Sound-induced differential motion within the hearing organ

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Hearing depends on the transformation of sound-induced basilar membrane vibration into deflection of stereocilia[1] on the sensory hair cells, but the nature of these mechanical transformations is unclear. Using new techniques to visualize and measure sound-induced vibration deep inside the moving organ of Corti, we found that two functionally crucial structures, the basilar membrane and the reticular lamina, have different centers of rotation, leading to shearing motion and rapid deformation for the mechanoreceptive outer hair cells. Structural relations within the organ of Corti are much more dynamic than previously thought, which clarifies how outer hair cell molecular motors can have such a powerful effect.

The high sensitivity and frequency selectivity of the hearing organ (**Fig. 1a**) is dependent on a specialized motor protein, prestin[2], which is localized to the outer hair cells (OHC). Force generated by prestin profoundly affects organ vibration. Consequently, mice lacking this protein have greatly reduced hearing ability[3]. Classically, the organ of Corti has been assumed to vibrate as a stiff unit, without structural changes, around the point where the basilar membrane attaches to the bony core of the cochlea[4] (asterisk in **Fig. 1b**). Indirect experimental data[5,6] support this idea, but a fundamental question remains: how can OHC molecular motors have such a large effect if the structure remains unaltered? One potential solution, also supported by indirect data[7–10], is that the OHCs deform such that the basilar membrane and the reticular lamina are drawn closer together through forces generated by prestin (**Fig. 1c**). There is no experimental data that directly proves or disproves either of these theories.

We used guinea pig cochlear explants[10,11] to get images of the organ of Corti during sound stimulation at 160 Hz, which is near the best frequency of the recording location (**Fig. 2a** and **c**). An acousto-optic modulator inserted in the beam path of the confocal microscope generated 125-μs laser pulses, locked to a specific phase of the sound stimulus, creating a stroboscopic effect that eliminated motion artifacts. Laser pulses were then locked to a sound stimulus phase-separated by 180° from that of the first image, and another image was acquired. Images within a pair were subtracted to highlight the motion (**Fig. 2b** and **d**). To analyze the motion (see **Supplementary Video** online), each frame was filtered by transformation with a two-dimensional wavelet transform. A differential motion constraint equation[10,12] was applied to all wavelet components, yielding an overdetermined linear system. The system was solved by least-squares inversion for the displacement between the frames at each pixel of the image.

Going along the reticular lamina in the direction of the OHCs, displacements increased linearly (**Supplementary Fig. 1**). Motion vectors had an angle of 85–105° relative to the long axis of the reticular lamina, implying absence of radial components. This pattern was seen in 15 image pairs from five different preparations. The linear increase of displacements and the direction of the vectors suggested that the reticular lamina moved around a point situated close to the inner hair cells. To estimate the location of this pivot point, a straight line was drawn down the long axis of the reticular lamina. Using custom software, each point on the line subsequently moved according to the optical flow map, and the displacement was compared to the predicted motion for rotation around a given axis by minimizing the mean-square error over the possible axis locations. Pivot points for 26 different such lines clustered within a confined region close to the apex of the inner hair cell (P_{RL} in **Fig. 2b** and **d**).

Moving along the basilar membrane from its attachment under the inner hair cells to the feet of the outer pillar cells, basilar membrane displacements also increased linearly (**Supplementary Fig. 1**). The amplitude was similar to the segment of the reticular lamina close to the inner pillar cells. Using computations as above, we found that the segment of the basilar membrane that we could visualize had its center of vibration (P_{BM} in **Fig. 2b** and **d**) at a point different from that of the reticular lamina. In the 15 image pairs, this pivot point showed little variability. It was found either at the feet of the inner pillar cells or under the nerve fibers contacting the inner hair cells, in the prolongation of the long axis of the basilar membrane. Pivot points for the

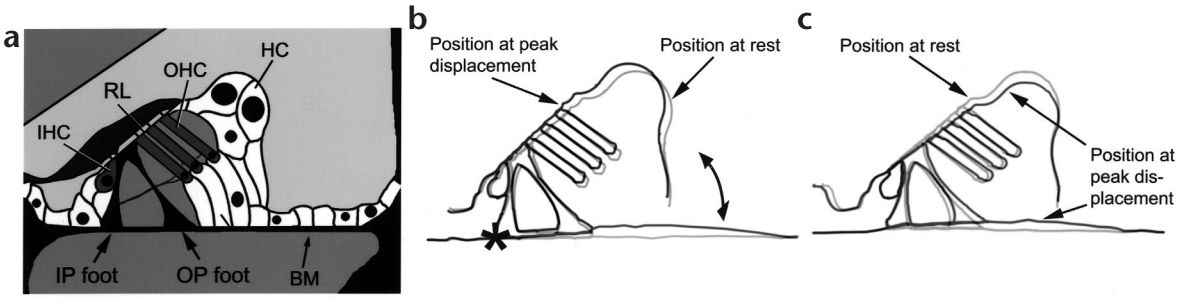

Fig. 1. Drawings of the hearing organ. (**a**) Important anatomical structures. OHC, outer hair cells; IHC, inner hair cell; BM, basilar membrane; HC, Hensen cells; IP, inner pillar cells; OP, outer pillar cells; RL, reticular lamina. (**b**, **c**) Schematic representations of two different theories of organ of Corti motion. The resting position is indicated by the gray outline, and position at peak displacement is indicated by the black outline. In (**b**), only a single degree of freedom is present, so all structures move in unison. In (**c**), at least two degrees of freedom exist, allowing complex deformation during sound-evoked vibration.

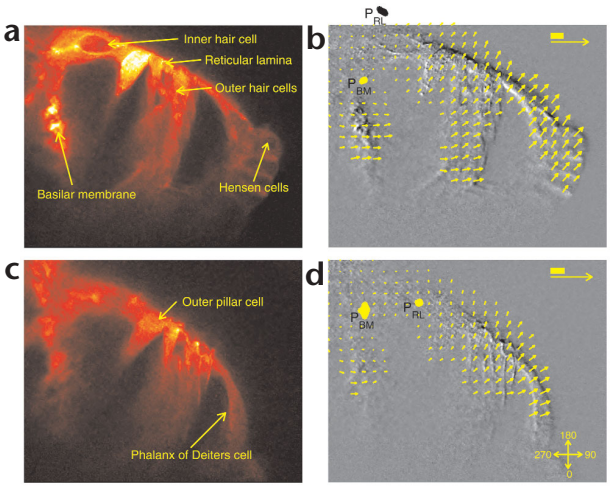

Fig. 2. Confocal images from the apical turn, obtained during sound stimulation. (**a**, **c**) Cells were stained with RH414 and calcein/AM, the fluorescence directed to one detector of a MRC 1024 confocal microscope (Biorad, Hemel Hempsted, UK). When using standard confocal microscopy, obvious blurring occurred. The stimulus level was 126 dB SPL (decibels sound pressure level; the effective level is reduced by opening of the cochlea and immersion of the preparation in tissue culture medium). The regional ethics committee approved all animal procedures. (**b**, **d**) Here, structures without motion appear neutral gray; moving structures are either white or black. The optical flow map was superimposed on the image (yellow arrows), with vectors scaled 3 × relative to the pixel size. Bars and arrows in top right corner show 10-μm distances in the image and 10-μm displacements, respectively. P_{BM} and P_{RL} denote center of rotation for the basilar membrane and the reticular lamina, respectively. Inset at lower right in (**d**) shows the coordinate system used for describing vector directions.

reticular lamina were located inside the inner hair cell or in the prolongation of the long axis of the reticular lamina.

Due to anatomical constraints, it was impossible to visualize the entire length of the OHC. Nonetheless, the stereociliary part could be seen and its motion reliably calculated. Close to the reticular lamina, OHC and reticular lamina vectors had the same orientation, but parts of the cell body closer to the basilar membrane showed an increasing deviation. Reticular lamina vector directions were 20–45° different from the point closest to the basilar membrane that could be measured (**Fig. 2d**; an almost identical pattern occurred for the preparation in **Fig. 2b**). Such motion was a consistent feature that occurred in all experiments. This implies that OHCs deformed, as vectors from different points along the cell membrane had orientations incompatible with rigid rotation around the same center of rotation.

Standard confocal images were acquired before and after acquisition of each image pair obtained during sound stimulation. Structural changes were not seen in any preparation. In a separate series of experiments, Reissner's membrane was penetrated with microelectrodes to measure the cochlear microphonic potential evoked by stimulation with the identical level, duration and frequency that was used during actual experiments. The stimulation caused no change of the microphonics.

The motion pattern was sensitive to manipulations known to affect OHC function. After 15–20 minutes of continuous acoustic overstimulation at levels 12–20 dB above those used during image acquisition, reticular lamina displacements increased in 7 of 8 image pairs (mean increase 76%, range –22 to +240%). Basilar membrane displacements also increased (mean +18%, range –4 to +78%). OHCs were deformed also after the overstimulation, although minor changes of vector orientation occurred. In one preparation, 20 mM of 2,3-butanedione monoxime was perfused through the scala tympani. This compound blocks OHC electromotility *in vitro*[13]. Thirty minutes after adding the drug, OHC vectors had the same orientation as vectors at the reticular lamina, implying absence of deformation. Reticular lamina vibration amplitudes increased after application of the drug (+32%), whereas basilar membrane displacement decreased by 50%. Thus, OHCs controlled a part of the vibration despite the relatively high stimulus level.

In summary, we found that sound stimulation caused cyclic deformation of OHCs and that the basilar membrane and reticular lamina had different centers of rotation. OHC deformation may have important implications, given that these cells respond to mechanical stimuli directed at the cell membrane[14], and that they possess a chloride current activated by membrane stretch[15]. By showing that the basilar membrane and reticular lamina are capable of independent motion, an important feature of the dynamic model (**Fig. 1c**) is confirmed, although we did not observe compression of the organ. The classical rigid-body theory of organ motion is thereby refuted. This differential motion also explains how forces generated by prestin can so profoundly affect hearing sensitivity. Contrary to previous assumptions, the hearing organ has a highly dynamic structure that provides a flexible framework for coupling force generation to vibration.

Note: Supplementary information is available on the Nature Neuroscience website.

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Competing interests statement

The authors declare that they have no competing financial interests.

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