Micromechanical Responses to Tones in the Auditory Fovea of the Greater Mustached Bat’s Cochlea

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Russell, I. J. and M. Kössl. Micromechanical responses to tones in the auditory fovea of the greater mustached bat’s cochlea. J. Neurophysiol. 82: 676–686, 1999. An extended region of the greater mustached bat’s cochlea, the sparsely innervated (SI) zone, is located just basally to the frequency place of the dominant 61-kHz component of the echolocation signal (CF2). Anatomic adaptations in the SI zone are thought to provide the basis for cochlear resonance to the CF2 echoes and for the extremely sharp tuning throughout the auditory system that allows these bats to detect Doppler shifts in the echoes caused by insect wing beat. We measured basilar membrane (BM) displacements in the SI zone with a laser interferometer and recorded acoustic distortion products at the ear drum at frequencies represented in the SI zone. The basilar membrane in the SI region was tuned both to its characteristic frequency (62–72 kHz) and to the resonance frequency (61–62 kHz). With increasing stimulus levels, the displacement growth functions are compressive curves with initial slopes close to unity, and their properties are consistent with the mammalian cochlear amplifier working at high sound frequencies. The sharp basilar membrane resonance is associated with a phase lag of 180° and with a shift of the peak resonance to lower frequencies for high stimulus levels. Within the range of the resonance, the distortion product otoacoustic emissions, which have been attributed to the resonance of the tectorial membrane in the SI region, are associated with an abrupt phase change of 360°. It is proposed that a standing wave resonance of the tectorial membrane drives the BM in the SI region and that the outer hair cells enhance, fine tune, and control the resonance. In the SI region, cochlear micromechanics appear to be able to work in two different modes: a conventional traveling wave leads to shear displacement between basilar and tectorial membrane and to neuronal excitation for 62–70 kHz. In addition, the SI region responds to 61–62 kHz with a resonance based on standing waves and thus preprocessed signals which are represented more apically in the CF2 region of the cochlea.

INTRODUCTION

The greater mustached bat, Pteronotus parnellii parnellii, is the only New World bat to emit constant frequency (CF) echolocation calls, a specialization it exploits to hunt insects within the forest canopy and thereby occupy an ecological niche not available to its close relatives (Schnitzler and Kalko 1998). The typical echolocation call of P. parnellii consists of a 15- to 25-ms-long CF component followed by a brief, downward sweeping FM. The fundamental frequency of the call is close to 30 kHz (CF1), and there are three harmonics, the loudest at ~60 kHz (CF2). By comparison with closely related species, P. parnellii has an extended cochlear duct that is used to analyze the Doppler-shifted echoes of the dominant 61 kHz (CF2) component of the CF calls. The echoes are Doppler-shifted to a few hundred hertz above the call frequency due to changes in the relative velocity between the bat and its target. The incredibly sharp frequency tuning of the expanded, echoreponse region of the cochlea enables P. parnellii to resolve fine FM s in the echoes to the CF calls caused by insect wing beats and to localize and distinguish the insects against the scattering of acoustic signals by the foliage of the bat’s hunting grounds. In order that the frequency of the return echo to the CF call falls precisely within the very narrow bandwidth of this ‘sweet spot’ in the echo-response region of the cochlea, the bat continually adjusts the frequency of its call (Doppler compensation behavior). The combination of extremely narrow frequency filtering in the echo-response region and the setting of the echo-response frequency to a few hundred hertz above the call frequency enables the cochlea to detect minute changes in the echo while strongly rejecting direct stimulation from the intense CF call (for review, Neuweiler 1990).

The sharply tuned neuronal responses to the echoes is due to mechanical processing within the echo response region of the cochlea. There are adaptations in cochlear anatomy that may serve to enhance frequency tuning to the CF2 echoes. On the basis of an inner hair cell (IHC) cochlear frequency map of the basilar membrane (BM), which was obtained by characterizing and dye labeling auditory neurons and observing where they terminated on the IHCs along the length of the BM (Kössl and Vater 1985a), the frequency representation of the 60-kHz range is expanded along the BM in the sparsely innervated zone (SI) and in the CF2 echo frequency response region (CF2) (Fig. 1A). The thickness of the BM in the SI region is increased (Fig. 1C), largely due to an extension of the pars pectinata on the upper surface. This thickening consists of extensive longitudinal fibers, indicative of strong longitudinal coupling of the BM in the SI region (for review, Kössl and Vater 1995). The tectorial membrane (TM) in the SI region is also highly specialized in that it is club-shaped in cross-section with a reduced attachment to the spiral limbus (Henson and Henson 1991; Vater and Kössl 1996). The frequency-dependent sensitivity of the inner ear can be assessed by measuring acoustic distortion products in the external ear canal (Gaskill and Brown 1990). It has been proposed that the mechanoelectrical transducer function is a dominant nonlinearity that could distort the cochlear response to sound in vivo (Frank and Kössl 1997; Lukashkin and Russell 1998; Patuzzi et al. 1989; Santos-Sacchi 1993) and give rise to

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quadratic and cubic distortion products. There is evidence that the emission of the distortion products from the cochlea is filtered by the TM (Allen and Fahey 1993; Brown et al. 1992). On the basis of distortion product measurements in the mustached bat (Kössl and Vater 1996a), it has been suggested that the TM in the SI region resonates close to the CF2 echo frequency (~62 kHz), and according to a model by Steele (1997), the motion of the TM would be like that of a hammer on an anvil (the thickened BM) for frequencies close to the CF2 echo. These anatomic features are thought to contribute to the strong spontaneous (SOAEs) and stimulus-frequency-evoked otoacoustic emissions (SFOAEs) that are an indirect indicator of mechanical resonance in this region of the cochlea (Henson et al. 1985; Kössl and Vater 1985b). The resonance frequency, as determined by measuring SFOAEs, is a few hundred hertz above the CF2 call frequency and close to the echo frequency (Fig. 1B). The strong mechanical resonance in the cochlea of the mustached bat is also evident in a threshold for the emission of the distortion products from the cochlea is measured in the cochleae of nonecholocating mammals. These experiments were, in part, initiated by Gale and Ashmore’s (1997) conclusion that the upper limit for outer hair cell (OHC) motility was 25 kHz and that bats, and presumably other mammals capable of detecting ultrasound (Brown 1970), must employ processes at ultrasonic frequencies other than those involving OHC motility (for review, Dallos 1992) for amplifying mechanical signals and for generating high-frequency resolution. Another aim was, indeed, to investigate the role of the SI zone of the cochlea in the generation of the cochlear resonance and the extraordinarily sharp frequency tuning in the echo-response region of the BM.

**METHODS**

Mustached bats (P. parrnelii parrnelii) were anaesthetized with an initial dose of 2 mg of pentobarbital sodium per 100 g and either 1.2 mg of ketamine hydrochloride per 100 g (Ketavet, Parke-Davis) or 0.13 mg of fentanyl dihydrogencitrate per 100 g (Hynporm, Janssen). The bats were maintained at 37°C, the level of anesthesia was monitored continuously, and every 40 min an additional Ketavet or Hypnorn dose was administered. At the end of the experiment, the animals were overdosed with pentobarbitol sodium. The middle ear was opened to expose the round window of the cochlea, which was left intact. Tone stimuli were delivered through the auditory meatus via a calibrated, closed sound system under computer control. The sound system incorporated two ½-in driving microphones (Bruel and Kjaer 4133) and a ½-in measuring microphone (Bruel and Kjaer 4135) that was used to measure OAEs. The system was compensated for constant sound pressure level (SPL) at the microphone membrane. Detailed methods are published elsewhere (Kössl 1994).

Displacements of the BM were measured by the self-mixing effect of a laser diode. This method of measuring basilar membrane vibration has been described previously (Kössl and Russell 1995) and involves reflecting back from the BM a small proportion of the light emitted by the laser into the laser cavity. The self-mixing effect has
The advantage that it is not necessary to place reflecting targets on the BM and it is essentially linear for displacements of <30 nm. The light emitted by the laser is monitored by an intrinsic photodiode, and the current from this is directly proportional to the BM displacement. The bandwidth of the displacement-dependent signal was 200 kHz. Calibration of the signal was achieved by displacing the interferometer by a known amount with a piezoelectric driver. The system is also self-calibrating in that the limits of the transfer characteristics of the interferometer are a quarter wavelength, which is 167.5 nm for the laser diode used in these experiments. A schematic of the interferometer is shown in Fig. 2. The output of the interferometer was fed into a spectrum analyzer (3561A, Hewlet Packard) and a pair of lock-in amplifiers (5210 EG&G Princeton) set in quadrature, and the phase and magnitude of the signal were calculated on-line by the computer.

Sound levels are given in decibels (dB) re 20 μPa. The beam of the laser diode interferometer was focused through the transparent round window membrane. Laser beam is detected by the integral photodiode and the photocurrent is amplified and converted to voltage for processing with a dual phase lock-in amplifier phase-locked to the driving voltage to the sound system. The signal from the photodiode is also low-pass filtered and amplified and serves as the error signal in an electromechanical feedback circuit driven by a piezoelectric stack. This feedback system compensates for heart beat, respiration, and other low-frequency movements of the basilar membrane relative to the interferometer.

To determine the cochlear resonance frequency of the individual bats, SFOAEs were measured in awake animals before conducting surgery to expose the cochlea for the BM recordings. The bats were restrained gently in a shaped, foam cavity and by a head holder (Kössl 1994). To elicit the SFOAEs, a pure tone stimulus generated by a B&K 4133 microphone capsule was used as a speaker was swept upward in frequency, and the resulting frequency response at the tympanum was recorded with a B&K 4135 microphone connected to a B&K 2670 preamplifier and a B&K 2610 measuring amplifier. At the frequency of cochlear resonance, the tone stimulus interferes with the SFOAE to produce a characteristic sequence of a sound pressure maximum and minimum associated with phase changes in the frequency response (Fig. 3A) (Kössl 1994). The transition between level maximum and minimum, where maximum phase change occurred, was used to define the SFOAE frequency (vertical line in Fig. 3A).

Distortion product otoacoustic emissions (DPOAEs) were recorded to monitor changes in cochlear mechanics during the anesthesia and surgery. To obtain DPOAE data, the ear was stimulated with two pure tone stimuli (f1, f2). Two B&K 4133 microphone capsules served as speakers and the 2f1–f2 DPOAE was measured with the B&K 4135 microphone. The stimuli wave-forms were generated by using a dual HP 8904A synthesizer, and the recorded response was fed into a HP 3561A dynamic signal analyzer for fast Fourier Transform (FFT) analysis. Figure 3B shows representative examples of so-called “distortion audiograms” measured before and during anesthesia and after opening the middle ear to gain access to the cochlea. To measure audiograms of the 2f1–f2 DPOAE, the two stimuli were delivered with a constant level difference, with the level of f1 10 dB above that of f2, and both stimulus frequencies were increased stepwise over the tested frequency range. During this procedure, the frequency ratio

![Diagram of laser diode assembly](http://jn.physiology.org/)

**FIG. 2.** Self-mixing effect interferometer. Beam from the laser diode (10 mW, 670 nm) is collimated and focused to a 5-μm spot on the basilar membrane. Laser beam is detected by the integral photodiode and the photocurrent is amplified and converted to voltage for processing with a dual phase lock-in amplifier phase-locked to the driving voltage to the sound system. The signal from the photodiode is also low-pass filtered and amplified and serves as the error signal in an electromechanical feedback circuit driven by a piezoelectric stack. This feedback system compensates for heart beat, respiration, and other low-frequency movements of the basilar membrane relative to the interferometer.

![Graph of SFOAE frequency response](http://jn.physiology.org/)

**FIG. 3.** OAEs in the mustached bat. A: stimulus-frequency OAE (SFOAE) evoked by a continuous pure tone sweep. At the frequency of cochlear resonance, the emitted emission interacts with the tone stimulus and a characteristic interference pattern consisting of sound pressure level minima and maxima (——) and a phase change (---) emerges. Frequency of the SFOAE is defined as maximum phase change and transition between level maximum and minimum (●). B: distortion-product OAE (DPOAE) “audiogram.” Two pure tone stimuli of different frequency (f1, f2, with the ratio f2/f1 = 1.02 and the f1- and f2-levels at 60 and 50 dB SPL, respectively) were stepwise increased in frequency for a f2 range of 55–96 kHz. The induced 2f1–f2 DPOAE is maximal close to the SFOAE frequency (†). By comparison with the awake animal (●), after anesthetic (○) and opening the middle ear (★), the high-frequency DPOAEs deteriorate in level. ···, average noise level. Data from bat P1.
f2/f1 was kept constant at a small value between 1.001 and 1.04 which, for the mustached bat, is known to elicit maximum levels of the 2f1–f2 DPOAE (Kössl 1994). There were clear influences of anesthesia and of middle-ear opening on the DPOAE levels. In the frequency range between 60 and 70 kHz, the DPOAE levels decreased by 3–15 dB, and for higher frequencies the loss of DPOAE levels could amount ≤50 dB. Furthermore in anesthetized animals, the cochlea resonance frequency, as measured by the SFOAEs, shifts by a few hundred hertz to lower frequencies.

In addition to the preceding measurements, we recorded DPOAEs in six awake bats that were not used for surgery to determine the phase and group delay behavior of these emissions for comparison with the BM data. To be able to measure the DPOAE phase, both stimuli have to be presented at a constant phase relationship; this is not possible with the HP synthesizer. Therefore we used two D/A output ports of a Microstar Dap 3200/c400 digital signal processing board sampling at a rate of 250–400 kHz to produce the stimuli. The microphone response was recorded with an A/D channel of the same board and FFT analysis was performed onboard. Software for data acquisition and processing was written in Asyst (Keithley).

The phase behavior of the 2f1–f2 DPOAE was measured by keeping the f2 stimulus constant at a certain frequency and the f1 frequency was varied stepwise. As a result, the 2f1–f2 frequency was swept across the frequency range to be tested. The measured phase of the 2f1–f2 DPOAE (\(\Phi_{2f1-f2}\)) was corrected for the stimuli phases according to Mills and Rubel (1997). The corrected DPOAE phase angle (\(\Phi_{2f1-f2}\)) is given by

\[ \Phi_{2f1-f2} = \Phi_{2f1-f2} - 2(\Phi_{f1} - \Phi_{f2}) \]

where \(\Phi_{f1}\) and \(\Phi_{f2}\) are measured phase angles for the f1 and f2 stimuli.

The corrected DPOAE phase angles for successive f1 and DPOAE frequencies then were unwrapped by a computer program and displayed. In some cases, for lower primary levels, the phase change between two consecutive data points was >180° and therefore the unwrapping is ambiguous. In such cases a more gradual phase change could be observed for higher sound levels, the low level data were unwrapped to yield an overall phase change similar to that observed at higher levels. The group delay of the 2f1–f2 DPOAE (\(T_{2f1-f2}\)) was calculated from the phase change (\(\Delta\Phi_{2f1-f2}\)) versus frequency change (\(\Delta f_{2f1-f2}\)) of successive data points (Kimberley et al. 1993; Mills and Rubel 1997), where

\[ T_{2f1-f2} = -\frac{\Delta\Phi_{2f1-f2}}{\Delta f_{2f1-f2}} \]

RESULTS

Frequency dependence of basilar membrane displacement at constant SPL

BM displacement measurements were made in nine individuals of P. parnellii by directing the beam of the interferometer through the transparent round-window membrane. From lesions made after each experiment, it was discovered that the measurements reported in the present study were made from the SI region of the cochlea. Examples of tone-evoked BM displacement recorded from a position between 35 and 40% of the BM length with respect to the base of the cochlea (see Fig. 4, inset) are shown in Fig. 4 for frequencies between 20 and 90 kHz and levels between 50 and 100 dB SPL. This region is near the apical extent of the SI region, and primary afferent neurons in this region have characteristic frequencies between 63 and 65 kHz (Kössl and Vater 1985a). For low-level stimuli (50 dB SPL), the response bandwidth is very narrow and centered around the resonance frequency of 61.125 kHz of the individual bat. This is slightly lower than the SFOAE of 61.99 kHz in the awake animal. At 70 dB SPL, a second peak appears at the characteristic frequency of the recording location (64.2 kHz). With increasing level, the region of response extends toward higher frequencies with peak responses to both 61 and 65 kHz. At the highest levels, there is a sharp high-frequency cutoff ~74 kHz. In addition, for levels <100 dB SPL, there is a sharp low-frequency cutoff of the response close to 60 kHz. The region of insensitivity extends down to ~50 kHz. BM displacements were recorded in response to frequencies <50 kHz and extended at least down to 20 kHz, which was the lowest stimulus frequency used in these experiments. In response to tones at 100 dB SPL, the low-frequency cutoff and the region of insensitivity disappeared.

Basilar membrane displacement as a function of sound level

It soon became apparent that each measurement point in the SI region responded sharply and sensitively to the SFOAE frequency and to a higher frequency (characteristic frequency) at which the response threshold reached a second minimum. The frequency of this second minimum corresponds to the characteristic cochlear frequency place as determined from a cochlear frequency map that is based on horseradish peroxidase labeling of the afferent innervation of IHCs (Kössl and Vater 1985a) (Fig. 1A). The displacement of the BM was plotted as a function of sound level for tones at frequencies close to and at the SFOAE frequency as an initial step in constructing BM iso-displacement frequency-tuning curves. Examples of the compressive nonlinear level-functions are shown in Fig. 5 for a single preparation for frequencies around the resonance frequency (61.25 kHz; Fig. 5A) and the characteristic frequency (64.2 kHz; Fig. 5B). The level functions are typically saturating, nonlinear curves with initial slopes close to unity and are most sensitive for frequencies close to the resonance frequency and the characteristic frequency. They are relatively insensitive and do not saturate for levels <90 dB SPL for frequencies ~1 kHz above and below the resonance frequency (Fig. 5A). The curves become compressive again 2 kHz below the characteristic frequency and remain compressive for frequencies 2 kHz above the characteristic frequency (Fig. 5B).

Basilar membrane frequency tuning curves

Iso-response frequency-tuning curves for four different animals are shown in Fig. 6. They are derived from displacement-level functions such as those shown in Fig. 5. The response criteria are different for each preparation and are taken just above the noise floor of the measurements. Three of the iso-response curves (Fig. 6, A–C) are characterized by a sharp threshold minimum at the resonance frequency, which was between 60.45 and 61.56 kHz in six preparations. At frequencies within 1 kHz below the frequency at the tip of the tuning curve there is a strongly insensitive region. Within 1 kHz above the frequency of the tip there is a high-frequency shoulder, which may appear as a distinct lobe (see Fig. 6, A and B). Where extended frequency measurements have been possible (Fig. 6, A–D), distinct threshold minima were observed at
frequencies corresponding to the characteristic frequencies of the recording locations, which were between 63 and 67 kHz in these experiments. The tuning curve shown in Fig. 6D was from a relatively insensitive preparation and was measured at a higher response criterion than the other curves. The characteristic resonance peak was absent, and the curve probably reflects the mechanical frequency response of the BM at the 67-kHz recording site.

Tuning curves Fig. 6, A–C, have sharp peaks for frequencies close to the SFOAE with very steep high and low frequency slopes. The sharpness of tuning is defined by the $Q_{10\,\text{dB}}$ (the center frequency divided by the bandwidth 10 dB above the tip). For measurements of the resonance in five preparations in the SI region, the $Q_{10\,\text{dB}}$ varied between 102 and 430. In four preparations where such measurements were possible, the low frequency slope of the tuning curve varied between 2,042 and 6,892 dB/octave (mean = 4,657 dB/octave) and the high-frequency slope was less steep and varied between 1,960 and 3,706 dB/octave (mean = 2,735 dB/octave). It was possible to measure the $Q_{10\,\text{dB}}$ of the characteristic frequency from three preparations and these were 11 (CF = 65.0 kHz), 25 (CF = 64.2 kHz), and 6 (CF = 67 kHz).

FIG. 4. Basilar membrane displacement as a function of stimulus tone frequency at different levels indicated against each trace. *Inset:* basal coil of the bat cochlea and the location of the recording site. Laser beam is projected through the round window membrane to form a spot on the apical part of the SI (sparsely innervated) region of the basilar membrane. Peaks at the resonance frequency (61.125 kHz) and the characteristic frequency (64.4 kHz) are shown by the vertical lines. Noise level of the measurement. Data from bat P2.
Basilar membrane iso-level magnitude and phase responses

Iso-level magnitude and phase functions at levels between 55 dB SPL and 95 dB SPL for a single preparation are shown in Fig. 7. With increasing level, the peak response to the resonant frequency becomes larger and broader and moves toward lower frequencies as is also apparent in Fig. 4. The response envelope tends to expand toward higher frequencies and toward the region of the characteristic frequency of the measurement site. The resonance is associated with a phase transition (a phase lag), which is seen in the accumulated phase curves in Fig. 7 right.

The phase lag, measured from the curves shown in Fig. 7 is $181.7 \pm 10.3^\circ$ (mean $\pm$ SE). Similar measurements in a second preparation yielded $183.0 \pm 17.9^\circ$.

The rate of change of phase in degrees per kilohertz is largest for low sound levels and amounts to $480^\circ$/kHz at 55 dB SPL. With increasing levels, the rate of phase change decreases and is $150^\circ$/kHz at a level of 95 dB SPL. If one extrapolates the slope of the phase change down to lower sound pressure levels, then at 20 dB SPL, the slope values would be $\sim 1,200^\circ$/kHz.

DPOAE measurements

Phase changes associated with mechanical cochlear resonance are apparent in the SFOAE recordings (Fig. 3A). In these measurements, however, the observed phase change in the interference pattern is subject to interaction between the OAE and the stimulus. Therefore the full range of phase change around the resonance frequency cannot be obtained unambiguously from SFOAE data.

If one uses a signal that is generated by nonlinear transduction in the cochlea itself and that does not coincide with the stimulus frequencies, then the phase behavior of the mechanical resonance is determined more easily. For this reason, we have used the 2f1–f2 DPOAE to probe cochlear mechanics. In the examples given in Fig. 9, the frequency of f2 was adjusted to a value between 63 and 70 kHz, which, according to the BM frequency map of the mustached bat, should be represented in the specialized SI region. By changing f1, the DPOAE frequency was swept across the frequency range of the cochlear resonance. Close to the SFOAE frequency, maxima and minima of the DPOAE levels coincided with a steep phase change of approximately $360^\circ$ (Fig. 9, bottom). For f2 frequencies between 62 and 66 kHz and for low stimulus levels (f1, f2 at 30/20 or 40/30 dB SPL), the steepest changes of distortion phase ranged between 1,600 and 2,900°/kHz. For louder stim-

FIG. 5. Top: BM displacement as a function of level for a single preparation at frequencies close to the resonance frequency (61.25 kHz). Bottom: same preparation for frequencies close to the characteristic frequency (64.2 kHz). Data from bat P2.

FIG. 6. Isoresponse tuning curves for 4 preparations: P3 (A), P4 (B), P2 (C), and P5 (D). \( \downarrow \), frequency of the SFOAE measured in the awake animal at the beginning of the experiment. Largely as a consequence of the anesthesia (see Fig. 3), these are at a higher frequency than the tips of the tuning curves. \( Q_{10} \) of the resonance peak and the response criterion for each figure are A: 203, 0.3 nm; B: 430, 0.3 nm; C: 177, 0.2 nm; and D: 0.5 nm.
ulus levels (f1, f2 at 50/40 or 60/50 dB SPL), the phase changes were more gradual and amounted to 800 – 1,500°/kHz. The frequency of the steepest phase transition moved to lower values with increasing stimulus levels. Within the range of levels used in the experiments (30/20 – 60/50 dB SPL), this shift of the frequency of steepest phase change could reach maximum values of 370 Hz (Fig. 9 A). The same behavior is also evident in the group delay maxima associated with the steep phase transitions. For stimulus levels of ≤40/30 dB SPL, group delay maxima of ≤8 ms were measured (Fig. 9, top). This compares with average values of 0.15–0.87 ms outside the range of the steep phase transitions.

Level-dependent changes in the resonance frequency, determined from the phase transitions and the group delay maxima, could be observed for f2 frequencies between 62 and 64 kHz. For f2 frequencies in the range between 65 and 66 kHz, the measured resonance frequency was level independent and stayed constant within the measurement frequency resolution of 122 Hz. In addition, for f2 frequencies of ≤74 kHz (not shown), phase transitions could occur when the distortion was close to the SFOAE frequency that were similar to those observed at lower f2 frequencies. Such discontinuities associated with the SFOAE could not be measured for f2 frequencies >74 kHz.

**Discussion**

Measurements of BM vibrations in the SI region of the cochlea, where the echoes produced by CF calls are preprocessed, reveal that each measurement location is tuned to the characteristic frequency of the location (62–72 kHz) and in addition resonates at the echo frequency (61–62 kHz), as indicated by the frequency of the SFOAE. The level and frequency dependency of the BM vibrations measured at ultrasonic frequencies in the bat cochlea are very similar to those that have been measured in the basal, high-frequency turns of the cochlear of nonecholocating mammals to tones within their auditory response range (e.g., Cooper and Rhode 1992; Murugasu and Russell 1995; Nuttall and Dolan 1996; Rhode and Robles 1974; Robles et al. 1986; Ruggero et al. 1997; Russell and Nilsen 1997; Sellick et al. 1982). Thus with increasing sound levels, the BM responses in the bat initially grow with a slope close to unity and saturate at high levels. BM level functions obtained at frequencies close to the resonance or to the characteristic frequency of the measurement location, are most sensitive and are strongly saturating. With increasing level, the resonance broadens, the center frequency moves to lower frequencies, and the phase changes associated with the resonance become more gradual (Fig. 7). These findings lead us to conclude that the SI region of the mustached bat cochlea transduces and amplifies mechanical signals in exactly the same way as in nonecholocating mammals. Our findings do not resolve the current controversy over the involvement of OHC motility in the amplification, compression, and sharpening of BM vibrations (Dallos and Evans 1995; Hudspeth 1997; Kolston 1995). However, if OHC motility is the basis for cochlear sensitivity and frequency resolution, then it appears to be able to act at ultrasonic frequencies. From measurements in isolated OHCs in the guinea pig, there is also disagreement over the upper frequency limit of OHC motility. While Gale and Ashmore (1997) have found that OHC motility has a frequency limit of ~25 kHz, Frank et al. (1999) have measured a corner frequency of 79 kHz in isolated OHCs.

**Mechanical versus neural responses from the SI region**

The responses of brain stem neurons with characteristic frequencies at or slightly below the SFOAE frequency are extremely narrowly tuned, with Q10 dB values between 100 and 400, while those with characteristic frequencies within the response range of the SI region (62–72 kHz) are more broadly tuned.
tuned and are less sensitive with $Q_{10\, \text{dB}}$ values of $\sim 20$ (Kössl and Vater 1990; Suga and Jen 1977). Furthermore unlike the BM responses in the SI region, the neurons are tuned only to the characteristic frequency and not also to the resonance. On the basis of the BM frequency-place map derived from labeling afferent dendrites (Kössl and Vater 1985a) and from our measurements, the BM responses at each measurement location in the SI region are tuned to tones within the frequency response range of the SI region. On the three occasions we were able to determine them, the $Q_{10\, \text{dB}}$ values of the mechanical tuning curves were similar to those of the nerve fibers and were at 6, 11, and 25, respectively. In addition to being tuned to their characteristic frequency, each measurement location within the SI region was found to resonate sharply to frequencies close to the SFOAE. The $Q_{10\, \text{dB}}$ values of the resonance varied between 102 and 430, which is within the range of the $Q_{10\, \text{dB}}$s measured from the auditory nerve fibers tuned to just above the CF2 frequency but less than the $Q_{10\, \text{dB}}$ of 610, which was measured previously (Kössl and Russell 1995) from a sensitive preparation. Furthermore the slopes of the BM tuning curves and of neurons tuned to just above the CF2 frequency are similar with the low-frequency slope being steeper than the high-frequency slope.

Although the mechanical tuning of the BM in the SI region recorded in anesthetized animals is as sharply tuned to the neural tuning recorded in awake animals to tones at both the resonance frequency and to tones within the frequency response range of the SI region, it is less sensitive. Several factors may contribute to the insensitivity of the micromechanical responses observed here. Anesthesia and opening the middle ear can reduce the sensitivity of OAEs by $\leq 30$ dB SPL (see Fig. 3), and one might expect a similar reduction in the sensitivity of BM vibration. In all recordings we have made from the SI region, the BM vibrates most sensitively to frequencies close to the SFOAE. The frequency of resonance, which was estimated from the peak BM displacements and the midpoint of the associated rapid phase-change, is a few hundred hertz below that of the SFOAE recorded in the awake animal. This discrepancy is probably due to anesthesia because it is known that the SFOAE can shift downward by $\pm 500$ Hz when the animal is anesthetized (Kössl and Vater 1985a).

Thus the most noticeable difference between the BM and neural responses in the SI region is that the nerve fibers are tuned only to their characteristic frequency, whereas the BM is tuned to the characteristic frequency of the measurement location and to the resonance. One explanation for this difference is that the specialized BM and TM in the SI region have two modes of vibration, one of which is in the transverse plane and the other in the radial plane. Accordingly, when driven by tones at the resonance frequency, the TM and BM vibrate together in the transverse plane (Steele 1997). The TM and the BM move as a single mass, radial shear between them is minimal (e.g., Geisler and Sang 1995; Markin and Hudspeth 1995) and hence there is no neural excitation. However, when excited by tones at frequencies in the response range of the SI region, it is suggested that the TM and the BM vibrate as a two-mass system so that there is relative shear between them. The shear displaces the IHC and OHC sensory hair bundles which ultimately leads to neural excitation (Davis 1965).

**Evidence for standing wave resonance in the SI region: acoustic distortion products**

From measurements of the 2f1–f2 acoustic distortion product it has been proposed that the TM in the SI region is a mechanical resonator that is sharply tuned to the SFOAE frequency throughout its length (Kössl and Vater 1996). With the exception of f2 frequencies between 65 and 66 kHz, the distortion phase, group delay and resonance frequency are labile and level dependent (Fig. 9). In the frequency range of the resonance, the distortion phase change amounts to 360°. With increasing stimulus levels, this phase change becomes less steep, the group delay becomes smaller and the resonance frequency shifts downwards. These findings are also reflected...
regions of the BM in the SI zone. It is proposed that reflections are generated at the two points of morphological, and probable mechanical discontinuity (as indicated by the sharp response cutoffs at 60 and 74 kHz in BM iso-level responses, Fig. 4) at the apical and basal extents in the SI region of the cochlear duct, leading to intracochlear pressure changes that generate standing-wave-like oscillations at a frequency close to 62 kHz in the SI region. Either the TM or the BM, or both together, could be the primary source for the resonance. In view of the morphological considerations described in the preceding text, one might expect standing waves to be generated differently in the two structures (Fig. 10). On the basis of the neural tuning and the characteristic frequency responses of the BM in the SI region (Fig. 6), the BM in this region is tuned tonotopically to frequencies between 62 and 72 kHz, whereas the TM appears to be tuned exactly to 62 kHz over the whole length of the SI zone. Therefore we suggest that the TM resonance drives the basilar membrane and that OHCs are critical for the interaction of both membranes. This view is supported by the observations that the BM resonance is labile (Kössl and Russell 1995) and level dependent and disappears in insensitive preparations (Fig. 6D). Similarly, BM displacements recorded in the SI region are insensitive to frequencies close to 65.5 kHz (Fig. 5). These observations may indicate that a standing wave node, either in terms of displacement or velocity, is located in this region.

Faulstich and Kössl (1997) and Kössl and Vater (unpublished results) have observed that the effects of aminoglycoside antibiotics and anesthetics on P. parrelli, which are presumed to selectively interfere with OHC function or can lead to their destruction (see Rybak 1986 for a review), can severely attenuate DPOAEs and cochlear neural responses by 10–20 dB SPL. However, during aminoglycoside treatment, evoked OAEs that are associated with cochlear resonance decrease in bandwidth and increase in level. Under anesthesia, these emissions even can convert to spontaneous OAEs. This surprising observation may indicate that, through negative feedback, the OHCs in the SI region act to stabilize the mechanical properties of the cochlear partition and to reduce evoked and spontaneous OAEs at the resonance frequency.

Thus it appears that at the resonance frequency the cochlear partition in the SI region behaves as a single-mass system with feedback from OHCs in phase with BM motion. Under these circumstances, OHC feedback actually can damp BM vibration rather than augment it (e.g., Markin and Hudspeth 1995). Hence the removal of OHC feedback after treatment with aminoglycosides might be expected to increase the amplitude and decrease the bandwidth of evoked and spontaneous OAEs associated with the cochlear resonance.

Cochlear resonance: product of an acoustic laser?

On the basis of our measurements of the resonance in BM vibrations and acoustic distortion, we suggest that the frequency spectrum of the cochlea’s mechanical response to the CF2 echo frequency (~62 kHz), at the CF2 echo place, is sharpened, fine tuned, and stabilized through feedback from a standing wave resonance generated in the SI region. The resonant vibrations in the SI zone cause the build-up of large intracochlear pressure changes at the CF2 echo frequency place in the BM resonance (Fig. 7) where the phase change is 180°. However, distortion at the resonance frequency measured when the site of distortion generation is located between the 65- and 66-kHz places on the BM (as determined by the f2 frequency) is remarkably resistant to level change (Fig. 9). The special characteristics of this location are also apparent from two other observations. The SFOAE is most sensitive to acoustic suppression by tones centered ~65 kHz (Kössl and Vater 1985a), and suppression tuning curves for the 2f1–f2 distortion product at the SFOAE frequency, where the primaries are located within the SI zone (62–72 kHz), are bilobed with minima near the SFOAE frequency and between 65 and 66 kHz (Frank and Kössl 1995). Thus the resonance evoked at the 65- to 66-kHz location is particularly stable as a function of level but very sensitive to suppression by tones at the frequency of the site. These properties might be explained if the 65- to 66-kHz location represented a node in a standing wave resonance present in the SI zone.

Generation of a standing wave resonance in the SI zone

If one views the BM and TM as strings, spanned in the longitudinal direction between the apical and basal extents of the SI region, then the TM string may be regarded as being fixed at both ends because the attachment of the TM to the spiral limbus increases abruptly at the extreme extents of the SI region (Fig. 10). In contrast, the BM string in the SI region may allow free movement at both ends because the arcuate thickening, and the longitudinal fibers within it, are limited in extent to the SI region. The longitudinal fibers also will tend to promote longitudinal mechanical coupling between adjacent
at the transition region between the SI zone and the CF2 region. In this region, the spiral ligament with its tension fibroblasts (Henson and Henson 1988) is enlarged greatly, probably to prevent damage to the organ of Corti and BM when this energy is released (see Kössl and Vater 1996). If vibration of the OHC hair bundles results in electromechanical feedback of energy to the cochlear partition, as it does elsewhere in the mammalian cochlea (reviewed by Dallos 1992), then this would contribute to local frequency tuning at the CF2 echo place. In addition, the local electromechanical feedback of energy by the OHCs also could pump energy into the cochlear resonance in the SI region, which in turn feeds back to the CF2 echo place. This pumping of energy is analogous to that produced by a diode laser with an extended cavity where the pumping of energy into the cavity provides frequency-selective feedback to reduce the laser’s line width and to improve its tunability (Bosheir et al. 1991). Fine structural features in the SI region may be responsible for setting the subtle frequency differences related to the age and sex in calls and SFOAEs of the mustached bat.

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