Medial efferent effects on auditory-nerve responses to tail-frequency tones. I. Rate reduction

Konstantina M. Stankovic
Eaton–Peabody Laboratory of Auditory Physiology, Department of Otolaryngology, Massachusetts Eye and Ear Infirmary, 243 Charles Street, Boston, Massachusetts 02114 and Harvard–MIT Division of Health Sciences and Technology, and Research Laboratory of Electronics, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

John J. Guinan, Jr. a)
Eaton–Peabody Laboratory of Auditory Physiology, Department of Otolaryngology, Massachusetts Eye and Ear Infirmary, 243 Charles Street, Boston, Massachusetts 02114 and Harvard–MIT Division of Health Sciences and Technology, and Research Laboratory of Electronics, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139 and Department of Otology and Laryngology, Harvard Medical School, Boston, Massachusetts 02115

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One way medial efferents are thought to inhibit responses of auditory-nerve fibers (ANFs) is by reducing the gain of the cochlear amplifier thereby reducing motion of the basilar membrane. If this is the only mechanism of medial efferent inhibition, then medial efferents would not be expected to inhibit responses where the cochlear amplifier has little effect, i.e., at sound frequencies in the tails of tuning curves. Inhibition at tail frequencies was tested for by obtaining randomized rate-level functions from cat ANFs with high characteristic frequencies (CF ≈ 5 kHz), stimulated with tones two or more octaves below CF. It was found that electrical stimulation of medial efferents can indeed inhibit ANF responses to tail-frequency tones. The amplitude of efferent inhibition depended on both sound level (largest near to threshold) and frequency (largest two to three octaves below CF). On average, inhibition of high-CF ANFs responding to 1 kHz tones was around 5 dB. Although an efferent reduction of basilar-membrane motion cannot be ruled out as the mechanism producing the inhibition of ANF responses to tail frequency tones, it seems more likely that efferents produce this effect by changing the micromechanics of the cochlear partition. © 1999 Acoustical Society of America. [S0001-4966(99)00908-X]

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INTRODUCTION

Stimulation of medial olivocochlear efferents that synapse on outer hair cells (OHCs) reduces activity in auditory-nerve fibers that contact inner hair cells (IHCs) even though there are no known neuronal connections between medial efferents and these afferents, or between OHCs and IHCs. It is now widely believed that the major mechanism for this inhibition is an efferent-induced reduction of cochlear-amplifier gain that reduces basilar-membrane motion in response to sound (reviewed by Guinan, 1996). If this is the only way in which medial efferents inhibit, then medial efferents would be expected to have little or no effect at sound frequencies in the broadly tuned, insensitive, low-frequency “tail” region of tuning curves because active cochlear mechanisms appear to have little or no effect on basilar-membrane motion an octave or more below CF (Rhode, 1973; Ruggero et al., 1991, 1996, 1997; Nuttall and Dolan, 1996).

Earlier investigations left the impression that efferents do not significantly affect responses of auditory-nerve fibers (ANFs) to tail-frequency stimuli, even in fibers that show large inhibitions at the characteristic frequency (CF) (Wied-erhold, 1970; Kiang et al., 1970; Guinan and Gifford, 1988c). This impression was further reinforced by the finding that efferent stimulation had little effect on IHC receptor potentials evoked by tail-frequency tones (Brown and Nuttall, 1984). Most recently, this impression was supported by the report that efferent stimulation “had no apparent effect on the basilar-membrane displacement in response to tones at frequencies more than one-half octave below CF” (Murugasu and Russell, 1996).

Despite this earlier work, a systematic study of efferent effects on responses to tail-frequency tones is lacking in the literature. All of the studies that reported the small efferent effects at tail frequencies were focused mainly on other aspects of efferent inhibition. The most relevant study—an exploration of efferent effects on ANF tuning curves (Guinan and Gifford, 1988c)—concluded that efferents sometimes significantly elevate tuning-curve tails (see their Fig. 1), but the inhibition at 1 kHz (the approximate center of the tails) was small, averaging only 1 dB. However, there are several limitations of tuning-curve studies. First, these studies measured the efferent effect only at threshold. Second, the prolonged efferent stimulation required to record a whole tuning curve with efferent stimulation (usually more than several minutes) complicates the interpretation because (1) there is adaptation in the effects of efferent stimulation, (2) the effer-
ent slow effect (Sridhar et al., 1995) may change the results depending on whether the tuning curve is measured by sweeping frequency up or down, and (3) the properties of the stimulating electrode may change with long stimulating runs (Mountain, 1978).

The current study is the first extensive examination of efferent effects at tail frequencies. To overcome the difficulties involved with measuring efferent effects on tuning curves and to extend the sound-level range sampled, we recorded sound level series at specific tail frequencies with and without efferent stimulation. By simultaneously randomizing the presentation of sound level and efferent stimulation—while keeping the sound-on and shocks-on duty cycles low—adaptation was minimized both in the responses of ANFs to sound, and in the effects of efferent stimulation (Guinan and Stankovic, 1996). Our results show that efferents can have an appreciable effect at tail frequencies and therefore that some current conceptions regarding cochlear physiology need revision.

I. METHODS

A. Surgical preparation

Healthy adult cats weighing 1.5–3.0 kg, with no signs of middle-ear infections, were used. Anesthesia was induced by intraperitoneal injection of 0.15 ml/kg diallyl barbiturate in urethane (100 mg/ml diallyl barbiturate, 400 mg/ml monothyurea, and 400 mg/ml urethane), and maintained by injections of $\frac{1}{10}$ of the original dose, as needed. Treatment of experimental animals was in accordance with protocols approved by the Committees on Animal Care at the Massachusetts Institute of Technology and the Massachusetts Eye and Ear Infirmary.

The surgical approach to the auditory nerve was as in Kiang et al. (1965). Briefly, after insertion of a tracheal tube and cannulation of a femoral vein for an intravascular drip of lactated Ringer's solution and cannulation of a femoral vein for an intravascular drip of lactated Ringer's solution, the ear canals were surgically exposed and the auditory bullae opened. The bony septum between the bulla and middle ear was removed and the tendons of the middle ear muscles were cut using cautery or a surgical argon laser with a “Megabeam Endo-ENT Probe.” A posterior craniotomy was performed and the medial and lateral parts of the cerebellum were aspirated to expose the floor of the fourth ventricle and the exit of the auditory nerve.

To increase the efficacy of efferent stimulation without evoking motion, some animals were paralyzed with intravenous atracurium in saline and artificially ventilated. Ventilator parameters were adjusted so as to maintain the breathing rate at 15–18 breaths per minute, and expired CO$_2$ at 3.5%–5%. Every animal’s physiological state was continuously monitored using a custom-LabView system for real-time monitoring of electrocardiograms, electroencephalograms, breathing rate, CO$_2$ content in expired air, and rectal temperature.

Cochlear sensitivity was monitored by recording auditory-nerve compound action potentials (CAPs) with an electrode on or near the round window and using an automated tone-pip audiogram (Guinan and Stankovic, 1996). Sometimes, CAP audiograms were elevated for a few hours after middle-ear-muscle cutting. However, for all animals reported here, CAP audiograms were in the normal range by the time data acquisition started—usually ten or more hours after middle-ear-muscle cutting.

B. Stimulation and recording

Efferents were stimulated along the midline of the floor of the fourth ventricle using a multiprong electrode (Gifford and Guinan, 1987) and shock timing as in (Guinan and Stankovic, 1996). Briefly, shocks (0.3-ms pulses at 200/s) were transformer coupled to the pair of adjacent prongs which yielded the greatest inhibition of the click-evoked CAP response without evoking movement. The CAP inhibition (measured as the equivalent reduction in sound level) ranged from 10–20 dB while gathering the data reported here.

Recordings of single ANFs were done as in Kiang et al. (1965) with broadband noise bursts as a search stimulus. Based on their spontaneous rate (SR), ANFs were grouped into three categories: low: SR$\leq$0.5, medium: 0.5$<$SR$\leq$18, and high: SR$>18$ spikes/s (Liberman, 1978). We studied fibers of all SRs but were biased toward high-SR fibers because they have the lowest tail thresholds. This enabled us to record the tuning curve tails and at least a 20-dB portion of the fiber’s dynamic range (sound levels at which rate increases with sound level) without causing acoustic trauma.

We concentrated on fibers with CFs$\geq$10 kHz because (1) they had clearly defined tips and tails, and (2) most of the tail could be studied without harmonic distortion of the tail-frequency stimulus eliciting a response in the tip region of a tuning curve. Some fibers with CFs$<10$ kHz were also studied and these gave the same qualitative results as fibers with CFs$\geq$10 kHz. Since harmonic distortion in the stimulus was an important issue, we determined it for every experiment. In the last four experiments, harmonic distortion was measured directly from Fourier transforms of the sound pressure in the ear canal recorded during spike-data acquisition. For earlier experiments, harmonic distortion was calculated from (1) the driver voltage attenuation, (2) measurements of harmonic distortion in a closed cavity at this attenuation, and (3) the sound pressure versus frequency calibration for the individual experiment. Data were rejected for any run for which the harmonic distortion fell within the tuning-curve tip.

C. Data gathering

After making contact with a fiber, data were gathered in the following sequence: (1) A tuning curve was obtained. In the tip region, standard tuning-curve parameters (30 points per octave, level steps of 3/4 dB) were used, but in the tail region, where threshold is not a strong function of frequency, the point spacing was changed to 6 points per octave to increase the speed. (2) Spontaneous firing was recorded for 20 s, and SR was calculated. (3) Randomized level functions with and without efferent stimulation were run at a tail frequency. Fiber contact time permitting, additional level functions were obtained at the same and/or at different tail frequencies and/or at CF.
Our level-function paradigm randomized both sound level and the presence/absence of efferent stimulation (as in Guinan and Stankovic, 1996) and yielded two rate-level curves—one "with efferents activated" and one "without efferents activated." Points were acquired every 3 s, and at each point the firing rate was obtained by averaging responses from ten sequential tone bursts (50 ms on, 50 ms off, 2.5 ms rise/fall time). Spikes were counted within a time window from 6 ms after the beginning of the tone-burst rise to 1 ms after the end of the tone-burst fall. For points "with efferents activated," a train of efferent shocks started 100 ms before the onset of tone bursts and lasted throughout the duration of the tone bursts (i.e., 1.1 s). At high frequencies, most randomized level functions covered the range from 40–100 dB SPL with 3-dB resolution. Exceptions include the first three experiments where some rate-level curves were 50–100 dB SPL with 2.5-dB resolution, 60–100 dB SPL with 2-dB resolution, or 70–100 dB SPL with 1.5-dB resolution. In the last three experiments, rate-level curves were 40–91 dB SPL with 3-dB resolution in order to minimize exposure to potentially traumatic sounds. At CF, randomized rate-level curves were from 0–100 dB SPL with 5-dB resolution.

Our most commonly used sound frequency was 1 kHz because (1) tuning-curve tails are usually most sensitive around 1 kHz, and (2) the harmonic distortion from an intense 1 kHz stimulus is usually outside of the tuning-curve tip for fibers with CFs > 10 kHz. Doing many runs up to 100 dB SPL at a single frequency often led to hearing loss (as evidenced by elevation of CAP thresholds to tone pips) near that frequency. However, these threshold shifts were for fibers with CFs near the tail frequency and thus are not directly relevant to fibers with CFs > 10 kHz. All of the data presented here are from fibers with CFs at frequencies with stable hearing, i.e., at which CAP thresholds remained within 5–15 dB (most commonly within 10 dB) of the initial measurements. Low-frequency hearing was preserved in the last three experiments in which rate-level curves were run only up to 91 dB SPL. The efferent effects in these cats were in agreement with the results from earlier experiments and suggest that the threshold losses near 1 kHz had little or no influence on our results. Because of this, we felt justified in pooling data across all cats (except two, see below), so that this report is based on ANFs from 17 cats. Data from two cats were not included: one because of audible noise associated with the ventilator, the other because the cat had extremely sensitive hearing. In the latter cat, efferent stimulation caused an unusually large (~80%) reduction of SR, suggesting that "spontaneous" activity was in fact a response to animal-generated noise (as suggested by Wiederhold and Kiang, 1970; Guinan and Gifford, 1988b; Kawase et al., 1993). Furthermore, rate-level curves in response to a single tail-frequency tone demonstrated dips characteristic of two-tone suppression (Abbas and Sachs, 1976), providing additional evidence that the cat was responding to sounds not present in the stimulus.

D. Data selection

In addition to the data selection criteria already stated, several other criteria had to be met. Only fibers with tail thresholds that exceeded tip thresholds by at least 35 dB were included. For each data set, peri-stimulus-time histograms locked to the shock times were inspected to detect possible missed or extra triggers due to shock artifacts. Only data free of such artifacts were included.

E. Data analysis

All data analysis was done with Matlab 5 software. For easier data handling in Matlab, the sound-level spacing in all tail runs was "standardized" to the most commonly used sound-level spacing of 3 dB. Rate-level curves with level spacing of 1.5 dB were down sampled (every-other point used) and those with 2 or 2.5 dB were linearly interpolated (these only covered ranges of 70–100 dB, 60–100 dB, and 50–100 dB, respectively).

To aid in evaluating data in the figures and for use in statistical tests, standard errors were calculated or estimated for firing rate points. When there were multiple sets of rate-level curves, the standard error (SE) of an average rate point was calculated in the usual way as 

\[ \text{SE} = \frac{\sigma}{\sqrt{n}} \]

where \( \sigma \) is the standard deviation of the data and \( n \) is the number of rate-level curves. When only a single set of rate-level curves was available, an arbitrary formula for SE was used: 

\[ \text{SE} = 10 \text{ spikes/s} \text{ if the driven firing rate (i.e., firing rate minus SR) was} \leq 20 \text{ spikes/s}; \text{ otherwise, SE} = 20 \text{ spikes/s} \text{ (SE estimates were needed because SE was used as a weighting factor for fitting data with straight lines for statistical tests, see below).} \]

This formula was arrived at by selecting fibers for which \( \sigma \) could be calculated from multiple measurements, and then plotting \( \sigma \) as a function of the driven firing rate. In comparison with the data, the simplified formula overestimates SE.

To aid detecting trends in scatter plots, a loess fit (also known as local nonparametric regression) was used (Cleveland, 1993). The loess fit is characterized by two parameters: (1) a smoothing parameter that is related to the size of the smoothing window, and (2) the degree of the locally fitted polynomial (here always set to 1). In figures with a loess fit, the smoothing parameter was normally 0.2, which means that the smoothing window included 20% of the data.

We tested for the significance of a statistic, without making any assumptions about underlying statistical distributions (e.g., that the data are normally distributed) by using permutation tests (Efron and Tibshirani, 1993). In a permutation test the actual data are used to provide an estimate of the underlying distribution space of possible data. The probability that a certain value of a statistic might arise from chance is then estimated by repeated trials in which the original data points are shuffled with the statistic calculated on each trial. For example, presume we had obtained data in \( N \) fibers and calculated statistic "\( S \)" which compares low-SR and high-SR fibers (e.g., \( S \) might be the difference in slopes of \( \Delta_s \) versus sound level plots as in Sec. II B). We want to know if the value of \( S \) might have occurred by chance, i.e., how often this value might occur in a population of \( N \) fibers.
II. RESULTS

We found that efferents can indeed affect ANF responses to tail-frequency tones and that the effect varied across fibers and stimulation parameters. Typically, the efferent effect at tail frequencies ranged from clear inhibition to no apparent effect, as shown in Fig. 1. This figure shows rate-level functions for tones at a tail frequency (1 kHz) and, for comparison, at fiber CF. Panels (a) and (c) show data from multiple runs at the tail frequency and illustrate the reproducibility of the data. The bottom panels show averages and standard errors derived from these multiple runs [Fig. 1(b) and (d)] or from the firing rate [Fig. 1(f), see Sec I]. The efferent inhibition is a rightward shift of the rate-level curve, meaning that a higher sound level was required with efferent stimulation to produce the same response as in the absence of efferent stimulation. Note that efferent stimulation can also change the slope of the rate-level rate-level curves, and the extent of this varies across fibers [compare Fig. 1(a) and (c)]. Figure 1(f) demonstrates that efferents can have a negligible effect at 1 kHz, even in fibers that show a significant shift at CF. Since there was no clear line of demarcation between fibers that showed efferent inhibition at tail frequencies and those that did not, we cannot make a precise statement as to what fraction of auditory-nerve fibers were inhibited at tail frequencies. Nonetheless, at least 80% of the fibers tested appeared to be inhibited [i.e., had tail-frequency rate versus level functions more like Fig. 1(a) and (b) than Fig. 1(c)]. Furthermore, inhibition at tail frequencies was found in every animal that was adequately tested.

A. Level shift, $\Delta L$

As a measure of the efferent effect on rate-level curves, we used the level shift, $\Delta L$ (Fig. 2). Level shift is the amount (in dB) by which the sound level must be increased with efferent stimulation to produce the same response as obtained without efferent stimulation. As noted above, $\Delta L$ was usually not constant in the rising phase of the level functions.
FIG. 2. Computing the level shift, $\Delta L$, and the normalized level shift, $\Delta L_n$. (a) Rate-level curves averaged from five pairs of curves with $\Delta L$, illustrated for the point at 82 dB SPL. $\Delta L$ was measured from each no-efferent-stimulation point in the rising phase of the response (visually determined) to the point at the same rate in the with-efferent-stimulation curve. The with-efferent-stimulation curve was determined by straight lines point to point and, if necessary, an extension past the highest point by a straight line through the highest three points (as in Guinan and Stankovic, 1996, Fig. 1). (b) $\Delta L$ and its standard errors calculated from the rate-level curves in panel (a). The main source of error in $\Delta L$ stems from the errors in determination of the firing rate, because errors associated with determination of the sound level are negligible in comparison. The error in $\Delta L$ was calculated from the errors in the firing rates taking into account the influence of the slopes of the level functions (Meyer, 1975). Details of the procedure used are given in Stankovic (1997). (c) $\Delta L$ normalized to a CAP inhibition of 20 dB by multiplying $\Delta L$ from (b) with 20/14. Note that standard errors were also normalized using the same factor (i.e., 20/14). Fiber TS37-17, CF = 10.59 kHz, SR = 1.2 spikes/s.

FIG. 3. The normalized efferent-induced level shift, $\Delta L_n$, versus sound level for auditory-nerve fibers from three CF regions (10–15 kHz, 15–20 kHz and 20–30 kHz), stimulated with a tail-frequency tone of 1 kHz. Each curve shows averages from one SR group. Bars indicate standard deviations; points without bars are from one fiber only. Differences in the lowest initial sound level among the SR groups reflect differences in tail thresholds. To clearly show error bars from different SR groups, medium-SR data were shifted to the right by 0.2 dB, and low-SR data were shifted to the left by 0.2 dB. The number of fibers in each SR group are: (a) 24 high, 18 medium, 13 low. (b) 25 high, 7 medium, 9 low. (c) 14 high, 5 medium, 1 low.

3 kHz (10 fibers, average $\Delta L_n = 7.1$ dB). These tests showed that, on average, $\Delta L$ (raw or normalized) was different from zero at a very highly significant level ($p < 0.0001$) for each sound frequency. However, these overall statistics ignore dependencies of $\Delta L_n$ on other variables such as sound level and the characteristics of individual ANFs (CF and spontaneous rate). These dependencies are summarized in the next two sections.

B. Dependence of $\Delta L_n$ on sound level and spontaneous rate

To explore the dependencies of $\Delta L_n$ on sound level and spontaneous rate—while controlling for the dependencies on sound frequency and CF—we considered one sound frequency at a time with fibers grouped in CF bands. The bands were determined by the amount of data available for a given sound frequency. The most data was obtained on fibers with CFs $\geq$ 10 kHz and tones at 1 kHz so three CF bands (10–15, 15–20, and 20–30 kHz) were used for the fibers responding to 1 kHz (Fig. 3). For other tone frequencies we had less data so one CF band (10–20 kHz) was used (2 kHz: Fig. 4; 3 kHz: Fig. 5; 500 Hz: not shown). For these frequencies, CFs above 20 kHz were not included because there were too few data and the efferent effects were considerably less for CFs over 20 kHz.

Figures 3–5 provide a visual appraisal of the dependence of $\Delta L_n$ on sound level and spontaneous rate in two ways: plots of averages and scatter plots. All three figures show plots of averages and standard errors for each SR
group, a display which allows a visual appraisal of average differences. In Figs. 4 and 5, where there are fewer fibers in each group, we have also included scatter plots which allow a better appraisal of the differences across individual fibers and the degree to which averages and standard errors summarize the data. Visual inspection of Figs. 3–5 suggests there is little difference in \( \Delta L_n \) across SR groups and that \( \Delta L_n \) depends on sound level.

This visual impression was confirmed by several statistical tests that indicated there were no significant differences in \( \Delta L_n \) across SR groups. Testing for changes across SR groups was complicated by the fact that the data from individual fibers often did not cover the same range of sound levels. In particular, low-SR fibers had higher thresholds than high-SR fibers and therefore had fewer measures of \( \Delta L_n \) at low sound levels. To overcome this, a straight line was fit (using a least squares criterion) to the data from each fiber, and the mean slope and intercept were calculated for each SR group. Permutation tests (Efron and Tibshirani, 1993; see Sec. I) were done by shuffling the fibers across SR groups and using the difference in the mean slope (or intercept) of two SR groups as a comparison statistic. Alternatively, fibers were shuffled among SR groups, for each resulting SR group a line was fit to all \( \Delta L_n \)'s, and comparison statistics were calculated from the difference in slope (or intercept) of the lines from two SR groups. In all of these cases, statistically significant differences across SR groups were not detected at the \( p = 0.05 \) level. Because there were no significant differences among SR groups, data were pooled across SR groups for subsequent tests.

Permutation tests also showed that \( \Delta L_n \) varied significantly with sound level, at least for most fiber groups. Our initial tests were done using the fiber groups in Figs. 3–5 by shuffling the \( \Delta L_n \)'s from each sound level and comparing the slopes of straight lines fitted through the resulting plots of \( \Delta L_n \) versus sound level. A significant dependence of \( \Delta L_n \) on sound level (\( p < 0.05 \)) was found for 1-kHz tones and CFs of 15–20 kHz, and for 2- or 3-kHz tones and CFs of 10–20 kHz. Although this test did not show a significant variation across all sound levels for 1-kHz tones and CFs of 10–15 kHz, visual inspection suggested that the greatest variation in this group was between 90–100 dB SPL [see Fig. 3(a)]. A permutation test restricted to 90–100 dB SPL for this group showed a very highly significant (\( p < 0.001 \)) dependence of \( \Delta L_n \) on sound level across this limited sound-level range.

C. Dependence of \( \Delta L_n \) on sound frequency

Perhaps the best way to study the dependence of \( \Delta L_n \) on sound frequency is to obtain level functions from many different tone frequencies on a single fiber. Two examples with particularly extensive data are shown in Figs. 6 and 7. For the fiber in Fig. 6, efferent inhibition was minimal at 500 Hz, small at 1 kHz, larger at 2–2.2 kHz, maximal at 2.5–3 kHz, and again minimal at 4 kHz. This fiber is also interesting because the without-efferent-stimulation rate-level curves at 2–3 kHz seem to have two rising portions separated by a small plateau (these features were shown consistently in multiple runs at each frequency). At these frequencies, efferents appear to inhibit the lower-level portion substantially more than the upper-level portion. For the fiber in Fig. 7 the overall efferent effect was small but the frequency dependence of the efferent inhibition can still be appreciated. Specifically, the inhibition was minimal at 500 Hz, small at 1–1.5 kHz, somewhat bigger at 1.7–2 kHz, and back to minimal at 3 kHz.

We were able to get data at many tone frequencies for only a few fibers, so to provide a more comprehensive view of
FIG. 6. Data from one auditory-nerve fiber demonstrating that efferent inhibition in the tail depends on tone frequency. Tone frequencies are stated in each panel and indicated by arrows on the tuning curve in the upper left panel. Every pair of rate-level curves in this figure (except the CF run) is an average of multiple runs. Bars on points at tail frequencies are the standard error of the mean calculated from these runs. Note that the sound-level axis is different on the \( f = \) CF panel. Fiber TS37-24, \( CF = 17.78 \) kHz, \( SR = 69.4 \) spikes/s. The number of runs at each frequency are 2 at 500 Hz, 3 at 1 kHz, 3 at 2 kHz, 3 at 2.2 kHz, 3 at 2.5 kHz, 2 at 3 kHz, 2 at 4 kHz, and 1 at 17.78 kHz.

The average normalized level shift, \( \Delta L_n \), on sound frequency we used data pooled from many fibers. This has already been done in Figs. 3–5 which show data from fibers in CF bands stimulated at one tone frequency. Visual inspection of these figures suggests that \( \Delta L_n \) is greater for 2- and 3-kHz tones than for 1-kHz tones across a variety of sound levels. To determine whether there was a statistically significant change in \( \Delta L_n \) across stimulus-tone frequencies of 1, 2, and 3 kHz at levels of 64–97 dB SPL for fibers with CFs between 10–20 kHz [i.e., using data from Figs. 3(a) and (b), 4, and 5], we used permutation tests (Efron and Tibshirani, 1993; see Sec. I). In these tests, no distinction was made between SR classes since previous statistical tests did not detect significant differences among SR groups. To use data from different sound levels and take into account that \( \Delta L_n \) varies with sound level, the \( \Delta L_n \) versus sound-level data for each fiber were fit with a straight line and the \( \Delta L_n \) value at 70 dB SPL (a level in the middle of the data) was used to characterize the individual fiber. Fiber data were shuffled across tone-frequencies groups and comparison statistics were obtained from the difference in average \( \Delta L_{n,70} \) values of two tone frequencies. These permutation tests revealed that the original data show statistically significant differences in \( \Delta L_n \) across tone frequency: 1 kHz vs 3 kHz \((p<0.001)\), 1 kHz vs 2 kHz \((p<0.001)\), and 2 kHz vs 3 kHz \((p<0.019)\). Only one of these differences across frequency was robust enough to show up when the comparison statistic was obtained by first shuffling the fiber data across tone-frequency groups, then fitting a \( \Delta L_n \) versus sound-level line to the pooled data of a tone-frequency group and obtaining a \( \Delta L_{n,70} \). A significant change was detected between responses at 1 and 3 kHz \((p < 0.01)\), but not 1 and 2 kHz \((p > 0.13)\), or 2 and 3 kHz \((p > 0.10)\). Nonetheless, the first test is sufficient to enable us to say that there are statistically significant differences in \( \Delta L_n \) across tone frequency.

To focus on the frequency dependence of \( \Delta L_n \), we averaged \( \Delta L_n \) across all sound levels <85 dB SPL and thereby collapsed the level dependence of \( \Delta L_n \) into a single, average \( \Delta L_n \) value—the “average normalized level shift” or \( \Delta L_{n,ave} \). Averaging was done only up to 85 dB SPL to focus on sound levels for which \( \Delta L_n \) tended to be large. Since fibers from different SR groups have different tail thresholds, \( \Delta L_n \) was typically averaged down to lower sound levels for high-SR fibers than for medium-SR and low-SR fibers. Also, since \( \Delta L_n \) tended to be larger at low sound levels than at high sound levels (Figs. 3–5), the averaging procedure introduced a bias in \( \Delta L_{n,ave} \) across SR groups, thereby precluding comparisons across SR groups. Because of this, the frequency dependence of \( \Delta L_{n,ave} \) was analyzed with fibers of all SRs considered together.

The average normalized level shift, \( \Delta L_{n,ave} \), as a function of tone frequency, is shown in Fig. 8(a) and (b) for fibers with CFs between 10–30 kHz.
scatter in values from individual fibers [Fig. 8(a)] but some clear trends in the average across fibers [Fig. 8(b)]. As tone frequency increased, the efferent inhibition grew from 500 Hz to 2–3 kHz, and then declined [Fig. 8(b)]. The downward trend at the highest frequencies should be interpreted with caution because it comes from only few fibers.

The average normalized level shift, \( \Delta L_{n,ave} \), as a function of tone frequency relative to CF, is shown in Fig. 8(c). Expressing tone frequency as \( f/\text{CF} \) aligns tuning curves at their tips. Plotted this way, the data show a trend similar to that in Fig. 8(a) and (b) with perhaps somewhat less scatter. In Fig. 8(c), \( \Delta L_{n,ave} \) peaks 2–3 octaves below CF. Again the downward trend at the highest frequencies should be interpreted with caution although we note that the trend is consistent with data from single fibers (e.g., Figs. 6 and 7)—data that are included in Fig. 8(c).

### III. DISCUSSION

Our data clearly show that efferent stimulation can produce substantial inhibition of auditory-nerve responses to tail-frequency tones. To insure the reliability of the data we applied strict criteria, selecting only data with excellent triggering, free of shock artifacts, and free of contamination from harmonic distortion. To a considerable extent the parametric dependencies in the present data argue against the possibility that these artifacts substantially affected the results. For instance, the efferent inhibition at tail frequencies decreased with sound level, opposite the expected result if harmonic distortion at high sound levels was producing excitation in the tuning-curve tip.

Although the previous literature on efferent inhibition at tone frequencies below CF has emphasized the small size of the effects seen, a closer examination shows considerable, but not complete, consistency with our results. Wiederhold (1970) and Brown and Nuttall (1984) tested almost exclusively at tone frequencies within 1 octave of CF and found little or no inhibition at frequencies \( \frac{1}{2} \) to 1 octave below CF. We do not have measurements at such frequencies, but an extrapolation of our data suggests that there are small effects at these frequencies (see Fig. 8). A somewhat greater, but mixed, discrepancy exists with efferent effects obtained from tuning curves. Kiang et al. (1970) showed one fiber with no efferent inhibition at tail frequencies, and Guinan and Gifford (1988c) showed many tuning curves, some with and some without substantial efferent inhibition at tail frequencies. These data could be considered consistent with our present results except that the average inhibition for threshold tones at 1 kHz was 1 dB in Guinan and Gifford (1988c) but considerably greater here (typically 5–10 dB at the 1-kHz threshold). In the initial work on the present project, we ran tuning curves with and without efferent shocks and found variable results, sometimes from the same fiber. We are not sure of the origin of this problem, but think it may be due either to adaptation in the efferent effect (a possibility suggested by Guinan and Gifford, 1988c) and/or inconsistent properties of electrical stimulation over the several minutes of continuous shock stimulation needed to monitor efferent effects on tuning curves. Our present use of short bursts of efferent stimulation (1.1 s) and randomized series produce consistent results [e.g., Fig. 1(a) and (c)], and we believe our present results give an accurate picture of efferent effects at tail frequencies.

#### A. The relation of efferent inhibition to the shape of the tuning-curve tail

A comparison of the frequency dependence of efferent inhibition with that of the tuning-curve tail shows that the inhibition is largest at frequencies above the most sensitive part of the tail (Fig. 9). In Fig. 9 we compare the efferent inhibition from Fig. 8(c) (plotted downward) with tuning curves averaged from three CF bands that span the range of CFs used. Figure 9 shows that the efferent inhibition is broadly tuned, similar to the frequency dependencies of the tuning-curve tails, but the inhibition peaks at a frequency above the most sensitive parts of the tails from each frequency band. Similar results were obtained from individual fibers (e.g., Figs. 6 and 7). Figures 6, 7, and 9 also illustrate...
that efferent inhibition can be very different at tail frequencies with similar thresholds on the upper and lower parts of the tail (e.g., in Fig. 9 compare points at 2.6 and 4 octaves below CF on the tuning curve labeled ‘‘CF≈11 kHz’’). The net effect of the efferent inhibition shown in Fig. 9 is to raise the threshold in the upper edge of the tail with little effect at the lower edge so that the tail seems to be shifted to lower frequencies.

Two previous papers are consistent with our results in indicating that the higher- and lower-frequency parts of tuning curve tails can be affected differently by various manipulations. Siegel and Relkin (1987) showed that perilymphatic perfusion of Mg$^{2+}$ in chinchillas caused a frequency-dependent elevation of tuning curve tails with the greatest elevation in the region near where the tail meets the tip. Sewell (1984) also found a frequency-dependent elevation of tuning curve tails from intravenous administration of furosemide in cats. These results, together with ours, call into question the idea that the tuning-curve tail is a single entity that is uniform in its properties across its frequency range.

B. The observed results are due to fast inhibition from shock-evoked activity in medial olivocochlear efferents

Before considering mechanisms further, it is important to point out that there are several compelling reasons to believe that the efferent effects reported here are due to medial olivocochlear (MOC) efferents that synapse on outer hair cells, rather than lateral olivocochlear (LOC) efferents that synapse on ANF dendrites contacting inner hair cells: (1) The use of extracellular shocks and high shock rates both strongly favor stimulation of the myelinated MOC fibers over the unmyelinated LOC fibers (Gifford and Guinan, 1987). (2) We recorded from neurons from the cochlear base but only a very low percentage of the crossed LOC fibers (the only ones near our stimulating electrode) go to the cochlear base (Guinan et al., 1984). (3) In an individual fiber, efferent inhibition varied with tone frequency which would not be expected from LOC synapses on the dendrite of an auditory-nerve fiber. (4) The LOC innervation varies considerably across fibers with different SRs (Liberman, 1980b), but the observed inhibition varied little across SR groups. In addition, the effects are not due to middle-ear-muscles because their tendons were cut. We also note that the cats in this study were kept in a deep anesthetic state so that it is unlikely that the efferent inhibition reported here was influenced substantially by sound-evoked efferent activity. As a test of this, in one cat we attempted to measure efferent inhibition evoked by contralateral sound (as in Warren and Liberman, 1989), but could not detect any, despite a prominent inhibition at tail frequencies when efferents were stimulated electrically.

Based on a variety of evidence, our results appear to be attributable to fast efferent effects rather than the slow efferent inhibition described by Sridhar et al. (1995). In our data, inhibition was evident on the first tone burst, just 100 ms after the onset of efferent stimulation. In addition, our stimulus paradigm presented efferent stimulation at a low duty cycle (1.1 out of 6 s, on average), about half of that used by Sridhar et al. (1995) to evoke efferent slow effects. Also, our randomized stimulation paradigm would average away any differences due to slow effects that lasted much longer than the 3-s trial time.

C. Could the efferent inhibition of ANF responses to tail-frequency tones be due to an efferent reduction of basilar-membrane motion?

There are only two published reports of efferent effects on basilar membrane motion in response to tail-frequency tones, and these provide opposing views (Murugasu and Russell, 1996; Russell and Murugasu, 1997). Murugasu and Russell (1996) reported that efferents did not affect basilar-membrane (BM) motion at frequencies more than $\frac{1}{2}$ octave below CF. However, the major focus of that study was not on tail frequencies and the tail-frequency observations appear to be very limited in scope. Motivated by our preliminary data showing efferent inhibition of ANF responses at tail frequencies (Stankovic and Guinan, 1997), Russell and Murugasu (1997) presented data showing efferent inhibition of BM motion (by 10 dB at 65 dB SPL, decreasing at higher levels) that is very reminiscent of our data (see Figs. 3–5). However, these basilar-membrane data are for only one frequency from one animal (4 kHz with CF=15 kHz). Therefore, it remains to be seen what fraction, if any, of the ANF inhibition at tail frequencies is produced by efferent reduction of basilar-membrane motion. To resolve this issue we need careful measurements of basilar-membrane motion not just at tail frequencies, but also at sound levels near the tail thresholds where we have found the largest efferent effects.

It is useful to make the tentative assumption that an efferent reduction of basilar-membrane motion completely accounts for the inhibition of ANF responses to tail-frequency tones, to see the implications of this assumption.
Since the efferents involved are medial efferents ending on OHCs, one implication would be that OHCs influence basilar-membrane motion at tail frequencies. This would be somewhat surprising since a variety of agents that are presumed to influence basilar-membrane motion primarily through OHCs (e.g., furosemide, acoustic trauma, death; Rhode, 1973; Ruggero et al., 1991, 1996, 1997; Nuttall and Dolan, 1996) have little or no effect on basilar-membrane motion at tail frequencies. In addition, evidence from several species indicates that, in the absence of efferent stimulation, basilar-membrane-motion grows linearly with sound level in response to tail-frequency tones (guinea pig: Sellick et al., 1982; Nuttall and Dolan, 1996; chinchilla: Ruggero et al., 1997; cat: Cooper and Rhode, 1992). In particular, Ruggero et al. (1997) would have been able to detect systematic deviations from linearity of 2 dB or more. This, with our finding that efferent inhibition changes across sound levels (Fig. 1-7), implies that for tail-frequency tones, BM motion with efferent stimulation would have to grow nonlinearly with a growth rate faster, on average, than sound level. Again, while not impossible, this would be surprising. This linearity argument, while weak, appears to us to be the strongest evidence available on this issue. In summary, the hypothesis that an efferent reduction of basilar-membrane motion entirely accounts for the inhibition of ANF responses to tail-frequency tones cannot be ruled out, but it seems more likely that inhibition of basilar-membrane motion accounts for little, if any, of the inhibition of ANF responses to tail-frequency tones.

D. If the inhibition is not due to a reduction of basilar-membrane motion, then what is it due to?

If, in response to tail-frequency tones, efferent stimulation does not change basilar-membrane motion but does change the firing of auditory-nerve fibers, then efferents must affect some stage in between. This implies a divergence between basilar-membrane motion and neural firing when comparing tip versus tail frequencies, but this is not surprising because (1) agents that affect the tips of neural or mechanical tuning curves do not always have the same effect in the tail [e.g., furosemide (Ruggero and Rich, 1991; Sewell, 1984), acoustic trauma (Ruggero et al., 1993; Liberman and Kiang, 1978), and death (Rhode, 1973; Ruggero et al., 1997; Nuttall and Dolan, 1996)]. (2) In the one case where direct measurements of basilar-membrane and auditory-nerve tuning curves were made on the same animal, the tips are very similar but the tails clearly diverge (Narayan et al., 1998). In theory, efferent inhibition which would affect tuning-curve tails of auditory-nerve fibers but not basilar-membrane motion might occur anywhere between basilar-membrane motion and the excitation of auditory-nerve fibers. There are two general mechanisms worth serious consideration for how efferent endings on OHCs might affect other parts of the cochlea: (1) electrical effects through the MOC potential, or (2) mechanical effects that change cochlear micromechanics (specifically, that change the coupling between basilar-membrane motion and the bending of IHC stereocilia).

1. Inhibition from the MOC potential

Activation of MOC efferents produces the MOC potential, an efferent-evoked voltage change that can be measured as a reduction of the endocochlear potential (EP) or as a positive potential within the organ of Corti (Fex, 1967; Brown and Nuttall, 1984; Gifford and Guinan, 1987; Guinan and Stankovic, 1995; see Guinan, 1996 for a review). Although changes in EP that influence OHCs and change the gain of the cochlear amplifier are important for CF stimulation, the effect of this OHC-based change appears to be negligible at tail-frequencies (at least on basilar-membrane motion, see Ruggero and Rich, 1991). One long-considered way that the MOC potential might inhibit auditory-nerve fibers is by reducing IHC transmembrane potential and/or IHC receptor potentials (Geisler, 1974; Guinan and Gifford, 1988b; Guinan and Stankovic, 1995). Brown and Nuttall (1984) found MOC potentials of 0 to 1.5 mV in IHCs, +0.5 to +2 mV in the organ of Corti near IHCs and −2 to −4 mV in scala media. These data suggest that efferents might produce an IHC transmembrane potential of 0.5 mV, which might be enough to produce a significant effect.

An estimate of the inhibition from the MOC potential can be obtained from the finding that furosemide injections reduced EP and increased auditory-nerve tuning-curve thresholds at tail frequencies by 0.3 dB for every 1-mV decrease in EP (Sewell, 1984). If the same ratio holds for an efferent-induced change in EP, then the 4-mV change in EP in the hook region of the cat (the largest reported; Gifford and Guinan, 1987) would increase tail thresholds by about 1.2 dB. The tail-threshold change for fibers with 10–15 kHz CFs should be larger because medial efferent innervation is larger in the 10–15-kHz CF region than in the hook region (Guinan et al., 1984; Liberman et al., 1990). A relevant index of this innervation difference is the efferent inhibition of spontaneous activity which is thought to be due to the efferent-induced change in EP (ΔEP) (Guinan and Gifford, 1988b; Guinan and Stankovic, 1995). Figure 6 of Guinan and Gifford (1988b) indicates that for 10-kHz CFs (the region with the largest inhibition of spontaneous activity), the inhibition is approximately twice that found for 30-kHz CFs (the CF region used to measure ΔEP). Thus, ΔEP might make tail threshold changes of 2.4 dB for fibers with CFs near 10 kHz. Since we found an average elevation of tail threshold of ∼5 dB, occasionally being as large as 25 dB, it seems likely that the efferent-induced reduction of EP plays a role, but perhaps only a minor role, in inhibiting auditory-nerve fiber responses near threshold at tail frequencies. However, ΔEP might be the major factor producing the much smaller inhibition seen at 100 dB SPL.

Another way in which the MOC potential might inhibit auditory-nerve responses is through changes in extracellular potential around IHC-afferent synapses or near the action-potential initiation sites of auditory-nerve fibers (see Russell and Sellick, 1983; Guinan and Gifford, 1988b; Hill et al., 1989; Cheatham and Dallos, 1998; Cody and Mountain, 1989). Such extracellular potential changes might change IHC transmembrane potentials near the IHC-afferent synapse and affect transmitter release, or change the probability of action potentials being initiated for a given transmitter re-
2. Inhibition from an efferent-induced change in cochlear micromechanics

An alternate mechanism by which efferents might inhibit auditory-nerve fibers at tail frequencies without substantially changing basilar-membrane motion is by changing cochlear micromechanics so that basilar membrane motion is less efficiently coupled to the bending of IHC stereocilia. Although it is conceptually possible that efferents, through the MOC potential, might change mechanical properties at a distant point (e.g., IHC stereocilia), it seems most likely that any mechanical change takes place in the OHCs or the OHC stereocilia. There is good evidence for an efferent-induced mechanical change on a slow time scale in guinea pig OHCs (Sziklai et al., 1996), so an efferent-induced mechanical change on a fast time scale would not be surprising. One appealing mechanism is an efferent-induced reduction of OHC stiffness (perhaps like that found by Sziklai et al., 1996) that leads to reduced motion of the upper part of the organ of Corti relative to the basilar membrane. Another possibility is that efferent activity changes OHC stereocilia stiffness (e.g., by hyperpolarizing the OHCs) and that this ultimately changes the coupling between basilar-membrane motion and the bending of IHC stereocilia. Since the efferent-induced change in auditory-nerve firing rate depends on sound level, the efferent-induced change in cochlear micromechanics must also depend on sound level. This could come about either because efferents produce a nonlinear mechanical change, or because the coupling between basilar-membrane motion and the bending of IHC stereocilia is nonlinear even without efferent modulation. Whatever the actual mechanisms involved, the requirements that the efferent-induced mechanical change reduce the bending of IHC stereocilia without substantially changing basilar-membrane motion, and that the greatest change be at sound levels near the tail threshold and sound frequencies 2–3 octaves below CF, greatly constrains the types of mechanical change that might account for efferent tail-frequency inhibition. All in all, our data indicate that current conceptions that auditory-nerve-fiber tuning-curve tails represent a passive, constant coupling of basilar-membrane motion to IHC stereocilia needs substantial revision.

E. Implications of tail-frequency efferent inhibition for audition

Although the main goal of the present work is to help understand basic cochlear mechanisms, it is worth noting that the efferent inhibition of responses to tail-frequency tones may also have significant implications for signal coding by auditory-nerve fibers. Efferent inhibition at tail frequencies can be substantial (e.g., 15 dB at tail threshold). Furthermore, the sound levels and frequencies at which this inhibition is substantial are important for conversational speech. Thus, efferent inhibition at tail frequencies seems likely to be relevant for all hearing people, but it may be particularly relevant for the hearing impaired who have reduced tuning curve tips.

IV. CONCLUSIONS

(i) Medial efferents can inhibit responses of ANFs to tail-frequency tones even though the cochlear amplifier may have little effect at frequencies far below CF.

(ii) Activation of medial efferents lowers responses more in the higher-frequency part than the lower-frequency part of ANF tuning curves, thus tuning-curve tails should not be regarded as single entities with frequency-independent characteristics.

(iii) Since basilar membrane motion at tail frequencies always appears to be linear but efferent inhibition of auditory-nerve fibers at tail frequencies is strongly dependent on sound level, it seems unlikely that efferent inhibition at tail frequencies is due entirely to a reduction in basilar-membrane motion.

(iv) The efferent-induced reduction of EP probably provides part of the inhibition of auditory-nerve fiber responses at tail frequencies. It seems most likely that the remaining inhibition is due to efferent-induced changes in cochlear micromechanics.

(v) The existence and characteristics of medial-efferent inhibition of responses at tail frequencies provide new constraints on concepts of how the cochlea works.
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1These data are reminiscent of the efferent inhibition of tone level functions in the tip region of low-CF fibers that also showed a low-level portion that was inhibited substantially by efferent stimulation, and a high-level portion that showed little or no inhibition (Gifford and Guinan, 1983). However, in the present data, the transition from the lower-level to the higher-level portion of the rate-level curves was not accompanied by an abrupt reversal in the response phase (phase of the first Fourier component of a period histogram) as were the transitions found by Gifford and Guinan (1983). Instead, the response phases gradually decreased with sound level and were similarly affected by efferent stimulation across all sound levels.


