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A differentially amplified motion in the ear for near-threshold sound detection

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The ear is a remarkably sensitive pressure fluctuation detector. In guinea pigs, behavioral measurements indicate a minimum detectable sound pressure of ~20 μPa at 16 kHz. Such faint sounds produce 0.1-nm basilar membrane displacements, a distance smaller than conformational transitions in ion channels. It seems that noise within the auditory system would swamp such tiny motions, making weak sounds imperceptible. Here we propose a new mechanism contributing to a resolution of this problem and validate it through direct measurement. We hypothesized that vibration at the apical side of hair cells is enhanced compared with that at the commonly measured basilar membrane side. Using *in vivo* optical coherence tomography, we demonstrated that apical-side vibrations peaked at a higher frequency, had different timing and were enhanced compared with those at the basilar membrane. These effects depend nonlinearly on the stimulus sound pressure level. The timing difference and enhancement of vibrations are important for explaining how the noise problem is circumvented.

The high sensitivity of the hearing organ is an important survival factor for most vertebrates. Behavioral measurements indicate that guinea pigs, an extensively studied species, can detect sounds at levels of around 20 μPa at 16 kHz[1–3]. Such weak sounds will vibrate the basilar membrane and all of the structures attached to it. Outer hair cells (**Fig. 1a**,**b**) improve hearing sensitivity by amplifying these vibrations. Despite this amplification, basilar membrane vibrations are less than 0.1 nm during near-threshold stimulation[4–6]—a distance similar to the diameter of a single hydrogen atom. To result in perception, these vibrations must be transmitted to hair cell stereocilia, the deflection of which opens mechanically sensitive ion channels. The only available data, originating from *in vitro* preparations in which outer hair cells do not amplify motion, indicate that stereociliary deflections are even smaller than basilar membrane vibrations[7,8]. This creates a fundamental problem, as direct measurements on isolated mouse[9] and amphibian hair cells[10], as well as theoretical considerations[11], indicate that the minimum stimulus that these cells can resolve is on the order of 5 nm. If this limit applied to the intact organ of Corti, weak sounds would be imperceptible. A potential remedy is to presume collaboration among hair cells, in effect an averaging of the response across cells, or to presume that hair cells detect displacement of their stereocilia over a smaller bandwidth when situated in the intact organ of Corti[9,12,13]. Although both propositions seem reasonable, they cannot be verified experimentally at present. Here we propose and validate an unconventional solution to this problem through direct measurement of vibration within the organ of Corti.

Great effort has been expended in probing basilar membrane vibration[14] because the amplification generated by outer hair cells is thought to be reflected in its motion. However, different cell types within the organ of Corti have different properties[15], which means that vibrations may differ among the various components of the hearing organ. For example, there is no reason why vibrations of the apical side of the organ of Corti, where stereocilia are located, must be fully reflected in the motion of the basilar membrane, which is known to be one of the stiffer components of the organ of Corti[16].

Our hypothesis is that vibration is enhanced at the side of the hearing organ opposite the basilar membrane. This, the reticular lamina or apical side, is where the initial stages of sensory transduction occur[17–19]. The problem of detecting faint vibrations could be circumvented if this part of the hearing organ has larger motions than the basilar membrane, as suggested by *in vitro* experiments that use electrical stimulation[20]. If differences in amplitude occur, the timing of vibrations may also differ, which is important because hair-cell force production must be correctly synchronized with the acoustic stimulus. To assess the potential relevance of these ideas, it is necessary to measure reticular lamina motion *in vivo*.

The behavior of the reticular lamina at the base of the cochlea, where amplification is most pronounced, remains unknown as no previous measurement method has been able to penetrate beyond the basilar membrane. We designed an optical coherence tomography system that allows vibration measurements from both the basilar membrane side and the reticular lamina in anesthetized guinea pigs.

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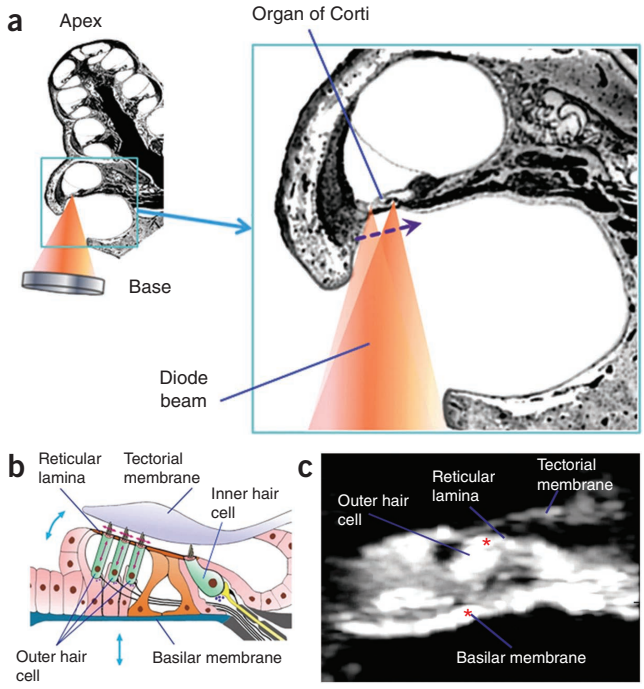

Figure 1 The cochlea and organ of Corti. (**a**) Cross-section of the guinea pig cochlea showing the method used to measure vibration. Right, scanning of the diode beam to obtain images of the hearing organ. (**b**) Schematic organ of Corti cross-section. Arrows, direction of motion of different structures. (**c**) OCT image of the organ of Corti *in vivo*. Asterisks, locations of vibration measurement.

We show that reticular lamina vibration has different frequency dependence, different timing with respect to the acoustic stimulus and a larger magnitude than the basilar membrane vibration. These features are important for explaining the remarkable sensitivity of mammalian hearing organs.

RESULTS

Here we used optical coherence tomography (OCT)[21,22] to image cochlear structures and to measure sound-evoked motion. In the OCT system, light from a superluminescent diode is divided into a reference and an object beam. The object beam reaches the organ of Corti through an opening in the cochlea (**Fig. 1a**). When scanning this beam across the tissue, the back-reflected light is used to generate images (**Fig. 1c**). Vibrations are measured by locking the object beam on structures in these images. Light that is reflected from the tissue is then recombined with the reference beam at the detector. The beams will interfere constructively if the length that they have traveled differs by <10 μm, corresponding to one-tenth of the distance separating the reticular lamina from the basilar membrane. Vibrations of the basilar membrane can therefore be differentiated from those of the reticular lamina.

Vibration of the basilar membrane and reticular lamina

In good preparations, basilar membrane vibrations were highly tuned. At a sound pressure level (SPL) of 20 dB (decibels relative to 20 μPa), a sharp peak in vibration amplitude was seen at 19 kHz and the vibration amplitude decays sharply on both sides of the peak (**Fig. 2a**). As the stimulus sound pressure level increased, the peak position moved to a lower frequency and grew flatter. The response amplitude did not grow in proportion to the increase in stimulus. This nonlinear feature is typical for a healthy cochlea; it is seen in many previous studies[23,24].

High-frequency reticular lamina vibration has never been measured *in vivo*. We found that reticular lamina displacements were highly tuned and had greater magnitude than those of the basilar membrane. At 20 dB SPL, peak reticular lamina displacement was approximately three times greater than that of the basilar membrane (**Fig. 2b**), a difference that decreased as the sound pressure increased. To illustrate this nonlinearity, we plotted displacement magnitudes as a function of stimulus intensity (**Fig. 3a**). Because successful measurements at sound pressures below 40 dB SPL are uncommon, we used data acquired at 40 dB SPL for computing average values. At this sound pressure and at the characteristic frequency, reticular lamina vibrations were a factor of 2.6 ± 0.3 (mean ± s.e.m.) larger than basilar membrane vibrations ($n = 9$; $P = 0.0045$, using the paired t-test with Bonferroni corrections for multiple comparisons). When the stimulus sound pressure level increased, the difference decreased, but it remained statistically significant at 80 dB SPL (1.8 ± 0.2, $P = 0.02$). Both basilar membrane and reticular lamina vibration showed nonlinear compression starting at 30 dB SPL (**Fig. 3a**). At the frequency evoking a maximum response (the best frequency; BF), reticular lamina vibrations evoked by low sound pressure levels grew at a rate similar to that of basilar membrane vibrations (in both cases, there was a 0.5- to 0.6-dB increase in response for every 1 dB increase in stimulus sound pressure level), but at higher stimulus sound pressure levels, the reticular lamina vibrations grew much more slowly (0.13 dB versus 0.3 dB per decibel SPL). As a result, the vibration amplitude of the two structures tended to converge between 70 and 90 dB SPL (**Fig. 3a**). The nonlinear compression was stronger at higher frequencies, but at lower frequencies the basilar membrane and reticular lamina responses tended to overlap. Post mortem, vibrations were much smaller and grew linearly with stimulus intensity, and different structures within the organ of Corti had nearly identical vibration amplitudes ($n = 4$; **Fig. 3b**). These data indicate that the amplified motion at the reticular lamina is due to processes that are sensitive to the physiological state of the cochlea.

Frequency differences

Apart from the amplitude difference, there was a difference in the frequency behavior of the reticular lamina and the basilar membrane. When examining the vibration of these two structures

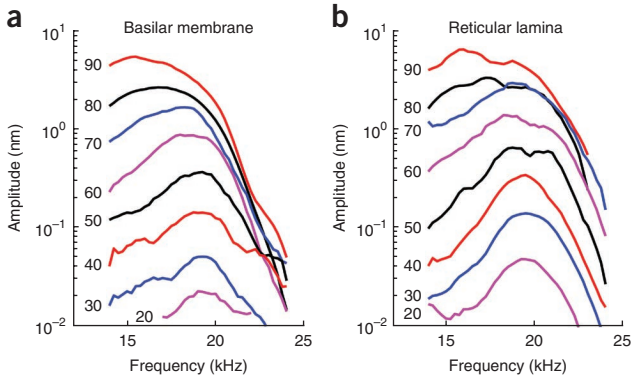

Figure 2 Vibration in the organ of Corti of a guinea pig cochlea. (**a**) Vibration of the basilar membrane. (**b**) Vibration of the reticular lamina. Displacement magnitudes of vibration are plotted as a function of stimulus frequency. An auditory sensitivity loss of 8 dB was caused by surgical procedures in the ear. The numbers against each curve represent sound levels (dB SPL) used to induce hearing-organ vibration. At 30 dB SPL, the maximum amplitude of basilar membrane vibration was 0.21 nm at 18 kHz. Reticular lamina vibration peaked at 18.75 kHz, with a displacement amplitude of 0.41 nm.

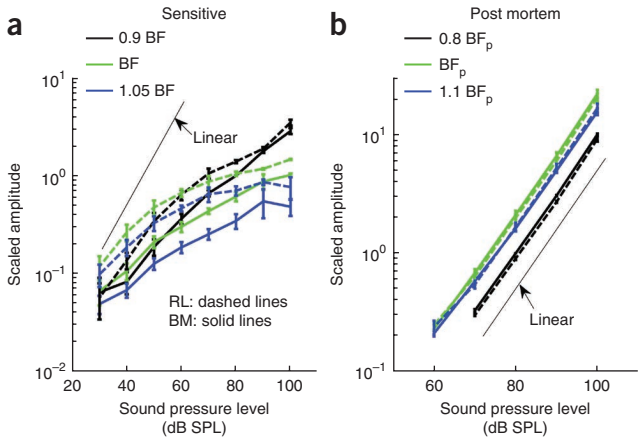

Figure 3 Displacement magnitude as a function of sound level (input–output function) measured from the basilar membrane and reticular lamina. (**a**) Input–output functions of basilar membrane (BM) displacement (solid lines) and reticular lamina (RL) displacement (dashed lines). The mean and s.e.m. from nine preparations are given. (**b**) Post-mortem input–output functions. Data plotted as mean ± s.e.m. The thin lines mark a linear relationship between the sound pressure level and vibration amplitude. BF_p, best frequency post mortem (~15 kHz in the experiments).

at exactly the same distance from the round window, peak frequency of reticular lamina vibration is higher than that of basilar membrane motion (frequency difference 440 ± 160 (mean ± s.e.m.) Hz; $n = 9$, $P = 0.02$ using the paired t-test; **Fig. 4a**,**b**).

The frequency differences were reflected in the timing of the response. Response timing, or phase, in the healthy cochlea depended on stimulus intensity. As the intensity increased, the slope of the phase-frequency curve decreased, a behavior observed both at the basilar membrane (**Fig. 4c**) and at the reticular lamina (**Fig. 4d**). This indicates the presence of dispersive traveling waves. These phase changes are more easily examined if the phase is plotted with reference to the basilar membrane phase observed at the highest stimulus intensity, 80 dB SPL (**Fig. 4e**,**f**). In these plots, the basilar membrane phase at 80 dB SPL is zero by definition (**Fig. 4e**). The crossover between the phase curves at different stimulus intensities occurred at a higher frequency for the reticular lamina, a disparity that is consistent with the higher BF for the reticular lamina described above. This difference in BF was accompanied by a timing difference between the two structures that is examined in detail below.

Timing differences

It is possible to calculate, by scaling the phase delay of a basilar membrane traveling wave, that if BF is 19 kHz for the reticular lamina and 18.5 kHz for the basilar membrane, a phase lead of about 80° for the reticular lamina with respect to the basilar membrane would result. Notably, and consistent with this calculation, reticular lamina vibration did not occur in synchrony with the basilar membrane motion but showed a difference in phase (**Fig. 5a**). This difference was dependent on the sensitivity of the preparation and the stimulus intensity. At a low sound level (40 dB SPL), peak reticular lamina displacement preceded peak basilar membrane displacement, resulting in a relative phase lead of about 86°. The phase systematically shifted as the sound level increased, and at 80 dB SPL, only 10° of phase lead remained. Notably, the reticular lamina phase lead occurred over the frequency range from 16 kHz to 19 kHz for a BF of 18 kHz. The average phase difference was 41 ± 11° at 40 dB SPL, which was significantly different from zero ($n = 9$, $P = 0.006$), decreasing to 11 ± 7° at 80 dB SPL ($n = 9$; not significant, $P = 0.12$). Post mortem, the phase and amplitude of the two structures were the same (data not shown).

These results demonstrated a mode of cochlear motion that was not anticipated by current models. Thus, confirmation of these findings with other techniques is essential. As no method other than OCT can directly quantify high-frequency reticular lamina motion, we measured extracellular receptor potentials within the organ of Corti in three guinea pigs. These potentials are an analog of outer hair cell receptor potentials[25] and result from stereocilia deflection; reticular lamina motion is therefore an important determinant of their phase and amplitude. We compared the potentials with basilar membrane motion, which we measured using a conventional laser velocimeter. Notably, we found a phase shift of extracellular receptor potentials with respect to basilar membrane motion of 76° at BF. This value is similar to that measured with OCT (**Fig. 5b**). The phase shift diminished with increasing sound level, giving rise to a negative slope in the plot of phase versus intensity. Phase changes were smaller at frequencies remote from the peak of the tuning curve and in guinea pigs with substantial loss of auditory sensitivity (data not shown).

DISCUSSION

To amplify a stimulus near to the part of the cell where it is detected makes sense. We have demonstrated that vibrations at the reticular lamina, where stereocilia reside, were enhanced, had different frequency dependence and a different timing from the commonly measured vibrations of the basilar membrane. It is known that outer hair cells produce force to boost the movement of the organ of

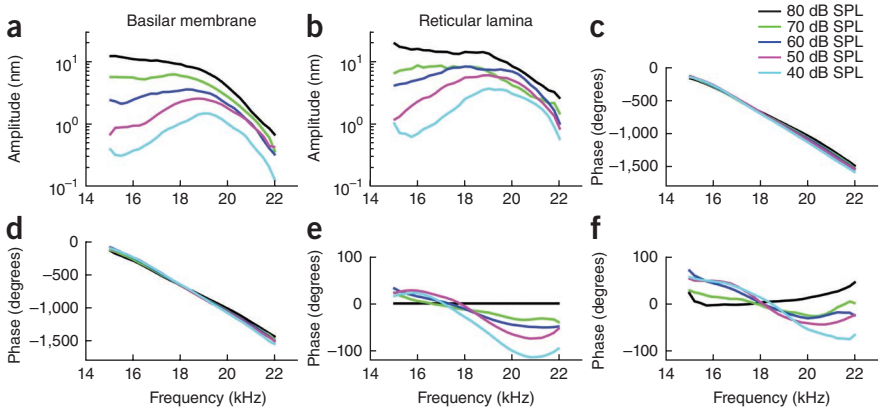

Figure 4 Sound-induced vibration of the basilar membrane and reticular lamina at the cochlear location giving a maximal vibration response at 19 kHz (BF) in a guinea pig with 7 dB sensitivity loss owing to surgical preparation. (**a**,**b**) Displacement amplitude versus frequency. (**c**,**d**) Displacement phase relative to the loudspeaker driving voltage versus frequency. (**e**,**f**) Relative phase versus frequency at different sound levels with respect to the phase at 80 dB SPL. Different sound levels were delivered in random order to avoid systematic errors. In panel **b**, the reticular lamina displacement magnitude at 70 dB SPL was affected by an additional sensitivity loss of ~6 dB. This was the last measurement of the experiment.

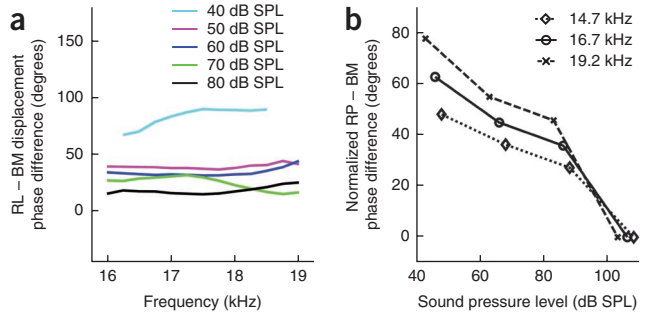

Figure 5 Phase differences of reticular lamina displacement and organ of Corti receptor potential compared with basilar membrane motion. (**a**) Phase of reticular lamina (RL) displacement minus phase of basilar membrane (BM) displacement at different sound levels plotted for a frequency range of 16–19 kHz and sound levels between 40 and 80 dB SPL. Numbers given next to the colored lines represent sound pressure levels. The best frequency was 18 kHz for BM and 18.5 kHz for RL. (**b**) Normalized relative phase of organ of Corti receptor potential (RP) and BM velocity for sound levels between 40 and 110 dB SPL. The best frequency was 18.5 kHz. Potentials were recorded in the fluid spaces within the organ of Corti, adjacent to the outer hair cells. The relative phase lead of electric potentials decreased with sound level, as it did for the phase of RL – BM displacement.

Corti[20,26–28]. This process depends on the motor protein prestin[29] and may involve force production by stereocilia[30,31]. This active process equips the ear with a capacity to detect basilar membrane displacements at the subangstrom level. Although direct comparison with stereociliary displacements is difficult, we note that this displacement magnitude is much smaller than the thermal noise at the hair bundle[10,32,33].

Two potential solutions emerge from the literature. The first requires hair cells to filter the incoming signal to a bandwidth of less than 100 Hz[9,11]. Such a process is difficult to verify experimentally, but if it occurs, the enhancement that we describe would relax the demands on the filter and allow its operation at bandwidths closer to psychophysically measured bandwidths[34]. The other potential solution is cooperation; the stimulation of hair cells in groups around the place of maximum vibration. Responses would be summed across several cells, and the averaged signal would provide a noise-reduced driving stimulus for outer hair cell motility. However, experimental support for this theory is lacking. Our experiments have shown vibration at the reticular lamina to be larger than at the basilar membrane. Because of this enhancement, stereocilia deflections will be closer to the noise-imposed limit found *in vitro*. The maximum difference between the reticular lamina and basilar membrane vibrations was close to a factor of 3, which is substantial and will contribute to solving the problem of detecting faint sound. However, the full resolution of this conundrum will probably require the development of new experimental techniques that can directly test the potential mechanisms mentioned above. The frequency differences are also important because they ensure that hair cell force production has the right timing for counteracting viscous drag[35], one of the main limitations on cochlear sensitivity. An erroneous timing would result in decreased rather than increased hearing sensitivity.

How is the enhanced reticular lamina motion generated? One important factor may be the interaction between the tectorial membrane and outer hair cell stereocilia. Experimental and theoretical studies indicate that the tectorial membrane is capable of resonant motion that enhances stereocilia deflections[36–38], and modeling studies indicate that the geometry of the organ of Corti may have a similar effect[39]. As the cochlea is a feedback system, these factors may lead to augmented force production from outer hair cells that would increase internal differences in motion within the organ of Corti. The lack of enhancement at high stimulus intensities, and the disappearance of the phenomenon post mortem, certainly imply a dependence on outer hair cells, as these are the only cells capable of generating force at the requisite speeds[27] and the effect of their activity is known to be highly dependent on the functional status of the cochlea[40].

Previous studies have found that auditory nerve fibers have tuning that is very similar to that of the basilar membrane[41], but because of the exceedingly demanding nature of such experiments, a frequency difference like that found here could easily escape detection.

All of our findings required the organ of Corti to be mechanically compliant[42–44]; otherwise, the motion of the reticular lamina would be fully reflected in basilar membrane vibrations. Measurements on isolated preparations do suggest gradients of compliance[20], which may lead to differences in motion amplitudes among structures in the organ of Corti[42]. A mechanically compliant organ of Corti would allow the cochlear amplifier to work efficiently to optimize the mechanical stimulation on hair cell stereocilia. Nanoscale differential motion therefore appears indispensable for the near-threshold behavior of the organ of Corti.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/natureneuroscience/.

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AUTHOR CONTRIBUTIONS

F.C., D.Z., A.F., J.Z. and N.C. conducted experiments. F.C., D.Z., A.F., J.Z., X.S. and A.L.N. analyzed and interpreted data. F.C., A.F., J.Z. and A.L.N. wrote the manuscript. F.C., N.C., S.L.J. and R.K.W. designed and built the OCT interferometer. F.C., A.F., J.Z. and A.L.N. designed the experiments. F.C., D.Z., A.F. and J.Z. contributed equally.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Optical coherence tomography. The OCT system uses light with a central wavelength of 1,310 nm and a special bandwidth of 94 nm for imaging and vibration measurement[21,22]. After spectral shaping in the post data processing to enhance the sensitivity of the system, the axial resolution at the cochlea was 6.5 in glass. When imaging, the beam scans across the tissue and reflected photons are used to generate images. With the scanning mirrors positioned on structures of interest, the system was operated as a homodyne interferometer to measure the displacement of the basilar membrane and reticular lamina. Because of the short coherence length of the light source, measurements are localized to within ~7 μm in tissue in the axial direction. The measurements presented here were conducted at the site showing maximum vibration along the radial axis of each structure.

Experimental procedures. Albino guinea pigs (250–350 g) with normal hearing were used. All procedures were approved by the Institutional Animal Care and Use Committee of Oregon Health & Science University. After anesthesia, the left cochlea was exposed and a small fenestra (~0.35 × 0.4 mm) was created in scala tympani. Tones were delivered using an acoustic coupler fitted to the ear canal. Vibrations were measured at the location corresponding to a best frequency near 19 kHz and at the radial location where outer hair cells reside (**Fig. 1c**). Measurements were only made in preparations in which image acquisition yielded a detailed map of organ of Corti structures. Signal generation for acoustic stimuli and data acquisition were performed using a lock-in amplifier (Stanford Research Systems, model SR830) using custom software. Pure tones ranging from 20 to 100 dB SPL at frequencies of 12 to 25 kHz were used for acoustic stimulation. Cochlear sensitivity was monitored throughout the experiment by measuring sound-evoked auditory nerve responses by means of a wire electrode on the round window membrane. Ears with surgically induced hearing loss of less than 15 dB were considered sensitive.

Electrophysiology. For measurement of the local potentials within the organ of Corti, a sharp glass microelectrode with a tip diameter of less than 1 μm was advanced through the opening used for recording mechanical responses. The position of the electrode was controlled by a motorized micromanipulator. Potentials were recorded using a BMA-200 amplifier (CWA Inc) connected to the SR830 lock-in amplifier. The frequency response of each electrode was calibrated while in position within the organ of Corti[45]. Electrode penetration caused an unavoidable 10- to 17-dB loss of auditory sensitivity. Comparisons were therefore performed using basilar membrane data acquired after electrode penetration. Basilar membrane vibration was measured with a conventional laser velocimeter, as previously described[24].

45. Baden-Kristensen, K. & Weiss, T.F. Receptor potentials of lizard hair cells with free-standing stereocilia: responses to acoustic clicks. *J. Physiol.* **335**, 699–721 (1983).

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