

Relations among motions of the reticular lamina (RL), tips of hair bundles, and endolymphatic surface of the tectorial membrane (TM) in the tectorial region of the excised cochlea of the alligator lizard (*Gerrhonotus multicarinatus*) were investigated. Sound stimuli from 105-140 dB SPL measured in the fluid adjacent to the basilar membrane, with frequencies from 40 Hz to 4 kHz, were used to excite cochleae in nine preparations. Images taken with a video microscopy system were analyzed to determine three-dimensional motions of cochlear structures. Motions of these structures were generally broadly tuned in frequency, with peak amplitudes smaller than 1 micrometer peak-to-peak.

Three micromechanical relations were investigated as functions of frequency: (1) TM/RL, the motion of the endolymphatic surface of the TM normalized to RL motion; (2) (TM-RL)/RL, the shear between the endolymphatic surface of the TM and the RL, normalized to RL motion; (3) (Tips-RL)/(TM-RL), the ratio of the deflection of the tips of hair bundles relative to the RL to shear between the endolymphatic surface of the TM and the RL. Although there was considerable variation across preparations, two trends were evident. First, the magnitude of TM/RL was less than 2. Second, none of the micromechanical relations showed sharp frequency selectivity.

The measured magnitudes of TM/RL are small compared to those predicted by resonant TM models of cochlear micromechanics. This difference suggests that TM resonance does not significantly contribute to the frequency selectivity of the lizard cochlea. The frequency selectivity of the measured micromechanical relations is broad compared to that seen in responses of auditory nerve fibers innervating the TM region. Thus, mechanisms other than mechanical resonance may contribute to frequency selectivity in the tectorial region of the alligator lizard cochlea.

# Experimental measurements of micromechanical transfer functions in the alligator lizard cochlea

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## INTRODUCTION

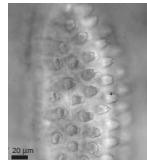
The alligator lizard cochlea has two regions. In the free-standing region, which has best frequencies from 1 to 4 kHz, hair bundles project directly into endolymph (Weiss et al. 1976). In the tectorial region, which has best frequencies from 200 to 800 Hz, hair bundles are capped by a tectorial membrane. The responses of auditory nerve fibers in the tectorial region are similar to those of mammals; tuning curves have  $Q_{10dB}$  values from 0.3 to 6.0 and high-frequency slopes from 40 to 700 dB/decade. Unlike the mammal, however, the lizard has a broadly tuned basilar membrane (Peake and Ling Jr., 1980). It has been suggested (Weiss et al. 1978) that sharp tuning in the tectorial region originates in micromechanics, as it does in the free-standing region (Holton and Hudspeth, 1983; Fishkopf and DeRosier, 1983).

To examine micromechanics in the tectorial region, we measured the gross motions of the tectorial membrane (TM), tips of hair bundles (Tips), and bases of hair bundles at the reticular lamina (RL). From these measurements we computed three micromechanical transfer functions: (1) TM/RL describes TM displacement normalized to that of RL; (2) (TM-RL)/RL describes the transformation from gross motion of the RL to shear of the TM; (3) (Tips-RL)/(TM-RL) describes the relation between TM shear and hair bundle deflection.

## METHODS

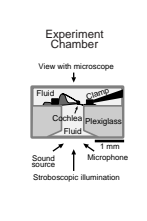
### Preparation

Motion measurements were performed on 140 hair bundles from cochleae of nine southern alligator lizards (*Gerrhonotus multicarinatus*) weighing 20-49g. The animals were sacrificed, and a dorsal approach to the cochlea was taken. The bone above the sacule was chipped away, and the sacculus otoconia removed by flushing saline or applying gentle suction. The eighth nerve was cut and the cochlear duct extracted. The duct was moved to an experimental chamber, in which it was clamped between two fluid-filled regions. The vestibular membrane was removed with fine forceps, and the entire chamber was placed on the stage of a microscope. Because the lizard cochlea is not coiled, its entire structure could be seen in the microscope. In these experiments, measurements were taken from the tectorial region (see figure at right).



### Experiment Chamber

The experiment chamber (see schematic, right) clamps the cochlea between two fluid-filled regions, both of which can be perfused. In early experiments, an artificial perilymph (171 mM Na<sup>+</sup>, 2 mM K<sup>+</sup>, 177 mM Cl<sup>-</sup>, 2 mM Ca<sup>2+</sup>, 3 mM glucose, 5 mM HEPES, pH 7.30±0.05) was perfused on both sides of the cochlea. In later experiments, the apical side was perfused with an artificial endolymph (2 mM Na<sup>+</sup>, 174 mM K<sup>+</sup>, 171.5 mM Cl<sup>-</sup>, 20 μM Ca<sup>2+</sup>, 0.5 mM H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, 2 mM SO<sub>4</sub><sup>2-</sup>, 5 mM HEPES, 4 mM L-glutamine, 20 mM MEM-amino acids 50x, 10 mL MEM-non-essential amino acids 100x, pH 7.30±0.05) while the basal side was perfused with Liebowitz L-15 medium, supplemented with 5 mM glucose and HEPES (pH 7.30±0.05). The sound stimulus was generated by a piezoelectric disk (EFR Series, Panasonic, NJ). A pressure transducer (EPX, Entran Devices, NJ) was used to determine the pressure under the cochlea. Two pairs of Ag/AgCl electrodes (E205, Warner Instruments, CT) were used to clamp the voltage across the cochlea to 16 mV and measure the resulting current.



### Microscopy

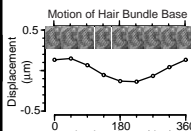
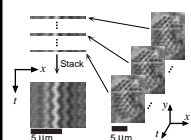
The cochlea was observed in brightfield Köhler microscopy using a Zeiss Achroplan 40x 0.75 NA water immersion objective (Carl Zeiss Inc, NY) with an additional gain of 2.5x, for a total magnification of 100x. Images were taken with a 12-bit cooled CCD camera (CH250A, Photometrics Ltd, AZ). Stroboscopic illumination using either a gas-discharge strobe (model 8440, Chadwick-Helmuth, CA) or an LED (AND190HP, Purdy Electronics, CA) was phase-locked to the stimulus, allowing us to image the positions of cochlear structures at specific phases of motion. This process was repeated at several focal planes, allowing us to observe the three-dimensional position of cochlear structures at each stimulus phase.

### Image analysis

Images were corrected for defects in the imaging system by a two-point correction technique (Davis and Freeman, ARO97). Motion between images was estimated by comparing changes in gray values within and across images. This estimation requires two assumptions: (1) the brightness of an object is constant regardless of its position in the image; (2) the object moves as a rigid body. Given these assumptions, it is possible to find the shift between two images that minimizes the mean squared change in brightness (Horn and Weldon Jr., 1988). We used this shift as an estimate of the displacement of an object between two images. By computing this displacement between images taken at successive phases of the stimulus, we determined the motion of cochlear structures at the stimulus frequency and its harmonics.

## RESULTS

### 1. Gross Motion

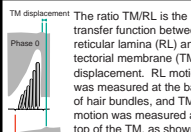


Images are obtained at different phases of the stimulus waveform using stroboscopic illumination. The figure at the left illustrates motions between images of a single hair bundle by extracting the same row of pixels from each image and stacking them. The resulting image shows changes in brightness as a function of position (x) and time (t). The sound stimulus was a 500 Hz, 120 dB SPL (equivalent to 94 dB SPL at the tympanic membrane) tone.

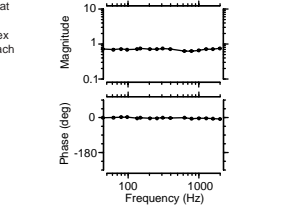
Quantitative motion estimates are obtained by analyzing all pixels in the bundle images using computer microvision. The plot to the left shows the motion of the base of one hair bundle in the x direction. Above the plot are the images from which motion was computed. The fundamental component of motion was 0.3 μm peak-to-peak. Higher harmonics were more than 26 dB smaller.

- The plot to the left shows the magnitude of the motion of the bases and tips of a typical hair bundle and of the overlying TM as a function of frequency at 120 dB SPL.
- Cochlear motions at the base, tip, and TM varied by less than 20 dB over the frequency range examined.
- TM motion is comparable to motion of the bundle tip; both the TM and the tips move less than the base of the bundle.

### 2. TM/RL Ratio

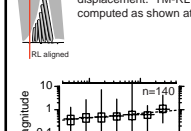
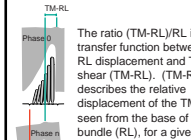


The ratio TM/RL is the transfer function between reticular lamina (RL) and tectorial membrane (TM) displacement. RL motion was measured at the bases of hair bundles; and TM motion was measured at the top of the TM, as shown at left. The fundamental component of the complex ratio was computed at each frequency.



- The plots below show the magnitude and phase of TM/RL vs. frequency for a typical hair bundle.
- The magnitude is less than one at all frequencies.
- The phase is near 0° at all frequencies.
- Both the magnitude and phase remain roughly constant with frequency.
- The left plots show the median, interquartile range, and range of TM/RL magnitude and phase vs. frequency for all bundles.
- The median ratio is less than one at all frequencies.
- The ratio was greater than 1 at most frequencies for only 10 out of 140 bundles.
- The phase is near 0°.

### 3. (TM-RL)/RL

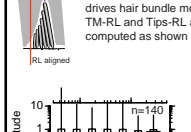
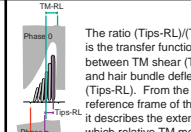


The ratio (TM-RL)/RL is the transfer function between RL displacement and TM shear (TM-RL). (TM-RL)/RL describes the relative displacement of the TM, as seen from the base of a hair bundle (RL), for a given RL displacement. TM-RL is computed as shown at left.

- The plots below show the magnitude and phase of (TM-RL)/RL vs. frequency for the same bundle shown in section 2.
- The magnitude is near 0.3.
- The phase is near -180°.

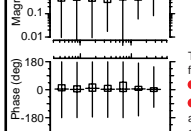
- The plots to the left summarize (TM-RL)/RL vs. frequency for all bundles.
- The median ratio is less than one at most frequencies.
- The ratio increases with frequency at about 3.8 dB/decade (dashed line). This increase was evident in the individual ratios for 44 out of 140 bundles.
- The phase is near -180°.

### 4. (Tips-RL)/(TM-RL)



The ratio (Tips-RL)/(TM-RL) is the transfer function between TM shear (TM-RL) and hair bundle deflection (Tips-RL). From the reference frame of the RL, it describes the extent to which relative TM motion drives hair bundle motion. TM-RL and Tips-RL are computed as shown at left.

- The plots to the right show the magnitude and phase of (Tips-RL)/(TM-RL) vs. frequency for the same bundle shown in section 2.
- The magnitude is near one.
- The phase is near 0°.
- Both the magnitude and phase remain roughly constant with frequency.



- The plots to the left summarize (Tips-RL)/(TM-RL) vs. frequency for all bundles.
- The median ratio is near one.
- The distribution is skewed; the ratio was less than 0.5 at most or all frequencies for 37 out of 140 bundles; it was greater than 1 at most or all frequencies for 7 bundles.
- The phase is near 0°.

## DISCUSSION

### For All Nine Cochleae:

- TM displacement was smaller than RL displacement; the TM did not resonate.
- TM shear was smaller than and out of phase with RL displacement.
- The deflection of the hair bundles was smaller than or equal to the shear of the TM.
- The RL, tips of hair bundles, and TM all moved in phase.
- None of the transfer functions or measurements showed sharp frequency selectivity.

### However,

- No electrical response to sound was detected from the excised cochleae. It is possible that some components of the transduction apparatus (e.g., tip links) were not functioning properly. These components may have affected the mechanical properties of the cochlea.
- Hair cells in our preparation often developed "blebs", or herniations of the membrane, adjacent to the kinocilium. Although we reversed the blebbing in some preparations by perfusion of artificial endolymph, the initial blebbing process may have caused invisible damage.

### Implications for Cochlear Mechanics

- In our preparations, motions were largest at the bases of the bundles, and decreased toward the tips of the bundles and the TM.
- Motions of the bases and tips of hair bundles and the TM were not resonant.

- The transfer functions examined are not sufficient to explain the  $Q_{10dB}$  values and high-frequency slopes of tuning curves measured in auditory nerve fibers innervating this region.

### References

1. Davis, C.Q. and Freeman, D.M., ARO 1997.
2. Fishkopf, L.S. and DeRosier D.J., *Hear Res* 12, 1983.
3. Holton, T. and Hudspeth, A.J., *Science* 222, 1983.
4. Horn, B.K. and Weldon Jr., E., *Int'l J Comp Vis* 2, 1988.
5. Peake, W.T. and Ling Jr., A., *J Acoust Soc Am* 67, 1980.
6. Weiss, T.F., Mulroy, M.J., Turner, R.G., and Pike, C.L., *Brain Res* 115, 1976.
7. Weiss, T.F., Peake, W.T., Ling Jr., A., and Holton, T., in *Evoked Electrical Activity in the Auditory Nervous System*, Academic Press, 1978.

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