

# Basilar Membrane Velocity in a Cochlea with a Modified Organ of Corti

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**ABSTRACT** Many cochlear models assign zero longitudinal coupling in the cochlea. Although this is consistent with the transverse basilar membrane (BM) fibers, the cochlear partition contains cellular longitudinal coupling. In cochlear models, longitudinal coupling diminishes passive BM tuning; however, it has recently been employed in theories of active mechanics to enhance tuning. Our goal in this study was to probe passive longitudinal coupling by comparing BM responses in damaged cochleae with passive responses in normal cochleae. The cochleae of gerbils were damaged with intratympanic neomycin followed by a waiting period to ensure that all of the cells of the partition were missing or severely disrupted. We then measured BM motion and examined the cochleae histologically. In comparison with passive responses in normal cochleae, we observed a downward shift in characteristic frequency, an expected consequence of reduced stiffness from cellular damage. However, we did not observe enhanced passive tuning in the damaged cochleae, as would be expected if longitudinal coupling were substantially greater in the normal cochleae. Thus, we conclude that cell-based longitudinal coupling is not large enough to influence passive cochlear mechanics. This finding constrains theories of active mechanics.

## INTRODUCTION

In mammalian hearing, incoming sound is mechanically sorted by frequency along a long narrow strip of sensory tissue within the cochlear partition (CP), which contains the cellular organ of Corti (OC). The passive substrate of CP stiffness and cochlear fluid mass gives rise to the cochlear traveling wave and mild peaking, and cell-based forces boost the local response by a factor of hundreds and sharpen the frequency response. Several models employ the longitudinal tilt of the Deiter cells processes and the outer hair cells (OHCs) to control the operation of this cochlear amplifier. (1,2). In these feed-forward models, longitudinal coupling works with the traveling wave to provide forces with the proper phase to pump up the traveling wave amplitude over a particular range of wavelengths and thus frequencies. This hypothetical utility of longitudinal coupling in active models is a departure from passive models in which increased longitudinal coupling broadens the tuning and makes the phase-versus-frequency curves less steep (3–5). Because the broadened tuning conferred by longitudinal coupling is inconsistent with the tuning that is present in passive preparations, in most passive models the CP is treated as transverse segments that are only coupled longitudinally via the cochlear fluid. The transverse collagen fibers of the basilar membrane (BM) (6,7) support this representation as long as the BM is the dominant stiffness. However, the cells do contribute to stiffness, especially the pillar cells (8–11).

In this study, we sought to determine whether longitudinal coupling plays a role in passive tuning. We used neomycin to severely damage the OC (12–14) and then compared the

BM motion responses in these cochleae with passive responses in undamaged cochleae. Based on the anatomy, Deiters cell processes and OHCs (Fig. 1) will impart longitudinal coupling. These cells are relatively fragile and thus are readily disrupted by neomycin damage. The tectorial membrane possesses longitudinal coupling (15,16) and is expected to detach from the OC once the OHCs are eliminated. Additionally, the stiff pillar cells are tightly coupled longitudinally at their heads. These cells are relatively robust, and our damage protocol was designed to also eliminate them.

The active process of cochlear mechanics is fragile (17), and in our untreated control cochleae the active process was present in some cases and absent in others, due either to a just-postmortem condition or to simple exposure of the cochlea. In active cochleae, we acquired passive-normal data by working at high sound pressure levels, where the contribution of the active process was expected to be small. In addition, because the phase excursion to the characteristic frequency (CF) is known to be nearly independent of the degree of activity (18), we used the phase to look for frequency shifts when comparing the two populations. Our treated cochleae all lacked hair cells but had varying degrees of overall damage, ranging from complete loss of the basal OC to transdifferentiated cells with pillar cells that were still apparent, though altered. Thus, our treated cochleae lie along a spectrum and their BM motions also lie along a spectrum. Nevertheless, they were able to inform us about the mechanical effect the cells of the OC have on passive cochlear tuning.

It would be interesting to specifically examine the role of longitudinal coupling in active mechanics by other methods, such as damaging the Deiters cells while leaving the OHCs intact, but this is not currently possible. Because the active

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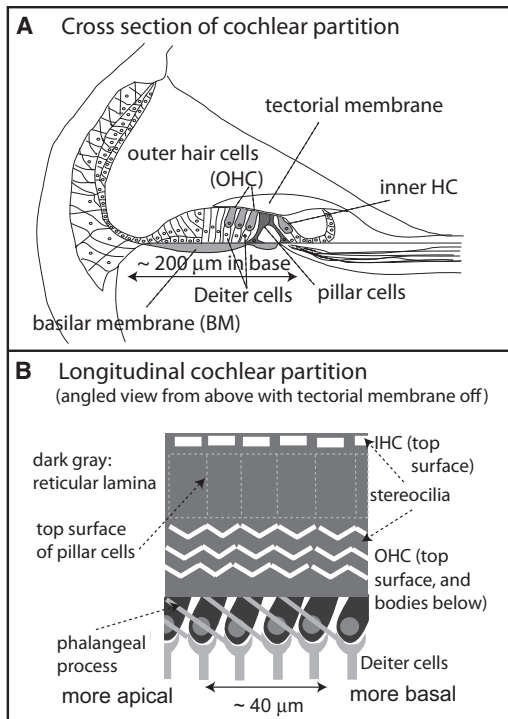


FIGURE 1 (A) Cross-sectional view of the CP. (B) A longitudinal view, angled from the top, with the tectorial membrane excluded. The longitudinal tilt of the Deiters cell phalangeal processes and OHCs is evident. The top surfaces of the pillar cells form a continuous sheet, part of the reticular lamina.

cochlea is built on the passive substrate, our findings impact on active cochlear mechanics.

## MATERIALS AND METHODS

### Preparation of animals with damaged cochleae

Experiments were conducted in accordance with the guidelines of Columbia University's Institutional Animal Care and Use Committee. Gerbils (*Meriones unguiculatus*), 50–70 g in mass, were used in the study. Anesthesia was induced with ketamine (40 mg/kg) and xylazine (5 mg/kg) and maintained with half doses of ketamine every hour. Yohimbine (1 mg/kg) was administered to reverse the xylazine at the end of the procedure. Core body temperature was maintained at 37°C with a heating blanket and rectal thermometer. After it was anesthetized, the gerbil was positioned on its dorsum. By using an aural speculum, we were able to visualize the tympanic membrane of the left ear with an operating microscope. We then placed 0.05 ml of 2% lidocaine into the ear canal and left it there for 10 min. After the lidocaine was absorbed, a tear was made in the pars flaccida with a minutiae pin. Using a micromanipulator and a 100  $\mu$ l Hamilton syringe, we injected the middle ear space with ~30  $\mu$ l of 10% neomycin sulfate to approximately fill the space. After surgery, the gerbils recovered with the left ear uppermost. They were observed for 2–3 h for signs of dizziness and distress, and were subsequently returned to the animal facility and monitored regularly for signs of dizziness, such as severe head tilt. One animal (out of 20) appeared to be severely dizzy and was euthanized; however, the others recovered and appeared unstressed by the procedure. It was previously shown that the pars flaccida can be opened without adversely affecting hearing at frequencies above a few kilohertz (19); moreover, in our animals, the pars flaccida had resealed by the time the BM motion measurements were obtained.

### Preparation for measurements of BM motion

After completion of the 6-month waiting period, BM velocity measurements were performed on the treated and untreated cochleae. The gerbils were sedated with ketamine (40 mg/kg) and anesthetized with sodium pentobarbital (60 mg/kg). Buprenex (0.2 mg/kg) was used every 6 h as an analgesic. Supplemental sodium pentobarbital was given as needed to maintain deep anesthesia. A tracheotomy was performed to maintain a patent airway. The animal's core body temperature was maintained at 37°C. The top of the gerbil's skull was exposed and fixed to a heated head-holder by dental cement. The head-holder was securely attached to a motorized three-axis stage with position readout. The left pinna was removed, leaving a short length of external auditory meatus to which a closed-field sound-delivery system was connected. The bulla was widely opened to allow good visibility of the round window (RW). The bony edge of the RW was shaved slightly with a sharp blade. Part of the RW membrane was removed with a minutiae pin. The fluid level stabilized without the use of a coverslip, and the BM was visualized for measurements with the laser interferometer. After the BM motion was measured, the animal was euthanized with pentobarbital.

### Histological preparation and assessment

After the mechanical measurements were completed all of the neomycin-treated cochleae and several of the control cochleae were assessed histologically. The cochleae were isolated and removed intact from the temporal bone. A needle was used to fashion a small hole in the apex to perfuse fixative into the cochlea. The excised cochleae were then immersed in fixative (2.5% glutaraldehyde, 1.5% formaldehyde in .065 M phosphate buffer) for 24 h. The cochleae were decalcified in a 120 mM EDTA, pH 7 solution over 5 days and washed in phosphate buffer. The cochleae were then immersed in 0.1% Osmium Tetroxide for 30 min and then washed with phosphate buffer. They were dehydrated with increasing concentrations of ethanol and then impregnated with increasing concentrations of Epon 812 epoxy resin in propylene oxide. The specimens were embedded in fresh Epon resin, put under vacuum for 24 h, and then kept in a 60°C oven for 24 h. Then 1.5  $\mu$ m thick sections were mounted on glass slides, stained with Toluidine blue, and examined by light microscopy.

### Stimulus presentation and data collection

A speaker coupled to the ear canal provided a closed-field acoustic system. The system used a plastic T-tube of 1/8 inch diameter, with one branch of the T connected to the ear canal and the opposite branch providing access to the microphone's probe tube. The middle branch of the T was connected to a super tweeter (Radio Shack, Fort Worth, Texas) by an 8 cm long tube. Acoustic stimuli were generated digitally with a System 3 signal processing system (Tucker Davis Technologies, Alachua, FL) operating with a sampling period of 5  $\mu$ s. Stimuli were controlled using programs written in MATLAB (The MathWorks, Natick, MA) and Visual Design Studio (Tucker Davis Technologies). The sound pressure level was calibrated in the ear canal within 3 mm of the tympanic membrane by means of a probe microphone (Brüel & Kjær, Nærum, Denmark).

### Compound action potential

The compound action potential (CAP) response of the auditory nerve was measured via an electrode placed on the bone surrounding the RW (20). Tone pips of 3 ms duration and frequencies between 0.5 and 40 kHz were used to determine the CAP threshold. The stimulus level was increased until a visually determined response level was established.

### Distortion product oto-acoustic emissions

Distortion product oto-acoustic emissions (DPOAEs) were measured as previously described (21). The f<sub>2</sub>:f<sub>1</sub> ratio was fixed at 1.25, with primary

levels usually at an 80 dB sound pressure level (SPL, referenced to a 20  $\mu$ Pa peak). Stimulus frequencies were swept from 2 to 30 kHz.

### Laser interferometer data collection

A Polytec laser vibrometer (OFV-534 and OFV-5000 VD06) was used to measure BM motion. Sound stimuli were swept from 0.5 kHz to 40 kHz at constant SPL. The stimuli were delivered one frequency at a time, with frequency steps of 0.5 kHz below 20 kHz and 1 kHz above. The stimulus was composed of continuous repetitions of a 4096 point waveform. Frequencies were fixed so that an integer number of periods fit within the 4096 points. The first 4096 points of the recorded signal were discarded to eliminate the transient response. The duration of the remaining 50 recorded repetitions was  $\sim$ 1 s. We time-averaged, stored, and Fourier-analyzed these signals using MATLAB to find the amplitude and phase at the stimulus frequency. By looking along the direction that defines the cochlear spiral, we were able to access a region with CF  $\sim$  20–25 kHz through the RW opening (22,23). The vibrometer's laser beam was focused on the BM, typically at a position that was approximately centered on the BM width. In most animals, several measurements were made over a longitudinal extent of  $\sim$ 200–300  $\mu$ m. The signal strength of the interferometer and a sharp image of the BM determined the focal plane of the laser beam. Data were collected over a range of SPL. The treated animals all had linear BM responses, and an 80 dB SPL was mainly used.

### Quantitative analysis

To compare the treated and untreated animals, we used three different measures as described below:

1. Q10dB. Q10dB, a measure of the sharpness of tuning, was found as  $f/\Delta f$ , where  $\Delta f$  is the full width of the amplitude curve 10 dB down from the maximum response, and  $f$  is the frequency where the response peaked. We determined Q10dB from a smoothed version of an amplitude curve because the amplitude curves had structure superimposed on an overall broad peak; it was the broad peak we wished to evaluate. We used responses at relatively high stimulus levels to compare normal-passive responses in our untreated preparations with responses in our treated preparations.

2. Characteristic frequency (CF(phase)). The CF is defined as the frequency at which the response peaks at low stimulus level (18). Many studies have observed that at the CF, the phase of the BM velocity response is  $\sim$ 1.5–2 cycles regardless of the stimulus level; in other words, the phase does not shift in frequency with the stimulus level (see Figs. 5 and 8). In contrast, the peak frequency does shift, and the higher stimulus level (less active) response peaks at frequencies below the CF (18). Because it was useful to include a metric that was not influenced by the degree of activity, we found the response CF using the phase. We used the point where the phase was  $-1.7$  cycles to find CF(phase), where we insert (phase) to emphasize that we find CF using phase. (The phase in our results was referenced to ear canal pressure and phase is sometimes referenced to stapes motion; the middle ear delay amounts to  $\sim$ 0.5 cycle at 20 kHz (24).)

3. Phase-frequency slope. The phase-frequency slope is steep in the region of the CF and theoretically will be steepened by a reduction in longitudinal coupling. Thus, our third measure for comparison is this slope, in units of cycles/octave. Because the phase slope at CF(phase) is influenced slightly by activity (see Figs. 5 and 8), we used responses obtained at relatively high stimulus levels to compare normal-passive responses in our untreated preparations with responses in our treated preparations. The results are presented with frequency on a log scale so that Q10dB and phase-frequency slope can be readily compared.

## RESULTS

We used 10 gerbils to determine the appropriate intratympanic neomycin damage procedure (12–14), and several

others to determine the approach for BM motion measurements. After we established the method, we treated 10 animals with neomycin (experiments 23–28, 30–32, and 35) and measured the BM motion. The BM motion measurement from exp. 32 was not successful, and exp. 27 was excluded because the approach was not the same as in the other experiments. As controls we used four additional ears: two with intratympanic saline (exps. 33 and 34) and two with no treatment (exps. 22 and 29). Even though exp. 33 did not have neomycin treatment, the observations (histology, CAP, DPOAE, and BM velocity) were all similar to those for a neomycin-treated ear. Therefore, either this cochlea was damaged severely through some other means or it was accidentally treated with neomycin instead of saline. We excluded it from the results. The intratympanic neomycin treatments were done in two groups (group 1: exps. 23–28; group 2: exps. 30–32 and 35). Even though the treatment and wait times were the same, the first group showed more extreme histological damage.

### Determining the damage protocol

The first set of histological experiments pointed to the need for a  $\sim$ 6-month waiting period after neomycin injection to eliminate or severely alter the relatively robust pillar cells. Cochleae evaluated at a 3-month time point retained pillar cells. Fig. 2 shows results from three of these cochleae (11GR = 6 months, 13GL = 5 months, and 15GL = 7 months). Also shown is a control cochlea (1GL) in which there was no injection or waiting period, and it appears normal. The photos are all from turn 1 of the 2.5-turn gerbil cochlea. In all of the damaged cochleae, turn-1 hair cells are completely missing, and the tectorial membrane is either missing or well lifted off from what remains of the OC. Very disfigured pillar cells are still apparent in 13GL but not in 11GR, and in 15GL the entire OC is missing in turn

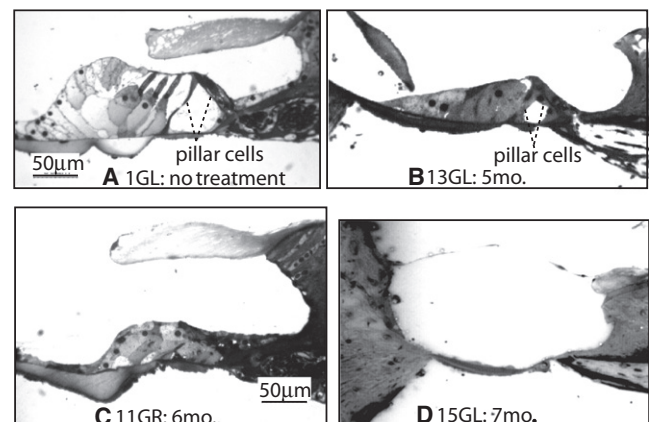


FIGURE 2 (A) Cochlea (turn 1) of an untreated animal. (B–D) Cochleae (turn 1) of animals treated with intratympanic neomycin after waiting periods of 5–7 months, showing variability in damage after an  $\sim$ 6 month waiting period.

1. Histological processing often results in distortions, such as bent pillar cells and distorted tectorial membrane (25). We are interested in gross changes to the cells of the OC, not the distortions that are apparent even in control ears, such as the double-bowed shape of the BM in cochlea 1GL. For our study, the damage to 11GR (Fig. 2 C) would have been optimal because although longitudinal coupling due to OHCs and pillar and Deiters cells would have been missing, the overall mass of the OC would have been close to normal. However, the damage was variable. Histological results are presented along with the BM motion results. After establishing the damage protocol, we initiated a multi-component study, starting with treatment and a wait period, followed by measurements of BM motion, CAP, and DPOAE, and histological evaluation.

### Multicomponent study

#### CAP and DPOAE

The CAP thresholds and DPOAEs for control exps. 34 and 22 were similar to control results from other studies (21). The control animal in exp. 29 died prematurely due to an inadvertent overdose of anesthetic, so data for that experiment are not available. In the neomycin-treated ears, CAP could not be elicited at any SPL, and DPOAEs were beneath the noise floor except at the lowest frequencies. Hair cells are relatively fragile, and, as expected, turn 1 hair cells did not survive the neomycin damage (Fig. 2). However, in this study we employed damage that went beyond hair cells to the passive cellular-structural properties of the OC. To relate this damage to changes in BM

motion responses, we emphasize the histological results because they are most pertinent. The CAP and DPOAE results provide independent verification of hair cell damage.

#### Histological results

Fig. 3 shows histological results from the eight neomycin-treated ears for which BM motion results are reported, and from control exp. 34, which was treated with intratympanic saline + a 6 month waiting period. Ears in the first set of experiments (Fig. 3, A–E) showed more extensive damage than those in the second set (Fig. 3, F–H). In all of the treated ears, the damage was extensive and hair cells were missing. In exp. 22 (Fig. 3 D) Reisner's membrane collapsed close to the CP. Pillar cells are still apparent in exp. 31 (Fig. 3 G), although they do not appear normal. In the saline-treated control (exp. 34; Fig. 3 I, dashed outline), hair cells are present and the pillar cells appear normal.

#### BM motion: further introduction to predictions

In the Introduction above, we described the predicted changes due to reduced longitudinal coupling and reduced stiffness. Before presenting the results, we show these predictions in Fig. 4. This highly schematized prediction is based on cochlear models in the literature, in particular, the studies of Steele and Taber (3,26). The solid black line corresponds to normal-passive tuning. Our study focuses on passive mechanics, and ideally we would like to compare our altered neomycin-treated cochleae with passive unaltered cochleae. In one of our control animals, the responses were measured just postmortem and thus were passive. In the other two control animals, high stimulus levels were used to approximate a passive condition. In addition, as

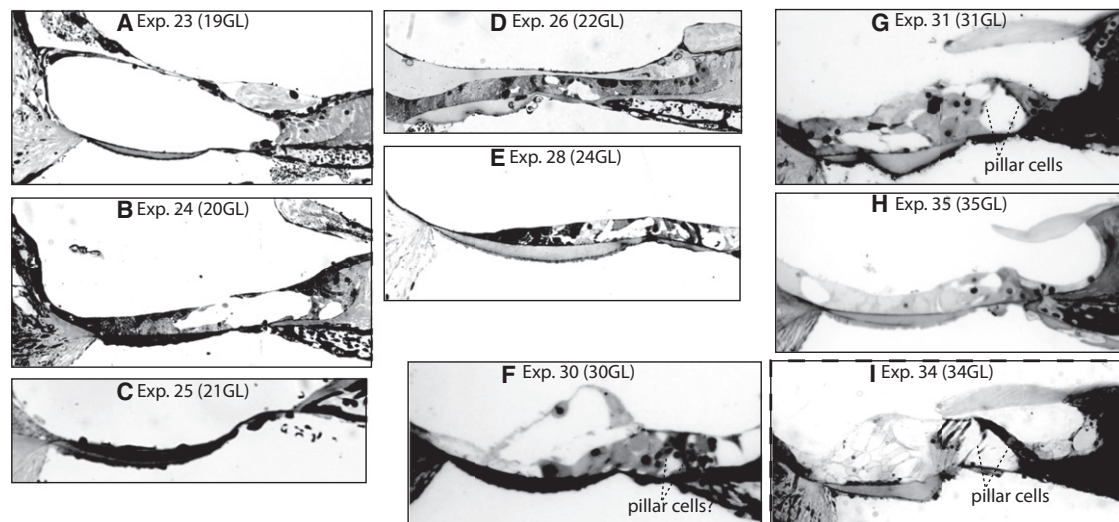


FIGURE 3 Histology in ears for which BM motion is reported in Fig. 7. The experiment number in the motion measurement is shown along with the histology number (in parentheses). (A–H) Cochleae (turn 1) of animals treated with intratympanic neomycin. The damage to the OC was extensive but variable, and generally more extreme in the first experimental group (A–E) than in the second group (F–H), where a remnant of pillar cells is apparent. (I) Cochlea (turn 1) of a control animal treated with intratympanic saline. The pillar cells, hair cells, and tectorial membrane appear normal. The CP is ~200  $\mu\text{m}$  wide at this location; see scale bars in Fig. 2.



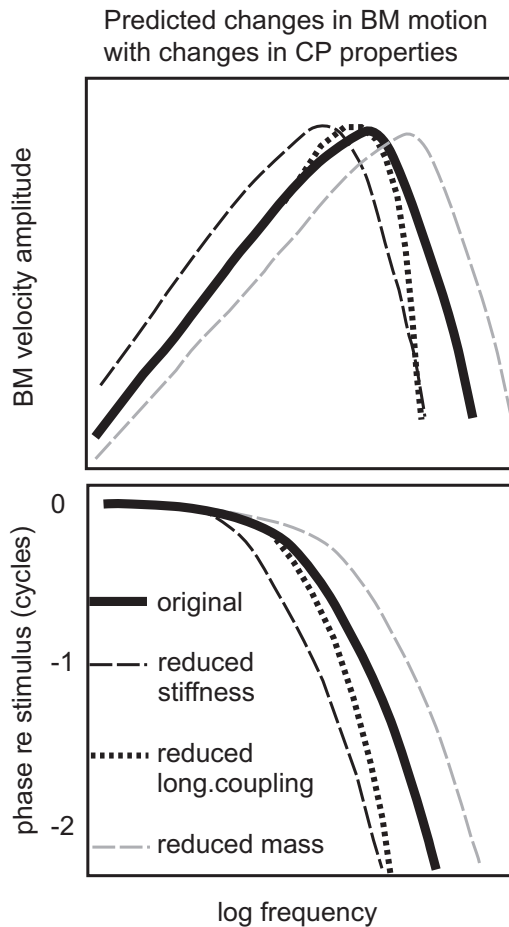


FIGURE 4 Predicted changes in BM motion with changes in CP properties. The solid black line indicates normal passive BM tuning. Black dotted lines show changes predicted due to decreased longitudinal coupling. Dashed lines show changes predicted due to reduced stiffness (*black*) or mass (*gray*). Neomycin damage may cause reduced stiffness and reduced longitudinal coupling, so those changes are what we look for in our results. When the OC is missing, we may see an effect due to reduced mass.

explained above, the CF(phase) metric is insensitive to the degree of activity.

Returning to the comparison curves in Fig. 4: The structural changes in the OC due to neomycin damage are expected to reduce the cellular stiffness (8,9,10) and longitudinal coupling. We are interested in whether the changes in longitudinal coupling will be apparent in the mechanical response to sound. The effect of reduced stiffness alone is to shift the response so that at a given location, the amplitude peak and the region of rapidly varying phase are shifted to lower frequencies; reduced mass alone has the opposite effect. Most of our histology results show that the damaged OC had approximately the same overall size as the normal OC, but in some cases the size was much reduced. On the other hand, the effective OC mass might be less than the actual mass (22), so changes in BM motion responses due to changes in OC mass might be small. (Changes in viscous damping in the OC tissue might occur with damage, but we

did not measure or consider resistance changes in this study. In untreated cochleae, the OC mechanical impedance due to stiffness dominates that due to resistance (27).)

The effect of reduced longitudinal coupling alone is to sharpen the amplitude peak and at the same time increase the slope of the phase-frequency curve. This is because reduced longitudinal coupling makes it easier for the wavelength to shorten. With no longitudinal coupling, there is nothing in the structure that impedes one longitudinal section from moving up while its neighbor moves down, whereas longitudinal coupling impedes this relative motion. A shorter wavelength corresponds to an increased phase-frequency slope.

In summary, a change in the tuning sharpness and phase-frequency slope implies a change in longitudinal coupling, and a change in CF(phase) implies a change in stiffness or mass.

#### BM motion: results

We begin by comparing two BM motion studies: exp. 22 and exp. 23. Exp. 22 involved an untreated nonlinear cochlea, and in Fig. 5 we show its normalized BM responses at several stimulus levels. This experiment was done early in

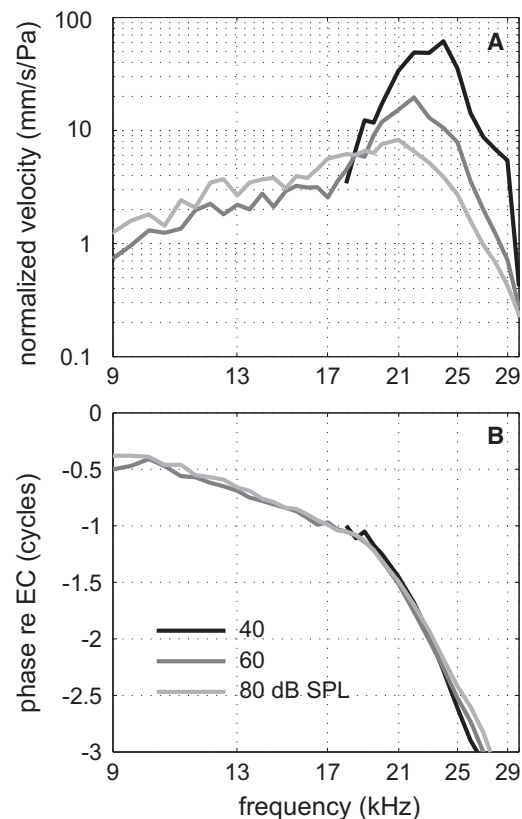


FIGURE 5 In vivo BM responses from an untreated preparation (exp. 22). The responses show sharper tuning at low stimulus levels than at high levels, the characteristic response of a healthy cochlea in which cochlear amplification is functioning. We used the 80 dB SPL response to approximate the passive case.

the study and we did not do histology on this cochlea. Its CAP and DPOAE responses were normal. The 80 dB SPL responses of exp. 22 were used for comparison with neomycin-treated ears. High-level SPL responses ( $\geq 80$ –90 dB SPL) show little of the nonlinear boosting observed at low-to-moderate levels, and approximate the passive responses of a just-postmortem preparation (17,23,28). Exp. 23 involved a treated cochlea (histology shown in Fig. 2 A). Fig. 6 compares the BM velocity responses obtained from the two animals, all taken at 80 dB SPL. The treated responses (exp. 23) were clearly shifted to lower frequencies: the amplitude peak shifted from  $\sim 20$  kHz (untreated) to  $\sim 13$  kHz (treated), and CF(phase) shifted from  $\sim 23$  kHz to  $\sim 16$  kHz. Motion was measured over a  $\sim 300$   $\mu\text{m}$  range of longitudinal locations in exp. 23, and passive tonotopic tuning was apparent. Even the highest-frequency curve of exp. 23 was substantially lower in frequency than the exp. 22 curves. In terms of tuning sharpness ( $Q$ ) of the amplitudes, the two cochleae were similar, and the slopes of the phase-frequency curves were also similar. Thus, the treated results appear to arise from a CP with reduced stiffness, but not reduced longitudinal coupling, compared with the normal cochlea. Because the cellular elements that we expect to give rise to longitudinal coupling were missing in the treated specimen (Fig. 3 A), we

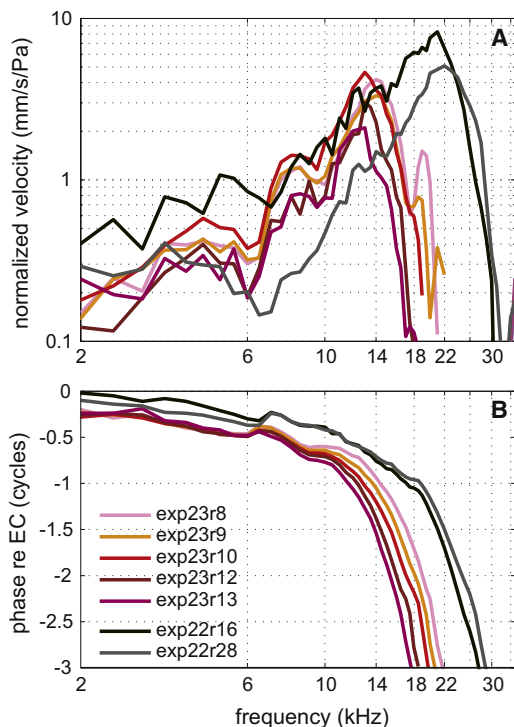


FIGURE 6 Comparison of BM responses in control (exp. 22) and neomycin-treated (exp. 23) animals. All data were collected at 80 dB SPL, which can be considered passive responses in the control animal. The different runs correspond to slightly different longitudinal locations, which in exp. 23 ranged over a longitudinal distance of  $\sim 300$   $\mu\text{m}$ .

conclude that these elements do not give rise to substantial passive longitudinal coupling in normal cochleae.

Further results are presented in Figs. 7 and 8. Fig. 7 shows grouped results from 12 animals (four untreated and eight treated). Several runs were made in each animal, and we selected one from each set of runs. When there were several runs at different longitudinal locations, we chose the middle one (as for exp. 23), and we selected runs for which the data were relatively clean.

The untreated animals are referred to as exps. 22, 29, 34, and ORexp8. ORexp8 is from a previous study (22) and was included to bolster the control results; however, the statistical analysis includes only the experiments performed in this study. These data, taken from the same approach as for this study, are very compatible with the results of this study. The data were obtained in vivo, but the basal cochlea was passive after exposure of the cochlea. Runs from two positions in exp. 22 are included to show that the size of the response can be quite variable (only run 16 is used in the statistical analysis). Such variation was previously documented in a study of the transverse profile of BM motion (28), and could also be related to variations in the angle between the laser and the BM surface at different positions. The size difference does not affect the  $Q$ -value. Exp. 29 involved the animal that died just before BM motion was measured. The similarity of the results obtained from exps. 22, 29, and ORexp8 attests to our ability to measure similar responses from active cochleae at high levels (exp. 22), from just-postmortem preparations (exp. 29), and from cochleae that were inadvertently linearized due to cooling or surgical trauma (ORexp8). The amplitude response from the active cochlea of exp. 34 had a more rounded shape, although the phase was similar to the others. In Fig. 8 we show a level series for this preparation, which showed substantial nonlinearity. The responses at 70–80 dB were more like the passive responses of the other, truly passive animals. However, we chose to use the 90 dB responses because, according to the general view of cochlear mechanics, the cochlear amplifier does less and less as the SPL increases, and thus the highest-level response will best approximate the passive response. An exception to this was observed in gerbils by Ren and Nuttall (23), who noted that in some cases the passive postmortem response was more similar to the 80 dB premortem response than to the broader premortem responses at 90 and 100 dB SPL. This interesting observation—that the cochlear amplifier can deamplify at very high levels and amplify at low and moderate levels—is worth further study.

Returning to the grouped comparison of Fig. 7, and eyeballing the  $Q$ , peak frequency, and slope of the phase-frequency curve, we can see that  $Q$  is similar in the two groups, except that the tuning of exp34r13 is milder, as discussed above. The peak frequency is clearly shifted down in the treated group, to  $\sim 14$  kHz from 20 kHz. As noted in Materials and Methods, guided by the established

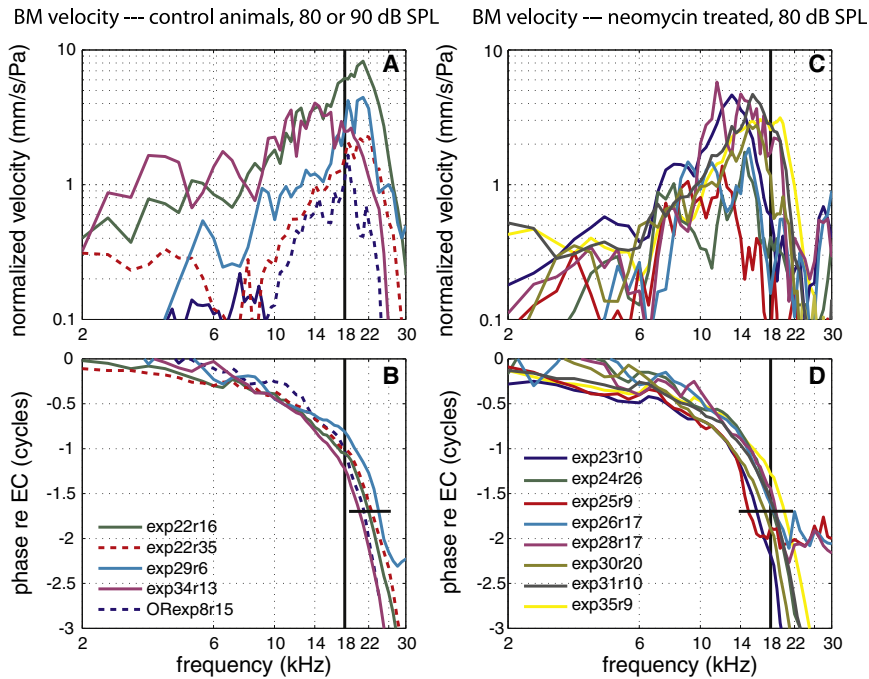


FIGURE 7 Comparison of BM responses in control (A and B) and neomycin-treated preparations (C and D). All data were collected at 80 dB SPL, except for exp34r13, which was collected at 90 dB SPL. These high-level responses can be considered passive responses in the control animals. exp29r6 was from a control animal that was just postmortem and thus passive. ORexp8r15 is from a different set of experiments and is included to bolster the passive-normal result. One representative curve per animal is shown, except for exp22, for which two curves are shown to demonstrate the range of response sizes that were measured in a single animal. ORexp8r15 and exp22r35 are shown in dashed lines to indicate that they were not used in the statistical comparison (ORexp8e15 because it is from a different study, and exp22r35 because we only included one result per animal). 18 kHz is indicated with a black vertical line to facilitate comparison of the two populations. A black horizontal line is drawn where the phase goes through  $-1.7$  cycles; this point is CF(phase). The slope of the phase-versus-frequency curve at this point was used as a representative measure of traveling wave wavelength.

relationship between CF and phase excursion (18), we chose the point where the phase went through  $-1.7$  cycles as our evaluation point (*bold horizontal line*), and refer to it as CF(phase). For the untreated cochleae, CF(phase) was 20–24 kHz, whereas for the treated cochleae it ranged from 15 to 20 kHz. The phase-frequency slope is predicted to steepen with reduced longitudinal coupling, and it was similar between the two groups. Thus, the only obvious change in the two groups was the clear downward shift in peak frequency and CF(phase). We noted that the damage varied in severity (Figs. 2 and 3), so we would expect changes in the BM responses of the treated cochleae to also show variability. In the treated cochleae, exp35r9 is the most like the untreated cochleae in terms of BM response, and from Fig. 3 H it can be seen that this preparation retained a loosely structured OC. Table 1 lists the CF (phase), phase-frequency slope (in cycles/octave) at the CF(phase), and Q10dB.

The average CF(phase) was 22 kHz for the three untreated cochleae, ranging from 20 kHz to 24 kHz, and for the treated animals it was 17.8 kHz, ranging from 15 to 20 kHz. The groups share a 20 kHz point but otherwise do not overlap. Results from a Student's *t*-test showed that the CF(phase) values for the two groups were significantly different. The phase-frequency slope was steeper for the untreated group ( $-3.87$  versus  $-3.07$  cycles/octave), but not significantly so, since each group had a standard deviation of  $\sim 1$ . The Q10dB values measured from the amplitude curves were similar in the two groups (1.8 for the treated and 1.95 for the untreated, with standard deviations of 1 and 0.4). Student's *t*-test showed that the two groups were not distinct in terms of the latter two measures.

## DISCUSSION

### Shift in CF

All of the treated cochleae exhibited traveling waves and tuning in BM motion, confirming that these characteristics are fundamental to the macromechanical system of the cochlea, which is a long flexible partition surrounded by fluid (29,30). The treated preparations showed a clear shift in CF to a lower frequency compared with the untreated preparations. Our CF(phase) metric was not sensitive to the degree of activity, and the CF shift signals a change in passive mechanics and indicates a significant reduction in CP stiffness. The cells of the OC are known to increase the CP stiffness, and the CF(phase) shift can be attributed to reduced cellular stiffness when the OC is missing or damaged.

We considered the possibility that we contributed to the observed change in CF(phase) by inadvertently measuring from different longitudinal locations in the different cochleae. Fig. 6 shows that a range of CFs can be detected within a given experiment. However, when measuring via the RW opening, it is unlikely that one could measure the region that in a normal cochlea has CF(phase)  $\sim 15$  kHz without using reflecting beads. In normal cochleae, we can just barely see the 20 kHz place, and the 15 kHz place would be  $\sim 650$   $\mu\text{m}$  further apical, down the cochlear tunnel (31). Ren and Nuttall (23) were able to reach the 14 kHz place, but only after removing the stapedial artery and further opening the RW opening to expose a large extent of turn 1. Thus, it is not possible that the CF(phase) separation we found between treated and untreated preparations was based on variability in the location of measurement.

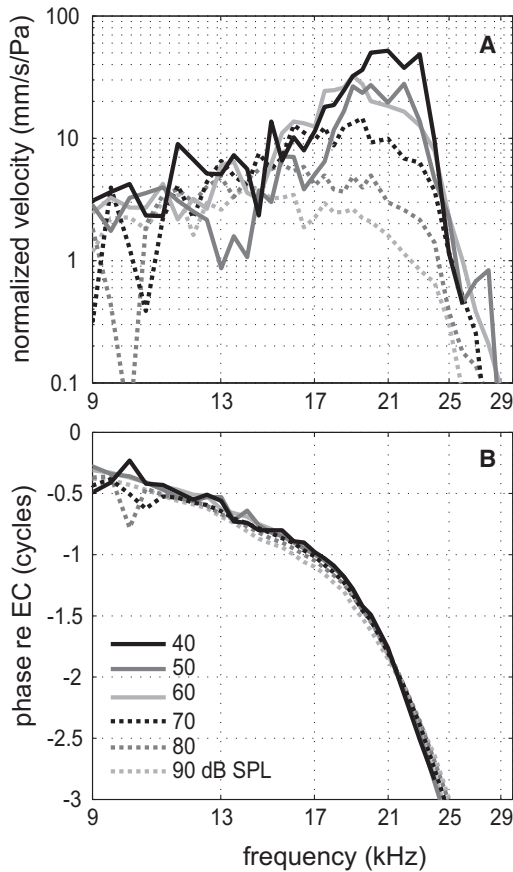


FIGURE 8 Exp. 34 was a control preparation in which intratympanic saline was injected, followed by a 6 month waiting period. In vivo BM measurements were obtained at the end of that period. The responses show sharper tuning at low stimulus levels than at high levels, the characteristic response of a healthy cochlea in which cochlear amplification is functioning. We used the 90 dB SPL response to approximate the passive case.

**No change in sharpness of tuning**

According to modeling predictions, a reduction in longitudinal coupling sharpens the tuning of the system and makes the phase-frequency curves steeper. The data show that there was no significant difference in Q10dB between the treated and untreated cochleae, and no significant change in the steepness of the phase-frequency curves. The fact that the CF(phase) changed without a change in tuning sharpness argues that the longitudinal coupling imparted by the cells of the OC is not large enough to impact passive mechanics.

**Considering the mass of the OC cells**

A point of uncertainty in cochlear models is whether the effective mass of the OC is equal to or less than the anatomical mass. Proponents of the latter view argue that the cells of the OC are like malleable bags of fluid that would appear mechanically like fluid, as opposed to a rigid mass. A previous experimental/theoretical study that addressed this

**TABLE 1 Statistical comparisons**

Identifier (experiment#run#)	CF(phase) = frequency where phase crosses -1.7 cycles (kHz)	Phase-frequency slope at CF(phase) (cycles/octave)	Q10dB
<b>Controls</b>			
exp22r16	22.0	-3.58	1.6
exp29r6	24.0	-4.91	2.8
exp34r13	20.0	-3.12	1.1
Average	22.0	-3.87	1.8
Standard deviation	2.0	0.93	0.87
<b>Treated</b>			
exp23r10	16.0	-3.55	2.0
exp24r26	18.5	-3.27	2.9
exp25r9	15.0	-1.77	2.0
exp26r17	18.5	-1.63	2.0
exp28r17	19.0	-4.83	1.4
exp30r20	17.0	-2.76	1.9
exp31r10	18.5	-3.00	1.7
exp35r9	20.0	-3.83	1.7
Average	17.82	-3.07	1.95
Standard deviation	1.65	1.05	0.4
Student's <i>t</i> -test results	<i>p</i> = 0.0063 ( <i>p</i> < 0.05: control and treated have different CFs.)	<i>p</i> = 0.28 ( <i>p</i> > 0.05: control and treated have similarly steep phase-frequency slopes)	<i>p</i> = 0.76 ( <i>p</i> > 0.05: control and treated have similar tuning sharpness)

question had equivocal results: whereas in the very base the effective mass coincided with (or was even greater than) the anatomical mass, the size of the effective mass was less than the anatomical mass in the more-apical regions of turn 1 (22). It was noted in that study that the analysis technique was relatively less reliable in the very base. In the study presented here, the mass of the OC was essentially missing in the basal turn of two of our experimental cochleae (exps. 23 and 25; Fig. 3, A and C), so the question regarding the role of mass can also be addressed. The predicted effect of reduced mass is a shift in CF(phase) to higher frequencies, whereas the predicted effect of reduced stiffness is a shift to lower frequencies. Because these two experiments had the clearest shift toward low frequencies, we conclude that the primary effect of the OC is to increase stiffness, not to increase mass. However, the histology usually did not indicate total loss of the OC and never indicated total loss throughout the cochlea, so it is difficult to say just how much the mass changed in the treated cochleae.

**Previous measurements**

Previous studies have probed longitudinal coupling by visualizing the response to static displacement, but the results were not definitive (32–34). To our knowledge, this is the first study to measure BM responses in neomycin-damaged cochleae; however, several previous studies measured BM



responses in cochleae in which the OC was damaged. In apical measurements in guinea pig, Cooper and Rhode (35) found little change in response after localized removal of the OC. Ulfendahl and colleagues (36) measured apical BM motion in a strain of guinea pig in which the OC underwent progressive deterioration. They found reduced  $Q$  compared with normal cochleae, and a downward shift of peak frequency. Apical measurements are not directly comparable with our basal measurements, and the results are not the same: we found a shift in peak frequency and CF(phase) to lower frequencies, but no change in  $Q$ . Kohlöffel (37) made BM displacement measurements and followed them several days postmortem. Over time, the peak shifted to lower frequencies and broadened, with a decrease in phase-frequency slope. Steel and Taber (38) surmised that these results were due to a postmortem decrease in the stiffness of the BM fibers and an increase in the longitudinal coupling of the BM's amorphous ground substance. Cooper (28) measured basal BM responses to high SPL stimuli at four time points relative to death:  $-1$  h, 0 h (just postmortem), 7 h, and 16 h. Between  $-1$  h and 0 h the amplitude diminished slightly, the peak frequency shifted down in frequency slightly and the phase changed very little. Over 7 and then 16 h, the peak frequency and CF(phase) shifted downward substantially. There was no notable change in  $Q$ . Cooper (28) interpreted these results as evidence that over hours postmortem, the stiffness of the CP decreased. Finally, BM motion measurements were recently obtained in a mouse with genetically modified tectorial membrane (16). With elimination of the *Tectb* protein, the fibrous structure of the tectorial membrane was disorganized compared with that of the wild-type. The tuning was sharpened and the phase-frequency curve steepened slightly. The authors attributed the sharpened tuning to reduced longitudinal coupling in the altered tectorial membrane (implying significant longitudinal coupling in the wild-type). This result contrasts with our findings in this study, in which changes in passive BM motion responses indicated that longitudinal coupling was not mechanically significant in normal cochleae. However, the analysis of the *Tectb* mouse emphasized active mechanics, so the results are not directly comparable.

## CONCLUSIONS

In this study, our main goal was to determine whether longitudinal coupling in normal cochleae influences passive cochlear responses. To that end, we compared passive BM responses in normal cochleae to responses in cochleae in which the cellular structure of the OC had been severely damaged by neomycin. The results indicated a lack of significant longitudinal coupling imparted by the cells of the OC. The BM responses in treated cochleae indicated a decrease in stiffness, likely due to loss of the OC cells. Even if the BM was also altered by the neomycin, the

apparent lack of a significant change in longitudinal coupling can only mean there was no substantial longitudinal coupling to begin with. Most passive cochlear models do not include longitudinal coupling, and our results argue that this is appropriate. In contrast, the results of the above-mentioned *Tectb* mouse study (16), which emphasized active mechanics, indicated that longitudinal coupling was reduced in modified cochleae compared with wild-type. A parsimonious conclusion is that even a very small amount of longitudinal coupling can influence active cochlear operation, and this is consistent with a recent cochlear model (39).

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