Threshold Tuning Curves of Chinchilla Auditory Nerve Fibers. II.
Dependence on Spontaneous Activity and Relation to Cochlear Nonlinearity

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Submitted 4 June 2008; accepted in final form 24 August 2008

INTRODUCTION

In cats, frequency threshold tuning curves (FTCs) are more sharply tuned in auditory nerve fibers (ANFs) with low spontaneous activity [spontaneous rate (SR)] than in high-SR ANFs (Kiang et al. 1976; Liberman 1978). SRs are associated with specific morphological features of cochlear neurons and their synapses on inner hair cells (Liberman 1980, 1982). Therefore because ANFs with the same characteristic frequency (CF), regardless of SR, must share the same frequency tuning of inner hair cell receptor potentials (which in turn is determined by the frequency tuning of the organ of Corti) and because organ of Corti vibrations (at least at the base of the cochlea) are more broadly tuned for intense stimulation than at threshold (Robles and Ruggero 2001), a dependence of ANF frequency tuning on SR must be viewed as somewhat paradoxical.

The finding of an SR dependence of ANF tuning in cats has not been replicated in other species, even though the SR dependence was explicitly sought in studies in gerbil (Oehlermillen and Echteler 1990; Schmiedt 1989). The companion paper presents evidence that the SR dependence of frequency tuning also applies to chinchilla ANFs (Fig. 1 of Temchin et al. 2008). The purpose of this paper is to expand on that finding and to show that the seemingly paradoxical dependence of tuning on SR actually reflects a previously unnoticed nonmonotonic variation of basilar membrane (BM) tuning as a function of iso-velocity criterion. A preliminary account of this work was published as an abstract (Temchin et al. 1997).

METHODS

All animal procedures, detailed in the companion paper (Temchin et al. 2008), were approved by the Animal Care and Use Committee of Northwestern University. In brief, conventional extracellular microelectrodes were used to record from individual ANFs, via an intracranial approach, in deeply anesthetized chinchillas. (At the end of each experiment, the chinchilla was killed by decapitation while still deeply anesthetized.) White noise bursts (50-ms duration, presented 3/s) were used as stimuli while attempting to isolate ANFs. On isolation of an ANF, a 10-s sample of spontaneous activity was recorded, and an FTC was measured using an automated adaptive procedure (Kiang et al. 1970; Liberman 1978) with threshold criterion equivalent to 20 spikes/s higher than spontaneous rate. Additionally, in many cases, thresholds were obtained from rate intensity function for responses to low-frequency (≤1,000 Hz) tones.

RESULTS

Here we analyze the relationship between spontaneous activity and the frequency tuning of responses to tones recorded in thousands of chinchilla ANFs (see Temchin et al. 2008).

Frequency distribution of spontaneous activity

Spontaneous activity (SR; the average firing rate in the absence of controlled acoustic stimulation) was measured in 4,184 ANFs recorded in 228 chinchillas. Figure 1, A and B, shows that there is a relative dearth of ANFs with medium and low SRs for CFs <3 kHz. This is quantified in Fig. 1C, which shows that the percentage of SRs <18/s changes abruptly at CFs around 3 kHz, almost doubling between 3 and 4 kHz.

The distribution of SRs for the entire population of ANFs is mildly bimodal (Fig. 2B), exhibiting a large peak for SRs <1 spike/s and a broader, much shallower, peak for higher SRs. However, when SRs for ANFs with CFs < and >3 kHz are plotted separately (Fig. 2A), it is apparent that the bimodality is confined to the former and absent from the latter. The bimodal frequency distribution of SRs for CFs <3 kHz justifies the classification of chinchilla ANFs into (only) two SR groups. Nevertheless, for the sake of comparison with other studies, we classified ANFs in three groups: low SR (SR <1 spike/s), middle-SR (SRs 1–18 spikes/s), and high-SR (SRs >18 spikes/s). Those groups made up 10.9, 23.0, and 66.2% of the ANF population, respectively.

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Figure 3 explores the relationship between SR and CF thresholds in a sample of 144 chinchillas in which 10-μV compound action potential (CAP) thresholds were determined using an automated procedure at frequencies separated by 0.5 octave from 0.5 to 16 kHz (see METHODS in Temchin et al. 2008). Data are shown in Fig. 3 only for ANFs with CFs between 0.5 and 12 kHz, a range in which CF thresholds of high-SR ANFs are relatively uniform (Fig. 4). CF thresholds are presented after normalization to CAP thresholds to attenuate the effects of threshold variations both as a function of CF (in individual animals) and across animals (in any single CF region). In logarithmic coordinates (log SR vs. SPL), data points spread out around a straight line that describes a power function (dashed line). The slopes of regressions calculated separately for low- and medium-SR ANF groups (solid lines) did not differ statistically from the regression for the entire population. On the other hand, a regression for the high-SR group did not differ statistically from a zero-slope line.

Regardless of CF or SR, 85% of all ANFs had thresholds within a 30-dB range. Figure 4 presents CF thresholds, computed separately for high-, medium-, and low-SR ANFs, averaged over 2/3-octave CF bands and plotted against CF. Average CF thresholds ± SD were 28.2 ± 12.4 dB SPL for low-SR ANFs (n = 448), 21.4 ± 12.3 dB SPL for medium-SR ANFs (n = 951), and 16.0 ± 11.4 dB SPL (n = 2785) for high-SR ANFs. The differences between thresholds for low- and high-SR ANFs were more or less constant across CFs, aver-
aging 13.8 dB in the 0.75- to 19-kHz CF range. The variance of CF thresholds in any single animal was substantially smaller than in the entire population. The average SD of the mean thresholds in the CF range 0.6–10 kHz, computed in 2/3-octave bands across the entire population of high-SR ANFs (Fig. 4, vertical black lines), was 10.1 dB. The corresponding average SD of CF thresholds was 8.2 dB after subtracting CAP thresholds individually for each animal, a procedure that tends to remove variance because of interanimal threshold differences. The average SD was even smaller, 3.9 dB, when computed as the grand average of SD for individual CF band across animals (Fig. 4, red vertical lines), a procedure that removes both interanimal variations and the effects of variations as a function of CF in individual animals.

**SRs and FTC shapes**

Figure 5 shows average tip-to-tail ratios (circles) for low- and high-SR ANFs with CF >3.5 kHz. The ratios in the two SR populations differ significantly (ANOVA: $P = 0.003$), being lower by 4.8 dB, on average, in low-SR than in high-SR ANFs. Averaged CF thresholds (squares) measured in the same ANFs were significantly higher for low-SR than for high-SR ANFs ($P = 0.005$). The CF threshold differences averaged 15.1 dB, i.e., nearly the same as in the general population of ANFs with CFs 0.75–19 kHz (13.8 dB). The difference in tip-to-tail ratios between the FTCs of low- and high-SR ANFs complements the results of Fig. 1 of the companion paper (Temchin et al. 2008), as well as Fig. 6, 7, and 8A of the present paper, which show that bandwidths, slopes and $Q$s of FTC tips also differ between ANFs with low and high SRs.

Figure 6, which shows FTC $Q$s as functions of CF for ANFs with low- and high-SRs, extends the findings of Fig. 1D of Temchin et al. (2008), confirming that low-SR ANFs are more sharply tuned than high-SR ANFs, particularly for high CFs. Although the differences in $Q$s between low-SR and high-SR ANFs are generally small, in many cases, they are statistically
significant (crosses). The largest average differences occur for $Q_{40\text{dB}}$ of ANFs with CF of 4.8 kHz (Fig. 6D), 1.35 versus 1.79 for high- and low-SR ANFs, respectively, or 32%.

In the companion paper, we showed that the lower and upper limbs of the FTCs and, consequently, the partial bandwidths below and above CF, varied differently as a function of CF.

**Fig. 6.** SR dependence of sharpness of tuning of FTCs. Sharpness of tuning is expressed as $Q_x$ (i.e., CF divided by bandwidth at $x\text{ dB}$ above CF threshold). $Q_{10\text{dB}}$ (A), $Q_{20\text{dB}}$ (B), $Q_{30\text{dB}}$ (C), and $Q_{40\text{dB}}$ (D) values were averaged separately for low- (open circles) and high-SR (closed circles) within 2/3-octave bands and plotted as a function of CF. Vertical bars are SD. Numbers of ANFs are indicated in each panel. Only data points for averages from 10 or more ANFs are plotted. Crosses indicate statistically significant differences ($P < 0.0005$) between low- and high-SR ANFs.

**Fig. 7.** SR dependence of partial bandwidths for FTC frequencies higher than CF. Partial bandwidths for FTC frequencies $>\text{CF}$ (see Fig. 1, B and C, of companion paper I; Temchin et al. 2008). Bandwidths were averaged separately for low- (open circles) and high-SR (closed circles) within 2/3-octave bands and plotted as a function of CF. Vertical bars are SD. Numbers of ANFs are indicated in each panel. Only data points for averages from 10 or more ANFs are plotted. Crosses indicate statistically significant differences ($P < 0.0005$) between low- and high-SR ANFs.
SR dependence of ANF sharpness of tuning derives from a nonmonotonic dependence of BM tuning on response criterion

The fact that the FTC tips are more sharply tuned in low-SR than in high-SR ANFs (Figs. 6, 7, and 8A; also Fig. 1 of Temchin et al. 2008) needs explanation, because SRs are determined at the synapses between ANFs and inner hair cells (Liberman 1980, 1982; Merchan-Perez and Liberman 1996), presumably devoid of intrinsic frequency tuning, and BM responses are broadly tuned for intense stimuli, i.e., a stimulus-level dependence seemingly opposite to that of the auditory nerve, in which low-SR ANFs, with higher thresholds, have sharper tuning.

An important clue to the origin of the dependence of tuning on SR is that when FTCs and other iso-rate tuning curves for the same ANF are displaced along the SPL axis, so that their lower limbs overlap, their upper limbs shift systematically to lower frequencies as a function of increasing criterion level. This is shown in Fig. 8B, redrawn from Fig. 3 of Geisler et al. (1974). On the reasonable assumption that thresholds for any single ANF corresponds to a constant magnitude of BM vibration, the fact that low-SR ANFs have higher thresholds than high-SR ANFs implies that the thresholds of low-SR ANFs correspond to higher magnitudes of BM responses than those of high-SR ANFs. Therefore, the upper limbs of average FTCs normalized to CF threshold should shift to lower frequencies as a function of SR. Figure 8A, showing synthetic FTCs with CFs of 7.4 kHz constructed from responses of low-SR, mid-SR, and high-SR ANFs, shows that the prediction is correct: regardless of SR, FTCs normalized to CF threshold have similar lower limbs near CF but the upper limbs shift to lower frequencies with decreasing SR. As a result, $Q_s$ vary inversely with SR. The FTCs of high-, mid-, and low-SR ANFs, respectively, have $Q_{20s}$ of 2.64, 2.80, and 2.90, $Q_{30s}$ of 1.94, 2.15, and 2.27, and $Q_{40s}$ of 1.45, 1.67, and 1.88.

Figure 9 shows iso-velocity tuning curves for BM responses to tones in the cochleae of chinchilla (A–C) and gerbil (D). To facilitate comparison among the tuning curves obtained with different velocity criteria, the curves in each panel have been normalized to CF. For low response criteria (solid lines), the iso-velocity curves overlap at most frequencies < CF. In contrast, the upper limbs of the tuning curves do not overlap. Starting with the lowest criterion velocities, bandwidths first decrease with increasing criterion velocity, until they reach a minimum (solid lines) at which $Q_s$ are maximal. For even higher response criteria (dashed lines), bandwidths increase and $Q_s$ decrease. This nonmonotonic behavior of BM tuning as a function of response criterion probably explains why FTCs of low-SR ANFs (Fig. 8A) are more sharply tuned than the FTCs of high-SR ANFs: FTCs of low-SR ANFs reflect BM iso-velocity curves with intermediate criterion velocities, which are more sharply tuned than iso-velocity curves with lower criterion velocities.

The differences between the CF thresholds (15.1 dB) and the tip-to-tail ratios (4.8 dB) of low- and high-SR ANFs are both consistent with the chinchilla BM data of Fig. 9 on the assumption that the thresholds of high-SR ANFs correspond to a BM velocity of 50 $\mu$m/s. In that case, low-SR ANFs would have thresholds corresponding to a BM velocity of 284 $\mu$m/s.

FIG. 8. SR dependence of sharpness of ANF FTCs and variation of tuning as a function of iso-response criterion. A: synthetic FTCs for high-SR (blue), mid-SR (green), and low-SR (red) ANFs, normalized to threshold at CF. B: iso-rate curves for responses of a single squirrel monkey ANF at the indicated percents of the maximum rate. Data from Fig. 3B of Geisler et al. (1974).

J Neurophysiol • VOL 100 • NOVEMBER 2008 • www.jn.org
and their tip-to-tail ratios could well be 4.8 dB lower than those of high-SR ANFs (due to the combination of BM linearity at tail frequencies and compression at CF).

The nonmonotonic dependence of BM tuning on iso-velocity criterion (Fig. 9) derives not from the compressive growth of BM vibrations per se, but rather from its uneven distribution across frequencies (Rhode and Recio 2000; Rhode 2007; Ruggero et al. 1997), at least for high CFs. At (tail) frequencies well below CF, responses are linear. Compression grows as CF is approached and surpassed and is maximal at a frequency higher than CF. For low velocities (e.g., 50–200 \mu m/s in Fig. 9A), the lower limbs of the BM tuning curves do not change as a function of criterion velocity because responses are linear. In contrast, the upper limbs shift to lower frequencies because the peaks of compression also shift systematically to lower frequencies as a function of increasing criterion velocity. The combined fixed lower limbs and shifting upper limbs result in sharper tuning as a function of increasing velocity for low criterion velocities. For higher criterion velocities (e.g., 400–1,600 \mu m/s), the upper limbs continue shifting to lower frequencies as a function of increasing velocity but so do the lower limbs, at an even faster rate. As a result, BM tuning becomes broader with increasing criterion velocity.

**DISCUSSION**

**Distribution of SRs in chinchilla and other species**

The range of SRs in this study (<1 to 140–150 spikes/s) is similar to ranges previously reported for chinchilla (Dallos and Harris 1978) and other species: cat (Kiang et al. 1965; Liberman 1978), gerbil (Müller 1996; Ohlemiller and Echteler 1990; Schmiedt 1989), guinea pig (Manley and Robertson 1976), and rat (el Barbary 1991a). Categorizing SRs with boundaries at 1 and 18 spikes/s, the percentages of low-, medium-, and high-SR fibers in chinchilla are 11, 23, and 66, respectively. With a similar definition, the percents are 12, 15, and 73 (Tsuji and Liberman 1997) or 20% for low-SR ANFs in guinea pig (Evans 1972). With the more commonly used boundaries of 0.5 and 18 spikes/s, low-, medium-, and high-SR ANFs constitute 8, 26, and 66% of the population in chinchilla, i.e., very similar to the 11, 23, and 66% reported previously for chinchilla (Salvi et al. 1982). These percents are also similar to those reported for cat (16, 23, and 61%; Liberman 1978) and gerbil (10, 30, and 60%; Schmiedt 1989).

**SRs as a function of CF in chinchilla and other species**

Contrary to results in chinchilla (Fig. 1), some studies of ANFs have found a gradual decline of the upper range of SRs (SR compression) as a function of increasing CF. Such compression seems to be well established in the case of gerbils (Ohlemiller and Echteler 1990; Schmiedt 1989; see also Müller 1996). Compression was also described in one study in cats (Liberman 1978) but not in another (Kim and Molnar 1979) and seems to be absent in rats (Fig. 3 of el Barbary 1991a). When it exists, SR compression might reflect basal cochlear dysfunction, either chronic, caused by overexposure to intense sounds (because the compression was not typically found in chamber-raised cats; Liberman 1978), or acute, as a result of cochlear cooling after opening of the bulla under anesthesia (Liberman and Dodds 1984b). However, compression was observed in gerbils even when cochlear temperature was maintained at 37–38°C (Ohlemiller and Echteler 1990). In this study, in which compression was not evident, chinchillas were “routine normals” (with uncertain history of exposure to intense sounds), and no special precautions were taken to avoid cooling of the cochlea. Thus the absence of SR compression...
may be one feature in which the chinchilla differs from gerbils (and possibly cats). In chinchilla, the percentage of low-SR fibers is much lower for CF <3 kHz than for higher CFs (Fig. 1C). A scarcity of low-SR ANFs with CF <3 kHz was also reported in gerbil (Ohlemiller and Echteler 1990; Schmiedt 1989). In contrast, the distributions of low-SR fibers in cat (Kiang et al. 1965; Liberman 1978) and rat (el Barbary 1991b) seem to be uniform across CFs. Computed for the entire ANF population, the frequency distribution of SRs varies widely between species and even among reports for the same species. The distribution is clearly bimodal in cat (Kim and Molnar 1979; Liberman 1978) and guinea pig (Tsuji and Liberman 1997), only mildly bimodal in chinchilla (Fig. 2B and Dallos and Harris 1978) and rabbit (Borg et al. 1988), but unimodal in rat (el Barbary 1991a) and mouse (Taberner and Liberman 2005). For gerbils and macaques, results are mixed; for gerbils, contrast Fig. 5 of Schmiedt (1989) with Fig. 3 of Ohlemiller and Echteler (1990); for macaque monkeys, contrast the results of Nomoto et al. (1964) and Joris et al. (2006).

**SRs and FTC features across species**

SRs and CF thresholds are inversely related in chinchilla (Fig. 3; see also Salvi et al. 1982) and in other mammalian species (cat, Kiang 1984; Kiang et al. 1976; Liberman 1978; Liberman and Dodds 1984a; guinea pig, Winter et al. 1990; Mongolian gerbil, Ohlemiller and Echteler 1990; Schmiedt 1989; and, to a lesser extent, rabbit, Borg et al. 1988). The SR dependence of CF thresholds in chinchilla (this study), cat (Liberman 1978), and guinea pig (Tsuji and Liberman 1997) can be compared with special confidence because they were obtained using essentially the same tuning curve algorithm. On average, low-SR ANFs in the present chinchilla sample have CF thresholds that are 13.8 dB higher than in high-SR ANFs (Fig. 4). In cat, the CF thresholds of low- and high-SR ANFs differ by 15–30 dB (Fig. 11 of Liberman 1978), a substantially larger amount than in chinchilla (Liberman 1978). The quantitative difference between the SR dependences in cat and chinchilla may reflect the fact that electric shocks were used as search stimuli in the cat study, thus ensuring the detection of ANFs with nearly zero SR. Thus it is possible that this sample in chinchilla underestimates the numbers of those ANFs, which presumably have especially high thresholds. In guinea pig, low-SR ANFs have CF thresholds ~19 dB higher than high-SR ANFs (Tsuji and Liberman 1997). In gerbil, low-SR ANFs have CF thresholds between 10 and 20 dB higher than high-SR ANFs.

The tips of FTCs are more sharply tuned in low-SR than in high-SR ANFs in chinchilla (Figs. 6, 7, and 8A; Fig. 1 of Temchin et al. 2008) and cat (Kiang 1984; Kiang et al. 1976; Liberman 1978). A dependence of sharpness of tuning on SR was sought, but not found, in gerbil (Ohlemiller and Echteler 1990; Schmiedt 1989) and has not been reported for any other species. In both chinchilla and cat, the differences of sharpness of tuning between low- and high-SR ANFs are relatively larger when measured at higher levels (30–40 dB) re CF thresholds. In chinchilla, the largest difference between $Q_{40}$ occurs for CFs around 5 kHz, with $Q_{40}$ being 40% larger in low-SR ANFs than in high-SR ANFs (Fig. 5D). In cat, the differences may be somewhat larger, to judge by Fig. 14 of Liberman (1978), which is the only quantitative account of differences of sharpness of tuning between low-SR and high-SR for cat ANFs. On average, for CFs between 800 Hz and 18 kHz, $Q_{40}$ are ~30% larger in low-SR than in high-SR ANFs.

The contrasting results regarding a link between SR and sharpness of tuning between studies on chinchilla and cat, on the one hand, and gerbil in the other, may reflect differences between the algorithms used for FTC determination. However, this explanation was disproved by showing that FTCs obtained with different algorithms (one of them the one used for both cat and chinchilla studies) yielded the same sharpness of tuning in low- and high-SR gerbil ANFs (Ohlemiller and Echteler 1990). Another explanation for the apparent species difference was based on the separations between CF thresholds of low- and high-SR ANFs, judged to be smaller in gerbil than in cat (Ohlemiller and Echteler 1990). Again, this explanation seems disproved by these results because the separations between low- and high-SR CF thresholds are similar in chinchilla and gerbil (cf. Fig. 4 with Fig. 4 of Ohlemiller and Echteler 1990).

**Conclusions**

The properties of spontaneous activity of chinchilla ANFs, including their relation to thresholds at CF, generally resemble those of other mammalian species. However, there are apparent species differences in the salience of the bimodality of the frequency distribution of SR.

In chinchilla, the FTCs of low-SR ANFs with CFs >3.5 kHz have lower tip-to-tail ratios, and have tips that are more sharply tuned, than the FTCs of high-SR ANFs. This finding is consistent with the (until now, exceptional) reports of SR dependence of ANF FTCs in cat (Kiang et al. 1976; Liberman 1978).

That the tips of FTCs are more sharply tuned in low-SR than in high-SR ANFs is probably explained by the (previously unnoticed) fact that BM tuning curves determined with mid-level iso-velocity criteria are sharper than those measured using lower (or higher) criterion velocities.

**Acknowledgments**

Many thanks to S. Dhar for carrying out the ANOVA.

**Grants**

This work was supported by National Institute of Deafness and Other Communication Disorders Grants DC-000110 and DC-000419.

**References**


