data of the relative-response index for six suppressor frequencies (50, 100, 200, 500, 1000 and 2000 Hz). The solid symbols indicate that the fiber CF is equal to or greater than 2000 Hz, while the empty symbols indicate that the fiber CF is below 2000 Hz. It is clear from the figure that, at all suppressor frequencies, regardless of fiber CF, the relative-response indexes for high-SR fibers are generally greater than those for lower-SR fibers, with virtually all lower-SR fibers having a relative-response index less than unity, and most high-SR fibers having one around or greater than unity.

Although lower-SR fibers generally showed relative-response indexes less than unity, there are some points that are above unity at the lower-SR sections of several panels in Fig. III-7 (e.g., suppressor frequencies between 100 and 1000 Hz). In Fig. III-7A to C (low suppressor frequencies, <= 200 Hz), such points are usually contributed by fibers with higher CFs (> 6 kHz). These fibers usually do not show much rate suppression, even at suppressor intensities of 95 dB SPL (i.e., the minimum two-tone rate had not been reached, cf., Fig. III-1). The points that are contributed by higher-CF fibers which showed incomplete rate suppression after intensities of 95 dB SPL, are labeled by stars in Fig. III-7. Figure III-8

Fig. III-7 The relative-response index as a function of fiber SR for different suppressor frequencies (50-2000 Hz). For graphing purposes, the points with 0.0/s SR were moved to 0.2/s. At all SUP frequencies, the relative-response index is SR-dependent, with the lower-SR responses generally less than unity, and the high-SR responses usually greater than unity. Stars (*) indicate that the point was obtained without a true response-rate minimum being obtained (see text). The solid symbols indicate points obtained from fibers with high CFs (>= 2 kHz), while the empty symbols indicate points obtained from fibers with lower CFs.

shows an example of those points (fiber D40-5, labeled by arrow in Fig. III-7B). There was no response to the suppressor tone alone at any intensity. At the highest suppressor intensity used (95 dB SPL), the fiber showed only phasic suppression, with the responses at some phases not affected at all (arrow). Thus a relative-response index around 1.0 is expected. If the suppressor intensity had been allowed to go beyond 95 dB SPL, we expect that there would have been more suppression and thus a relative-response index less than unity.

Some high-SR fibers showed relative-response indexes below unity, mainly for suppressor frequencies 500 Hz or below (Fig. III-7B-D). In Fig. III-9, we show an example (J77-4) of one such point (labeled arrow in Fig. III-7B). A 100-Hz suppressor was used in this case. At a suppressor intensity of 80 dB SPL, the fiber showed two suppression phases in the two-tone period histogram (middle column), a major one centered at a phase of about 0.75T (arrow; T is the period of the suppressor tone), and a minor one at about 0.25T. The two response peaks are at phases of approximately 0.42T and 1.0T respectively. As the suppression developed with increasing suppressor intensity, both suppression phases became deeper and broader, but the response peaks become higher at 85 dB SPL. Since there was no response to the suppressor tone alone at 85 dB SPL and the peak of response to the suppressor tone alone at higher intensities is at about half a cycle from the middle peak (at phase of 0.42T) in the two-tone response at 85 dB SPL, the enhancement of the two-tone peaks at the lower intensities does not seem to be related to the excitatory contribution of the suppressor tone. A further increase of the suppressor intensity to 90 dB SPL resulted in more suppression and reduction of both response peaks. At 95 dB SPL, the entire response is reduced considerably. At this intensity, the general shape of the two-tone period histogram resembles its counterpart in the SUP-alone case, except for a small peak (marked with arrow), which occurs in the trough at phase 0.4T, causing the peak two-tone level to fall

below the CF response level. This figure also demonstrates temporally the complex additive and suppressive interactions of the two tones.

The example shown in Fig. III-9 is typical for the high-SR fibers producing relative-response indexes below unity (Fig. III-7). There are also a small number of cases where the peak in the response to the suppressor alone coincides with the phase of maximum suppression in the two-tone segment (with a "phase-lead" near zero), producing a low maximum two-tone level. This near-zero phase relationship (between the phase of response to the suppressor and the phase of suppression) is quite unusual (Chapter 2). In the cases of zero phase-lead, the SUP-alone peak "filled" the trough in the two-tone segment, instead of the two-tone peak "falling" into the SUP-alone trough as in the example shown in Fig. III-9.

There is an apparent dependence upon suppressor frequency in the data shown in Fig. III-7. To see this point better, we plotted the data in a different way. In Fig. III-10, the averages and ranges for the relative-response indexes are shown as a function of suppressor frequency. It is clear that, although there is large variation, the relative-response index gradually decreases with suppressor frequency for both SR groups. The comparable behavior at still higher suppressor frequencies is not clear. However, in a few examples, with a 4000 Hz suppressor, there was no phase-specific suppression in either high- or lower-SR fibers, and both classes showed relative-response indexes below unity.

2. The responses of a high- and low-SR fiber pair

The data shown in the previous figures were taken at many different stimulus conditions. Therefore, before drawing general conclusions, it would be important to see how fibers from

similar cochlear locations but having different SRs behave in response to two-tone stimuli having identical stimulation parameters. If the basic response pattern were determined solely by the IHC stage in the excitation process, then both the lower-SR and high-SR fibers should have similar responses. Otherwise their responses might be different. Technically this has proven to be somewhat difficult, since low-SR fibers generally have higher thresholds and shallower rate-intensity curves. Thus, intensities (of the CF tone) which drive the lower-SR fiber at moderate rates produce saturation in the discharge of the high-SR fiber. However, we did record from several pairs of high- and low-SR fibers from the same animal having similar CFs. In one example, these two fibers came from successive recordings on the same electrode path, and their frequency tuning curve shapes and CFs are extremely similar. We believe that they at least innervated neighboring IHCs, if not the same IHC. Their small threshold differences allowed us to study them with the same stimulus conditions.

Figure III-11 shows the period histograms of the responses of this pair of fibers to a 500-Hz suppressor and a 12-kHz CF tone. There are several things to note. First, the general *shapes* of the responses of the two fibers in both the SUP-alone segment and the two-tone segment are extremely similar. Their phases of responses to the suppressor tone alone at 90 dB SPL, for example, are virtually identical: both showed two response peaks at the same phases (arrows). The suppression patterns in the two-tone segments are also similar, and the phases of suppression are the same (arrows). Second, by looking at the responses to the suppressor (500 Hz) alone, a threshold difference of about 5 dB is observed: the high-SR fiber has a threshold of about 80 dB and the low-SR fiber has a threshold of about 85 dB. This 5 dB threshold difference is similar to the threshold difference between these two fibers at CF, as determined from their tuning curves. The threshold difference is small (although not unusual, cf., Liberman, 1978; Geisler et al., 1985), but it was exactly this small difference that made

the use of the same CF intensity range possible. A third point of interest concerns the rates of response. The response rate of the low-SR fiber is much lower than that of the high-SR fiber. Thus, the histograms of the low-SR fiber look like scaled-down versions of the high-SR fiber's histograms. In fact, if we compare other histograms presented in this paper, we notice that fibers with lower SRs generally have lower discharge rates than those with higher SRs, although in both cases the CF tone was placed at about 10-30 dB above their respective thresholds (Liberman, 1978).

A final important feature in the responses of Fig. III-11 is the relative-response indexes. In the high-SR case, the maximum response level in the two-tone segment is always equal to or above the CF-alone response level, while in the low-SR case, the maximum response level is below the CF-alone response level for both the 80 and 85 dB SPL intensities. This result agrees with the general findings described before (Fig. III-10). It is significant that both fibers obtained their maximum overall suppression (i.e., minimum average discharge rate) at the same suppressor intensity (85 dB SPL).

Figure III-12 shows the two-tone rate-intensity curves for the fiber pair. They are very similar in that both are non-monotonic and reach a minimum at 85 dB SPL. For the high-SR fiber (I16) in the pair, although the minimum two-tone rate is reached at suppressor intensity of 85 dB SPL, the main response in the two-tone segment is contributed by the suppressor (cf., Hill and Geisler, 1992), as can be seen by comparing the temporal waveforms in the two-tone segment and the SUP-alone segment at that intensity (Fig. III-11A). The responses due to the CF tone must have been suppressed. We don't know if the similarity of the rate-intensity curves for this fiber pair holds in general for fibers that have similar basilar membrane (BM) origins but different SRs. Since the CF tone was generally presented at different intensities

for fibers with different SRs during our experiments, the data from fibers with different SRs are usually not directly comparable.

3. Threshold and strength of suppression

In Fig. III-13, we have plotted the thresholds of suppression with respect to fiber SR. The top panel (A) shows the thresholds obtained when a 100 Hz suppressor was used, while in the middle and bottom panels, the pooled data for three lower-frequency suppressors (50, 100 and 200 Hz) and for three higher-frequency suppressors (500, 1000 and 2000 Hz) are plotted, respectively. The solid lines are the regression lines. For the lower-frequency suppressors (Fig. III-13A-B), the average threshold difference was about 7-8 dB while for the higher-frequency suppressors it was about 4-5 dB. However, these differences are probably due to the analysis window we used to measure the CF-alone response rate and determine the rate suppression threshold, rather than to any real differences between high and low SR fibers (cf., Chapter 2). In contrast, the excitation threshold to the suppressor tone varies greatly with fiber SR (e.g., Figs. III-4 and III-5). Thus, despite significant differences in the excitation thresholds to the suppressor tone alone between fibers of different SR classes, the thresholds for *suppression* are similar for all fibers.

Figure III-14 shows the percentage of fibers that showed a fractional-response index equal to or less than a certain level (e.g., <= 0.2) for both high- (upper panel) and lower-SR (lower panel) fibers. Each symbol represents the percentage of fibers that showed a fractional-response index less than or equal to a certain value (at that suppressor frequency). There are several observations to be made. First, at any given level of fractional-response index, there is a much higher percentage of lower-SR fibers displaying responses not greater than that

Fig. III-13 Threshold of rate suppression as a function of fiber SR. For graphing purposes, the points with 0.0/s SR were moved to around 0.2/s. Top panel: suppressor frequency 100 Hz. Middle panel: suppressor frequencies 50, 100 and 200 Hz. Bottom panel: suppressor frequencies 500, 1000 and 2000 Hz. The suppression threshold is weakly dependent upon fiber SR for all suppressor frequencies. The solid lines are the regression lines.

Fig. III-14 Percentage of fibers showing certain levels of the fractional-response index, for both high- and lower-SR groups. For any given level of fractional-response index, there is a larger percentage of lower-SR fibers below that level. Very few high-SR fibers showed fractional-response indexes below 0.05, while the indexes of as many as 44% of lower-SR fibers fell below that at a suppressor frequency of 500 Hz. The data for the lower suppressor frequencies contain contributions from fibers of all CFs. Although higher-CF, lower-SR fibers generally show less suppression with lower frequency suppressors at the suppressor intensities which we used (maximum 95 dB SPL), compared to lower-CF, lower-SR fibers, no effort is made to distinguish between different CF groups due to the relatively small number of higher-CF, lower-SR fibers in our sample.

level than of the high-SR group, for all suppressor frequencies. Second, for higher frequency suppressors, a greater percentage of fibers show a particular fractional-response index than for lower frequency suppressors. This holds true in both SR groups. Third, at the extreme level of suppression, 20% or more of the lower-SR fibers showed fractional-response indexes of 0.05 or less at almost all suppressor frequencies (with the exception of 50 Hz), and a lot of them showed fractional-response indexes of 0.02 or less (i.e., virtually no response in the two-tone segment, Fig. III-5). By contrast, very few high-SR fibers show fractional-response indexes of 0.05 or less, and none showed fractional-response indexes of 0.02 or less.

Discussion

1. Summary of results and comparison with other AN data

We report two main findings in this paper. First, the relative-response index, which is defined as the ratio of the peak two-tone response level to the CF-alone response level, at the suppressor intensity for which the two-tone average rate is minimum, is strongly dependent upon fiber SR (Fig. III-7). Almost all lower-SR fibers show relative-response indexes of less than unity, while most high-SR fibers show relative-response indexes near or greater than unity. This is true at all suppressor frequencies (50-2000 Hz). There is also a slight dependence upon suppressor frequency (Fig. III-10). Our second main finding is that, although there is no appreciable difference in the average threshold of suppression for different fiber groups (Fig. III-13), lower-SR fibers generally show greater magnitudes of suppression than high-SR fibers do (Fig. III-14).

Although two-tone suppression has been studied for several decades, there has been little systematic study of the two-tone responses of AN fibers with different SRs. Among the studies that compared the two-tone responses of high- and lower-SR fibers, a study by Fahey and Allen (1985) was probably most relevant to this study. In that study, they noted that there was basically no difference in suppression threshold between high- and lower-SR fibers in cats, but that lower-SR fibers tend to suffer more rate suppression. Moreover, in some low-SR fibers, complete suppression was achieved. Schmiedt (1982) also observed only insignificant differences in suppression threshold between high- and lower-SR fibers in guinea pig. Our results (Figs. III-13, III-14, III-11 and III-4) are fully consistent with these findings. But to our knowledge, there have been no prior attempts to quantify, as we have done, the temporal responses in two-tone and CF-alone segments.

2. Relative-response index: comparison with BM and IHC data

The first principal finding in this study is the SR-dependence of the relative-response index. Since two-tone suppression has been demonstrated at the BM (e.g., Rhode, 1977) and the IHC stages (Russell and Sellick, 1979), for comparison purposes, it would be interesting to know the relative-response index at these stages as well. It has been shown that the IHC is responding to BM velocity at low frequencies (Sellick and Russell, 1980 and Nuttall et al., 1981). Since the velocity sensitivity at low frequencies can be represented as a "highpass" filter, the BM data may not be directly comparable to the IHC or AN data at these low frequencies (< 200-600 Hz). However, the data at higher frequencies are comparable. It is difficult to estimate the relative-response index from the early BM data collected using Mössbauer method, mainly due to the limited resolution and the lack of temporal data. Recently, there have been several groups studying the two-tone responses on the BM with

low-side suppressors, using laser interferometry. So far there has been no report that a relative-response index of less than unity occurs on BM for low-side suppression. Rhode and Cooper (1993) used displacement-sensitive laser interferometry to measure the two-tone responses in the hook region of cats and guinea pigs. They noticed that, at the intensity at which the suppressor (1000 Hz) causes suppression of the responses to the CF tone (34 kHz), the BM is responding strongly to the suppressor. The combined responses (to the suppressor plus to the CF tone) are much bigger than the CF-alone responses. Ruggero et al. (1992) used velocity-sensitive laser interferometry to measure BM movement. Since the velocity is the first order derivative of the displacement, the BM velocity corresponding to a certain displacement d due to a tone with frequency f is, assuming linearity, $2\pi fd$. Taking into account the difference in intensity level for the suppressor tone in the two-tone case and the SUP-alone case in their data (with assumption of linearity for the SUP-alone response), even a conservative estimate of the data shown in their Fig. 7 (with a CF of 8 kHz and a suppressor of 300 Hz) would give a relative-response index greater than one.

All studies of two-tone suppression at the IHC level have been done in one species, the guinea pig. Cheatham and Dallos (e.g., 1990, 1992a, 1992b) studied IHCs from the apical turns. They usually showed waveforms with the AC responses to both the low-frequency suppressor and the CF tone filtered out, leaving only DC responses. Occasionally they showed the unfiltered waveforms, where the peak of the two-tone response pattern is greater than the CF-alone response level (Cheatham and Dallos, 1990, 1992a). In one case they showed both the filtered and the raw waveforms (Fig. 3 of Cheatham and Dallos, 1992a). While in the filtered waveform the DC level in the two-tone segment is decreased as compared to the CF-alone segment, in the raw waveform the peak of the two-tone responses is greater than the CF-alone response level.

There are several groups who studied two-tone suppression in basal-turn IHCs. In an early study, Sellick and Russell (1979) used frequency-modulated sweep as the suppressor. Their use of a lock-in amplifier and data display make it inconvenient to tell directly the relative-response index from their data. But due to the large difference in the scales for the AC responses (which were locked to the 17-kHz probe tone) and the DC responses used in their plot (1 mV vs. 20 mV), it may still be safe to conclude that in their case the peak of the two-tone responses was greater than the CF-alone response level, at least for relatively low frequency suppressors. In another study, Patuzzi and Sellick (1984) used arbitrary scales for the AC waveforms in their plots, thus it is impossible to estimate the relative-response index from their data. It is impossible to estimate the relative-response index from the data of Nuttall and Dolan (1993), where no waveforms are shown. Recently, Russell and Kössl (1992) investigated the two-tone responses of basal turn IHCs with a 100-Hz suppressor. All of the relevant figures in that study seem to demonstrate that the peak of the two-tone responses are greater than the CF-alone response levels, except that at very high CF intensities both responses are about equal. Thus it seems that from the published BM and IHC studies, wherever we can estimate the relative-response index, it is always greater than unity.

3. Differences in the responses of high- and lower-SR fibers and possible mechanisms

One major difference between the responses of high- and lower-SR fibers to two-tone stimuli is the different ranges of their relative-response indexes. It seems to be quite common in our data that the usual relative-response index above unity in a high-SR fiber is often accompanied by a vigorous response to the suppressor tone alone (e.g., Figs. III-3 and III-5), while the usual relative-response index below unity in a lower-SR fiber is often accompanied

by a lack of responsiveness to the suppressor tone (e.g., Figs. III-2 and III-4). However, a significant response to the suppressor tone is not a necessary condition for a relative-response index above unity. For example, fiber D4 (Fig. III-3) showed a peak in its two-tone responses greater than the CF-alone level at low intensities, for which there was not much response to the suppressor tone (similarly, fiber J34-6 in Fig. III-6). Since each IHC is innervated by fibers with both high- and lower-SRs (Liberman, 1980a, 1982), the fibers innervating the same IHC must be sensing the same IHC potential, which contains a large low-frequency component (see part 2 of this section). Thus all the fibers innervating the same IHC (regardless of SR) should carry the same basic waveforms present in the IHC receptor potential, and the responses from both the high- and the lower-SR fibers should have a relative-response index above unity (as do the IHCs). However, our data clearly showed that this is not the case (Fig. III-7).

Putting aside the specific mechanisms that cause the obvious discrepancy between the experimental data and the prediction based on the basic anatomy and physiology of IHC-AN fiber synapses, this discrepancy seems to suggest that there are stages after the IHC that treat the "slow-changing" [2] and "fast-changing" components in the IHC potential differently in fibers with different SRs, whether the slow-changing component is due to a low-frequency tone stimulation or due to some other mechanisms before the IHC stage (e.g., two-tone suppression at the BM level, Figs. III-9 and III-11, at lower intensities). Lower-SR fibers

[2] The slow-changing information in the IHC potential can be carried by low-frequency components in the IHC receptor potential or by the envelope of the AM-like components, as in the suppression pattern produced at BM or IHC stage, which is actually high-frequency in nature (cf., Chapter 4).

must have lower sensitivities to the slow-changing components than high-SR fibers do (cf., Cheatham and Dallos, 1995). Although this idea of differential sensitivity has some support (cf., Cheatham and Dallos, 1995; Zagaeski et al., 1994), it seems to contradict the finding that threshold differences between high- and lower-SR fibers are about the same in both the tip and tail regions of their tuning curves (Liberman, 1978).

It is also shown in this study that the fractional-response index differs for fibers with high- and lower-SRs (Fig. III-14). If we assume that a high-SR fiber is relatively more sensitive to a low frequency suppressor, it begins to respond to the suppressor tone at a lower intensity, before the response to the CF component is suppressed sufficiently at or before the IHC stage. If at the intensity at which the CF component is severely suppressed, the response due to the suppressor becomes very large (Figs. III-3 and III-5), the peak level in the two-tone segment may go up instead of down with increased intensity. In those cases, the minimum two-tone rate is relatively large (thus a large fractional-response index), and the minimum is reached at relatively low intensities. Under the same stimulus condition, a lower SR fiber with the same IHC innervation as the high-SR fiber, due to its relatively low sensitivity to the low frequency suppressor, responds to the suppressor at a higher intensity, allowing the CF responses to be sufficiently suppressed before responding to the suppressor tone. Thus the minimum two-tone rate can be very low (which results in a small fractional-response index), and the minimum is reached at a relatively high intensity. The rate responses of the fiber pair (Figs. III-11 and III-12) do not seem to fit with this reasoning. Clearly, more data from such fiber pairs studied with the same stimulus conditions are needed.

There are at least two other possibilities for the differences in the responses of fibers from different SR groups, neither of which is completely satisfactory. One is the different

dynamics of adaptation in the high- and lower-SR fiber synapses, the other is the influence of extracellular potentials upon the different types of afferent fibers.

Adaptation

It has been shown that high-SR fibers adapt faster and recover faster than lower-SR fibers do (e.g., Rhode and Smith, 1985; Relkin and Doucet, 1991). Cooper et al. (1993) studied the responses of guinea-pig AN fibers to amplitude-modulated (AM) stimuli and observed different response characteristics between high- and lower-SR fibers, which they thought were due to different dynamics of high- and lower-SR fiber synapses. Although the AM stimuli and the two-tone stimuli are different, the IHC receptor potentials generated by both stimuli should be similar, in that both contain periodic peaks and valleys. Due to the stimulus paradigm we used, the fibers in our experiments approached an adapted state while they were responding to the CF-tone alone. The suppression due to the low-frequency suppressor caused cyclic reductions of the discharge rate of the AN fiber in every cycle of the suppressor tone. This temporary reduction of the discharge rate allowed the fiber to recover somewhat from the adapted state, and an "enhanced" response (overshoot) immediately followed the suppression. Thus, the peak response level in the two-tone segment (caused by the adaptation-enhancement sequence) should exceed the response level in the CF-alone segment (adapted, no recovery and thus no enhancement) in high-SR fibers. For lower-SR fibers, due to the longer time constants for adaptation and recovery, the recovery is not fast enough to produce the response enhancement immediately following the suppression, which results in a relative-response index below unity. While this mechanism seems plausible, especially in light of the data of Cooper et al. (1993) where modulation frequencies of 50-320 Hz were used, it cannot account for all of our data, for several reasons. First, the time allowed for the fiber to

recover is too short. When the suppressor tone was 2000 Hz, the recovery time allowed is less than 0.25 ms, which is much shorter than the fastest time constant of adaptation (about 2-3 ms) identified so far (Yates and Robertson, 1980). Secondly, it is also difficult to account for the situation in Fig. III-6, where most of the responses in the two-tone segment are suppressed, leaving a single peak high above the average. Thirdly, since the adaptation is viewed as some sort of lowpass filtering (e.g., Schroeder and Hall, 1974; Smith and Brachman, 1982; Cheatham and Dallos, 1992b), the different recovery time constants in high- and lower-SR fibers must be associated with different corner frequencies of the "lowpass filters". If this association is correct, the lack of phase differences (Chapter 2, also Fig. III-11) in the low-frequency responses and in suppression between lower- and high-SR fibers does not support this idea that adaptation is the main mechanism. Finally, since the peak of the two-tone response in the IHC receptor potential is always above the CF-alone level, there is probably no need to invoke the "adaptation-recovery" mechanism to justify the above-unity relative-response indexes seen in the high-SR fibers. From the above arguments, we conclude that adaptation must play only a secondary role here.

Electrical influence upon afferent fibers

Another possible mechanism to account for the differences in responses of fibers from different SR groups is the interaction between the organ of Corti potentials and the IHC/AN-fiber complex (Hill et al., 1989; Hill and Geisler, 1992). Although similar ideas have been proposed before (e.g., Sellick et al., 1982) to account for other nonlinear phenomena in the cochlea, the particular proposal by Hill et al. (1989) differs from the rest in that the interaction happens post-synaptically, at the AN fiber terminal. In their model, the receptor potential of the IHC causes the release of neurotransmitters at the afferent synapse and leads

to excitatory post-synaptic potentials (EPSPs) on the post-synaptic membrane. The EPSPs in turn initiate spikes on the AN fiber. Since the portion of the afferent fiber distal to the habenula is unmyelinated, it is possible that the organ of Corti electrical potentials affect the spike initiation. Specifically, the model (Hill et al., 1989) assumes that there is a DC potential, which is derived from the extracellular current flow, serving as a suppressive factor to counteract the excitatory factor (the EPSPs). Presumably, lower-SR fibers are more affected by this DC potential (Fig. III-14).

The advantage of this model is that it would predict no phase difference between the responses of fibers from different SR groups, either to suppressor alone or to two-tone stimuli. This independence of phase responses upon fiber SR fits well with our data (Fig. III-11, also Chapter 2) and data from Patuzzi et al. (1984a). Since the SUP component in the IHC potential is usually quite large for those higher frequency suppressors (500-2000 Hz), the effect of such DC potential on lower-SR fibers must be quite strong to produce a relative-response index below unity. Thus, the suppression of CF response due to this mechanism must also be very strong in those fibers, since presumably the extracellular DC potential affects the CF component and the SUP component equally. If this is the case, a difference in suppression threshold between high-and lower-SR fibers is expected. But both our study (Fig. III-13) and that of Schmiedt (1982) showed only insignificant difference in the suppression thresholds of high-and lower-SR fibers. However, the major weakness of this model is that so far no large DC organ-of-Corti potentials have been found to support this idea. On the contrary, the extracellular potential contains large AC components, which are thought to be mainly generated by outer hair cells (e.g., Cheatham and Dallos, 1994). These AC potentials are not usually in phase with IHC receptor potentials at low frequencies (because of phase shifts due to the hydrodynamic filtering in the sub-tectorial space). Thus,

these AC potentials do not affect AN fiber firing at the right phase. In conclusion, whether the extracellular AC potential is involved or not, there are unsolved problems with this scheme.

4. Relationship to mechanisms of two-tone suppression

We observed no *phase* differences between the suppressor effects upon the lower-SR and high-SR fiber pair (Fig. III-11), which agrees with the general SR-independence of the phases of suppression and excitation in our data (Chapters 1 and 2). We also did not see any apparent differences in the suppression thresholds of high- and lower-SR fibers (Fig. III-13). These results support the idea that the basic response and suppression patterns of all fibers are formed at or before the IHC stage. A major finding in this study is the different *amplitude* behavior (i.e., relative-response index and fractional-response index) of the high- and lower-SR fibers. Although we don't have satisfactory explanations for it, this difference, however, suggests that, besides the basic suppression pattern which is determined at or before the IHC stage, there must be other mechanism(s) somewhere between the IHC receptor potential and the auditory-nerve fiber discharges, which act differently for high- and lower-SR fibers. The importance of these post-IHC mechanism(s) in shaping the response patterns at the neural level cannot be overemphasized.

5. Functional significance

Each IHC is innervated by a number of fibers with different SRs, which are basically sensing the same receptor potential. One may question the functional necessity of this arrangement. Our results shows that, although the fibers receive the same basic information, they further process the information before sending it to the higher center. One major difference is their

sensitivity to slow-changing components in their inputs. Lower-SR fibers are less sensitive to the slow-changing components than the high-SR fibers. They tend to respond less vigorously to the slow-changing components of the IHC potential. This property, together with their higher thresholds and slower adaptation/recovery characteristics, makes them ideal for encoding higher-level, time-varying signals. Such roles of lower-SR fibers has been suggested before (Greenberg, 1986), an idea that is supported by their better phase-locking abilities (e.g., from AM studies) and timing accuracy. Since an essential function of the auditory system is to detect changes in the acoustical space, one would assume that one role of lower-SR fibers is to detect changes at higher sound levels, while high-SR fibers are responsible for detecting changes at low sound levels.