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Tectorial Membrane Porosity Controls Spread of Excitation and Tuning in the Cochlea

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Abstract. Modifications of genes that encode proteins found exclusively in the tectorial membrane (TM) alter mechanical properties and produce a wide range of hearing deficits. However, the changes in TM physical properties responsible for these deficits remain unclear. In particular, the cochlear tuning of *Tectb*^{-/-} mice is significantly sharper than that of *Tecta*^{Y1870C/+} mice, even though the stiffnesses of *Tecta*^{Y1870C/+} and *Tectb*^{-/-} TMs are similarly reduced relative to wild-type TMs. Here we show that differences in TM wave properties that are governed by shear viscosity account for these differences in tuning. The shear viscosity of the TM results from the interaction of interstitial fluid with the porous structure of the TM's macromolecular matrix. In basal regions of the cochlea, nanoscale pores of *Tecta*^{Y1870C/+} TMs are significantly larger than those of *Tectb*^{-/-} TMs. The larger pores in *Tecta*^{Y1870C/+} TMs gives rise to lower shear viscosity (by ~70%), which in turn, reduces wave speed and increases wave decay constants relative to *Tectb*^{-/-} TM wave properties. These results demonstrate the importance of TM porosity in cochlear tuning and that TM porosity, not stiffness, underlies the striking differences in hearing between *Tecta*^{Y1870C/+} and *Tectb*^{-/-} mice.

Keywords: tectorial membrane, traveling waves, porosity, viscosity, cochlear tuning

INTRODUCTION

The development of genetic models of hearing disorders has provided opportunities to study molecular mechanisms underlying the remarkable frequency selectivity and sensitivity of mammalian hearing. Of the mutants with hearing impairments developed to date a surprising number affect genes that target the tectorial membrane (TM). Although TM mutants display an enormous range of hearing deficits, the physical mechanisms underlying those deficits remain unclear.

For example, *Tecta*^{Y1870C/+} and *Tectb*^{-/-} mutant mice exhibit distinctly different hearing phenotypes: *Tectb*^{-/-} mice have sharpened basilar membrane (BM) tuning by a factor of two to three at mid to high frequencies [14], while *Tecta*^{Y1870C/+} mice have normal BM tuning and even broader neural tuning [9]. Although this difference in tuning is fundamental to our understanding of the distinctive properties of mammalian hearing, the mechanism is not known.

Here we investigate a mechanism based on TM traveling waves [3, 4, 7, 8]. We show that the effect of loss in waves is characteristically different from the effect of loss in conventional cochlear models. Furthermore, these studies show that porosity plays a key role in determining loss, and thereby spread of excitation, in both normal and mutant TMs. The TM wave measurements outlined here demonstrate that TM porosity, and not stiffness, underlies the striking differences in *Tecta*^{Y1870C/+} and *Tectb*^{-/-} hearing.

METHODS

Measuring TM wave properties

Isolated TM segments were suspended between vibrating and stationary supports in a wave chamber and stimulated as previously described [3, 4]. TM shear viscosity was altered by adding PEG (Sigma-Aldrich) to artificial endolymph (AE) surrounding the TM in the wave chamber. To ensure adequate equilibration of PEG, the bath (5 mL) was exchanged 4 times over a time course of approximately 5 minutes. This process was repeated for PEGs with a variety of molecular weights, and with concentrations chosen so that the viscosity of the bath was the same for each molecular weight: i) 4 μ M, 900 kDa; ii) 12 μ M, 600 kDa; iii) 35 μ M, 400 kDa; iv) 70 μ M, 300 kDa; v) 158 μ M, 200kDa; vi) 630 μ M, 100 kDa; vii) 15 mM, 8 kDa. Viscosity was also altered by adding 9-11 kDa dextran (Sigma-Aldrich) to the

AE bath with a concentration (24 mM) chosen so that the viscosity matched that of PEG solutions. To capture motions at high frequencies, a stroboscopically pulsed light emitting diode was synchronized to the audio frequency stimuli. To reconstruct wave motions, the TM was illuminated and images were captured at 8 evenly spaced stimulus phases over several stimulus cycles. The collected images were then analyzed to determine the magnitude and phase of TM displacement at multiple regions along the TM's surface between the supports.

RESULTS

TM traveling waves in *Tecta*^{Y1870C/+}, *Tectb*^{-/-} and wild-type mice

To characterize differences in *Tecta*^{Y1870C/+} and *Tectb*^{-/-} TMs, we measured wave motions [3] of isolated TMs from each mutant. TM segments were excised from the basal turn of the mouse cochlea and suspended between two supports immersed in AE. Forces were applied in the radial direction to these TM segments by driving one of the supports at audio frequencies (10-20 kHz).

Figure 1 shows the frequency dependence of speed (A-C) and decay (D-F) for *Tecta*^{Y1870C/+} ($n = 7$ preparations), *Tectb*^{-/-} ($n = 4$ preparations), and wild-type ($n = 5$ preparations) TMs pooled across a range of audio frequencies (10-20 kHz). Wild-type TM segments exhibited the highest wave speeds over the measured frequency range, while *Tectb*^{-/-} and *Tecta*^{Y1870C/+} TM speeds were significantly lower by $\sim 20\%$ and $\sim 40\%$, respectively.

Decay constants tended to decrease with increasing frequency (Figure 1). *Tecta*^{Y1870C/+} and wild-type TMs had similar decay constants with ranges spanning 135–400 μm between 10-20 kHz. In contrast, decay constants for *Tectb*^{-/-} TMs were significantly smaller (by as much as a factor of 2.25) than those of *Tecta*^{Y1870C/+} or wild-type TMs.

TM material properties in *Tecta*^{Y1870C/+}, *Tectb*^{-/-} and wild-type mice

We developed a lumped model consisting of a distributed series of masses coupled by viscous and elastic elements [3] and used this model to determine the relationship between wave properties and material properties.

Estimates of shear storage modulus, G' , are similar for *Tecta*^{Y1870C/+} (23.8 ± 3.5 kPa; $n = 5$ TM preparations, 15-20 kHz) and *Tectb*^{-/-} (20.2 ± 8.1 kPa; $n = 4$ TM preparations, 15-20 kHz) TMs. However, these stiffness are significantly reduced from that of wild-types (47.7 ± 8.8 kPa; $n = 5$ TM preparations, 15-20 kHz). While G' is similar in *Tecta*^{Y1870C/+} and *Tectb*^{-/-} mutant TMs, there are significant differences in TM shear viscosity, η . *Tecta*^{Y1870C/+} TMs have significantly lower η values (0.073 ± 0.033 Pa·s; $n = 5$ TM preparations, 15-20 kHz) compared to both *Tectb*^{-/-} (0.23 ± 0.033 Pa·s; $n = 4$ TM preparations, 15-20 kHz) and wild-type TMs (0.22 ± 0.048 Pa·s; $n = 5$ TM preparations, 15-20 kHz), indicating that the basal regions of the cochlea, the key difference between *Tecta*^{Y1870C/+} and *Tectb*^{-/-} TMs is in their shear viscosity.

Porosity is greater in *Tecta*^{Y1870C/+} TMs than in *Tectb*^{-/-} or wild-type TMs

To understand the mechanisms underlying this difference in shear viscosity between *Tecta*^{Y1870C/+} and *Tectb*^{-/-} TMs, we also probed poroelastic properties. To characterize the porous nature of the TM, we increased the viscosity of the AE bath using PEGs with a range of molecular weights from 8-900 kDa, chosen to provide a range of radii of gyration [10]. Figure 2 shows that adding large molecular weight PEGs (right panel) had negligible effect on wave speed and decay, but adding small molecular weight PEGs (left panel) increased speeds and decreased decay constants, suggesting that only PEGs small enough to permeate TM pores are able to alter the TM's internal shear viscosity and impact wave properties. Medium size PEGs (200-400 kDa, middle panel) only altered wave properties of *Tecta*^{Y1870C/+} TMs, suggesting that the porosity of *Tecta*^{Y1870C/+} TMs is greater than those of *Tectb*^{-/-} and wild-type TMs.

To verify that the changes in wave properties observed were due to changes in viscosity, we also characterized TM waves in the presence of dextran at the same viscosity and M.W. as in the PEG experiments. In this case, wave speed increased $\sim 42\%$ and decay constants decreased $\sim 46\%$ versus a speed increase of $\sim 37\%$ and decay constant decrease of $\sim 47\%$ in the presence of PEG (Figure 3).

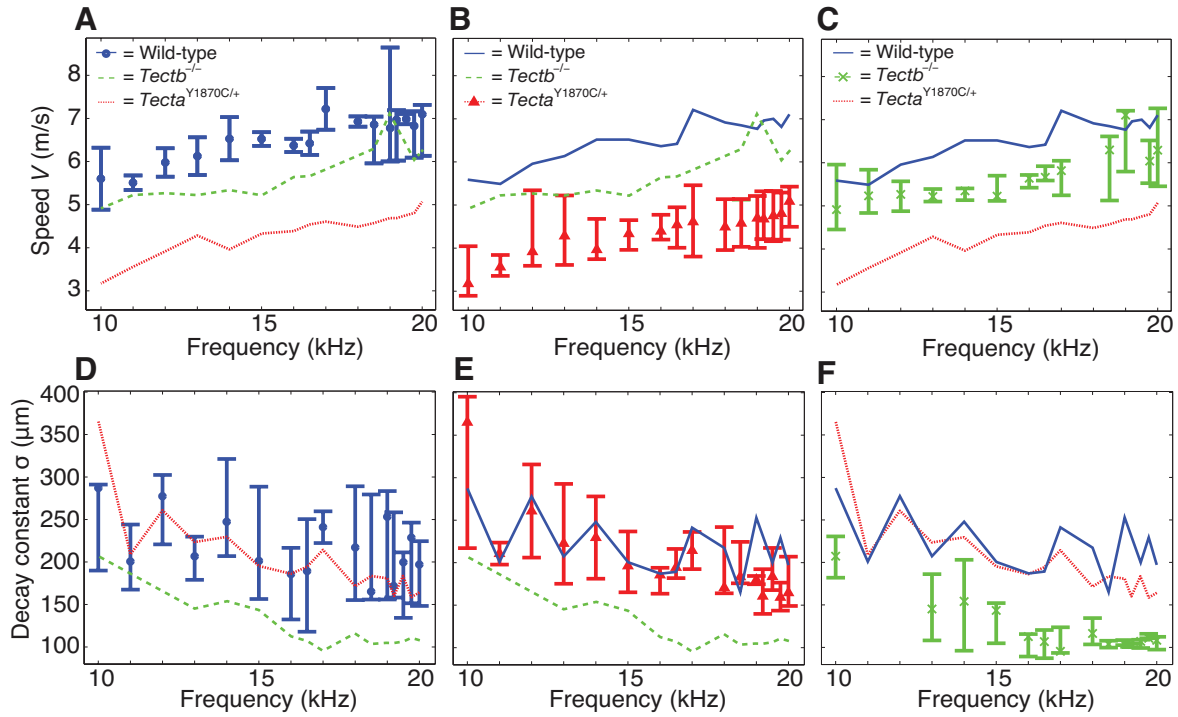


FIGURE 1. TM traveling waves in tectorin mutant and wild-type mice. (A-C) Wave speed measurements pooled across multiple TM samples. Median TM wave speeds of *Tecta*^{Y1870C/+} (red triangles) (*n* = 7 preparations), *Tectb*^{-/-} (green crosses) (*n* = 4 preparations), and wild-types (blue circles) (*n* = 5 preparations). (D-F) Wave decay constant measurements pooled across multiple TM samples. *Tecta*^{Y1870C/+} (*n* = 7 TM preparations; red triangles) and wild type (*n* = 5 TM preparations; blue circles) had similar wave decay constants (σ) (135-325 μm for wild types and 140-400 μm for *Tecta*^{Y1870C/+} mutants), whereas *Tectb*^{-/-} segments had significantly lower σ estimates (80-225 μm).

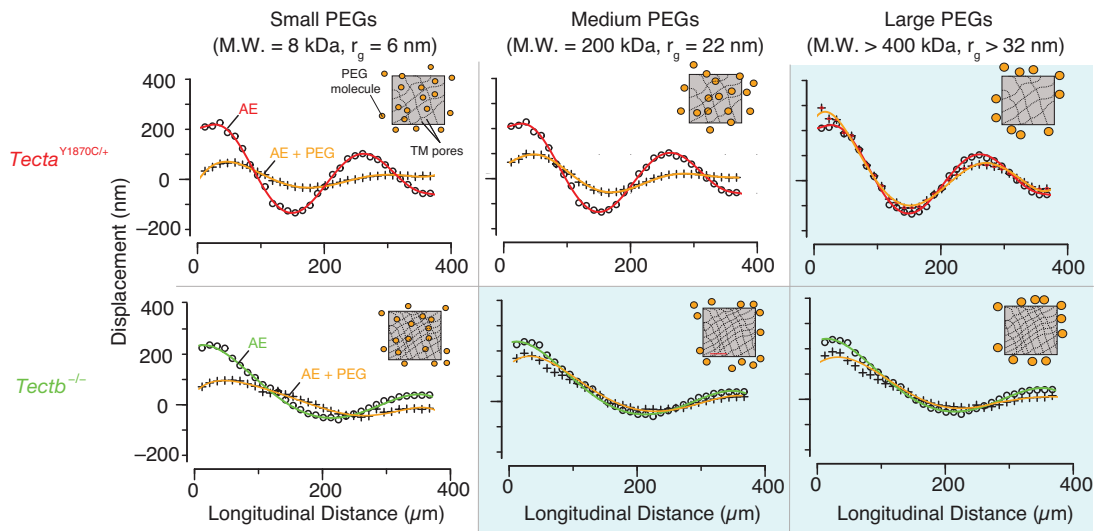


FIGURE 2. Polyethylene glycols (PEGs) with varying molecular weights (M.W.) and radii of gyration (r_g) added to AE surrounding *Tecta*^{Y1870C/+} and *Tectb*^{-/-} TMs caused changes in wave speed and decay. Only the smallest molecular weight PEGs changed wave properties in both mutants. Only in *Tecta*^{Y1870C/+} TMs did larger PEGs alter wave properties. Blue shaded panels indicate cases where PEG did not alter TM wave properties.

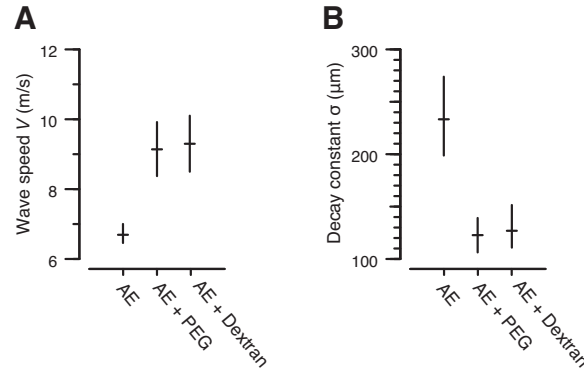


FIGURE 3. TM porosity and viscosity in wild-type mice. TM wave speeds (A) and decay constants (B) measured in AE, AE with 15 mM PEG (8 kDa), and AE with 24 mM dextran (9-11 kDa) at 15-20 kHz (medians and interquartile ranges).

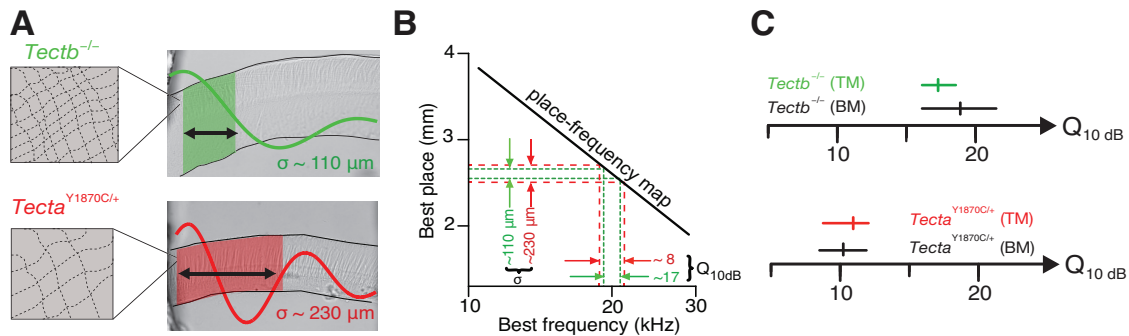


FIGURE 4. Relation between TM wave decay and quality of tuning. (A) Schematic drawings (left) and images (right) of *Tectb*^{-/-} (top) and *Tecta*^{Y1870C/+} (bottom) TMs. Shaded regions illustrate the spatial extent of TM waves. (B) Relation between TM decay constants and frequency tuning. The solid black line represents the relation between best place and best frequency given by the cochlear map of the mouse [11]. (C) Qualities of tuning ($Q_{10\text{dB}}$) calculated as shown in panel B for *Tectb*^{-/-} *Tecta*^{Y1870C/+} and *Tecta*^{Y1870C/+} TMs perfused with PEG (median and interquartile range at 20 kHz) and compared to measurements of BM tuning (mean and standard deviation at 50 kHz, Russell et al. [14]).

DISCUSSION

Importance of TM shear viscosity

While previous studies have demonstrated the importance of TM stiffness in cochlear mechanics [2, 3, 5, 6, 13, 15], our results suggest that shear viscosity is also an essential material property of the TM. Specifically, our results show that TM stiffness alone cannot explain the hearing phenotypes of *Tecta*^{Y1870C/+} and *Tectb*^{-/-} mutant mice. In addition to stiffness, shear viscosity determines TM wave properties. Our measurements demonstrate that TM shear viscosity is significantly lower in *Tecta*^{Y1870C/+} TMs than in *Tectb*^{-/-} and wild-type TMs. Reducing TM shear viscosity reduces transmission loss in longitudinally propagating waves, which in turn, allows TM waves in *Tecta*^{Y1870C/+} mutants to propagate further (larger wave decay constants) than those in *Tectb*^{-/-} mice (smaller wave decay constants).

Importance of TM porosity

Given that shear viscosity arises from the interaction of water with TM macromolecules, we tested the relation between viscosity and porosity by introducing PEG molecules with different radii of gyration to the bath surrounding the TM. We found a significant increase in the TM's shear viscosity only when the radius of gyration of PEG was sufficiently small to permeate the pores of the TM. The increase in internal shear viscosity induced by PEG molecules alters both the speed and decay of TM waves. In particular, we show that 200-400 kDa PEGs (28-32 nm radii of gyration) are able to alter the wave properties of *Tecta*^{Y1870C/+} TMs, but not those of wild-type and *Tectb*^{-/-} TMs,

which are only affected by PEGs smaller than 200 kDa (22 nm radii of gyration) (Figure 3). Therefore, differences in porosity account for differences in tuning between these mutants.

Implications for cochlear tuning

The effect of viscosity on TM waves and classical TM models is strikingly different. Classical models have suggested that viscous damping in the subtectorial space plays a critical role in determining frequency tuning and sensitivity in mammalian hearing [1, 12, 16]. In particular, fluid viscosity, such as that in the subtectorial space, limits sensitivity and sharpness of cochlear tuning. Our results suggest that viscous loss in the TM has the opposite effect on tuning. *Tecta*^{Y1870C/+} TMs exhibit less loss (shear viscosity), which in turn, increases the spatial extent of traveling waves relative to *Tectb*^{-/-} mutants. When combined with scaling symmetry and the cochlear map of the mouse cochlea, this increase in spread of excitation would lead to broader tuning (Figure 4). Thus, TM waves may compensate (at least in part) for the dissipative effects of fluid damping in the subtectorial space. Ultimately, our results demonstrate that porosity plays a key role in determining the cochlear phenotypes of *Tecta*^{Y1870C/+} and *Tectb*^{-/-} mutants and that porosity represents a fundamental material property of the TM. This porosity, in combination with shear storage modulus, determines the speed and decay of TM waves, contributing to the remarkable sensitivity and frequency selectivity of mammalian hearing.

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