Does the Apical Organ of Corti act as an Electromechanical Transistor?

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The inner ear not only transduces sound-induced vibration into electrical signals, it also amplifies weak sound to boost its detection. The actuators of this active process are sensory outer hair cells in the organ of Corti, whereas the inner hair cells ensure that the sound-induced signals reach the auditory nerve. However, how the outer hair cells modulate the stimulus to the inner hair cells remains unclear. Using theoretical modeling and experimental measurements, we find that the stimulation of the inner hair cells depends on the resting length of the outer hair cells. When these are in an elongated state, sound causes minimal stimulation of inner hair cells, whereas outer hair cell contraction leads to large amplification of sound-evoked motion. This novel mechanism for regulating the sensitivity of the hearing organ is analogous to an electromechanical transistor, and could be important for our ability to listen selectively to speech-frequency sounds.

Our ability to hear is due to an intricate mechanotransduction process that takes place inside the inner ear. Sound-evoked waves on the basilar membrane, an elastic structure stretching along the cochlear canal, cause the deflection of mechanosensitive hair bundles of the sensory cells, thus gating ion channels in the cell membrane and producing electrical signals that are ultimately transmitted to the brain. The transfer of basilar-membrane motion to deflection of the hair bundles is shaped by the structurally complex organ of Corti, where the outer hair cells have a prominent role. Changes in transmembrane voltage cause these cells to change length, a phenomenon referred to as electromotility. Furthermore, the hair bundles of outer hair cells can also generate mechanical force. Both mechanisms may contribute to an active modulation of the sound-evoked motion of the organ of Corti. This active mechanical feedback by outer hair cells is essential for the extraordinary sensitivity, tuning, and dynamic range of mammalian hearing organs, and damage to the outer hair cells consequently results in hearing loss. However, the way in which outer hair cells modulate the motion of the organ of Corti is a subject of intense research. Moreover, the operation of outer hair cells in the base of the cochlea, where very high-frequency sounds are transduced, appears to be different from that in the low-frequency region of the cochlea, the apical one. Although the cochlear apex is responsible for detecting frequencies below a few kHz that are most important for speech and music, the micromechanics of the organ of Corti in this region of the inner ear remains particularly poorly understood.
Recent in vitro experimental studies have shown that the apical organ of Corti deforms in a complex and unexpected way. When stimulated electrically, the outer hair cells contract and pull the reticular lamina, where the mechanosensitive hair bundles are located, downwards towards scala tympani. Surprisingly, the lateral portion of the organ of Corti composed of the Hensen cells moves in the opposite direction with an amplitude larger than that of the reticular lamina, while no motion could be detected on the adjacent portion of the basilar membrane. Such a pattern of motion has not been observed from the cochlear base, and it raises questions about its biophysical origin and functional significance. However, direct imaging of the underlying internal motion of the organ has so far not been possible.

Here we set out to identify the origin and the functional role of the complex motion of the organ of Corti at the cochlear apex. We show that a plausible assumption about the apical organ of Corti, namely that each cross-section is incompressible, highly constrains the organ’s internal motion. The deformation of the organ of Corti that results from length changes of the outer hair cells can then be described through a mathematical model that is largely based on the organ’s geometry. We develop this model and verify it through comparison with existing as well as newly acquired experimental data. Our results reveal that the apical organ of Corti can function as an electromechanical transistor where the resting length of the outer hair cells can sensitively determine how much of their motion is transferred to the reticular lamina, thus providing a novel mechanism for outer hair cells to regulate hearing sensitivity.

Results

Local incompressibility of the apical organ of Corti

Sound elicits a traveling wave on the basilar membrane which triggers the deflection of hair bundles and thus the electromotile response of the outer hair cells. As the outer hair cells contract, the reticular lamina and basilar membrane are pulled towards each other. This can potentially reduce the cross-sectional area of the fluid-filled space of Nuel, causing fluid to be displaced longitudinally, that is, along the cochlear canal. The volume of displaced fluid is proportional to the change in cross-sectional area, multiplied by the longitudinal extent of the organ that contracts. For a traveling wave, this longitudinal extent is approximately half the wavelength, and the amplitude of the evoked fluid velocity is proportional to the displaced volume and thus to the wavelength.

Near the cochlear apex, low-frequency sound elicits a wave with a long wavelength of several millimeters. The longitudinal extent over which the organ of Corti deforms similarly thus far exceeds the width and the height of the space of Nuel, which are of the order of 100 µm. Longitudinal fluid flow would thus require high velocities, far above those required for fluid displacement within a transverse section, and would hence be countered by viscous friction. We conclude that longitudinal flow is suppressed and that the cross-sectional area of the organ of Corti in the apex is accordingly approximately conserved when the outer hair cells change length. The same reasoning holds for in vitro experiments using electrical stimulation, due to the long effective range of the electrodes, but not for deformation of the organ of Corti near the cochlear base where the wavelengths in the peak region of a traveling wave can be much shorter, below one millimeter.

We further notice that the cross-section of the organ of Corti can be divided into two components, namely a fluid-filled space on the neural side of the organ, and a portion representing the body of Hensen cells on the abneural side. The cross-sectional area of each component needs to be conserved separately: the fluid space because of our argument above, and the Hensen cells because their cytoplasm cannot escape longitudinally.

Description of the geometric model

The motion of the cochlear partition can be decomposed into a passive component, where all structures follow the sound-evoked displacement of the basilar membrane, and an active component that
involves internal deformation of the organ of Corti caused by outer hair cell forces. Here we are interested in the latter and determine the motion of various structures of the organ of Corti—in particular of the Hensen cells, the reticular lamina, and the outer hair cells—with respect to the basilar membrane.

We use the constraint of a conserved cross-sectional area to estimate the active deformation of the organ of Corti on geometric grounds. We hereby characterize the length change of outer hair cells by a variable $\epsilon$, such that the length of an outer hair cell is given by $L_{OHC}(\epsilon) = (1 - \epsilon)L_{OHC,0}$, where $L_{OHC,0}$ is the resting length of the cell (Fig. 1b). A length change of the outer hair cells can result from electromotility as well as from hair bundle motility that can exert force on the reticular lamina. Experiments on isolated outer hair cells indicate that $|\epsilon| \lesssim 0.02^4$. Other anatomical elements of the organ of Corti are assumed to have constant length, except for the Deiter’s cells and the contour of the Hensen cells. Motion of the reticular lamina can be approximated as pivoting about the top of the pillar cells, which is why we summarize the three rows of outer hair cells and Deiter’s cell in a single, effective row, located approximately at the location of the third row.

Since we consider small deformations only, we assume linear relationships between the length change of the outer hair cells and the length $L_{DC}(\epsilon)$ of the Deiter’s cells as well as the length $L_{HC}(\epsilon)$ of the contour of the Hensen cells. We can therefore write $L_{DC}(\epsilon) = (1 + \epsilon \Delta)L_{DC,0}$ and $L_{HC}(\epsilon) = (1 + \epsilon \Gamma)L_{HC,0}$ with the resting lengths $L_{DC}(\epsilon = 0) = L_{DC,0}$ and $L_{HC}(\epsilon = 0) = L_{HC,0}$. The two parameters $\Delta$ and $\Gamma$ describe the extensibilities of the Deiter’s cells and of the Hensen cell contour, respectively. We assume that the Deiter’s cells can pivot around their attachment on the basilar membrane and that they do not bend. The arc of Hensen cells is treated as an elastic rod that deforms around a preferred shape, characterized by its local curvature along its length. Details of the model calculations are given in the Online Methods.

Realistic values for the two model parameters $\Delta$ and $\Gamma$ depend on geometrical and structural properties that are not a priori known. However, our modeling analysis reveals that different parameter values lead to qualitatively different deformation patterns of the organ (Figs. 2, 3). Through comparison with available experimental data we can therefore highly restrict these values (SI). In particular, the parameter values determine the vertical and horizontal displacement of key points along the Hensen cells upon outer hair cell contraction (Figs. 2c-f), as well as amount
Figure 2. Predicted motion of the Hensen cells for different parameter values. (a) We characterize the motion of the Hensen cells through the displacements of two points on the top and on the side of the Hensen-cell contour. (b) The motion pattern of our organ of Corti model that is consistent with experimental data involves large displacements at the base of the outer hair cell upon outer hair cell contraction. (c-f) Displacement components of the two points on the Hensen-cell contour for a hair-cell contraction $\epsilon = 0.005$ and different choices of $\Delta$ and $\Gamma$. (c) The top of the organ moves always upward when outer hair cells contract. (d) The radial displacement of the top position shows a more complex behaviour: both motion towards and away from the stria vascularis can occur under outer hair cell contraction, depending on the values of the extensibilities. (e,f) The direction of both the vertical and the radial motion of the side point depend on the values of the extensibilities as well. However, this motion was not experimentally accessible. The parameter values that are identified as biologically realistic through comparison with experimental data are indicated through an asterisk and are used in (b).
(Figs. 3a) and nonlinearity (Figs. 3b,c) of reticular-lamina and Hensen-cell motion as a function of outer hair cell contraction $\epsilon$. Comparison of model behavior with data on electrically-evoked motion of the Hensen cells shows that only values of approximately $\Delta = 1.15$ and $\Gamma = 0.1$ yield a model consistent with the observed motion (SI). Surprisingly, we find that the motion pattern observed at the surface of the organ suggests very large internal motion through large displacements of the bases of the outer hair cells (Fig. 2b).

**Displacement of outer hair cells**

Having constrained both free parameters of our model, we compared the resulting model predictions to additional known features of apical micromechanics. Recent *in vitro* experiments have shown that outer hair cells essentially pivot around their attachment at the reticular lamina when stimulated electrically. Outer hair cells were first subjected to a negative current, yielding a reference state, and then to a positive current of equal magnitude. The change in current leads to contractions of the outer hair cells $\epsilon$ implies that the absolute value of the derivative of $D_{RL}$ with respect to $\epsilon$ varies with $\epsilon$. The relative change is particularly strong for a large extensibility $\Delta$ of the Deiter’s cells, which has important functional implications.

(Figs. 4a) The angle of this rotation was quantified for different amplitudes of electrical stimulation; it increases linearly for small stimulation amplitudes and saturates at larger ones (Fig. 4b). In our model, the reticular lamina moves much less than the length change of the outer hair cells which is consistent with the essentially rotational motion of the outer hair cells found experimentally. Direction and amount of the rotation depend on the size of the outer tunnel of Corti as parametrized by the angle $\varphi$ between the outer hair cells and the arc of the outer tunnel (Fig. 1b, Fig. 4c). For simplicity, we here consider the reticular lamina as fixed and regard the organ at the hyperpolarized state of the outer hair cell as the reference position. The amount of contraction in Fig. 4c therefore ranges from $\epsilon = 0$ to approximately $\epsilon = 0.04$, rather than from $\epsilon = -0.02$ to $\epsilon = 0.02$ as before. Our model correctly predicts the direction of outer hair cell rotation when the outer tunnel is large, which agrees with the geometry commonly seen in micrographs: the realistic geometry is arguably...
Figure 4. The effect of geometry on outer hair cell displacement. (a) In vitro experiments show that, under current stimulation, the outer hair cell pivots around its apex. This motion can be characterized by the angle $\alpha$ of somatic rotation. (b) The somatic rotation angle $\alpha$ increases with the size of the current stimulation and saturates for high values (black line, dashed lines show the 95% confidence intervals). The displacement is directed towards the stria. The data are reused from earlier experiments$^{18}$. (c) The predicted angle $\alpha$ of rotation of the outer hair cells varies with the size of the arc of the outer tunnel. A positive angle corresponds to a counter-clockwise rotation. The largest arc of the outer tunnel (blue) represents a realistic geometry and implies an outer hair cell rotation that agrees well with measurements in both direction and magnitude.

Displacement of Hensen cells at different focal levels

While our model does not explicitly describe displacements of internal points of the Hensen cells, it suggests a motion pattern in which the entire body of Hensen cells is essentially displaced as one by the contracting outer hair cells with little internal deformation. As a consequence, structures at different depths within the organ are expected to show approximately constant vertical displacement, and the displacement should decrease only close to the basilar membrane. In particular, the direction of displacement should remain the same throughout the entire height of the organ. In contrast, if the observed motion was caused by fluid being pressed into the outer tunnel, vertical displacement would vary markedly and change direction as a function of depth.

We interferometrically determined current-evoked displacements from positions at different depths. We found that the direction of Hensen-cell displacement, as well as the displacement amplitude, vary little with depth (Fig. 5a). While the direction of the displacements with respect to the applied current was consistent in all preparations, the amplitude of the evoked displacements varied considerably between preparations, as well as with time in a given preparation. For this reason, results shown in Fig. 5b have been normalized to the average displacement at the surface of the Hensen cells. The displacement amplitude exhibited a small but significant decrease with increasing depth (13% on average; a linear mixed model reveals a negative slope of $-0.0008/\mu m$ in normalized displacement units, $p = 0.0014$). This agrees with our model that revealed that the counter-intuitive direction of Hensen-cell motion under electrical stimulation is due to large motion at the bases of outer hair cells.

As the measurement became increasingly noisy with increasing depth inside the tissue, we were not
Figure 5. Displacement at different depths in the organ of Corti under current stimulation. (a) Representative recordings at different depths under the arc of Hensen cells under negative and positive current stimulation (bottom). (b) Displacements of cumulative data from various pulse protocols are shown. The data have been normalized with respect to the average displacement at the surface of the organ, due to high variability in absolute values between preparations, and with time for a given preparation. Positive and negative current stimuli are shown by ‘(+)' and ‘(o)' symbols, respectively. Presence and absence of sound stimuli are shown by color codes ‘red’ and ‘black’, respectively. Mean values for each set of data with respect to depth only are shown by a flat line.

able to determine the location of the basilar membrane. The fact that large displacement amplitudes persist with depth suggests, however, that some basilar membrane motion occurs underneath the Hensen cells. In contrast, such motion was not detectable in the portion of the basilar membrane lateral to the organ of Corti. This is consistent with recent in vivo measurements obtained using optical coherence tomography.

Functional implications of the predicted reticular-lamina motion

Inner hair cells are responsible for detecting the mechanical sound vibrations and transducing them into electrical signals that are then forwarded to the brain. The hair bundles of the inner hair cells are deflected by the fluid flow between the reticular lamina and the tectorial membrane, and this fluid flow is proportional to the reticular lamina vibration. The nonlinear reticular-lamina displacement upon length change of the outer hair cells that is predicted by our model has therefore striking implications for the functioning of the inner ear (Figs. 3b,c).

Sound vibration at a frequency $f$ leads to an oscillating length change of the outer hair cells around some resting position $\epsilon^{(0)}$:

$$\epsilon(t) = \epsilon^{(0)} + \epsilon^{(osc)} \sin(2\pi ft).$$

(1)

This length change elicits an oscillating reticular-lamina motion $D_{RL}(t)$ at an amplitude $D_{RL}^{(osc)}$ around the steady displacement $D_{RL}^{(0)}$ that is set by the outer hair cell’s steady contraction $\epsilon_0$:

$$D_{RL}(t) = D_{RL}^{(0)} + D_{RL}^{(osc)} \sin(2\pi ft).$$

(2)

The amplitude of an oscillating length change of an outer hair cell for sound pressures in the hearing range is small, $|\epsilon^{(osc)}| \ll 0.02$. The amplitude of the resulting reticular-lamina vibration $D_{RL}^{(osc)}$ can thus be approximated by a linear expansion around the resting amplitude $D_{RL}^{(0)}$:

$$D_{RL}^{(osc)} = \left. \frac{dD_{RL}}{d\epsilon} \right|_{\epsilon^{(0)}} \epsilon^{(osc)}.$$  

(3)
For the Deiter’s cell extensibility $\Delta = 1.15$ that we identified from a qualitative and quantitative comparison of the model with experimental results, the derivative of reticular-lamina displacement with respect to hair-cell contraction, $dD_{RL}/d\epsilon$, is found to vary monotonically from approximately zero at a resting length change $\epsilon^{(0)} = -0.02$ of the outer hair cells to a value of approximately -7 at $\epsilon^{(0)} = 0.02$. As a result, an oscillating length change of the outer hair cells around a maximally elongated resting length defined by $\epsilon^{(0)} = -0.02$ produces virtually no oscillation of the reticular lamina. On the other hand, a vibration of the outer hair cell length around a maximally contracted resting length defined by $\epsilon^{(0)} = 0.02$ leads to a leverage of a small superimposed oscillation of the outer hair cell length into a vibration of the reticular lamina at a seven-fold higher amplitude.

Figure 6. Operation of the apical organ of Corti as a mechanoelectrical transistor. How much of an oscillatory length change in outer hair cell motion translates into reticular-lamina vibration depends critically on the operating point set by the static length change of the outer hair cell. An oscillatory length change of an outer hair cell around an elongated state, characterized by a negative value of $\epsilon$, leads to only a very small motion of the reticular lamina (blue). The vibration of the reticular lamina becomes increasingly larger for outer hair cells that oscillate around a progressively more contracted length (red and green).

For what frequencies does our argument hold? In our experiments, the organ of Corti deforms rapidly in response to current stimulation. Observed relaxation times in vitro from a deformed state to the resting configuration are of the order of a few milliseconds, suggesting that forcing frequencies of a few hundred Hertz can be considered low enough for the organ of Corti to act as if in a quasi-static regime where its impedance is dominated by stiffness, rather than viscosity. This is also consistent with earlier results which suggest that for frequencies up to 500 Hz the compressional impedance of the organ of Corti that is due to stiffness exceeds viscous impedance by a factor of at least three in the cochlear apex.

The resting length of the outer hair cells thus sensitively determines how much vibration of the reticular lamina is elicited by an oscillating length change of the outer hair cells at low frequencies. The organ of Corti can thus act as an electromechanical transistor: the oscillating length change of the outer hair cells corresponds to an alternating voltage at the transistor’s input terminal, the vibration of the reticular lamina represents the output voltage, and the resting length change of the outer hair cell sets the operating point, controlling the amplification of the output compared to the input.


Discussion

We have developed a model for the deformation of the organ of Corti that is based on the organ’s geometry as well as on the plausible assumption that the organ of Corti near the cochlear apex is incompressible. The model involves only two parameters that are not derived from the organ’s geometry, namely the extensibility of the Deiter’s cells and of the outer edge of the Hensen cells. Qualitative comparison of model predictions with experimental data on the vibration of the Hensen cells highly constrains these parameters, and the resulting model predictions agree excellently with further data on the displacement of the outer hair cells and the vertical vibration at different depths in the organ.

Our model generically produces the counterphasic motion of the reticular lamina and the Hensen cells that was recently observed experimentally. Importantly, our analysis suggests that this behaviour does not result from perilymph being pressed against the Hensen cells, as hypothesized recently. Instead, our model and our measurements evidence that the entire body of Hensen cells is being pulled upwards by the contracting outer hair cells. Generally, the experimental data is reproduced if the base of the outer hair cell is allowed to move somewhat more than its apex, such that the largest displacements then occur inside the organ of Corti. Intriguingly, this is corroborated by our own recent in vivo measurements using optical coherence tomography.

What is the origin of this internal motion? In our model, this pattern is achieved through Deiter’s cells that are fairly compliant, at least in response to quasi-static or low-frequency outer hair cell forcing. Alternatively, or in addition to that, large displacements at the bases of outer hair cells could also occur as a consequence of a locally very compliant basilar membrane. We have not detected basilar-membrane motion lateral of the organ of Corti in response to current stimulation. However, our interferometric measurements from different depths inside the Hensen cells indicate that some basilar-membrane motion is present in a limited region underneath the organ, while the decrease in amplitude with depth suggests that some stretching occurs as well. Cross-sectional area conservation of the organ of Corti would then require counterphasic displacement of the arcuate zone of the basilar membrane, as observed by Nuttall et al. in more basal regions in response to electrical stimulation. We did not include this mode of deformation in our model, as no corresponding data is available for the cochlear apex, and as this would necessitate additional fitting parameters. Including a bimodal motion of the basilar membrane may lead to smaller radial motion at the side of the organ in order to conserve the cross-sectional area of the organ. To directly determine the precise deformation pattern of the organ of Corti experimentally, and to understand the unusual deformation of the basilar membrane remains a subject for future research.

Current theories of cochlear function suggest that the mechanical activity of outer hair cells serves to amplify the motion of the basilar membrane or the reticular lamina in order to render faint sounds more easily detectable for the stereocilia of inner hair cells. In this light, it seems surprising that the largest motion would occur in the interior of the organ. However, our geometrical analysis and experiments suggest that this motion pattern is associated with a nonlinear dependence of the reticular-lamina motion on the length of the outer hair cells. In consequence, we find that the organ of Corti can behave as a mechanoelectrical transistor in which the resting length of the outer hair cells can control the vibration of the reticular lamina that is evoked by an oscillating length change of the outer hair cells. Experimental evidence for this effect comes from the observed nonlinear dependence of sound-evoked motion on an imposed endocochlear potential in vitro.

It has been suggested previously that static length changes of the outer hair cells might influence the operating point of hair bundles, or of the micromechanics of the organ of Corti as a whole, but the details of such a mechanism have remained unclear. Our analysis shows that the incompressibility of the organ of Corti together with a high level of compliance at the base of outer hair cells yields a novel and intriguingly simple mechanism for the outer hair cells to very precisely regulate hearing sensitivity through their static length change. While we have thus shown the availability of such a mechanism, further experimental work and improved imaging techniques are needed to verify it in the living cochlea.

Our geometrical model quantifies the internal motion of the organ of Corti. The actual sound-
evoked and active motion of the cochlear partition is a linear combination of the internal deformation and an overall net displacement. While internal motion is due to active amplification by outer hair cells, the net displacement of the organ can be caused both by sound stimulation as well as by the mechanical activity of outer hair cells. In a recently proposed ratchet mechanism, or unidirectional amplification, the outer hair cells may cause only internal deformation of the organ of Corti without displacement of the basilar membrane\textsuperscript{24}, in agreement with some recent experimental observations\textsuperscript{14}. Further modeling that integrates the geometric model presented here with an analysis of the different forces produced by outer hair cells and their effects on the overall motion of the organ of Corti, as well as further experimental results on the linear or nonlinear response of the reticular lamina and the basilar membrane to varying sound intensity, are needed to clarify these issues.

Our findings are particularly relevant for two lines of further research. First, our results could shed new light on the role of a static and frequency-dependent motile response of outer hair cells to acoustic stimulation that was identified almost thirty years ago, but whose biophysical origin and function in the cochlea remain poorly understood\textsuperscript{35,36}. Indeed, outer hair cells exhibit predominantly static contractions, and sometimes elongations, when stimulated with sound. Moreover, the amount of contraction is largest at a particular sound frequency, and this frequency matches the characteristic frequency of the cochlear location of the outer hair cell. Our model shows that a frequency-selective sustained length change of outer hair cells can serve as an effective tuning mechanism which can circumvent the poor mechanical tuning of the basilar membrane in the cochlear apex\textsuperscript{2}. As set out above, elongated outer hair cells will transfer only little of their oscillating length change to the reticular lamina. The mechanical sound signal elicited by a pure tone may, however, cause outer hair cells at the characteristic position to contract such that their additional oscillatory response to sound is leveraged into a large vibration of the reticular lamina and thus of the hair bundles of the inner hair cells. This effect can thus endow the motion of the reticular lamina with a frequency selectivity that is independent of the mechanical tuning of the basilar membrane which is comparatively poor in the cochlear apex\textsuperscript{2}.

Second, the discussed principle could present a potential mechanism for efferent medial olivocochlear (MOC) nerve fibers that innervate the outer hair cells to modulate the auditory stimulus\textsuperscript{37}. This efferent feedback is thought to play an important role, for example, in our ability to understand speech in noisy environments. Signals from these fibers, communicated by the neurotransmitters acetylcholine (ACh) and gamma-aminobutyric acid (GABA), are known to cause hyperpolarization of the outer hair cells\textsuperscript{38}. Although experiments on isolated outer hair cells did not detect such an effect\textsuperscript{38}, such hyperpolarization is usually associated with an elongation of the cell body. It has been suggested previously that efferently induced changes in the configuration of outer hair cells could modify the transfer function from basilar-membrane motion to inner hair cell stimulation. What we suggest here, in contrast, is a modification of the transfer of outer hair cell activity to reticular-lamina motion that might be achieved through efferently mediated length changes of the outer hair cells. Recent experiments have indeed observed efferently induced modifications in the auditory nerve signal that is not found in the mechanics of the basilar membrane, suggesting that inner hair cell stimulation is in part directly due to outer hair cell activity\textsuperscript{39}. This effect was present throughout the cochlea, and was particularly prominent in low-frequency regions. A mechanism as the one described here could underlie these observations.

References


Online Methods

Model Geometry

Detailed morphometry of the guinea pig’s organ of Corti in the cochlear apex has been performed by Kelly\(^40,41\) and Teudt and Richter\(^42\). We use their data in conjunction with high-quality micrographs from other authors (Lenoir, Fridberger) as a basis for our geometrical model (see Fig. 1). Relative sizes and orientations of different structures in the organ of Corti show a high level of consistency, so that our derived geometry of the organ of Corti cross section can be considered realistic. The contour of the Hensen cells is represented by a polynomial curve approximating the shape seen in micrographs. Since we assume the reticular lamina to pivot as a stiff rod around its attachment near the inner hair cell\(^8,9\), we have for simplicity summarized the three rows of outer hair cells and Deiter’s cells into a single one, located at the position of the outermost row.

Model Equations

Deformation of the fluid space. The fluid space of the organ of Corti can be further decomposed into three subcomponents (Supplementary Fig. 1): the triangle formed by the basilar membrane and the pillar cells (the tunnel of Corti) with cross-sectional area \(A_{TC}\), a polygonal space defined by basilar membrane, reticular lamina, the outer pillar, the outermost outer hair cell, and the Deiter’s cell (the space of Nuel) with cross-sectional area \(A_{SN}\), and the outer tunnel adjacent to the Hensen cells with cross-sectional area \(A_{OT}\). We now give the equations determining the configuration change of the fluid space when the outer hair cell changes its length by a given amount, such that its total cross-sectional area remains conserved.

The elements shaping the tunnel of Corti are comparatively stiff\(^43\), so that we assume its shape to be unaffected by outer hair cell forces and accordingly \(A_{TC} = \text{const.}\).

The outer tunnel is assumed to be of circular shape with arc length \(a\). When it deforms due to motility of the outer hair cell, it deforms into another circular segment with the same arclength. This is motivated by the fact that its outer wall is supported by stiff polymer cables\(^13\). Let \(\varphi\) be the angle between the outer-tunnel arc and the adjacent outer hair cell (Supplementary Fig. 1). The length \(L_{OHC}\) of the outer hair cell, corresponding to the chord length of the circular segment representing the outer tunnel, is given by

\[
L_{OHC} = a \frac{\sin \varphi}{\varphi}. \tag{4}
\]

The area \(A_{OT}\) of the outer tunnel as a function of \(a\) and \(\varphi\) is

\[
A_{OT}(a, \varphi) = a^2 \left( \frac{1}{4\varphi} - \frac{\sin 2\varphi}{8\varphi^2} \right). \tag{5}
\]

Let now \(L_{OHC,0}\) be the initial length of the adjacent outer hair cell and assume it changes to \(L_{OHC}(\epsilon) = (1 - \epsilon)L_{OHC,0}\) for some small value \(\epsilon\) with \(|\epsilon| \ll 1\), which in the following we will call the contraction, as \(\epsilon > 0\) corresponds to a shortening, and \(\epsilon < 0\) to an elongation of the outer hair cell. If initially \(\varphi(0) = \varphi_0\), then we find the new angle \(\varphi(\epsilon)\) using equation (4), which yields the relation

\[
\frac{\sin \varphi(\epsilon)}{\varphi(\epsilon)} = (1 - \epsilon)\frac{\sin \varphi_0}{\varphi_0}. \tag{6}
\]

To account for the possibility that the Deiter’s cell might deform elastically\(^30\) rather than just rotate in response to outer hair cell contractions, we introduce the Deiter’s-cell extensibility \(\Delta\) as a free parameter such that the length of the Deiter’s cell is given by \(L_{DC}(\epsilon) = (1 + \epsilon\Delta)L_{DC,0}\). A linear relation is justified since experiments on isolated outer hair cells have established that \(|\epsilon| \lesssim 0.02\)^4.

In contrast, we assume that the reticular lamina is stiff enough such that its length remains unaltered. Its motion is thus constrained to rotations around its attachment at the apex of the outer pillar cell.
The area $A_{SN}$ of the polygonal space of Nuel is found using the general formula for the area of a polygon with $N$ vertices $x_i = (x_i, y_i)$,

$$A_{poly}(x_1, x_2, \ldots, x_N) = \frac{1}{2} \text{abs}\left( \begin{vmatrix} x_1 & x_2 & x_3 & \cdots & x_N & x_1 \\ y_1 & y_2 & y_3 & \cdots & y_N & y_1 \end{vmatrix} \right),$$

(7)

where $\text{abs}(\cdot)$ denotes the absolute value and $|\cdot|$ are determinants. In our model, the only vertices that are not fixed by assumption are the two endpoints $a$ (the cellular apex) and $b$ (the base) of the outer hair cell, so that $A_{SN} = A_{SN}(a, b)$. We see that given an outer hair cell contraction $\epsilon$, we require four equations to determine the deformed configuration of the fluid space. Three equations arise because the lengths of the reticular lamina, the outer hair cell, and the Deiter’s cell are given. The fourth equation comes from the constant-area condition and reads

$$A_{SN}(a_0, b_0) + A_{OT}(a, \varphi_0) = A_{SN}(a_\epsilon, b_\epsilon) + A_{OT}(a, \varphi(\epsilon)).$$

(8)

The system of equations is then solved for the unknown endpoints of the outer hair cell $a_\epsilon$ and $b_\epsilon$.

Note that in all our calculations we consider only internal deformation of the organ of Corti and keep the basilar membrane fixed as a reference. Sound can elicit basilar-membrane vibration which is then superimposed to the motion considered here.

**Shape deformation of the Hensen-cell body.** The Hensen cells form the abneural portion of the organ of Corti that runs approximately along the midline of the basilar membrane. Rather than modelling the detailed mechanics of the Hensen cells, we take a simplified approach and model the contour of the Hensen cells as an elastic rod deforming around a preferred shape. The allowed deformations are constrained by the requirement that the cross-sectional area of the Hensen-cell region remains constant. Hence, the deformations are determined by minimizing with appropriate boundary conditions a functional of the form

$$E[\gamma] = \frac{1}{2} \int_0^{L_{HC}} ds \left[ \kappa_\gamma(s) - \kappa_{\gamma_0}(s) \right]^2,$$

(9)

where $L_{HC}$ is the length of the contour, $\gamma(s)$ and $\gamma_0(s)$ are arbitrary arc-length parametrisations of the deformed and initial contours, respectively, and $\kappa_\gamma(s)$ denotes the signed curvature given by

$$\kappa_\gamma = \frac{\det(\gamma', \gamma'')}{||\gamma'||^3}.$$

(10)

The expression (9) is in fact a one-dimensional version of the Helfrich Hamiltonian used to determine the shape of elastic membranes with spontaneous curvature. This way, however, the contour is implicitly assumed to be inextensible. Clearly, the contour in reality being the outer boundary of a viscoelastic body, this need not be true and its length may change from an initial length $L_{HC,0}$ to a new length $L_{HC}(\epsilon) = (1 + \epsilon \Gamma)L_{HC,0}$. The amount of length change is determined by the mechanical properties, as well as the geometry of the Hensen cells and is not a priori known. The extensibility $\Gamma$ thus presents another free parameter of our model. To take the length change of the contour into account, we modify the above functional to

$$E[\gamma] = \frac{1}{2} \int_0^{L_{HC}(\epsilon)} ds \left[ \kappa_\gamma(s) - \kappa_{\gamma_0}(s/\Gamma_\epsilon) \right]^2,$$

(11)

where we used the shorthand notation $\Gamma_\epsilon = 1 + \epsilon \Gamma$. We hereby make the simplifying assumption that the contour is stretched uniformly along its length. As $\Gamma_\epsilon$ is always close to one, we also make the approximation of comparing curvatures between the position $s$ of the new curve and $s/\Gamma_\epsilon$ of the initial curve.
It is convenient to parametrise the contour in terms of its local angle \( \phi(s) \) with respect to an arbitrary but fixed axis, which we take to indicate the \( x \)-axis in a cartesian coordinate system (Supplementary Fig. 1). In this coordinate system, the curve is given as \( \gamma(s) = (x(s), y(s)) \) with

\[
x(s) = \int_0^s d\tau \cos \phi(\tau),
\]

\[
y(s) = \int_0^s d\tau \sin \phi(\tau).
\]

The curvature now takes the very simple form

\[
\kappa_e(s) = \phi'(s).
\]

The endpoint \( s = 0 \) corresponds to the fixed outer edge of the Hensen cell body and lies at the origin. The position \( (x_e, y_e) \) of the endpoint at \( s = \LHC \), coinciding with the apex of the outer hair cell, is determined from the deformation of the fluid space of the organ. This introduces two additional constraints to the variational problem. Extending accordingly equation (11), our complete functional reads

\[
E[\phi] = \int_0^{\LHC} ds \left\{ \frac{1}{2} \left[ \phi'(s) - \Gamma_e \phi_0'(s/\Gamma_e) \right]^2 \right. \\
+ \lambda_x [x_e/\LHC - \cos \phi(s)] + \lambda_y [y_e/\LHC - \sin \phi(s)] \right. \\
+ \lambda_A [\alpha/\LHC - y(s) \cos \phi(s)] \left. \right\}, \quad (15)
\]

where \( \lambda_x, \lambda_y, \) and \( \lambda_A \) are Lagrange multipliers to account for the endpoint and area constraints, and \( \alpha \) is an appropriate constant to ensure the constant-area condition. We require two boundary conditions for the angle function \( \phi(s) \). On the abneural side, i.e. at \( s = 0 \), there is no evident physical constraint imposed on \( \phi \). The appropriate boundary condition is then given by the natural boundary condition \( \phi'(0) = \Gamma_e \phi_0'(0) \). At \( s = \LHC \), where the Hensen cells join with the reticular lamina, we require that the angle between the contour and the outer tunnel arc remains constant. For an appropriate value \( \phi_e \), the boundary condition hence reads \( \phi(\LHC) = \phi_e \).

To solve the problem computationally, we derive it in discretized form. For convenience, we divide the arc length \( \LHC \) of the contour into \( N \) equal elements of finite size \( \Delta s \). The function \( \phi(s) \) becomes an \( (N+1) \)-dimensional vector \( \phi = (\phi_i = \phi(s_i)) \) of function values at the discrete locations \( s = (s_i) \) along the contour. Introducing \( \lambda \) as the vector of Lagrange multipliers and \( \phi_0 = (\phi_0 = \phi_0(s_i/\Gamma_e)) \) as the vector of initial function values, the functional (15) above becomes the Lagrange function

\[
\mathcal{L}(\phi, \lambda) = \sum_{i=0}^{N-1} \frac{\Delta s}{2} \left( \frac{\phi_{i+1} - \phi_i}{\Delta s} - \Gamma_e \frac{\phi_0_{i+1} - \phi_0_i}{\Delta s} \right)^2 \\
+ \lambda_x \left( \sum_{i=0}^N \Delta s \cos \phi_i - x_e \right) + \lambda_y \left( \sum_{i=0}^N \Delta s \sin \phi_i - y_e \right) \\
+ \lambda_A \left( \sum_{i=0}^N \Delta s \left( \sum_{j=0}^i \Delta s \sin \phi_j \right) \cos \phi_i - \alpha \right) \\
+ \lambda_{s=0} \left( \frac{\phi_1 - \phi_0}{\Delta s} - \phi_0'(0) \right) + \lambda_{s=\LHC} (\phi_N - \phi_e), \quad (16)
\]
where the boundary conditions for $\phi(s)$ at $s = 0$ and $s = L_{HC}(\epsilon)$ have entered as constraints with corresponding Lagrange multipliers $\lambda_{s=0}$ and $\lambda_{s=L_{HC}(\epsilon)}$. The optimizing vector $\phi_{\text{opt}}$ is finally found as the solution to the system of nonlinear equations

$$\nabla_{\phi, \lambda} \mathcal{L}(\phi_{\text{opt}}, \lambda) = 0. \tag{17}$$

Together with the equations derived for the deformation of the fluid space, this is readily solved iteratively employing the MATLAB routine \texttt{fsolve} and using the initial configuration as initial guess for the solution.

**Experimental Methods**

Young guinea pigs weighing 200 to 400 g were used in the current study. Using procedures approved by the local ethics committee (permit N32/13), the temporal bones were removed, attached to a custom holder, and the bulla opened to expose the cochlea. The preparation was then immersed in oxygenated tissue culture medium (Minimum Essential Medium, Invitrogen, Carlsbad, CA, USA) and a small opening created over scala vestibuli in the apical turn. This opening provided optical access to the organ of Corti and also allowed the tip of a beveled glass microelectrode to be pushed through the otherwise intact Reissner’s membrane. The electrode was used throughout the experiment to monitor the sound-evoked potentials produced by the sensory cells. Data collection was aborted if these potentials underwent sudden changes, or if their initial amplitude was abnormally low. The electrode was also used for injecting electrical currents into scala media. The currents were generated by an optically isolated constant current stimulator (A395, World Precision Instruments, Sarasota, FL, USA). Scala tympani was continuously perfused with oxygenated tissue culture medium at a rate of $\sim 0.6 \text{ ml/h}$, starting within 10 minutes of decapitation, and the perfusion system was also used to introduce the dye RH795 (5 micromolars, Biotium, Howard, CA, USA), which provides fluorescent labeling of the cell membranes of sensory cells and neurons. All experiments were performed at room temperature ($21 - 24^\circ \text{C}$).

**Interferometry and confocal imaging.** A displacement-sensitive interferometer (noise floor $< 0.1 \text{ nm/} \sqrt{\text{Hz}}$ at frequencies above 10 Hz) was used for measuring organ of Corti motion. Cells in the organ of Corti had sufficient optical reflectivity to allow measurements in the absence of artificial reflectors. All displacement data were averaged 10 to 20 times, but traces where the carrier signal of the interferometer had low amplitude were automatically rejected by the Labview-based data acquisition software. The interferometer provides a high-precision measurement of organ of Corti motion, usually from the Hensen cells, but the system can only detect motion directed along the optical axis. To assess the direction of motion, it is necessary to withdraw the electrode, reposition the preparation and the stimulus electrode, and then repeat the interferometric measurements. The complexity of this experiment meant that it was only occasionally successful.

To provide additional data on the direction of electrically evoked motion at the reticular lamina, we used the rapid confocal imaging method described previously\(^45\). In these measurements, RH795 was applied to the hearing organ as described above, and the dye excited with 488-nm light from a confocal microscope (LSM510, Zeiss, Jena, Germany). A 40x, NA0.8 water immersion lens was used to detect the fluorescence emitted from the dye, using appropriate optical emission filters. To visualize electrically evoked motions, a sequence of 25 - 37 confocal image frames were acquired without interframe delay. The microscope generated a $+5 \text{V}$ electrical pulse each time a pixel was acquired; this clock signal was used to drive the generation of the electrical stimulus, a square wave at a frequency of 5 Hz. Since the electrical stimulus is applied at a frequency many octaves below the best frequency of the recording location, it is considered static. The method of stimulus generation means that the exact phase of the stimulus with respect to each individual pixel is known, which makes it possible to reconstruct the motion of the sensory cells using custom Matlab scripts. Processing of the image sequence resulted in a new sequence of images, where each frame was specific for one phase of the stimulus. To quantify the motion seen in these image sequences, we used a
wavelet-based optical flow calculation method described earlier\textsuperscript{46}. Detailed performance evaluations of these techniques\textsuperscript{45} have shown that cochlear motion patterns can be accurately quantified down to a motion amplitude of approximately 0.5 pixels. The pixel size was adjusted to allow measurement of motions down to approximately 30 nm.
Supporting Information

Supplementary Figure 1

Supplementary Figure 1. Sketch of the organ of Corti cross-section to support the Online Methods.

Determining parameter values

Experimental measurement of the radial motion of the Hensen cells. To determine parameter values that yield realistic motion patterns in our model, we performed in vitro experiments to establish the radial motion component of the Hensen cells upon hair cell contraction. This was achieved through confocal imaging of the reticular lamina, as well as interferometry.

Experiments using confocal microscopy show that the reticular lamina often exhibits a pivot point between the second and third row of outer hair cells when an external current is applied (Supplementary Fig. 2a; data replotted from a previous study\textsuperscript{18}). The displacement of the third row of outer hair cells presumably follows the motion of the adjacent Hensen cells and suggests a motion predominantly towards scala vestibuli. Only a very small radial component is found which on average points towards the modiolus (Supplementary Fig. 2b,c).

We then used interferometry to determine the radial motion further away from the reticular lamina. While the side of the organ of Corti facing the stria vascularis was not accessible, it was possible to estimate radial motion at the top of the organ by tilting the preparation with respect to the interferometer beam (Supplementary Fig. 2d). These measurements suggest that the Hensen cells move with a small component towards the modiolus and away from the stria vascularis when positive current is injected, while the major displacement component is directed towards scala vestibuli, consistent with data from the reticular lamina.

The polarity of the radial Hensen-cell motion corresponds to model parameter values for which radial displacement at the top of the organ upon contraction of outer hair cells is negative. This restricts the values of the Deiter’s cell extensibility $\Delta$ and the Hensen cell contour extensibility $\Gamma$ to either $\Gamma \lesssim 0.2$ or $\Delta \gtrsim 1.2$ (Fig. 2d). A small value for $\Gamma$ is plausible, but too small values can prohibit deformations consistent with our assumption of conserved cross-sectional area of the organ of Corti for physiological values of outer hair cell contraction. In this case, no solution exists for our model equations for all $|\epsilon| \leq 0.02$. We therefore use $\Gamma = 0.1$ in the following.
Supplementary Figure 2. Direction of motion of the Hensen cells. (a) Confocal microscopy shows the motion of the reticular lamina when a negative externally-applied current is switched to positive current of equal magnitude, causing contraction of the outer hair cells. A pivot point is found between the second and third row of outer hair cells, with the third row outer hair cells following the displacement of the Hensen cells. (b) Colored bars depict the orientation of displacements of third-row outer hair cells. The dashed line indicates the orientation of the basilar membrane according to morphometric measurements by Kelly which is inclined by 37.26° on average with respect to the reticular lamina. Our own measurements from anatomical 3D-reconstructions indicate that this inclination is slightly, but significantly larger in the undamaged organ of Corti of our in vitro cochlear preparation (42.77° on average, continuous black line; N = 13, p = 0.009 by two-tailed t-test). (c) The first row of outer hair cells (squares) moves only little, as these cells are located close to the pivot point near the top of the pillar cells. The larger displacement of third-row outer hair cells (circles) mirrors the large displacement of the Hensen cells. Error bars indicate the standard error of the mean. (d) The radial component of Hensen-cell displacements was measured directly by tilting the preparation with respect to the interferometer beam. The largest motion occurs in a direction with a small component towards the modiolus (red) for positive current injections, consistent with the reticular-lamina data shown in a, b.
Displacements of reticular lamina and Hensen cells as functions of outer hair cell contraction. The direction of reticular-lamina displacement that is consistent with cross-sectional area conservation of the organ of Corti depends on the value of $\Delta$. For values smaller than a critical value $\Delta_C \approx 1.2$, the reticular lamina is pulled towards the basilar membrane, and pushed away from it for values larger than $\Delta_C$ (Fig. 3a). The latter is inconsistent with experimental observations$^{14-19}$. We find that for values of $\Delta$ smaller but close to the critical value $\Delta_C$, both the displacements of the reticular lamina and the Hensen cells become nonlinear functions of the outer hair cell contraction $\epsilon$ (Fig. 3b,c). The displacements plateau for cell hyperpolarization (negative values of $\epsilon$), but continue to grow for increasing depolarization (positive values of $\epsilon$). This is comparable to the experimentally observed dependence of Hensen-cell displacement on an externally-applied current$^{14,18}$. Moreover, the ratio between displacement amplitudes of Hensen cells and reticular lamina resembles that found experimentally, where the larger motion occurs at the Hensen cells$^{18}$ (see also Supplementary Fig. 2c).

Hence, we find that the parameter values $\Delta = 1.15$ and $\Gamma = 0.1$ yield good agreement with qualitative features of experimental data, whereas other regions in the parameter space yield qualitatively different behaviour that does not match with the observations. We therefore use these parameter values for our analysis.