Response properties of single auditory nerve fibers in the mouse

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Abstract

The availability of transgenic and mutant lines makes the mouse a valuable model for study of the inner ear, and a powerful window into cochlear function can be obtained via recordings from single auditory nerve (AN) fibers. This study provides the first systematic description of spontaneous and sound-evoked discharge properties of AN fibers in mouse, specifically in CBA/CaJ and C57BL/6 strains, both commonly used in auditory research. Response properties of 196 AN fibers from CBA/CaJ and 58 from C57BL/6 were analyzed, including spontaneous rates (SR), tuning curves, rate-vs-level functions, dynamic range, response adaptation, phase-locking and the relation between SR and these response properties. The only significant inter-strain difference was the elevation of high-frequency thresholds in C57BL/6. In general, mouse AN fibers showed similar responses to other mammals: sharpness of tuning increased with characteristic frequency, which ranged from 2.5 to 70 kHz; SRs ranged from 0 to 120 sp/sec, and fibers with low SR (< 1 sp sec) had higher thresholds, and wider dynamic ranges than fibers with high SR. Dynamic ranges for mouse high-SR fibers were smaller (< 20 dB) than those seen in other mammals. Phase-locking was seen for tone frequencies < 4 kHz. Maximum synchronization indices were lower than those in cat but similar to those found in guinea pig.
Introduction

The availability of transgenic and mutant lines with interesting cochlear phenotypes makes the mouse a valuable model for study of the auditory system. Minimally invasive measures of auditory function are often used to assess cochlear phenotype in mice. Such measures include distortion product otoacoustic emissions, auditory brainstem responses, and compound action potentials. Although these measures can rapidly provide useful information about cochlear sensitivity as a function of cochlear location, there are many aspects of cochlear physiology that cannot be unambiguously inferred from these gross responses, because they represent the summed activity of numerous cochlear generators.

Although requiring more invasive procedures, recordings from single auditory nerve (AN) fibers can provide more detailed insight into the functional state of the inner ear. The vast majority of AN fibers in the mammalian ear make synaptic contact with only a single inner hair cell, by means of one synaptic complex (Liberman 1980; Spoendlin 1969; Spoendlin and Schrott 1988). Thus, recording from a single AN provides a sensitive window into the microenvironment of a single sensory cell in the inner ear and a sensitive functional metric of the transducer apparatus on the specific inner hair cell contacted and the neighboring outer hair cells which influence its local cochlear mechanics. Analysis of the fine timing patterns of spike discharges (e.g. the degree of phase locking or the response adaptation) can provide insight into the processes involved in synaptic transmission between the hair cell and its primary sensory neuron. By sampling from numerous fibers in the same animal or mutant strain, a detailed picture can be assembled of the outputs of the sensory transduction and synaptic transmission machinery all along the cochlear duct.
There have been only two previous reports of single-fiber activity from the mouse AN. These pioneering studies were not primarily interested in understanding cochlear mechanisms. One was aimed at validating the use of the galvanic skin response as a minimally invasive measure of cochlear sensitivity (Finck and Berlin 1965) and thus mainly collected data on single-fiber thresholds. The other was aimed at understanding the neurophysiological bases for critical bands and thus collected data on single-fiber thresholds and on the masking of tone responses by noise bands (Ehret and Moffat 1984). In contrast, our aim is to describe those fundamental aspects of auditory nerve response that provide the most insight into the mechanisms of transduction and synaptic transmission in the inner ear, especially when coupled with the use of targeted genetic modification in the mouse model. Thus, our study was designed to provide a systematic description of the most fundamental response properties of the mouse AN response, including the distribution of spontaneous rates, tuning curves, rate-vs-level functions, dynamic range, response adaptation, degree of phase-locking and the relations between spontaneous rate and the aforementioned response properties.

In the present study, we describe the spontaneous and sound-evoked discharge properties of a large number of AN fibers recorded from the CBA/CaJ strain and somewhat smaller set of recordings from the C57BL/6 strain. The former strain is important, because it is commonly used in auditory research, and it retains excellent cochlear sensitivity throughout its life span (Jimenez et al. 1999; Li and Borg 1991; Zheng et al. 1999). The latter strain is of interest because it is often the background strain in which knockout mice have been produced (Mullen and Ryan 2001), and because it has also been extensively studied due to the presence of a type of age-related hearing loss (Ding et al. 1999; Henry and Lepkowski 1978; Li et al. 1993; Parham 1997; Willott 1986)
Comparisons between mouse AN physiology and that of other mammals is also of interest, because the mouse cochlea is specialized for higher frequency stimuli than cat, guinea pig, chinchilla, or gerbil, the other mammalian species in which AN responses have been well studied. Despite this important difference, we show that the fundamental aspects of AN response are qualitatively similar in the mouse. We further show that the responses of these two commonly used mouse strains are quantitatively similar as well.

**Methods**

Single-fiber recordings were made from the auditory nerve in CBA/CaJ mice, aged 8 - 17 weeks, or in C57Bl/6 mice aged 16 - 17 weeks. Animals were anesthetized with xylazine (5 mg/kg) and urethane (1.32 mg/kg). Animal temperature was maintained near 38°C with a heating pad and by maintaining the ambient temperature in the experimental chamber at about 33°C. For the surgical approach, the cartilaginous ear canals were removed, scalp reflected, skull opened, and a semi-cerebellectomy performed to expose the left cochlear nucleus. Glass microelectrodes filled with 2M KCl and 4% methyl blue were directed at the surface of the cochlear nucleus, ~1 mm medial to the edge of the temporal bone, angled anteriorly at 24° in the sagittal plane and laterally at 10° in the coronal plane. All animal procedures were approved by the IACUC of the Massachusetts Eye and Ear Infirmary.

The sound system comprised dual electrostatic sound sources (TDT ED-1) and a Knowles electret microphone coupled to a probe tube. The sensitivity of the probe-tube microphone was calibrated for frequencies between 1.0 and 73 kHz using a calibrated Bruel & Kjaer 1/4” condenser microphone in a coupler.
Distortion-product otoactoustic emissions (DPOAEs) were monitored throughout the experiments to assess cochlear stability. At the beginning of the experiments, before cerebellar aspiration, DPOAEs were measured with $f_2$ frequencies from 5.6 to 45.2 kHz in half octave steps. During single-fiber recordings, the DPOAEs were re-checked at the $f_2$ nearest the characteristic frequency (CF) of each fiber studied. Data are included in the final database only if the thresholds for DPOAEs at $2f_1-f_2$ were $\leq 5$ dB re initial values. The DPOAE at $2f_1-f_2$ was recorded in response to primary tones $f_1$ and $f_2$, with $f_2/f_1=1.2$ and $L_2 = L_1 - 10$ dB. The ear-canal sound pressure waveform was amplified and digitally sampled at 4 µs intervals. Fast-Fourier transforms were computed from averaged sound pressure waveforms, and the DPOAE and surrounding noise floor were extracted.

50-msec noise bursts were used as search stimuli. Tone bursts were used for all other sound-evoked measures (except phase-locking): they were 50 msec in duration, with 2.5 msec rise-fall and a repetition rate of 10/sec. Tuning curves were measured under computer control, and represent isorate contours for response magnitude of 10 sp/sec $>$ spontaneous rate. Spontaneous rates were calculated from 10 sec samples.

Because both cochlear nucleus cell types and AN fibers are encountered along the electrode track, a classification scheme based on post-stimulus time histogram (PSTH) shape, first spike latency (FSL), and the coefficient of variation (CV) of the interspike intervals, was developed to distinguish the two cell types. PSTHs used to characterize response type were based on 150-250 tone burst presentations at CF and were always presented at 30 dB above threshold. A measure of FSL, i.e. the mode of the FSL distribution, and the CV, i.e. the ratio of the standard deviation to the mean of the interspike interval distribution, were both obtained from responses to 150 CF tone bursts at 30 dB above threshold. CV measures were derived only from spikes occurring 20-
40 msec after tone-burst onset (i.e., excluding the initial and final 10 msec, where some spike intervals may include spontaneous discharge).

Rate-level functions were measured at CF with 10 - 20 tone bursts per level. Levels (in 5 dB steps) were presented in random order, and if contact time permitted, also in sequential order. Rate-vs-level functions were fit using a modified version of a previously published model (Sachs et al. 1989). The input to the saturating nonlinearity was given an exponential β (a free parameter) which allowed the function to have steeper slopes. The fitting procedure was done in Matlab® using the function \textit{fminsearch}, which performs a multidimensional unconstrained nonlinear minimization (Nelder-Mead). The root-mean-square-error (rms error) between the response data and the best-fit curve was used to determine goodness of fit. To eliminate "noisy" data runs, a maximum allowable rms error of 14 sp/sec was arbitrarily set for inclusion into the final data base. Dynamic range was defined as the difference between SPLs evoking 10% and 90% of the (model fit) maximum driven rate.

Measures of synchrony were obtained from post-zero crossing (PZC) histograms derived from the presentation of single-tones of 15 sec duration. The tone frequencies were constrained (f = 1.0, 1.6, 2.0, 2.5 or 4 kHz) so that an integer number of cycles fit in the digital buffer. When measuring phase-locking in AN response, care was taken to eliminate artifactual synchrony due to microelectrode pick-up of cochlear microphonic potentials. A model developed by Johnson (Johnson 1978) was used to compute a synchrony noise floor below which synchrony may be artifactual. According to the model, the synchronization index (SI) due to the microphonic artifact is given by:

\[ SI_{\text{artifact}} = \sqrt{8 \pi f \gamma t} \]
assuming a randomly-distributed spike train contaminated by an additive sine wave at frequency $f$; $\gamma$ is the ratio of the rms stimulus artifact amplitude to the peak spike amplitude, and $t$ is the rise time of the spikes. Rise time $t$ and ratio $\gamma$ were calculated from measurements of digitized spike trains obtained during each synchrony measurement: the amplitude of the sine wave (microphonic) component at the stimulus frequency was extracted via FFT. Only the data for which the measured SI was at least 2 times greater than $S_{\text{artifact}}$ are included in this paper.

Results

A. Distinguishing auditory nerve from cochlear nucleus

Because the auditory nerve (AN) in mouse is difficult to expose directly without compromising cochlear function, the AN was reached via electrode penetrations which first travel through the cochlear nucleus (CN), which can be readily exposed. Electrode angles and insertion area were chosen to avoid the anteroventral CN, which contains cells with AN-like responses in other mammalian species (Rouiller and Ryugo 1984). Based on surface landmarks, most electrodes penetrations entered through the anterior portions of the DCN. Cell types encountered along the electrode track yielded post-stimulus-time histograms (PSTHs) with "chopper", "onset", "pri-notch", and “primary-like” shapes. As expected, cells with chopper (figure 1C) tended to be seen superficially in the electrode tracks, whereas "primary-like" (AN) responses were almost exclusively seen at electrodes sites deeper than 1 mm from the point of penetration.

In addition to qualitative classification of PST shapes, two more quantitative measures were used to distinguish AN fibers from CN cells: 1) the coefficient of variation (CV) of interspike
intervals and 2) first spike latency (FSL) (Young et al. 1988), both of which were measured in response to tone bursts at the characteristic frequency. In cat, where AN and CN can be separately accessed by microelectrodes (with visible superficial landmarks identifying the Schwann-glial border separating these two structures), AN responses are more irregular (i.e., larger CV for interspike intervals) and show a smaller FSL than most CN cells with similar characteristic frequencies and spontaneous rates (Young et al. 1988).

In early experiments to develop effective criteria for differentiating AN fibers from CN cells, tone-burst responses were obtained and analyzed from all fibers encountered along each electrode pass. Figure 1A shows the relationship between CV and FSL in fibers subjectively classified by PST shapes. Results suggest that mouse AN fibers have CVs $\geq 0.5$ and FSLs $\leq 5$ msec, which are similar to values reported for cat (Young et al. 1988). As in cat, there appears to be some overlap of CV and FSLs between AN and CN (e.g., for the “pri-notch” CN units) (Young et al. 1988). Nevertheless, the data in Figure 1 suggest that the following criteria adopted for classification as AN fiber should provide the best trade-off between sensitivity and specificity: 1) CV $\geq 0.5$, (2) FSL $\leq 5$ msec, (3) electrode depth $\geq 1000$ and 4) “primary-like" PSTH.

In later experiments, the superficially located CN cells were bypassed in order to increase the yield of AN fibers in each animal studied. By the end of the experimental series, the mean yield of AN recordings had grown to 7/animal (with a maximum of 16/animal). Termination of the recording session was typically dictated by decreasing cochlear sensitivity leading to DPOAE threshold elevation beyond the 5 dB limit on threshold shift arbitrarily imposed to maintain data "purity".
B. Rate Threshold and Tuning

A fundamental property of AN fibers is their frequency selectivity, which is often quantified via "threshold" tuning curves which track iso-response contours in the frequency-intensity plane (Figure 2). Tuning curves were obtained for all mouse AN fibers encountered. As in other mammalian species, mouse AN tuning curves show sharply tuned "tips", defining a characteristic frequency (CF: frequency of maximum sensitivity) and broadly tuned low-frequency “tails”. The data in Figure 2 show populations of tuning curves obtained from 28 CBA/CaJ mice, clustered according to CF. Tuning curve shapes are remarkably similar across animals. As reported for other species, tuning curves from lower CF regions tend to be more "V-shaped", with a less obvious low-frequency tail. Tuning curves from C57BL/6 mice showed similar features.

Plotting thresholds at CF for all the AN fibers obtained in the present study provides an overview of the sensitivity and frequency range of the peripheral auditory system in these strains. In this study, CFs ranged from 3.1 – 69.8 kHz for 196 fibers obtained in 28 CBA/CaJ animals and from 2.5 – 22.6 kHz for 58 fibers from 13 C57BL/6 animals (Figure 3A). A local threshold minimum in both strains was seen among fibers in the middle of that range, i.e. 12 - 24 kHz. In both strains, minimum thresholds rose for CFs below that region and the spread in thresholds at any one region of CF was on the order of 30 dB. In the CBA/CaJ, threshold sensitivity near 0 dB SPL could be seen in fibers with CF as high as 60 kHz. In C57BL/6, minimum thresholds rose dramatically for CFs > 15 kHz, as expected given the early onset of high-frequency threshold shifts in this strain (Li and Borg 1991)

The sharpness of AN tuning in mouse increases with CF. This relationship can be seen qualitatively in Figure 2 and quantitatively in Figure 3B, which shows $Q_{10dB}$ (ratio of CF to
bandwidth at 10 dB above threshold at CF) for all AN fibers in the present study. No striking
differences between the two strains were visible in regions where response thresholds overlap.

C. Spontaneous rate

Auditory nerve fibers discharge spontaneously in the absence of sound (Kiang et al. 1965).
Figure 4A shows the spontaneous rate (SR) distribution for the fiber populations sampled from
the two strains in the present study. SRs ranged from 0 - 120 sp/sec in both strains, with 49%
(CBA/CaJ) and 67% (C57BL/6) of the fibers having SRs < 20 sp/sec. In contrast to many other
mammalian species studied, e.g. cat (Kiang et al. 1965) or guinea pig (Tsuji and Liberman 1997),
the SR distribution in mouse is not clearly bimodal. While there is no clear relationship between
fiber SR and CF (Figure 4B), there is a clear relationship between fiber threshold and SR. As
seen in other mammalian species (e.g. Liberman, 1978), fibers with the lowest relative thresholds
(with respect to the most sensitive fibers of similar CF) also had the highest SRs (Figure 4C).
There are no striking differences between data from CBA/CaJ vs. C57Bl/6 mice.

D. Response adaptation

PST histograms of AN fibers show a peak in discharge rate at tone-burst onset, which decays
to a steady-state response (Kiang et al. 1965; Smith 1977). The ratio of peak to steady-state rate
is one measure of response adaptation. In the present study, adaptation of mouse AN responses
was studied using PST histograms of responses to CF tone bursts at 30 dB above threshold. The
histograms were normalized by the number of tone burst presentations and the histogram bin
width (0.5 msec) to give the instantaneous discharge rate.
Response adaptation is a function of both SR and CF in the mouse AN (Figures 5A and 5B). In CBA/CaJ mice, fibers with SR ≥ 1 sp/sec had peak-to-steady state ratios from 2.2 - 7.4, while fibers with SR < 1 sp/sec had ratios between 1.6 and 4.2 (Figure 5A). Fibers with the largest peak-to-steady state ratios had CF > 20 kHz (Figure 5B). The increase in peak-to-steady state ratio is due to an increase peak rate with CF. This difference can be seen from the superimposed PST histograms for high-SR (SR ≥ 1) fibers with CF < 20 kHz vs. CF > 20 kHz (Figures 5C and D, respectively). All features of response adaptation appeared similar in CBA/CaJ and C57BL/6 strains.

E. Rate-vs.-Level Functions and Dynamic Range

Dynamic range of single AN fibers in mouse was investigated by measuring discharge rate vs. sound level for tone bursts at CF. The rate-vs.-level data were fit by a modified version of an existing model (Sachs et al. 1989). Figure 6 shows examples of two rate-level functions, their model fits and calculated dynamic ranges. The rms errors of the model fits in these two cases were 13.2 and 13.9 sp/sec. Of 156 rate-level functions obtained from CBA/CaJ mice and 45 from C57BL/6, 92 and 41, respectively, had model fits with "acceptable" root-mean-square error (≤14 sp/sec) and were included in the study.

Among mouse AN fibers there was a tendency for fibers with low-SR (< 1 sp/sec) to show lower maximum discharge rates than those with SR ≥ 1 sp/sec (Figure 7A): group mean differences were statistically significant (P = 0.026, student’s t-test). There did not appear to be any relation between maximum discharge rate and CF (data not shown); and there were no obvious differences between the two strains studied.
Dynamic ranges of most AN fibers were less than 15 dB (Figure 7B: dynamic range defined as the dB range between the points corresponding to 10 and 90% of the (model fit) maximum driven rate). However, larger dynamic ranges were sometimes seen among fibers with SR < 1 sp/sec. Furthermore, dynamic ranges were more heterogeneous for this "low" SR group, ranging between 8.4-43.0 dB. In contrast, for high SR (≥ 1 sp/sec) fibers, the largest dynamic range was only 22.8 dB.

In data from guinea pig AN (Winter et al. 1990), rate-level functions have been categorized as “hard saturating”, “sloping saturating”, and “straight”. In data from mouse, the great majority of rate-level functions, and all those with dynamic range < 20 dB, showed hard saturation in response to tone bursts (Figure 8A,B). In contrast, fibers with dynamic ranges > 20 dB showed "sloping saturation" or "straight" functions (Figure 8 C,D). In both strains of mice, fibers with the largest dynamic range had SR < 1 sp/sec and relative threshold > 10 dB.

F. Response Synchrony

When AN fibers respond to low-frequency tones there is a correlation between the stimulus periodicity and the timing of spikes (Johnson 1980), as illustrated by the post-zero crossing histogram in Figure 9C. In cat, where it has been most systematically studied, this phase-locking, or synchrony, falls off dramatically as stimulus frequency approaches 4 kHz (Johnson 1980). In the mouse AN, there are relatively few fibers with rate threshold < 60 dB SPL at frequencies ≤ 4 kHz (e.g. Figure 2). Thus, given that high SPL tones elicit large cochlear microphonic potentials that can produce artifactual synchrony when picked up by the microelectrode, response synchrony could only be studied systematically in a few fibers. A model developed by Johnson (Johnson 1978) was used to compute a synchrony noise floor for each recording (based on the
ratio of microphonic size to spike size) to prevent pollution of data by these microphonic-based artifacts (See Methods).

Although the sample size is limited by the general lack of low-frequency responsiveness in the mouse AN, the fundamental features of response synchronization are similar to those reported in other mammalian species. Data from one of the lowest-CF fibers in the present study illustrate the main trends of the synchrony data from mouse AN (Figure 9A,B). For this fiber (CF= 3.1 kHz), rate and synchrony data were obtained for stimulus-level functions at 5 frequencies from 1 to 4 kHz. No synchrony was demonstrable in the response to 4 kHz, and maximum synchrony increased systematically as stimulus frequency decreased towards 1 kHz (Figure 9B and 9C). As reported in other species, the AN discharge can synchronize to the stimulus period at lower SPLs than those at which the average rate increase (e.g. data at 1 or 2 kHz), although this is not always the case (e.g. 1.6 kHz). Note that the maximum synchronization seen across all mouse AN fibers studied in both strains was lower than the values reported for similar stimulus frequencies in the cat (gray region in Figure 9C) but similar to guinea pig (dotted region in Figure 9C), another rodent species.

Discussion

A. Robustness of criteria for identifying AN fibers

In the present study, PST shape, response regularity, response latency, and location along the electrode track, were used to distinguish AN fibers from CN units. Based on this classification scheme, the fiber population studied must be dominated by AN responses. However, CN units with “primary-like” responses could also be included, given that, in cat, there is some overlap in response latency and regularity measures between these CN cells and AN fibers (Young et al.
CN cells that can generate primary-like responses include spherical and globular bushy cells (Blackburn and Sachs 1989; Smith et al. 1993). Spherical bushy cells are located in the anterior AVCN. Our electrode angle and insertion point in the DCN is such that anterior AVCN is not traversed. Thus, it is not likely that any of the present results are from spherical bushy cells. Globular bushy cells are located more posteriorly in the AVCN. Because globular cells are located near the AN root in mouse (Webster and Trune 1982) and have tone burst FSLs comparable to AN fibers (Young et al. 1988), it is possible that some globular cell responses were classified as AN fibers in the present database. Such inclusion would have minimal affect on the conclusions, since globular cell responses are generally so similar to those of AN fibers. The greatest differences include a tendency towards larger SRs, maximum driven rates and peak-to-steady state ratios in globular cells (Rhode and Smith 1986). Thus, these aspects of mouse AN response may be slightly skewed towards higher values in the present study.

B. Relevance to other data on auditory function in mouse

1. CF limits and cochlear frequency maps

Information used by the central nervous system in processing auditory stimuli comes through the ~20,000 fibers of the AN. Thus, the superposition of tuning curves obtained from mouse AN provides an informative overview of the threshold sensitivity of the auditory periphery (Figure 10). Not surprisingly, the minimum envelope of these single fiber data basically tracks the published behavioral thresholds obtained in a variety of mouse strains, at least for frequencies below 50 kHz. Since “threshold” is defined differently in each study, and since the behavioral studies use an intact pinna and free-field acoustics, absolute comparisons of threshold values are
not warranted. The discrepancy above 50 kHz between single-fiber and behavioral data is likely due to the difficulties in accurately specifying the sound pressure at the eardrum in both cases.

Knowing the range of single-fiber CF present in the mouse AN is key to constructing an accurate cochlear frequency map. CFs encountered in our sample of mouse AN fibers ranged from 2.5 to 69.8 kHz (Figure 3). The high-frequency limit was artificially imposed: the probe-tube microphone response was too attenuated at high frequencies to justify calibration above 70 kHz. The low-frequency CF limit of 2.5 kHz is subject only to sampling errors: i.e. low-CF fibers were rare, and the true low-CF limit may be somewhat lower. Accurate CF measurement is sometimes difficult at very low CFs, because tuning curves can be very broad. However, this is not an issue in mouse AN data, as these "low-frequency" tuning curves are quite sharply tuned (See highlighted tuning curve in Figure 10B).

A low-frequency CF limit of 2.5 kHz is significantly higher than the value predicted by two older cochlear frequency maps for mouse (Ehret 1975; Ou et al. 2000), however, it is ~ 1 octave lower than that suggested by the most recent map (Mueller et al., 2004). The oldest map (Ehret 1975) was derived by choosing values for the upper and lower limit of mouse hearing from behavioral measures and then fitting a power function of the type inferred for the human cochlear map based on psychophysically derived critical bands (Greenwood 1961). The lower limit chosen for mouse hearing was 0.8 kHz. As shown in Figure 10, our data suggest that the perception of frequencies < 2.5 kHz is mediated by excitation of the "tails" of the apical-most AN fibers, not by fibers with CFs of 0.8 kHz.

A second cochlear map for mouse was derived by comparing the patterns of noise-induced hair cell lesions to patterns of threshold shift seen in ABR responses (Ou et al. 2000). The data set did not include any frequency points below ~6 kHz, but the best-fit logarithmic function
extrapolated to a value of 1.5 kHz for the apical-most CF in mouse. According to the present results, this value may also be too low by ~1 octave. A similar discrepancy between the cat cochlear map inferred by correlating lesion site to cochlear sensitivity changes was detected when a definitive map was derived by intracellular labeling of single physiologically characterized nerve fibers (Liberman 1982a).

Recently, a mouse map was more directly derived by injecting HRP extracellularly into the CN after recording neuronal responses and correlating CF with the locations of labeled terminals in the cochlea (Mueller et al. 2004). Although there were no data points for the apical cochlea (lowest CF was ~7 kHz); the best-fit logarithmic function extrapolated to a minimum CF value of 4.8 kHz. The present study shows that tuning in the cochlear apex extends at least down to 2.5 kHz. Thus, the simple log-frequency-to-linear-distance relation suggested Mueller et al. (2004) should be modified to a function of the Greenwood type, such that frequency representation in the apical turn changes more slowly with distance, as is the case in cat (Liberman 1982a).

2. Other studies of AN response and behavioral thresholds in mouse

Two previous studies, Finck and Berlin (1965) and Ehret and Moffat (1984), describe recordings from mouse AN fibers, using the CBA/J strain and the (outbred) NMRI strain, respectively. In both studies, electrode tracks traversed the CN to access the AN, thus the possibility for unit mis-classification exists. This possibility was acknowledged, but not addressed, by Finck and Berlin; Ehret and Moffat developed a classification scheme based on average FSL. Thus, as in the present study, their database may include a small population of globular bushy cells in addition to the majority population representing AN response.
Aspects of AN physiology characterized in these previous studies included: response areas (i.e., tuning curves), thresholds at CF, masked thresholds to tone bursts in noise, and critical ratio bands. Since the present study did not examine noise masking, and previous studies did not directly quantify the sharpness of tuning curves, the only point of comparison among the three studies concerns the distribution of thresholds at CF. Threshold data from the three studies are compared in Figure 10A, where all tuning curves from CBA/CaJ mice in the present study (gray lines) are superimposed on curves representing the minimum thresholds at CF for AN fibers sampled in the CBA/J (filled diamonds) and the NMRI strains (filled circles). Minimum thresholds for the NMRI strain are lower at most CF regions than those seen in the present study. However, data from the present study do not show the precipitous loss of sensitivity for CFs > 30 kHz (see below). Minimum threshold envelopes for the CBA/J data and the present CBA/CaJ data are remarkably similar for CFs < 30 kHz. For higher CF regions, CBA/CaJ maintains low minimum thresholds out to the high-frequency limits of our acoustic system (~70 kHz); whereas, the CBA/J data show steeply sloping loss of threshold sensitivity. It is not clear whether these differences in high-frequency behavior reflect true interstrain differences in high-frequency sensitivity, differences in acoustic calibration procedure or artifactual loss of sensitivity in the CBA/J due, for example, to cochlear cooling in the anesthetized preparation (Brown et al. 1983).

Comparisons of single-fiber thresholds from the present study with behavioral thresholds for a number of mouse strains are also shown in Figure 10. With the exception of an extremely low value for 1-kHz behavioral threshold in the NMRI mouse, all behavioral threshold functions agree reasonably well with the minimum single-fiber thresholds measured in CBA/CaJ in the present study (Figure 10A). Note that none of the behavioral curves show the precipitous threshold elevation for frequencies > 30 kHz seen in the CBA/J single-fiber study. The reason
for the discrepancy in high-frequency sensitivities between the behavioral and neural data for C57BL/6 (Figure 10B) is not clear; however, it probably does not arise from incomplete sampling in the present study, since there was a clear trend among fibers sampled showing threshold elevations for CFs above 15 kHz (e.g. Figure 3A).

3. Inter-strain differences and the choice of CBA/CaJ and C57BL/6

The availability of transgenic and mutant lines with interesting auditory phenotypes makes the mouse a valuable model for the study of the auditory system; and recording from single auditory nerve fibers provides a powerful tool for assessing the cochlear phenotype associated with any genetic manipulation. However, the interpretation of hearing results from mutant mouse lines is often complicated by the fact that these lines are created or maintained in several inbred mouse strains (e.g. C57BL/6, 129/SvEv and FVB/N), and there can be clearcut interstrain differences in threshold sensitivity, especially as animals age, that complicate the selection of control animals. Although CBA/CaJ is not one of the inbred strains in which mutant or transgenic lines are typically available, it was chosen for the present study because it is one of the most commonly used strains for the study of hearing, since it does not show the premature age-related cochlear degeneration seen in these other strains.

A challenge in the use of single-fiber assays for phenotypic analysis in mouse is the time-consuming nature of the data collection, averaging only 5 - 10 units per 6 hr experiment. Thus, it is of practical importance to understand the nature of inter-strain differences in AN response, so that informed decisions can be made as to the appropriateness of different "control" datasets. For example, is the present data set appropriate for comparison to data obtained in a knockout line maintained in a different background? Setting aside issues related to the premature onset of
high-frequency threshold shifts in some of these non CBA strains, the answer appears to be a cautious "yes". As described in more detail below, results from this work suggest that several fundamental aspects of mouse AN response are quantitatively similar to those of other mammalian species, e.g. the relation between sharpness of tuning and CF (Figure 11) and the relation between SR and threshold sensitivity (Figure 4C). Other properties are qualitatively similar, e.g. the relation between SR and dynamic range. Thus, the likelihood of significant inter-strain differences in these general properties seems remote. More directly relevant to the question, the limited comparison between CBA/CaJ and C57BL/6 AN data in the present study (e.g. Figures 3 - 9) fails to reveal any obvious differences between these two strains.

C. Comparison to data from other mammals

1. Tuning-curve tips vs. tails and rate vs. synchrony coding

Tuning curves recorded from the mouse AN were qualitatively similar to those recorded in other mammalian species. They showed sharply tuned "tips" near the characteristic frequency and broadly tuned low-frequency “tails” (Figure 2). As in other mammals, mouse AN fibers showed increasing sharpness of tuning with increasing CF (Figure 3B). A more quantitative comparison of mean Q10dB values obtained from several mammalian species is shown in Figure 11. The combined data suggest a common relationship between Q10dB and CF, with similar sharpness of tuning seen across several mammalian species, where CF regions overlap.

A more quantitative comparison of tuning-curve "tails" shows that low-frequency thresholds are significantly elevated in mouse compared to other mammalian species (e.g., cat (Liberman 1978), guinea pig (Evans 1972), chinchilla (Dallos and Harris 1978), and gerbil (Ohlemiller and Echteler 1990)). For example, a direct comparison of mouse and cat tuning curves for fibers
with CF near 15 kHz (Figure 12) shows that, whereas thresholds in mouse increase monotonically for frequencies below CF and rise above 90 dB for frequencies < 4 kHz, thresholds in cat show a second minimum at low frequencies and do not rise above 90 dB SPL until frequencies fall below ~0.2 kHz. These differences in low-frequency responses of high-CF fibers are likely due to the roll-off in middle-ear transmission for frequencies <10 kHz in the mouse (Rosowski et al. 2003).

In mammals with better low-frequency hearing than mouse, significant information is carried in the fine timing of AN discharge, in addition to the information carried by changes in average rate. Maximum response synchrony in cat AN is constant for frequencies < 1 kHz, rolls off dramatically above 1 kHz and is essentially absent for stimulus frequencies > 4 kHz (Johnson 1980). Thus, in cat or guinea pig, and probably humans, low-frequency sensitivity is such that AN fibers spanning many octaves of CF will show phase-locked response to a moderate-level (80 dB), low-frequency (e.g. 1 kHz) tone. In mouse AN, by contrast, there would be almost no fibers responding to such a stimulus (See Figure 10). This raises the question as to whether this mammalian ear specialized for high-frequency hearing has also developed specializations to shift the frequency range of AN phase-locking. Data in the present study suggest that this is not the case: the relation between maximum synchronization index and stimulus frequency appears to be fundamentally similar to that seen in other mammals (Figure 9). Indeed, if anything, the high-frequency limit of synchronization occurs at a lower frequency than in cat. Thus, the data further suggest that phase-locking of AN response is not a particularly important component of mouse hearing.
2. Spontaneous Discharge and SR groups

In cat and guinea pig, where it has been most exhaustively studied, differences in SR are strongly correlated with differences in other response properties such as threshold sensitivity (Liberman 1978), adaptation (Rhode and Smith 1985), susceptibility to forward masking (Relkin and Doucet 1991) and dynamic range (Winter et al. 1990), among others.

In cat, where data from hundreds of units can be obtained from individual animals under highly stable recording conditions, analysis of the relation between SR and threshold sensitivity suggested that three SR groups could be defined: high-, medium and low, with SR ranges of >18, 0.5 to 18 and < 0.5 sp/sec respectively (Liberman, 1978). Subsequent structure-function correlations using intracellular injection of neuronal tracers revealed that there are systematic differences among these three groups, in 1) locus of peripheral terminal on the IHC circumference (Liberman 1982b), 2) size and complexity of the synaptic apparatus within the IHC (Merchan-Perez and Liberman 1996), 3) degree of branching of the central axon (Fekete et al. 1984) and 4) the CN subdivisions to which they project (Liberman 1991, 1980). Many of the same structure-function relationships have been corroborated in the guinea pig, suggesting that the subdivision of AN fibers into SR-based groups is part of the fundamental mammalian plan (Tsuji and Liberman 1997).

In cat, guinea pig, chinchilla and rabbit (Figure 13A) the SR distribution is fundamentally bimodal, and fibers from the lower peak in the SR distribution have higher thresholds than those from the high rate peak (Tsuji and Liberman 1997). Although the SR distribution in the mouse is not as clearly bimodal as in these other species, the general relation between SR and threshold sensitivity is maintained (although the evidence for discrete SR groups rather than a continuum of response is not clear). Recent ultrastructural work in mouse suggests that, in this species, as
previously reported in cat and guinea pig, the AN terminals contacting the modiolar side of the IHC are lower in mitochondrial content than those on the pillar side (Francis et al. 2004). In cat, these mitochondrion-poor afferents have been definitively identified as corresponding to the high-threshold low-SR group. Thus, the same anatomical differences may underlie the observed SR-based heterogeneity of response in mouse.

With respect to the shape of the SR distribution, mouse is similar to rat (el Barbary 1991), another high-frequency animal, and to high-CF AN fibers in gerbil (Ohlemiller and Echteler 1990), where the SR distribution varies dramatically with CF. As can be seen in Figure 13B, for rat, mouse and the basal turn of gerbils, the SR distribution is not clearly bimodal and is shifted towards lower spontaneous rates. This association between a compressed SR distribution and high-frequency hearing is interesting given that robust background discharge may be most useful at frequencies < 4kHz, where response synchronization occurs, and where synchrony rises at lower SPLs than average rate (Figure 9A,B).

As for cat (Rhode and Smith 1985) and guinea pig (Muller and Robertson 1991), response adaptation in mouse AN is related to fiber SR (Figure 5A) and CF (Figure 5B). In both cat and guinea pig, response adaptation for fibers with CF < 4 kHz was smaller than response adaptation in fibers of higher CF (Muller and Robertson 1991; Rhode and Smith 1985). Given that phase locking occurs for frequencies < 4 kHz (Johnson 1980), Rhode and Smith (Rhode and Smith 1985) hypothesized that the lower peak rates observed in cat low CF fibers were due to an interaction between phase locking and rapid adaptation. In mouse, there was a difference in response adaptation between fibers with CF below vs. above 20 kHz (Figure 5). This CF-related difference clearly cannot be correlated with presence or absence of synchrony; rather, it likely arises from CF-related changes in the kinetics of synaptic transmission.
3. Dynamic range in the auditory periphery

Rate-vs.-level functions for AN responses to CF tone bursts have been best studied in cat (Sachs et al. 1989), guinea pig (Winter et al. 1990) and gerbil (Ohlemiller et al. 1991). In all three of these mammals, dynamic ranges depend strongly on SR: low-threshold, high-SR fibers show hard rate-saturation and small dynamic ranges; whereas high-threshold, low-SR fibers show sloping saturation or non-saturating rate-vs.-level functions with significantly larger dynamic ranges. Data in the present study show that the mouse AN has fundamentally the same behavior (Figure 7); thus these SR-related differences in dynamic range also appear to be part of a general mammalian plan.

A striking difference between AN properties in mouse vs. other mammals is the small dynamic range of the low-threshold, high-SR fibers. For example, figure 14 compares dynamic ranges for high-and low-SR AN fibers from the present study to data from cat (Guinan and Stankovic 1996) which were derived according to exactly the same stimulation and analysis protocols. The great majority (> 90%) of high-SR fibers in the mouse had dynamic range values less than 18 dB, while the majority (>70%) of high-SR fibers in cat showed dynamic ranges greater than 18 dB. Gerbil high-SR fibers (Ohlemiller et al. 1991) have been shown to have even higher dynamic range values (25-40 dB). Quantitative data for guinea pig are not available, however visual inspection of rate-level functions shown by Winter et al. (1990) suggest that dynamic ranges in this species for high-SR fibers are comparable to those in cat.

The small dynamic ranges seen among mouse AN fibers, compared to those measured in other mammalian ears, may be related to the fact that the mouse ear is specialized for high frequencies: most of the hearing range is above stimulus frequencies at which response phase-locking can be used to carry information. In the gerbil AN, there is a CF-dependence to dynamic
range (Ohlemiller et al. 1991): high-frequency fibers, with CFs above the limits of phase locking, show a smaller mean dynamic range (~25 dB) than low-CF fibers (~35 dB). The existence of steeper rate-vs.-level functions in mouse high-SR fibers might be expected to subserve an enhanced ability to detect small changes in stimulus intensity at levels near threshold. However, existing measurement of intensity difference limens in mouse do not suggest any extraordinary abilities compared to other mammalian species investigated (Fay 1988).

The steepness of rate-vs.-level functions in mouse could arise at several stages in cochlear processing. Comparison of basilar membrane displacement-vs.-level functions for tones at CF near the base of the cochlea in mouse (Legan et al. 2000) compared to other mammals (Robles and Ruggero 2001) do not suggest striking differences in slope (Figure 15). Thus, the basis for the observed differences in dynamic range must arise in within the inner hair cell, the auditory nerve, or the synaptic transmission between the two.

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Figure Legends

Figure 1: Criteria for distinguishing auditory nerve (AN) units from cochlear nucleus (CN) units. Panel A: relation between the coefficient of variation of the interspike intervals (CV) and the mode of the first spike latency (FSL) for all units encountered in the preliminary study. CV is the ratio of the standard deviation to the mean of the interspike interval distribution obtained from responses to 150 CF tone bursts, 30 dB above threshold. Symbols represent the subjective classification based on PST shapes (see legend). An acoustic delay of 0.5 msec should be subtracted from the FSL values shown in order to obtain time re arrival of acoustic signal at the eardrum. Panels B and C: examples of a “primary” and a “chopper” PST, respectively, each situated above its respective interspike interval distribution.

Figure 2: Tuning-curve shapes in different regions of characteristic frequency for AN fibers from CBA/CaJ. Data from 28 mice were segregated into groups according to characteristic frequency (half-octave bins) from 3.1 kHz (upper left) to 54 kHz (lower right). Each tuning curve was normalized by characteristic frequency and placed in the middle of its respective half-octave bin. Only data from fibers with high spontaneous rate (i.e., SR > 1 sp/sec) are shown.
Figure 3: Thresholds at CF (Panel A) and sharpness of tuning (Panel B), as a function of characteristic frequency for 196 AN fibers from 28 CBA/CaJ mice and for 58 fibers from 13 C57BL/6 mice. Arrow in panel B indicates a Q_{10dB} value that was off-scale. In this and all subsequent figures, CBA/CaJ data are shown as circles while data from C57BL/6 mice are plotted as triangles.

Figure 4: Spontaneous rate (SR) data from the same fiber populations shown in Figure 3. Panel A: frequency distribution of the SRs observed. Panel B: SR as a function of characteristic frequency. Panel C: relation between SR and threshold sensitivity. Relative threshold is defined by referring the threshold at CF of each fiber to the threshold for the most sensitive fibers sampled in the same ear in the same CF band. In this and all subsequent figures with SR on a logarithmic scale, SR values of 0 are plotted as 0.05 sp/sec. AN fibers from C57BL/6 with CF > 15 kHz are not included, because threshold elevation in this region indicates the presence of cochlear pathology (see Figure 3).

Figure 5: Response adaptation as a function of SR (Panel A) and CF (Panel B). Adaptation was derived from PST histograms based on 150-250 CF tone bursts, 30 dB above threshold. Peak to steady state (SS) ratio was obtained by dividing the maximum rate by the average rate during the last 10 msec of the tone burst. Panels C and D: superimposed PST histograms all from CBA/CaJ high-SR (> 1 sp/sec) fibers with CF < 20 kHz and CF > 20 kHz respectively. Histograms are normalized by the number of stimulus presentations and the histogram bin width (0.5 msec) to give instantaneous discharge rate.
Figure 6: Model fitting of rate-vs-level functions using a modification of the formula developed by Sachs et al (1989). Panels A and B: raw data (filled symbols) and model fit (dashed lines) for the rate-level function for a fiber with CF = 18.8 kHz and SR = 47.6 sp/sec (A) and CF = 23.7 kHz and SR = 0.1 sp/sec (B). Dynamic range (paired vertical lines in each panel) was defined as the difference between SPLs evoking 10% and 90% of the (model fit) maximum driven rate. The root-mean-square-errors for the model fits were 13.2 sp/sec and 13.9 sp/sec in panels A and B, respectively.

Figure 7: Relation between SR and maximum rate (Panel A) or dynamic range (Panel B). Dynamic ranges were extracted from the model-fit data as described in the caption for Figure 6.

Figure 8: Shapes and dynamic ranges of all rate-vs.-level functions obtained from CBA/CaJ or C57BL/6 fibers, divided into those with small (< 20 dB: Panels A,B,C) or large (> 20 dB: Panels D,E,F) dynamic range. Superimposed rate-vs-level functions for CBA/CaJ are shown in the first column and functions from C57BL/6 in the middle column: raw functions were normalized by (1) dividing the vertical axis by the maximum rate and (2) by shifting along the horizontal axis according to model-fit thresholds. The dynamic range distribution for each group is shown in the histograms in the right column (Panels C,F): as shown in the key, data from CBA/CaJ are shown as filled circles, data from C57BL/6 as filled triangles.

Figure 9: Phase-locking in mouse AN fibers. Panels A and B show data from one well-studied fiber (CF = 3.1 kHz, SR = 22.4 sp/sec). Synchrony (Panel B) and rate (Panel A) measures were extracted from post-zero-crossing histograms obtained from 15-sec continuous tones. The
synchronization index (SI: panel B) was calculated as described by Johnson (1980). **Panel C:** Comparison of maximum SI vs. tone frequency for mouse data from panel B and for one AN fiber from a C57BL/6 mouse (CF = 5.81 kHz, SR = 37.5 sp/sec) compared to published cat data (shaded area (Johnson 1980)) and guinea pig data (dotted area Palmer and Russell 1986)), excluding an outlier at 1.68 kHz. SI values marked by asterisks are underestimates of the maximum, since SI-vs.-level functions had not saturated at the highest sound level presented.

**Figure 10:** Superimposed tuning curves from all CBA/CaJ (Panel A) or C57BL/6 (Panel B) fibers in the present study (gray) compared to threshold data from other mouse studies, including both behavioral (dotted lines and open symbols) and AN data (solid lines and filled symbols). Behavioral data are from the following sources: CBA/CaJ mouse (Prosen et al. 2003), CBA/J mouse (Birch et al. 1968), feral house mouse (Heffner and Masterton 1980), NMRI mouse (Ehret 1974) and C57BL/6 (Mikaelian et al. 1974). AN data are from the following sources: NMRI mouse (Ehret and Moffat 1984), CBA/J (Finck and Berlin 1965). Error bars in panel B represent one standard deviation.

**Figure 11:** Mean Q_{10dB} as a function of CF for AN tuning curves in five mammalian species (see key). Data from each species were segregated into groups according to characteristic frequency (octave bins), and the average Q_{10dB} for each bin was placed at the mean fiber CF. Data are only shown for frequency bins containing at least 5 data points. Data for mouse (present study), cat (Liberman 1978), guinea pig (Tsuji and Liberman 1997) and chinchilla (Liberman, unpublished) are all from our laboratory; thus the methods for data aquisition and
analysis are identical. Data for gerbil are from other investigators (Ohlemiller and Echteler 1990).

**Figure 12:** Comparison of tuning curves from cat and mouse AN fibers in a similar CF range (11 - 16 kHz) illustrates the difference in low-frequency sensitivity. The cat tuning curves were obtained in a previous study (Liberman, 1978).

**Figure 13:** Comparison of SR distributions for AN fibers recorded from a number of different mammalian species. Bimodal distributions are grouped in **Panel A:** cat (Liberman 1978), guinea pig (Liberman, unpublished), rabbit (Borg et al. 1988), chinchilla (Liberman, unpublished), and the low-CF region of the gerbil cochlea (Ohlemiller and Echteler 1990). Non-bimodal distributions are grouped in **Panel B:** mouse (present study), rat (el Barbary 1991) and the high-CF region of the gerbil (Ohlemiller and Echteler 1990).

**Figure 14:** Comparison of dynamic ranges for cat and mouse AN fibers, separated into high and low SR groups (Panels A and B respectively). Mouse data are from the present study. Cat data are from a previous study (Guinan and Stankovic 1996) using an identical data acquisition paradigm, i.e. 50 msec tone bursts at CF presented in random level order.

**Figure 15:** Comparison of basilar membrane responses to CF tones recorded in cat, chinchilla, guinea pig, and mouse at locations in the basal turn. Data for the cat, chinchilla and guinea pig were replotted from Robles and Ruggero (2001). The mouse data were re-plotted from Legan et. al. (2000).
References Cited


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