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Supplemental Information

Tectorial Membrane Traveling Waves Underlie Sharp Auditory Tuning in Humans

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Supplementary material for 'tectorial membrane traveling waves underlie sharp auditory tuning in humans'

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MATERIALS AND METHODS

Extraction of human TM samples

Human temporal bones were obtained from the Massachusetts Eye and Ear Temporal Bone Bank. Temporal bones were removed within 24 hours post mortem and were refrigerated in 0.9% normal saline for several hours before being transferred to a bath of artificial endolymph (AE) containing 174 mM KCl, 5 mM Hepes, 3 mM dextrose, 2 mM NaCl, and 0.02 mM $CaCl_2$. The bath was titrated to pH 7.2 using small quantities of KOH or HCl as necessary. Preparation of the specimen was performed using universal precautions. After opening the facial recess, the round window and stapes were exposed, and the incudo-stapedial joint was severed to allow removal of the tympanic membrane and middle ear cavity without disrupting the inner ear. Surgical drilling took approximately three hours to expose the cochlea and thin the bone near the region covering the organ of Corti while the specimen was kept moist with AE. Once the outline of the cochlear spiral was sufficiently thinned and the specimen well rinsed of bone dust, the remaining bone around the cochlear spiral was removed using a scalpel blade (no. 11) and curved surgical scissors under a dissection microscope (Wild Hexagon, Stockholm, Sweden). Once the bone around the cochlea was opened, the cochlea was kept in AE. Stria vascularis was removed using fine forceps, and a needle (26 ga) was used to extract the organ of Corti from along the cochlear spiral starting from the base. The TM was gently removed from the surface of the organ of Corti using a sterilized eyelash. TM segments were photographed to identify their origin along the cochlea then were cut into 1-2 mm segments using a needle. TM segments from the basal turn were transferred to a clean AE bath using a glass-tipped pipette, then were used for wave measurements as described below. In one human bone that was used for training, the entire cochlear spiral was exposed (Suppl. Fig 1) showing the basal, middle, and apical turns of the human cochlea. A total of 15 temporal bones were used in the development of the measurement techniques, and results were obtained from an additional 3 temporal bones.

Human TM wave properties could not be measured immediately after death. All measurements shown here were performed roughly 48 hours post mortem. We performed a study in mice to measure the changes in mouse TM wave properties when treated in a similar fashion to humans. We found that mouse TM wave decay constants were almost indistinguishable in samples dissected 48 hours post mortem compared to samples measured 1 hour post mortem (Suppl. Fig. 2).

Extraction of mouse TM samples

Mice were euthanized by carbon dioxide asphyxiation, followed by decapitation. All mouse TMs were extracted in a similar manner to that used in human TM extraction. To this end, heads were refrigerated overnight after which temporal bones were extracted and bullae were opened and placed in 0.9% saline. After several hours, cochleae were dissected and placed in an artificial endolymph bath as described above for humans. An example mouse cochlea is included in Supplementary Figure 1. The cochleae were refrigerated in AE until approximately 36 hours after the animal's time of death, at which point the cochleae were dissected using a scalpel blade (no. 11) and TM samples were extracted using a sterilized eyelash. TM wave measurements were performed approximately 48 hours post mortem to mimic the human condition as closely as possible.

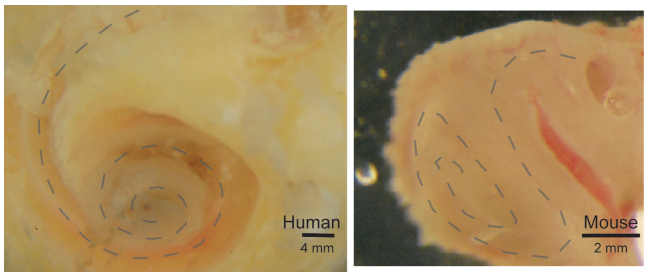

Figure 1: Light microscope images of human (left) and mouse (right) cochleae.

Measurement of TM wave properties

TM waves were measured optically as described by Ghaffari et al (1). Briefly, isolated TM segments were suspended between two supports using Cell Tak bioadhesive (Collaborative Research, Bedford, MA). One of the supports was glued down and thus remained stationary while the other was attached to a piezo-electric

actuator (Thorlabs Inc., Newton, NJ). The TM was stimulated in the radial cochlear direction, and motions along its surface were measured at frequencies in the basal range for mice (>10 kHz) and humans (>5 kHz), subject to the maximum frequency of our amplifier (<20 kHz). Samples were optically inspected to eliminate those that were damaged during the isolation process or improperly mounted. Experiments were performed at MIT and approved by MIT's Committees on Animal Care and Environmental Health and Safety.

Motion amplitude and phase were measured using a stroboscopic computer vision technique that allows images to be captured at several phases of motion (2). Radial TM displacement and phase were determined from a one-dimensional fast Fourier transform (FFT) taken at evenly spaced points along the TM. Spatial decay constant, σ, was defined as the distance in μm along the TM over which the wave magnitude decays by a factor of *e*. The σ values for each TM were determined by fitting an exponential to the overall magnitude of the response along the TM. Speed, *v*, was determined by fitting a straight line to the phase as a function of distance along the TM and multiplying the verse slope by angular frequency.

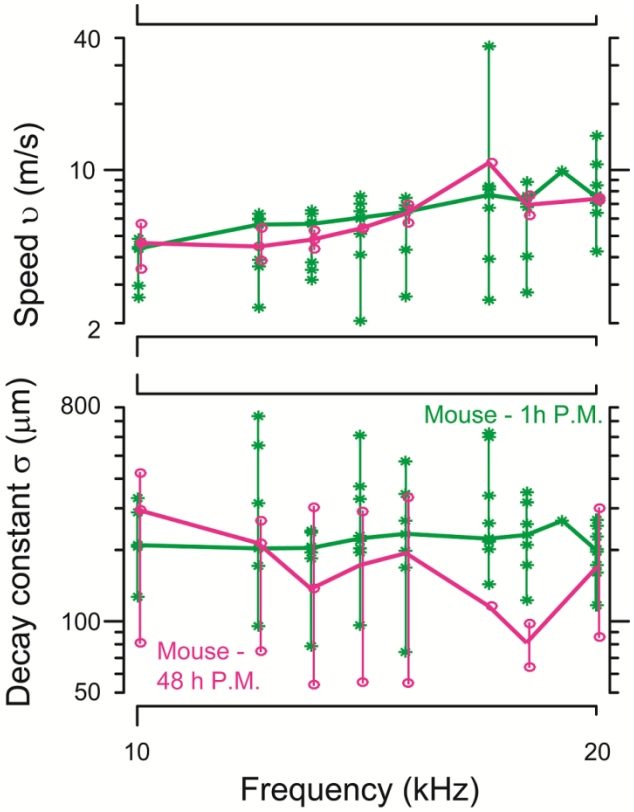

Figure 2: Wave speeds (top) and decay constants (bottom) in freshly dissected (1 hour post-mortem, n = 10) and aged (48 hours post-mortem, n = 4) mouse TMs from 10 to 20 kHz. Symbols indicate all data points. Thick horizontal lines indicate medians, and vertical lines indicate range of all data points at a single frequency.

SUPPLEMENTARY FIGURE AND VIDEOS

Human TM measurements were performed approximately 48 hours post-mortem in this study. To study the impact of this aging on TM wave properties, we aged several mouse temporal bones in a similar manner to the human temporal bones and compared these measurements to freshly dissected mouse TM waves. As shown in Supplementary Figure 2, we did not see significant differences in wave properties due to this aging of mouse temporal bones ($p = 0.01$). We therefore concluded that the human TM measurements shown in this study were a close representation of the wave properties we would see if it were possible to dissect and measure human TMs sooner after death.

Also included here are two videos of human and aged mouse TM waves from samples that were included in this study. In these videos, we magnified the TM motions by a factor of 20 using a phase based method (2) to see the motions more easily. The videos show motions at 20 kHz. These videos were used for comparison with measurements of TM wave properties presented in Figure 1, which were analyzed using computer microvision algorithms (3).

References

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