

Vibration of beads placed on the basilar membrane in the basal turn of the cochlea

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Abstract: Interferometric recordings of sound-evoked vibrations in the basal turn and hook regions of the guinea-pig cochlea are used to show that reflective microbeads (i) follow the motion of the structures on which they are placed, and (ii) do not affect this motion dramatically. Extrapolating these findings to other types of reflective or radioactive material lends support to the findings of numerous studies of cochlear mechanics.

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1. Introduction

Experimental studies of cochlear mechanics have provided insight into the manner in which sounds are processed in the peripheral auditory system. Most of the observations in these studies have been made at the level of the basilar membrane, and almost all have been facilitated by the placement of small objects (either radioactive sources, reflective microbeads, or mirrors) on the cochlear partition. The effects that these objects might have on the vibrations of the cochlear partition have often been questioned, but have very rarely been tested [Kliauga and Khanna, 1983; Sellick *et al.*, 1983].

One recent report has highlighted the potential problems of using reflective microbeads in studies of cochlear mechanics; Khanna *et al.* (1998) reported that beads that had been placed on the basilar membrane did not follow the motion of the cochlear partition. Moreover, the presence of the beads affected the motion of the underlying partition quite dramatically. These observations were made in the apical turns of the cochlea in an isolated temporal bone preparation, but there is little reason to prevent their extrapolation to other regions of the cochlea. Such an extrapolation would clearly cast doubt on the relevance of almost all other observations in the field of cochlear mechanics.

The present study was designed to investigate the issue of using beads in the more basal turns of the living cochlea, where the vast majority of previous observations have been made. The principle aims of the study were to find out whether the reflective microbeads follow the motion of the basilar membrane, and/or whether they affect this motion.

2. Methods

2.1 Surgical preparation

Ten young, pigmented guinea-pigs provided substantial data in this study. The animals were anesthetized using a combination of pentobarbital sodium (25-30 mg/kg i.p.) and Hypnorm (6 mg/kg fluanisone + 200 µg/kg fentanyl i.m.). Supplementary doses of each agent were given as needed to maintain a state of deep areflexia, and the animals were overdosed with pentobarbital on completion of the experimental procedures. Tracheotomies were performed, and end-tidal CO₂ concentrations were monitored using an infra-red gas detector. Artificial ventilation was provided when necessary. Core temperatures were maintained near 37.6°C using a thermostatically-controlled heating blanket monitored by a rectal probe thermistor.

The animal's scalp and right pinna were retracted, and the skull was fixed into a combined head-holder and earpiece. The postero-lateral bulla was opened to gain access to the cochlea, and a small opening was made into the scala tympani of the cochlea's basal turn. The opening was made either (i) by gently shaving through the bone ~3.5 mm from the basal end of the cochlear partition (n=1), (ii) by picking through the bone from the apical border of the round window ~2 mm from the basal end of the partition (n=2), or (iii) by carefully tearing through the round window membrane between 1 and 2 mm from the basal end of the partition (n=7). The temperature inside the bulla was maintained near 37.6°C using heat from a thermostatically-controlled light bulb.

Gold-coated polystyrene microbeads (15 or 25 μm diameter, mean density range 1.1 to 1.5 $\text{g}\cdot\text{cm}^{-3}$) were introduced onto the perilymphatic meniscus using a stainless-steel pick. The beads were manipulated to fall through the meniscus and onto the underlying basilar membrane. Once an acceptable bead placement had been made, the perilymphatic meniscus was covered with a small glass cover-slip. The cover-slip minimized any optical interference caused by movements of the meniscus, but it did not form an acoustic seal [Cooper and Rhode, 1992].

2.2 Stimulus generation and control

Stimuli were generated using a computer-controlled DAC and attenuators, with a reverse-driven condenser microphone cartridge serving as a loudspeaker. Multiple presentations of a 30-ms-duration tone-pip were made with repetition periods of >100 ms. The tone-pips were gated on and off using half-periods of a raised-cosine envelope (1-ms duration).

Stimuli were coupled into the dissected ear canal through an earpiece that formed part of the animal's head-holder. Closed-field sound pressures were monitored less than 1 mm from the tympanic membrane using a condenser microphone equipped with a calibrated 1-mm-diameter probe tube. All experiments were performed on a vibration-isolated workstation in a soundproof chamber.

2.3 Measurement techniques

Vibrations were measured using displacement-sensitive heterodyne laser interferometry [Cooper, 1999a]. This technique has been shown to be linear over a wide dynamic range (object velocities from 0 to 13 $\text{mm}\cdot\text{s}^{-1}$) and was sensitive enough to measure low-level displacements from the untreated cochlear partition (the system's noise-floor was ~10 $\text{pm}\cdot\text{Hz}^{-0.5}$ for objects that reflected just 1 ppm of the incident light). In the present study, displacement responses were averaged across 8-64 presentations of the 30-ms-long stimuli. The steady-state portions of the averaged responses (between 1 and 29 ms peri-stimulus-time) were Fourier analyzed to determine the magnitude and phase of the response component at the stimulus frequency.

Compound action potentials (CAPs) were used to monitor the physiological condition of the cochlea. These were recorded from a silver wire electrode placed in the round window niche. CAPs were amplified (x1000) and band-pass filtered (0.1-10 kHz) before being displayed on an oscilloscope and digitized and averaged in a computer. Baseline CAP audiograms [Johnstone *et al.*, 1979] were determined prior to opening the cochlea in each experiment. Subsequent checks on the CAPs were made whenever there was reason to suspect a change in the physiological condition of the cochlea.

3. Results

Comparisons were made between the responses observed (i) on and (ii) within 20-190 μm of individual microbeads in six living cochleae and two dead cochleae. The responses from each site were stimulus-dependent and varied with the physiological condition of the

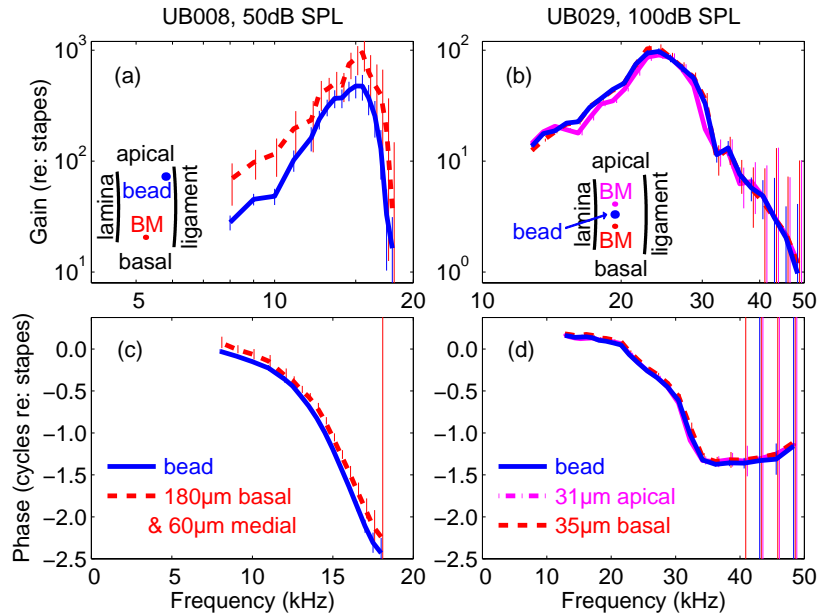


Fig. 1. Tuning characteristics measured on and around individual microbeads in the basal turn and hook regions of the guinea-pig cochlea. Response amplitudes (a,b) and phases (c,d) are expressed with respect to those measured from the stapes in each experiment. Solid lines show bead responses; dashed lines show responses measured from adjacent sites on the cochlear partition. Vertical bars indicate 95% confidence intervals. Bead locations were ~ 3.5 and ~ 1.8 mm from the basal end of the cochlear partition, ~ 140 and $56 \mu\text{m}$ from the osseous spiral lamina, in UB008 and UB029, respectively. Relative locations of other recording sites are shown in the insets of (a) and (b) and specified in (c) and (d).

preparation, but the comparisons between the responses on and around the beads were always similar. Typical results are illustrated in Fig. 1: in each panel of this figure, the tuning curves observed on and around the beads have very similar shapes. To be more specific, more than 90% of the variance observed in the bead responses could be accounted for by the variance observed at adjacent sites on the cochlear partition. (This statement applies to the findings in every experiment.) Systematic differences between the absolute sensitivities of the individual curves were often observed (the largest difference is illustrated in Fig. 1a), but these could always be accounted for by differences in the radial locations of the recording sites [Cooper, 1999b]. On three occasions, there were also systematic differences between the response phases observed on and around the beads (the largest of these is illustrated in Fig. 1c). These phase differences were always consistent with differences in the longitudinal locations of the sites studied, assuming that the displacement waves travel from the base to the apex of the cochlea. In general, the closest matching responses on and around the beads were observed when the distance between the two recording sites was minimized and the recordings were made at similar radial positions (cf. Figs. 1 b, d).

Attempts to make recordings before and after placing single reflective microbeads in individual preparations were unsuccessful (the final locations of the microbeads were too difficult to predict). However, two lines of indirect evidence suggest that the actual placement of the microbeads had little effect on the mechanics of the cochlear partition. First, as shown in Fig. 2, comparisons between preparations that were known to be in good physiological condition revealed very similar characteristics when microbeads were used

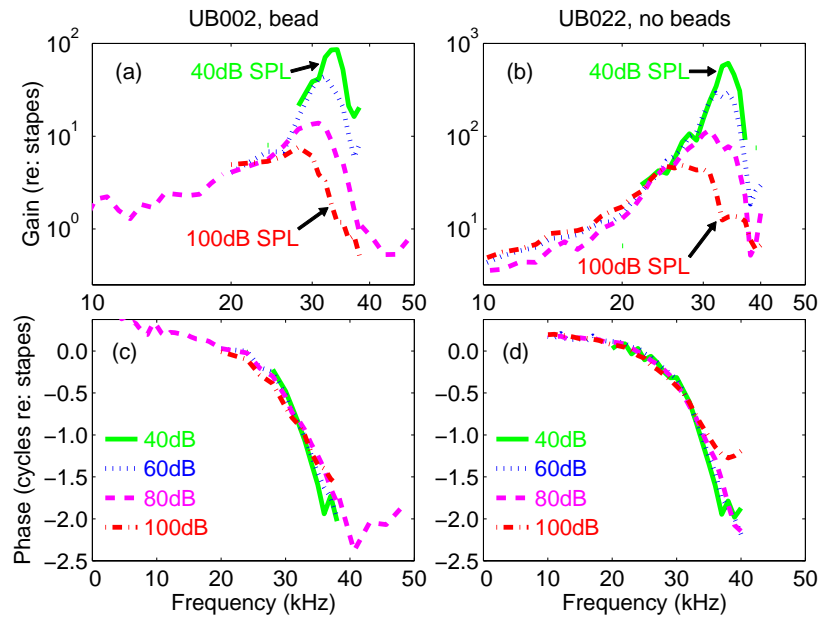


Fig. 2. Level-dependent tuning characteristics measured with (a,c) and without (b,d) the use of microbeads in two separate experiments. Sound pressure levels (dB re: $20 \mu\text{Pa}$) ranged from 40 to 100 dB in 20 dB steps. Recording were made ~ 1.5 mm from the basal end of the cochlear partition, ~ 20 and $\sim 60 \mu\text{m}$ from the osseous spiral lamina in UB002 and UB022, respectively.

(Figs. 2 a, c) and were not used (Figs. 2 b, d). Once again, there were differences (in this case on the order of 20 dB) between the absolute sensitivities of the different preparations, but these could probably be accounted for by interanimal variability (including variations in the radial locations of the recording sites on the basilar membrane). The most important features to note in Fig. 2 are the similarities between the *shapes* of the tuning curves and their nonlinear variation with sound pressure level. These similarities are consistent with the suggestion that the beads have little effect on the ‘normal’ operation of the cochlea (see discussion).

The second line of evidence that the beads had little effect on the operation of the cochlea is illustrated in Fig. 3: very little difference (typically $< \pm 3$ dB) was observed between the CAP thresholds recorded before and after placement of the beads on the basilar membrane. The supra-threshold amplitudes of the CAP and other potentials (e.g., summing potentials) often varied more than the CAP thresholds themselves (although not in the example illustrated in Fig. 3). The most likely reason for this is that the current paths in the cochlea changed when the cochlea was opened and/or the perilymph was manipulated.

An overall comparison between the sharpness of tuning observed with and without the use of reflective microbeads is shown in Fig. 4. The $Q_{10\text{dB}}$ values in this figure quantify tuning sharpness by dividing each preparation’s peak frequency by its bandwidth at points 10dB below the peak. The fact that the reference line (showing $Q_{10\text{dB bead}} = Q_{10\text{dB BM}}$) intersects all of the error bars in Fig. 4 indicates that the data are consistent with a hypothesis that motion of the beads follows that of the basilar membrane.

4. Discussion

The results of this investigation indicate that reflective microbeads (i) follow the motion of the structures on which they are placed, and (ii) do not affect this motion dramatically. These

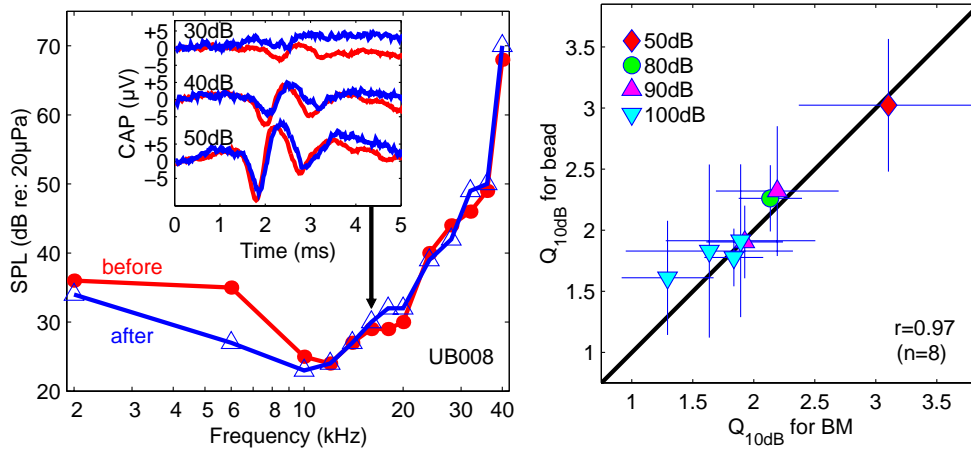


Fig. 3 (left). CAP audiograms and responses to 16 kHz tones (inset) before and after placing a microbead ~3.5 mm from the basal end of the cochlear partition (cf. Fig. 1 a, c).

Fig. 4 (right). Sharpness of tuning on the basilar membrane (BM) and on individual microbeads in 8 experiments. Q_{10dB} values quantify the sharpness of tuning by dividing peak frequencies by the bandwidths 10 dB below the peak. Error bars depict 95% confidence intervals (cf. Fig. 1). Sound pressure levels (see key) are expressed in dB re: 20 μPa.

findings lend direct support to the findings of previous studies that have used similar types of microbead [e.g., Cooper and Rhode, 1997]. The consistencies between these studies and studies that have used different types of microbead [e.g., Ruggero *et al.*, 1997], or even radioactive sources [e.g., Rhode, 1971; Sellick *et al.*, 1982], allow this support to be extended to numerous previous observations. Perhaps the most important of these observations are that basilar membrane responses are sharply tuned, nonlinear, and physiologically vulnerable.

The major weakness of the data in this report is the lack of ‘before and after’ observations from individual preparations that were known to be in good physiological condition. This is unfortunate, and may well be overcome in subsequent experiments. In the meantime, the strongest evidence that the beads have little effect even under near-optimal physiological conditions (Figs. 2 and 3) is indirect. It should be noted that the data of Fig. 2 (either with or without beads) demonstrate much more nonlinearity and better tuning than those in previous studies of the hook region basilar membrane [e.g., Cooper and Rhode, 1992]. One reason for this is that these preparations were in much better condition than those reported previously; their CAP thresholds of 35-40 dB SPL at ~34 kHz were 10-15 dB lower than those observed in most experiments (cf. Fig. 3). The fact that the beads led to little or no deterioration in the CAP thresholds when the thresholds were so low to begin with is the strongest evidence to date that the beads have little effect on the operation of the cochlea.

4.1 Comparison with previous studies

The conclusions of the present study diametrically oppose those of the only other investigation of this kind to be performed to date [Khanna *et al.*, 1998]. Various methodological differences may mean that the two sets of conclusions are not actually inconsistent with one another, however. In the view of the present author, the most important of these differences are likely to relate to the longitudinal positions of the sites studied. This issue will be expanded below. Other methodological differences, such as the

exact type of microbead used, the orientation of the recording sites when the vibration measurements are made, and the physiological condition of the preparations are likely to be less important. (This statement is based on the author's own observations using various types of microbead in a wide range of cochlear preparations.)

The most obvious difference between the studies of Khanna *et al.* (1998) and those reported here is that they were performed in different parts of the cochlea. This may have profound consequences. The placement of beads in the apical turn of the cochlea involves contact with the luminal surfaces of specialized epithelial cells known as Claudius cells. Both Khanna *et al.* (1998) and Rhode and Cooper (1996) have commented on the difficulty of getting a microbead to 'stick' to these cells. In contrast, the placement of beads in the more basal turns of the cochlea involves contact with cells that line the tympanic face of the basilar membrane. Anecdotal evidence suggests that these cells provide much more grip for the microbeads. It is rare for a bead to slip or drift away from its initial landing place in the basal turns of the cochlea, whereas it is rare for one *not* to run away in the apical turns. Another difference between the approaches to the basilar membrane in the apical and basal turns of the cochlea concerns optical access. It is relatively simple to obtain near perpendicular access to the basilar membrane in the basal turn of the cochlea, whereas perpendicular access in the apex is hindered by the highly reflective surfaces of the lipid droplets in the Hensen's cells just above the basilar membrane. The presence and orientation of the bony septum just below the basilar membrane can also be problematical in the apical turn of the cochlea. These features make unambiguous recordings of basilar membrane motion very difficult to achieve (without the use of microbeads) in the apical turn of the cochlea, and prevent the present author from making direct comparisons with the data of Khanna *et al.* (1998).

Acknowledgments

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