SUPPLEMENTARY INFORMATION

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Section 1. Experimental preparation, data acquisition and analysis

Experimental preparation

The surgical procedures have been described\(^1\). Briefly, sacculi were dissected from adult bullfrogs (*Rana catesbeiana*) following a protocol approved by the Institutional Animal Care and Use Committee and maintained in oxygenated saline solution comprising 120 mM NaCl, 2 mM KCl, 1 mM CaCl\(_2\), 10 mM D-glucose, and 5 mM HEPES at pH 7.3. After a 30-60 min digestion at room temperature in 1 mg·ml\(^{-1}\) collagenase (type XI, Sigma Chemical Co.), each sensory epithelium was peeled with an eyelash from the underlying connective tissue and the otolithic membrane was removed. The epithelium was then folded in its plane of mirror symmetry and secured in an experimental chamber by a 100-mesh gold electron-microscopic grid. All experiments were performed in the same saline solution that we used for the surgery. In the solution that we used to remove the top connectors, NaCl and CaCl\(_2\) were substituted by the equivalent amount of mannitol. All experiments were performed at a room temperature of 20 °C to 25 °C.

Data acquisition and analysis

We used dual-beam interferometry to measure stereociliary motions with a sub-nanometre spatial and a sub-millisecond temporal resolution\(^1\). The measurements were sets of twenty 100-ms- or 1-s-long records sampled at 10-µs intervals. Each record corresponded to a double time series, acquired simultaneously and independently with the two laser beams directed at different positions on the hair bundle (Figure S1). Between each of these records, each beam of the detection
apparatus was recalibrated independently. The presence of small debris in the optical path and pointing instabilities in the lasers occasionally corrupted records. Such outliers were rejected on the basis of unstable root-mean-square amplitudes, abnormal cross-correlation amplitudes, or pronounced drift in the time series.

Figure S1: Structure and movement of a hair bundle. (a) Left: a scanning electron micrograph of a hair bundle from the bullfrog’s saccus illustrates an array of closely apposed, cylindrical stereocilia separated by small gaps which in a living hair bundle are filled with viscous liquid. The calibration bar corresponds to 2 µm. Right: a schematic diagram depicts a single file of stereocilia in a hair bundle’s plane of symmetry, connected by tip links (orange) and horizontal top connectors (violet). The red and green laser beams of the interferometer, focused to diffraction-limited spots, record the motion of the short and tall edges of the bundle. (b) Simultaneous records of the movement at the two edges of a thermally excited hair bundle (top) convey the impression of highly coherent motion, which is confirmed by the calculated coherence spectrum (bottom). The mean value of the coherence is shown in orange and the 95 % confidence interval in light orange.
Data structures were investigated using frequency-domain representations. A central assumption underlying these techniques is that the time series are stationary. Nonstationarities in the data were controlled by three means. First, we acquired relatively short records between which the measurement apparatus was recalibrated. Next, our criteria for outlier rejection guaranteed that strongly nonstationary records were eliminated from the data pool. Finally, we used multitaper spectral-estimation techniques to derive estimates of spectral densities. These techniques prevent low-frequency artifacts from contaminating the results.

The multitaper method of spectral estimation performs a local weighted average over a frequency bandwidth in order to reduce the bias between different frequency channels and to provide several statistically independent estimates for the same time series. We typically generated eight orthogonal Slepian data tapers, but used only the first six to reduce the bias. Jackknife variance statistics was applied to derive confidence intervals of our estimates, a procedure that is statistically conservative.

The similarity between simultaneously recorded time series was characterized by the complex coherency function, which was split into its modulus—the coherence—and its phase. The coherence is a dimensionless quantity that measures for each frequency the quality of the phase estimate. It is bounded by one for perfectly correlated signals and by zero for totally uncorrelated ones. Multitaper spectral estimates and jackknife confidence intervals for coherence spectra were derived following Thomson and Chave (1991). We used the same spectral analysis and coherence estimates both for the experimental data and the data from the numerical stochastic model (see Section 5).
**Estimation of stiffness and drag coefficients**

To determine the stiffness and the drag coefficient of the hair bundles, we first removed the shaft connectors, basal links, kinociliary links, and tip links by treating each preparation for 1 h at room temperature with 20 µg·ml⁻¹ subtilisin (protease type XXIV, Sigma) and then briefly with 5 mM BAPTA. Hair bundles without tip links are passive objects whose fluctuations satisfy thermodynamic equilibrium. We used the dual-beam interferometer to record 100-ms time series of Brownian motion. We estimated the mean-squared displacement \( \langle x^2 \rangle \) and used the equipartition theorem to calculate the associated stiffness as \( \kappa = k_B T \langle x^2 \rangle \), in which \( k_B \) is the Boltzmann constant and \( T \) is the temperature. The stiffness for six hair bundles that had their kinocilia, tip links, and lateral links except for the top connectors removed was 19±12 µN·m⁻¹ (everywhere where it applies in the Supplementary Information and in the main text, results are presented as mean ± standard deviation). Such a small value is in accord with the observation that removing all elastic connections except for the horizontal top connectors eliminates more than 90 % of a hair bundle's stiffness. Our value for the flexural rigidity of 13 kinocilia, 0.63±0.3 zN·m² (range 0.3–1.4 zN·m²), is in good agreement with the value for the cilia of echinoderm spermatozoa, 0.3–0.7 zN·m². When active movements of disconnected kinocilia were observed, the measurements were discarded.

The drag coefficient was calculated as the product of the stiffness and the decay time of the normalized autocorrelation function. Because the tip links were removed, any contribution of the phenomenological friction assigned to channel
gating\textsuperscript{11} could be excluded. We used for this analysis only hair bundles that moved with a coherence exceeding 0.95 between 100 Hz and 5 kHz.
Section 2. Use of a scaled physical model to estimate the drag coefficient of a stereocilium separating from a hair bundle

Using data on hair bundles from the bullfrog's saccular macula\textsuperscript{12}, we constructed a simplified model of a bundle enlarged twelve thousand times (Figure S2). The stereocilium at the tall edge of the hair bundle was represented as a pivoting rod, 6.35 mm in diameter, which could move only in the plane of symmetry of the model, separating from the rest of the stationary hair bundle. This stationary unit was a cone with the upper part cut off at an angle, creating a sloping surface. In a groove on the longest side of the model, three rods, 6.35 mm in diameter and 94 mm to 97.85 mm in height, with a 0.66 mm wall-to-wall distance at the bottom, mimicked stereocilia surrounding the longest stereocilium (101.7 mm in height) and generated space where liquid could flow around the movable rod. The four rods made contact at their tips; the tip of the movable rod was pressed against the three stationary rods by a leaf spring at its base.

The model hair bundle was mounted on a frame, whose basal plate was 76 mm wide and 9 mm thick (not shown). The centre of the model was mounted at a distance of 460 mm from the vertical part of the frame. From the tip of the moving rod a braided nylon string 0.2 mm in diameter led to a custom-built force transducer including a half of a Wheatstone bridge and two strain gauges (SGT-3N/350-DY11; Omega Engineering, Stamford, CT, USA). The string passed over a pulley positioned at the same height as the tip of the pivoting rod, redirecting the string vertically. At a height of 710 mm above the force transducer, the string could be wound at a constant speed onto a shaft with a circumference of 20 mm and driven by a direct-
current, permanent-magnet motor (Model 800, M. H. Rhodes/Cramer Co., South Windsor, CT, USA). The speed of uptake could be 0.17–1.11 mm·s$^{-1}$ for a gearbox ratio of 10:1 or 0.015–0.101 mm·s$^{-1}$ for a ratio of 110:1.

**Figure S2:** Schematic of the scaled hair-bundle model in three projections. The moveable stereocilium is shown in red. All dimensions are in millimetres.
The force transducer was connected to the second half of the Wheatstone bridge and a differential amplifier. To reduce drift from temperature shifts, the instrument board was placed in a plastic-foam box. The signal progressed through a low-pass filter with a cutoff frequency of 100 Hz to a computer at which data were acquired with LabVIEW (National Instruments, Austin, TX, USA). All further analysis, including noise reduction with a 60 Hz notch filter, was accomplished with MATLAB (The MathWorks, Natick, MA, USA).

During experiments the model was submerged in a basin, measuring 730 mm by 300 mm and filled to a depth of 190 mm with a 2.2 % methylcellulose solution displaying a kinematic viscosity of $5.1-5.4 \times 10^{-3} \text{ m}^2\text{s}^{-1}$ measured by a Cannon-Fenske viscometer (Cannon Instrument Co., State College, PA, USA). Starting with the tip of the mobile rod pressed against the others, the string was then reeled in. To ensure that the rods were touching, some slack was allowed in the string at the outset. Activation of the motor produced a small spike in the recorded signal that marked zero time; for each speed, 13 to 20 replicate measurements were aligned with this signal and averaged. As a control, this procedure was repeated in water with the same density and a kinematic viscosity of $10^{-6} \text{ m}^2\text{s}^{-1}$ at room temperature. The reason for performing the control in water rather than in air was to keep the nylon wire wet to minimize possible stiffness changes.

Data were obtained at speeds of 0.015, 0.04, 0.08, 0.26, 0.55, 0.83, and 1.1 mm·s$^{-1}$. Upon activation of the motor, the control experiments in water showed a small and relatively fast rise in force while the slack was removed and some tension accumulated in the string (data not shown). The force increased steadily and linearly thereafter as the tension increased in the leaf spring at the base of the movable rod.
When the motor was activated during experiments in methylcellulose, the force peaked immediately after the slack was removed from the string; the force then displayed again the linear relation attributed to the leaf spring. For the three lower speeds of movement, the linear coefficient from a string position of 5 mm and greater was subtracted from the experimental records. For the four higher speeds, the force owing to the viscosity of methylcellulose could be found by subtracting the control signals obtained in water from those measured in methylcellulose. We also checked whether the elasticity of the wire might alter the results. We estimated this stiffness as 105 N\cdot m^{-1} using the Young's modulus of nylon (3 GPa), a diameter of 0.2 mm, and a length of 1.11 m. Correcting the displacements at the rod's tip for the wire elasticity yielded for the lower velocities a maximum drag coefficient of 5915 nN\cdot s\cdot m^{-1} at the scale of a hair bundle. For these velocities the drag coefficient was not dependent on velocity, which is consistent with the Reynolds number being much smaller than one. At higher speeds, the drag coefficient with the correction was overestimated, likely because of the increased Reynolds number, whereas without the correction it was underestimated due to possible plastic deformations in the wire caused by greater forces. The final results are shown in Figure S3.
Figure S3: Maximal drag coefficient for the separating stereocilium. The results are shown both with correction for the wire's elasticity (blue) and without it (red). The black line shows the average of the corrected values at the four smallest velocities, for which the plastic deformations in the wire are minimal.
Section 3. Finite-element formulation, model, and coherence evaluation

This section describes the implementation and the validation of the finite-element model of the hair bundle used to compute the results presented in Fig. 1 of the main text. More information can be found in J. B.’s PhD thesis at:

http://nbn-resolving.de/urn:nbn:de:bsz:14-qucosa-63810

Context

In classical engineering, fluid-structure interaction problems are solved by coupling the fluid mechanics based on the variables velocity and pressure to the solid mechanics based on displacements. At the interface between the solid and the fluid, the two sets of variables are linked by the continuity of momentum and displacement. Because in the classical approach the fluid and solid domains are solved separately, an iterative procedure has to be implemented to link the two, at the cost of computation time. A way to circumvent this difficulty is to use a single set of dynamic variables for the whole domain and account for the different materials solely by different material laws. Several studies have used this approach based on velocity variables. To our knowledge, no such unified approach has been presented for displacement and pressure variables. These variables, however, turn out to be the most appropriate ones for the small-amplitude motions involved in hearing mechanics. These are the variables we used in our finite-element model.

Governing equations

The fluid-dynamical problem under investigation is characterized by values of the Reynolds number well below one; furthermore, the hair bundle moves by only a
small fraction of its size. Therefore, the nonlinearities originating from the convection of the fluid, which are important when relatively large displacements occur as in classical engineering problems, can be neglected here. This simplifies the conversion from velocity to displacement and allows a unified description for fluid and solid by a single set of variables, which we chose to be the displacement field \( \mathbf{u} \), the pressure \( \rho \), and the force field \( \mathbf{f} \) from external loads. Furthermore the density variations are small and can be neglected. Under these circumstances, the momentum balance of the Newtonian fluid reads

\[
\rho \frac{\partial^2 \mathbf{u}_i}{\partial t^2} = -\frac{\partial \rho}{\partial x_i} + \eta \frac{\partial}{\partial x_j} \left( \frac{\partial^2 \mathbf{u}_i}{\partial x_j \partial t} + \frac{2}{3} \delta_{ij} \frac{\partial^2 \mathbf{u}_l}{\partial x_l \partial t} \right) + \mathbf{f}_i, \quad (3.1)
\]

in which \( t \) is the time, \( \rho \) the density, \( \eta \) the dynamic viscosity, \( i, j, l \) are the coordinate directions, \( u_i \) are the displacement components, \( f_i \) are the components of the external force, and \( \delta_{ij} \) is the Kronecker delta function. Summation over repeated indices is assumed. To this description must be added a constitutive equation, which is given here by the linear relation between pressure and displacement:

\[
p = -K \frac{\partial u_i}{\partial x_i}, \quad (3.2)
\]

in which \( K \) is the bulk modulus of the fluid. For a solid material, the set of equations reads:

\[
\rho \frac{\partial^2 \mathbf{u}_i}{\partial t^2} = \frac{\partial}{\partial x_j} \left[ \mu \left( \frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i} \right) + \lambda \delta_{ij} \frac{\partial u_l}{\partial x_l} \right], \quad (3.3)
\]
in which the two Lamé coefficients of the solid $\mu$ and $\lambda$ are linked to the Young’s modulus $E$ and Poisson ratio $\nu$ by $\mu = \frac{E}{2(1 + \nu)}$ and $\lambda = \frac{E\nu}{(1 + \nu)(1 - 2\nu)}$.

Appropriate boundary conditions must be added to these equations, given in terms of either displacements or forces. The wall of the solid is impermeable to the liquid in normal direction. In the tangential direction, the flow usually sticks fully to the wall. Nevertheless, on scales of a few nanometres, a partial slip can occur at the interface between liquid and solid, depending on the material properties and the geometry of the interface\textsuperscript{15}. Because the slip has not been investigated for hair bundles, it is not considered here explicitly. Instead, we use a gap width with a no-slip boundary condition that might be understood as an effective gap distance for the liquid, taking into account the liquid slip at the stereociliary wall. At the outer boundary of the finite domain, the liquid experiences a zero force because displacements are not constrained there. The excitation is computed by setting the imposed displacement to a fixed amplitude in the excitatory direction and to zero in the other directions. The motion in the normal direction is blocked in the symmetry plane.

\textit{Finite-element formulation}

The finite-element method is employed to solve the equations given above for the fluid and solid materials. Figure S4 illustrates the discrete degrees of freedom for the displacement and pressure on a hexahedral geometry of an individual finite element. For the fluid the pressure is approximated by linear basis functions, and for both the fluid and solid the displacement is approximated by quadratic basis functions. The
implementation follows Zienkiewicz et al. (2005) for the two-field incompressible elasticity. The basis functions have no nodes on the centre of the faces (serendipity family). Using a harmonic ansatz of the form \( \exp(-i\omega t) \) for the time dependence of the quantities involved, in which \( i \) is the imaginary unit, allows one to formulate the problem in the frequency domain. Using a weak formulation and a discretization by the shape functions, the system of equations for the fluid and solid domain reads:

\[
-\omega^2 M_{ij} x_j - i\omega C_{ij} x_j + K_{ij} x_j = b_i, \tag{3.4}
\]

in which \( M_{ij}, \ C_{ij}, \) and \( K_{ij} \) are respectively the mass, damping, and stiffness matrices. The vector \( x \) contains the displacement degrees of freedom for the whole domain and additionally the pressure degrees of freedom in the fluid domain. The vector \( b \) includes the external excitation forces for the displacement degrees of freedom for which the pressure load corresponds to the normal forces, and is zero for the pressure degrees of freedom.

**Figure S4**: Hexahedral finite element. (a) Location of the nodes representing the displacement degrees of freedom by quadratic shape functions as used for both fluid and solid. (b) Location of the nodes representing the pressure degrees of freedom by linear shape functions as used for the fluid only.

**Implementation**

The finite-element software Ansys (Version 11, Ansys Inc., Canonsburg, PA, USA) was used for the computations. The fluid was implemented as a user-defined
element. For the solid material, the SOLID186 element was used with hexahedral geometry and quadratic shape functions for the displacement. The links in the model were represented by the COMBIN14 element, which is a linear spring that exerts a force in the direction of its length only. The amplitude of the force is equal to the spring length change times the assigned stiffness coefficient.

Hair-bundle model

Our approach to model the fluid-structure interactions in a hair bundle by means of the finite-element method differs in a fundamental way from the numerical models in the literature. Indeed, although several numerical studies of some of the forces that are at play in a hair bundle have been proposed, they have described only the elastic elements\textsuperscript{17–22}, sometimes adding liquid-flow estimations\textsuperscript{23}, or investigated the interactions of a solid hair bundle with its environment in the inner ear\textsuperscript{24–31}. None of these studies however attempted to resolve simultaneously both scales involved in hair-bundle geometry: the large scale associated with external liquid motion, corresponding to several tens of micrometres, and the small scale associated with liquid motion between the stereocilia, which corresponds to gap spaces as tiny as about 10 nm. Thus, unlike all the previous studies, we place a model hair bundle with all its individual stereocilia and the linkages between them explicitly in its liquid environment.

A comprehensive finite-element model of the full hair bundle allows a detailed analysis of the liquid motion along the whole auditory frequency spectrum covering four decades. The three-dimensional geometry of the hair bundle of the bullfrog sacculus is set up based on data from Jacobs and Hudspeth (1990)\textsuperscript{12} and the
meshed model is displayed on Figures S5 and S6. Stereocilia are quasi-rigid cylinders of radius $r = 190$ nm that can pivot around their bases. They are distributed on a hexagonal array of nine stereocilia along its maximal length and five along the side edges, which gives a total of 61 stereocilia. Their height decreases from 7.8 $\mu$m for the tallest stereocilium in steps of 325 nm between next neighbours. They are separated at their bottom by 400 nm above the tapered rootlets and at their tips by 10 nm. On the taller side of the hair bundle is located the kinocilium, which is a true cilium without a tapering rootlet section. It is 8.45 $\mu$m tall and is placed farther apart from the stereocilia as shown on Figure S7.

In the model, the top-connector stiffness represents the collective stiffness of all lateral linkages and we assume that the stereocilia are quasi-rigid. For example, the basal links, which are mechanically in parallel with the top connectors, are not explicitly represented. The explicit representation of top connectors was preferred to that of other lateral linkages because top connectors have the longest lever arm with respect to the center of rotation.
Figure S5: Meshed geometry of the finite-element hair-bundle model. Both the liquid and the hair bundle are shown on the half space, the other half being completed by symmetry.
Geometrical parameters of the bundle are summarized in Table S1. The mesh is multi-block structured and uses only hexahedral elements with quadratic shape functions for the geometry. The bundle, whose maximum diameter is 6 µm, is placed into a cylinder of liquid with a diameter of 62 µm and a height of 37 µm (Figure S5). The material properties are adjusted to reproduce the experimental observations and are given in Table S2. This arrangement results in a mesh with approximately 800,000 degrees of freedom. On a single processor, the computation for one single-frequency data point took about ten hours. About 100,000 hours of CPU time were spent to obtain the final results, which was possible because we used a PC farm with
2576 cores equipped with AMD Opteron Dual Core processors, each with at least 4 GB of main memory.

**Figure S7:** Sketch of the hair-bundle model in the symmetry plane with geometrical parameters as given in Table S1. Tip links (orange) and top connectors (violet) are depicted as lines.
Table S1: Geometrical parameters of the finite-element model.

| Name                                           | Variable | Value  | Ref.     |
|------------------------------------------------|----------|--------|----------|
| height of kinocilium                           | $h_{kc}$ | 8.45 µm | **       |
| height of kinociliary bulb                     | $h_{kb}$ | 6.5 µm  | **       |
| height difference between adjacent stereocilia  | $h_t$    | 325 nm  | **       |
| height of rootlet                              | $h_r$    | 975 nm  | *        |
| diameter of the stereocilia and kinocilium     | $d_o$    | 380 nm  | *        |
| diameter of the stereocilia at the cuticular plate | $d_b$ | 125 nm  | *        |
| gap distance at the tip                        | $g_t$    | 10 nm   | estimated|
| gap distance at the bottom without taper        | $g_b$    | 400 nm  | estimated|
| spacing of the stereocilia on the cuticular plate | $s$  | 780 nm  | *        |
| angle between bundle's edges                   | $\beta$  | 96°     | *        |
| number of stereocilia along one edge           | $n_s$    | 5       | *        |
| total number of stereocilia                    | $n_{tot}$| 57      | **       |

** Jacobs and Hudspeth (1990)\textsuperscript{12}

* this study
Table S2: Material properties of the finite-element model.

| Name                                      | Variable | Value     |
|-------------------------------------------|----------|-----------|
| density of liquid and solid               | $\rho$   | 1 Mg·m$^{-3}$ |
| viscosity of liquid                       | $\eta$   | 1 mPa·s   |
| bulk modulus of liquid                    | $K$      | 1 GPa     |
| Young's modulus of rootlet                | $E_r$    | 111 kPa   |
| Young's modulus of stereocilium           | $E_{sc}$ | 1 GPa     |
| Young's modulus of kinocilium             | $E_{kc}$ | 585 kPa   |
| Poisson ratio of solid material           | $\nu$    | 0.3       |
| stiffness of tip link                     | $k_{tl}$ | 1 mN·m$^{-1}$ |
| stiffness of top connector                | $k_{tc}$ | 20 mN·m$^{-1}$ |
| stiffness of kinociliary link             | $k_{kl}$ | 1 N·m$^{-1}$  |
To validate the results, the mesh was refined and the total liquid domain enlarged until the results were independent of these quantities. The implementation of the method to model the fluid-structure interaction was also checked by comparison with analytical solutions of related problems. First, a finite-element calculation was performed to validate the drag by the external liquid, using the same mesh as the one for external liquid in the hair-bundle model. The bundle is replaced by a 8 $\mu$m-tall cylinder, pivoting in a single direction around its contact point with a rigid plane orthogonal to its main axis. The rigid plane represents the cuticular plate of the cell. The reaction moment is computed with respect to the bottom centre of the cylinder. The division by the cylinder height and the velocity at the cylinder tip provides an equivalent drag coefficient from the finite-element computation. The liquid properties are as given in Table S2. The frequency of motion is 1 Hz. This gives a characteristic dissipation length for the liquid of about 400 $\mu$m, a value much larger than the computation domain, such that the problem is quasi-static.

Figure S8 shows the drag coefficient in the finite-element solution when the outer liquid boundary is either fixed or free to move. The latter corresponds to the case used for all other hair-bundle computations in this work. The two finite-element solutions align well with each other. This implies that the external liquid domain is sufficiently large down to frequencies of 1 Hz. The finite-element solution aligns as well with the analytical approximation of the cylinder drag. Because the cuticular plate is not included in the analytical calculation, this match suggests a minor effect of the cuticular plate on the drag.
We also compared the solution obtained for two stereocilia moving to their common centre with the corresponding analytical estimate, and found a good agreement (see Section 6 for details).

**Figure S8**: Drag coefficient of a cylinder displaced by a linear profile over the height on a fixed plate. The height is $h = 8\, \mu m$. The drag coefficient is given for an equivalent force at the cylinder’s tip. For the finite-element results the liquid domain has an outer diameter of 62 $\mu m$ and a height of 37 $\mu m$. The outer boundary is either free to move (FEM free) or fixed in position (FEM blocked). For comparison we show the analytical solutions for a translating sphere (anal. sphere), for a rotating half ellipsoid (anal. ellipsoid)$^{32}$, and for a cylinder (anal. cylinder)$^{33}$. The latter is provided within the valid range of $r < 0.22\, h$. 


Estimation of the coherence from FEM integration

To compare the results of the finite-element model with the coherence spectra that we obtained from the experimental data, we computed the coherence for the finite-element model. This computation is possible because the fluctuation-dissipation theorem relates the two-point correlation functions to the linear response functions at thermodynamic equilibrium\(^{34}\). The coherence is directly linked to the former statistical quantities, whereas the finite-element model allows for the computation of the latter deterministic ones.

The finite-element model of the hair bundle was stimulated by oscillatory forces applied in succession to the first and to the fifth stereocilium located in the central row, and their displacements were recorded, mimicking experimental acquisition of positional data. Because small stimuli were applied, the system operated in the linear regime. Thus, four linear response functions were obtained for each frequency of stimulation. Labelling \(f_t\) and \(f_s\) the stimuli applied respectively to the tall and short edge of the hair bundle, and \(x_t\) and \(x_s\) the corresponding measured displacements, these linear response functions can be summarized by the linear response matrix \(\chi\) defined as

\[
\begin{pmatrix}
\chi_{tt} & \chi_{ts} \\
\chi_{st} & \chi_{ss}
\end{pmatrix}
\begin{pmatrix}
\mathbf{f}_t \\
\mathbf{f}_s
\end{pmatrix}
= 
\begin{pmatrix}
x_t \\
x_s
\end{pmatrix}
\]  

(3.5)

For positional variables, this matrix should be symmetric\(^{34}\). The ratio

\[
\varepsilon = 2 \frac{\chi_{ts} - \chi_{st}}{\chi_{tt} + \chi_{ss}}
\]  

(3.6)
provides a measure of the relative error due to numerical integration. In the cases investigated, this ratio was never larger than 0.7 %. The two-point correlation function matrix is then given by the fluctuation-dissipation theorem as\textsuperscript{34}:

\[
G_i(\omega) = \frac{2k_B T}{\omega} \chi''_i(\omega),
\]

in which \(\chi''_i(\omega)\) is the imaginary part of the linear response matrix \(\chi_{ij}\) at the circular frequency \(\omega\) and the indices \(i\) and \(j\) refer to either \(t\) or \(s\). Finally, the coherence spectrum is given as a function of \(\omega\) by:

\[
\gamma(\omega) = \frac{|G_s(\omega)|}{\sqrt{G_n(\omega) \cdot G_{ss}(\omega)}} = \frac{|G_t(\omega)|}{\sqrt{G_n(\omega) \cdot G_{ss}(\omega)}},
\]

the two expressions being equal up to rounding errors quantified by \(\varepsilon\). The final result is displayed in Fig. 1c.
Section 4. Robustness of spectral quantities: a parameter-variation study

By testing the role of three properties in the spectral observables displayed in Fig. 1, we have investigated with numerical models the robustness of our conclusions. Because this parameter has been characterized the least by experimentation, we tested the top-connector stiffness. To investigate the role of inertia, in particular on the drop of coherence that is observed at high frequencies both experimentally (Figure S1b) and in the finite-element model (Fig. 1c), we varied the value of the liquid and solid densities. Finally, we tested whether the specific geometry of the bullfrog's saccular hair bundle restricts the applicability of our results by changing the geometry to that of a mammalian hair bundle. In each instance, we investigated the associated consequences on the hair bundle's drag, coherence, and stiffness spectra.

The effect of top-connector stiffness is documented in Figure S9, in which the spectral quantities are displayed for different values of this parameter, namely 0, 0.02, 0.2, 2, 20, and 200 mN·m⁻¹. Only if the top connectors display a stiffness of 20 mN·m⁻¹ or greater do the coherence and drag in the model match the experimental values over the whole range of frequencies. In particular, if the top connectors are too soft, the bundle's drag increases at low frequencies.

In the second computation, we varied the density of the liquid and solid material simultaneously by decreasing or increasing it tenfold with respect to water's density. The results are displayed in Figure S10. Increasing the density tenfold significantly decreases the coherence and increases the drag at high frequencies.
Figure S9: Effect of top connectors on the drag, coherence, and stiffness spectra as derived from the finite-element model. Different values of the top-connector stiffness $k_{tc}$ are investigated, as shown in the legends of the plots on the left. The default parameter values are as given in Tables S1 and S2. Results are shown in the absence of tip links (top row), and in the presence of tip links with a stiffness of 1 mN·m$^{-1}$ (bottom row).

The effect on the drag can be understood from the fact that the characteristic decay length of the shear waves created by the oscillating bundle decreases with increasing density, increasing the local velocity gradient at the bundle’s edge. On the other hand, decreasing the density tenfold has a very small effect on both measures. These results show that inertia plays a significant but small role at high frequencies, as described in the main text.
The stiffness of the bundle is computed from the real part of the response function. Above the resonant frequency of the system this quantity becomes negative and therefore cannot be displayed in a logarithmic plot. Plotting instead the phase of the response function at the kinociliary bulb reveals a smooth transition over several decades from the low-frequency regime dominated by elasticity, for which the phase is near zero, to the high-frequency regime dominated by viscous forces, for which the phase is close to -π/2 (Figure S11). The phase continues to decay for high
frequencies such that a regime dominated by inertia can be expected for frequencies above 20 kHz. The smooth transition of the phase suggests an overdamped system: with estimated values for the bundle stiffness, drag coefficient, and mass of respectively 500 $\mu$N·m$^{-1}$, 100 nN·s·m$^{-1}$ and 50 pg, the quality factor is 0.05 so the bundle is indeed overdamped.

**Figure S11:** Phase of the response function at the kinociliary bulb as a function of frequency, obtained from the finite-element model as presented in Fig. 1 of the main text, for four different configurations of the hair-bundle links: with only pivotal stiffness and hydrodynamic drag (blue downward triangles); adding horizontal top connectors with a stiffness of 20 mN·m$^{-1}$ (violet squares); adding instead tip links with a stiffness of 1 mN·m$^{-1}$ (orange upward triangles); and adding both top connectors and tip links (red circles).

Finally, we tested whether our general conclusions apply to mammalian hair bundles, which have only three rows of stereocilia and in inner hair cells are thought to lack top connectors$^{35}$. For that purpose, we constructed a model of a mammalian hair bundle from the basal, high-frequency region using the geometry from Zetes and Steele (1997)$^{23}$. The model comprises linear elastic Euler–Bernoulli finite-element beams and linear springs and dampers$^{16}$. For simplicity, only a single stereociliary column is included and the resulting drag and stiffness values are multiplied by the number of columns, here assumed to be 30, to obtain the properties of the entire
bundle. This approach allowed us to evaluate a two-dimensional model, a schematic representation of which is displayed in Figure S12a. The model contains three stereocilia of radius 100 nm and of heights 1.6, 1.3, and 0.9 µm. The finite radius of the stereocilia is modelled by inertialess beam elements, which offset the end points of the coupling springs and dampers away from the stereociliary axis. At rest, the stereocilia are parallel and separated by a wall-to-wall distance of either 1, 10, or 100 nm, providing three different geometrical sets.

The mass per unit length of the central beams constituting each stereocilium is defined such that it contains the whole mass per unit length of the cylindrical stereocilium, based on a density of 1 Mg·m⁻³ and using the stereociliary radius. The flexural rigidity is computed based on the same cross-section and with a Young's modulus of 1 GPa. The flexural rigidity of all the other beams is the same as that of the bulk stereocilia and their mass is zero. The linearly elastic Euler–Bernoulli finite-element beams exhibit three degrees of freedom at each end, two displacements and one rotation. At the points connecting different beams the displacements as well as the rotations are continuous and thus the beams are clamped at their point of connection. At the cuticular plate all displacements are blocked and a pivotal stiffness of 10 aN·m·rad⁻¹ as well as pivotal damping are added to the rotational degree of freedom.

Horizontal top connectors are split into two equivalent springs, each with half of the reported stiffness, and located one at the tip of the shorter stereocilium and the other 100 nm below. Liquid coupling is represented as a damper located in the middle of the two top-connector springs. Tip links connect on the axis upper end of one stereocilium to the side of its taller neighbour at an angle of 45°. The tip-link and
top-connector stiffness values are the same as those used in the finite-element model of the bullfrog hair bundle (Table S2). The liquid damping coefficient is estimated from Eq. (6.3) based on the height of the intermediate stereocilium, which yields 4100, 130, and 4.1 nN·s·m⁻¹ for wall-to-wall distances of respectively of 1, 10, and 100 nm. The bundle’s drag coefficient is estimated from Eq. (5.2) as 77.5 nN·s·m⁻¹ based on the height of the tallest stereocilium and a radius of 3 µm. To ensure this damping coefficient for the coherently moving bundle, a pivotal damping coefficient of 2.16 zN·s·m·rad⁻¹ is applied to all stereocilia at their insertion point to the cuticular plate.

The results with and without top connectors are displayed in Figure S12. For the stiffness and drag computations, the force is applied to the tips of all stereocilia in the tall row, which is the physiological mode of stimulation for an outer hair cell. Applying instead a force equivalent to a pressure load for a moving liquid, which models an inner hair cell, produces approximately the same results. The locations at which the coherence is assessed are the tips of the tallest and shortest stereocilia.

The results show the same pattern of behaviour as that observed for the bullfrog’s hair bundle. The coherence is high and the drag is low over the whole auditory spectrum except when the wall-to-wall distance between stereocilia exceeds 100 nm, whereupon liquid coupling becomes weak. It is noteworthy that knockout of the gene encoding stereocilin, a putative component of top connectors, disorders the hair bundles of outer hair cells, causing abnormally large variations of the distance between their stereocilia, and attenuates cochlear nonlinearities³⁵,³⁶. Our results suggest that the damage to top connectors and the ensuing hair-bundle disorder would reduce the coherent motion of stereocilia that is important for active
hair-bundle motility\(^1\). Moreover, when stereocilia move in disarray, the substantially increased viscous drag would thwart the active process.

Figure S12: Linear model of a mammalian hair bundle with finite-element beams, springs, and dampers. (a) Schematic representation of the model. The stereocilia are represented by beam elements along their axes (black diamonds) and their finite radius is introduced by inertialess beam elements (grey disks). The stereociliary pivots are assigned rotational damping and stiffness at their bases (green circles). The stereocilia are interconnected by tip links (orange upward triangles), top connectors (violet squares), and dampers to mimic liquid coupling (blue downward triangles). (b) Associated coherence spectra obtained using the procedure described in Section 3 and shown for three indicated gap widths, each in the presence or absence of top connectors. The associated drag (c) and stiffness (d) spectra reflect two forcing conditions: either stimulation at the tips of all stereocilia in the tall row as occurs physiologically in outer hair cells (solid lines), or force application equivalent to a pressure load of a moving liquid on all stereocilia as occurs physiologically in inner hair cells (dashed lines). These results were obtained for the three different gap widths indicated in panel (b), in both the presence and absence of top connectors.

With the normal distance between stereocilia, however, top connectors in this geometry have a negligible effect both on the coherence at all frequencies and on
the drag for frequencies above 1 kHz, that is for the frequencies to which these cells are physiologically responsive. The stiffness of the bundle is slightly smaller than that measured experimentally$^{37}$, with values around 1 mN·m$^{-1}$. This might reflect stiffer rootlets in mammalian hair bundles. Increasing the pivotal stiffness by a factor of five results in a bundle stiffness of about 2.5 mN·m$^{-1}$ (data not shown).

The cuticular plate in the amphibian hair cells may be more curved than in the mammalian hair cells, which could have an effect on the interactions between stereocilia. We have explored specifically the influence of the bowl-like shape of the cuticular plate by lowering the anchor point of the rootlet of the intermediate stereocilium by one stereociliary radius. This results in a curvature variation that is as large as that typically observed in the bullfrog (our unpublished data) or the alligator lizard$^{38}$. We calculated the associated coherence, drag, and stiffness spectra. With this variation, the spectra do not differ by more than 10 %. This indicates that the curvature of the cuticular plate is not a crucial factor in the coupling between stereocilia with the mechanical properties used in this work.
Section 5. Stochastic computations for the hair bundle in a thermal bath

This section describes the stochastic model and its numerical implementation to compute the results presented in Fig. 2 of the main text.

General description of the model

The hair-bundle geometry on which the stochastic simulations are based is identical to the geometry used for the finite-element model, but projected in two dimensions as follows. The simulated stereocilia are represented by the positions of their centres at a common given height, here the height of the kinociliary bulb (Table S1). Time evolution is described by a Langevin dynamics. The equations include the interactions with the surrounding liquid and the elastic interactions between the stereocilia and with the cuticular plate. The latter constitutes a fixed reference frame to which individual stereocilia are connected through elastic rootlets that have pivoting stiffness. Interactions with the liquid around and between the stereocilia cause viscous friction forces and stochastic forces induced by the thermal bath at room temperature. We also computed the coherence of the model taking into account the inertial mass of the stereocilia, and found a contribution in the coherence spectrum of less than 2.2% up to 5 kHz. We investigated the role of inertia in more detail in the full three-dimensional finite-element model by varying the density of the liquid and solid materials (see Section 4).

Because experimentally measured displacements are typically of a few tens of nanometres for stereocilia lengths of a few microns, and ignoring for the moment the position-dependent friction coefficients (see below), the overall dynamics of the system can be described by a linear Langevin dynamics:
\[
C_{ij} \frac{dx_j}{dt} + K_{ij} x_j = \xi_i, \tag{5.1}
\]

in which \((x_i)_{i=1,2 \times N}\) represents the displacements of the stereocilia relative to their resting positions at the height of the kinociliary bulb. Summation over repeated indices is assumed. Each stereocilium can move into two independent directions and thus the vector with all displacements has twice the number of stereocilia \(N\) as entries. \(C_{ij}\) and \(K_{ij}\) are respectively the matrix of damping and stiffness coefficients and \(\xi_i\) represents the stochastic forcing terms originating from thermal noise. The off-diagonal terms of the matrices directly couple the force exerted on the degree of freedom number \(i\) to a motion at the degree of freedom number \(j\). Because interactions are reciprocal and the system is passive, the damping and stiffness matrices are both symmetric and positive definite.

The stiffness matrix \(K_{ij}\) is constructed as follows. Each stereocilium is anchored into the cuticular plate of the cell by rootlets with pivotal stiffness \(k_{\text{piv}}\), which appears on the diagonal of the matrix. The stereocilia interact with each other through the top connectors, described as elastic Hookean elements of stiffness \(k_{\text{tc}}\) between all adjacent stereocilia in the hexagonal two-dimensional arrangement that they form seen from above (see schematic representation of these positions on Figure S13). Tip links are excluded from this model, which is intended to simulate cells in which the tip links were removed. Therefore, only the top connectors contribute here to the off-diagonal elements of the stiffness matrix.
Figure S13: Schematic diagram showing the arrangement of the stereocilia (SC) and horizontal top connectors (TC) as used for the stochastic computations. The coupling liquid is modelled by dampers in parallel with the top connectors. Five stereocilia are located along the edges, and nine are positioned in the central symmetry row.

The damping matrix $C_{ij}$ contains on its diagonal the individual friction coefficients $c_{ij}$ of the different stereocilia with the surrounding liquid, which are all supposed to be equal for simplicity. Although liquid-mediated interactions between stereocilia exist a priori over the whole structure, direct interactions between adjacent stereocilia are the most important ones. They contribute to each off-diagonal coefficient of the damping matrix associated with a pair of adjacent stereocilia by a coefficient $c_{\text{squeeze}}$, as estimated analytically in Section 6. As illustrated in our final results displayed in Fig. 2b of the main text, these interactions suffice to capture the essential liquid coupling effects in the hair bundle. Note that the dampers representing the coupling liquid lie in parallel with the springs representing the top connectors, thus the matrices $C_{ij}$ and $K_{ij}$ have the same structure.
**Individual parameters and overall bundle drag**

Individual parameters are estimated as follows. The stiffness of the stereociliary pivots is such that the overall bundle stiffness without the tip links corresponds to the experimentally measured average value of 20 $\mu$N·m$^{-1}$, leading to $k_{piv} = 0.3$ $\mu$N·m$^{-1}$ for each of the 61 stereocilia. The top-connector stiffness is fixed from the finite-element model to $k_{tc} = 20$ mN·m$^{-1}$ (see Sections 3 and 4). The drag coefficient per stereocilium is $c_{piv} = 0.5$ nN·s·m$^{-1}$. When multiplied by the number of stereocilia, it gives the experimentally measured overall drag of the entire bundle without the tip links, roughly 30 nN·s·m$^{-1}$. This estimate assumes that nearly no internal dissipation contributes to the overall hair-bundle drag, which is the case if the relative motion in the hair bundle is negligible. In this situation, the hair-bundle drag should correspond to the drag of a solid object of the same dimensions. To check this correspondence, we estimated the drag of an elongated half-ellipsoid whose major axis equals the bundle’s height $h$ and whose minor axes equal the bundle’s radius $r$ at the cuticular plate. As the half-ellipsoid rotates around one of its minor axes, the force-to-velocity ratio at the tip is:

$$c_{ell} = \frac{8\pi\eta}{3} \frac{\sqrt{h^4 - h^2r^2}(h^4 - r^4)}{(2h^2 - r^2)\arccosh\left(\frac{h}{r}\right) - \sqrt{h^4 - h^2r^2}h^3}. \quad (5.2)$$

With height $h = 8$ $\mu$m, radius $r = 3$ $\mu$m, and dynamic viscosity $\eta = 1$ mPa·s, the calculation yields 28.8 nN·s·m$^{-1}$. This estimate agrees well with the experimentally measured value for the whole hair bundle without the tip links, buttressing the idea
that the hair bundle has no other significant sources of drag and justifying our choice for the value of $c_{\text{pv}}$.

**Characterization of the additive noise term in the Langevin dynamics**

Because the size of the gaps between the stereocilia influences the experienced hydrodynamic drag, our Langevin description contains friction coefficients that depend on the positions of the stereociliary tips. With such a dynamics, both a position-dependent noise term (or multiplicative noise) and an additional drift term are in principle required such that the final stochastic dynamics satisfies detailed balance and reaches a proper thermodynamic equilibrium distribution of positions at steady state\textsuperscript{39}. Expressions for both the drift and multiplicative noise terms depend on the discretization of the Langevin dynamics used for numerical integration. Among several conventions used in the literature, we chose the Ito convention, which estimates the noise at the location of the particle before the discrete integration time step is performed. To derive the associated drift and noise terms, we followed closely Lau and Lubensky (2007)\textsuperscript{39} for multi-component systems, which led us to the following final discrete Langevin dynamics:

$$x_{t+\Delta t} = \left( x_t - \Delta t C_{\dot{y}}^{-1} K_{jk} \dot{x}_k - k_B T \Delta t C_{\dot{y}}^{-1} \frac{\partial C_{jk}}{\partial \dot{x}_k} C_{\dot{y}}^{-1} + 2 \sqrt{k_B T \Delta t} G_{\dot{y}} \xi_{\dot{y}} \right) t. \quad (5.3)$$

Here $k_B$ is the Boltzmann constant, $T$ the temperature, $t$ the discrete time, and $\Delta t$ a step of time discretization. $G_{\dot{y}}$ is the square root of the inverse of the damping matrix $C_{\dot{y}}$, such that $G_{\dot{y}} \cdot G_{\dot{y}}^T = C^{-1}$. Imposing $G_{\dot{y}} = G_{\dot{y}}^T$, the matrix $G_{\dot{y}}$ is unique. Finally, $\xi_t$ is an array of independent Gaussian white noises of variance one half.
Because computations with the position-dependent damping matrix and its associated drift term represent a computational burden, we checked for their relative importance for the coherence in the hair bundle. In a coherent hair bundle, the gap distances between adjacent stereocilia vary relatively little, so the damping matrix is expected to be nearly constant and the drift term negligible. To test this hypothesis, we computed the coherence with and without this position dependence in a minimal system of two apposed cylinders with the geometrical parameters corresponding to the two stereocilia at the centre of the hair bundle. We generated 20 100-ms-long data sets for the positions of the centres of both stereocilia tips, and computed the two resulting coherence spectra $\gamma_1$ and $\gamma_2$, respectively with and without position dependence. The same realization of white noise was used for both computations.

We characterized the difference between the two by their relative difference $\frac{2|\gamma_1 - \gamma_2|}{(\gamma_1 + \gamma_2)}$. When the two cylinders were coupled by elastic links whose stiffness was 20 mN·m$^{-1}$, which is our estimate of the stiffness of the horizontal top connectors, this ratio was less than $3 \times 10^{-6}$ between 10 Hz and 5 kHz (Figure S14).

**Figure S14:** Relative difference between the coherence spectra with and without position dependence in the damping matrix.
This result confirms that, in hair bundles with intact top connectors, the gap sizes between adjacent stereocilia are virtually constant during motion. Taking this into account, we simulated the stochastic dynamics of the hair bundle using constant damping coefficients to generate the results displayed in Fig. 2b. In the case of intact top connectors, the resultant coherence spectra are therefore expected to be quantitatively correct.

*Numerical integration of the stochastic equations*

Discrete time-integration was performed following the Euler scheme with particular attention to two aspects. First, independence of the results with respect to initial conditions was ensured by letting the system evolve for a time longer than five times the largest eigenvalue of the matrix $K_{ii}^{-1} \cdot C_{ij}$ before the data were acquired for the analysis. This procedure guarantees that the initial conditions are forgotten by more than 99 %, such that the dynamics is already in a steady state when the data are acquired. Second, the integration time step was chosen sufficiently small such that the smallest eigenvalue was resolved and to ensure that, during one time step, the gaps between the two cylinders never changed by more than 5 %. This allowed us to use constant friction coefficients during each integration step.
Section 6. Hydrodynamic friction coefficient between two adjacent stereocilia

This section describes the analytic derivation of the friction coefficients between adjacent stereocilia that enter the damping matrix in the stochastic model presented in Section 5, as well as the comparison of this analytic estimate with the estimate derived from the finite-element model presented in Section 3.

Hydrodynamic interactions of two cylinders

The stochastic model presented in Section 5 relies on the liquid-mediated interactions of pairs of adjacent stereocilia. These friction coefficients entering the damping matrix in the Langevin dynamics are used to describe the time evolution of the hair bundle in a thermal bath at room temperature. For small amplitudes of motion, a linear regime is assumed. The pivotal motion of two neighbouring cylinders can be decomposed into an orthonormal basis of four independent elementary motions. Two of these four modes correspond to global translations of the two cylinders’ tips along the plane in the excitatory direction and in the perpendicular direction, and the other two modes correspond to squeezing and shearing displacements of the two cylinders’ tips with respect to one another. Among these, the squeezing mode represents the major contribution to the overall dynamics, such that only this mode is considered in the final damping matrix $C_{ij}$ that enters the stochastic model presented in Section 5.

To estimate the friction coefficient associated with the squeezing mode of two cylinders pivoting around their base, we exploit the fact that the stereociliary length is much larger than the variation of the gaps between them from bottom to tip. Therefore, the force per unit length at any given height can be considered equal to
that between two infinite parallel cylinders separated by the corresponding gap and pulled apart with a velocity orthogonal to the common direction of their axes.

Gaps at the tips of stereocilia in a hair bundle are much smaller than their length and diameter. Because most of the hydrodynamic forces originate from this region, these forces can be estimated from the Stefan-Reynolds equations under the lubrication approximation. Within this approximation, the main contribution to the hydrodynamic drag comes from the region of closest approach of the two infinite parallel cylinders. This region can be characterized by the radius $r$ of the cross sections of the cylinders with a plane orthogonal to their axes and by the distance $g$ separating these disks (Figure S15). In this region, the disks' profiles can be replaced by parabolas of the same tip curvature $1/r$, for which analytic estimates can be derived. For that, we integrate the lubrication equation, which in the case of a separation normal to the symmetry axis reads

$$\frac{\partial g}{\partial t} = \frac{1}{12\eta} \frac{\partial}{\partial x} \left[ g^3 \frac{\partial p}{\partial x} \right]. \quad (6.1)$$

Here the $x$-axis corresponds to the symmetry axis as depicted in Figure S15, $\eta = 1$ mPa·s is the dynamic viscosity of water at room temperature, $g(x,t)$ is the local normal separation between the two disks' edges at location $x$ at time $t$, and $p(x,t)$ is the fluid pressure at that location, whose dependence in the normal direction can be ignored within the lubrication approximation. The solution of Eq. (6.1) leads to an expression for the pressure $p$ as a function of $x$ and the relative velocity $v$ of the two disks. Integrating this pressure field along the $x$-axis leads to the force per unit length acting on the cylinders as
Figure S15: Two orthogonal two-dimensional representations of a pair of stereocilia used to derive Eqs. (6.2) and (6.3). (a) The geometry used for Eq. (6.2) consists of two solid disks in a two-dimensional liquid, positioned symmetrically with respect to the $x$ axis. The closest distance between the two disks is $g$. Eq. (6.2) is derived for two such disks approaching each other with a relative velocity $v$ and in the limit $g$ much smaller than $r$. (b) The geometry used to derive Eq. (6.3) consists of two closely apposed stereocilia with radius $r$ and height $h$, displayed projected on the plane defined by their axes. The wall-to-wall distance measures $g$, at their tips and $g_b$ at their bottom. They pivot with respect to their bottom insertions with velocities directed towards their respective axes. Eq. (6.3) is derived from a balance of momenta along the height $h$ that is based on Eq. (6.2). It is derived to leading order in the limit of small angles between the two stereocilia axes.

\[ F_{\text{squeeze}} = \frac{3\pi}{2} \eta v \left( \frac{r}{g} \right)^{3/2}, \]  

(6.2)

whose sign is such that the resulting force always opposes motion. Integrating this force times the lever arm along the two tilted cylinders gives the momentum on the stereocilia as they pivot at their bases in this squeezing mode. Dividing this by the cylinder's height $h$ provides an equivalent drag coefficient at the cylinder tips,

\[ c_{\text{squeeze}} = \frac{\pi\eta h \frac{3}{2}(3 + \varsigma)\chi^3}{(1 + \varsigma)^3}. \]  

(6.3)
Here $\zeta = \sqrt{g_t / g_b}$ and $\chi = \sqrt{r / g_t}$, where $g_t$ and $g_b$ are the gap distances between the two stereocilia at respectively the tip and the bottom.

Comparison between analytic and FEM estimates

To validate this expression, we constructed a finite-element model for this situation. We present in Figure S16 a comparison between the analytic result and this numerical finite-element solution. We see that both estimates agree especially well in the small-gap regime, as expected because the analytic derivation presented above is valid only in the lubrication approximation of very small gaps. We present in the same plot the finite-element solution with liquid motion blocked in the direction parallel to the cylinders' long axes. Indeed, our analytic estimate does not take into account liquid motion in this axial direction. The two corresponding finite-element solutions are very close to each other, confirming that the contribution of the axial flow is very small and further validating our assumption.
Figure S16: Drag coefficient for pivoting cylinders moving in the opposite directions in the squeezing mode with different wall-to-wall distance at the tip (gap). The height is 8 µm, the diameter 380 nm, the wall-to-wall distance at the bottom 400 nm, and the viscosity 1 mPa·s. The finite-element results are for a liquid free to move in all directions (free) and for a two-dimensional constrain where the flow in the cylinders’ axial direction is blocked (blocked). For comparison, the analytical approximation from Eq. (6.3) is given (anal.).
Section 7. Measuring the relative mode of stereociliary motion at distortion-product frequencies

General approach and control experiments

Our interferometric studies indicate that relative mechanical motions in a hair bundle are insignificant compared to the common mode of motion of the whole structure and the quantitative modelling described in the main text of the present communication corroborates this finding. The magnitude of the relative modes of motion depends on the balance between the forces that act to displace individual stereocilia with respect to one another and the forces that resist such motion. To fully appreciate this balance, one must identify conditions under which the relative motions can be unambiguously detected and inferences about the underlying forces can be made. We therefore devised an experiment whose outcome depends critically on the presence of individual motions of stereocilia in a hair bundle.

Because gating of the mechanotransduction channels confers mechanical nonlinearity, a hair bundle stimulated sinusoidally at two frequencies generates distortion products, namely spectral components at frequencies absent from the original stimulus. If a hair bundle acts as a mechanical system with a single degree of freedom, displacing any particular stereocilium should cause the entire array to follow faithfully the imposed movement. In contrast, if stereocilia can move independently, the spectral content of the stereociliary movement might change across the stereociliary array. For example, if the stimulus is applied at the bundle's tall edge with a stiff probe that is tightly coupled to it, the tall edge must follow the probe because all other motions will be suppressed by it. If one measures the motion
at this location and analyses its harmonic content, one would detect only the primary
frequencies constituting the stimulus. But if the measurement is performed on the
opposite, unconstrained, short edge of the hair bundle, one might observe nonlinear
distortion products in addition to the primary frequencies. The presence of these
distortion products would then indicate relative motion. In this case, the related force
acting between adjacent pairs of stereocilia could be used to quantify the strength of
the mechanical coupling between them, as explained below.

At the level of a single hair cell, distortion products were originally
demonstrated by stimulating a hair bundle simultaneously at two frequencies with a
flexible glass fibre and measuring the force exerted by the bundle in response\textsuperscript{40}. The
hair bundle indeed produced forces at the quadratic, cubic, and higher-order
distortion frequencies. Our initial control experiment was to evoke distortion products
using a similar protocol. Hair bundles were stimulated with a flexible glass fibre
whose stiffness was determined from the power spectrum of its thermal fluctuations
to be \textit{circa} 100 $\mu$N m$^{-1}$. Because distortion products are generated by a nonlinear
process, it was necessary to establish the range of amplitudes over which the
stimulation and measurement instruments functioned linearly. When the free fibre
was driven with a piezoelectric actuator (P-835.10, Physik Instrumente, Germany) at
90 Hz and 115 Hz, it displayed periodic motions with a root-mean-square (RMS)
amplitude of $46 \pm 3$ nm ($n = 20$). The spectrum obtained from the recorded time
series showed no nonlinear combination of frequencies (Figure S17a). Moreover, the
higher-order bispectrum betrayed no evidence of a quadratic nonlinearity, which
became apparent only when the RMS amplitudes exceeded 60 nm (data not shown).
Figure S17: Control experiments demonstrating the distortion products at the two edges of a hair bundle. (a) Thermal motions and sinusoidal oscillations of the soft probe driven at 90 Hz and 115 Hz. The scale bars shown here apply to panels (a) through (d). The expected value of the associated power spectral density (PSD) is shown in blue, and the 95% confidence interval is shown in orange. (b) A time series from a spontaneously oscillating hair bundle and the associated spectrum. (c) Stimulation of the hair bundle shown in (b) with the oscillating probe shown in (a) results in prominent distortion products at the short edge of the hair bundle, indicated on the spectrum by the dashed arrows. (d) Same as (c) but for the tall edge of the hair bundle.

We accordingly limited the maximum amplitude of the evoked hair-bundle movements to the linear regime of the stimulation and detection apparatus.

To guarantee that mechanotransduction was functional, we used for these experiments hair bundles that oscillated spontaneously. Recording from a
spontaneously oscillating hair bundle revealed motions of about 45 nm RMS (Figure S17b); the associated power spectrum showed substantial power at the frequency of oscillation near 10 Hz. The glass fibre was then attached to the kinociliary bulb and the same mechanical stimulus as the one used to drive the free fibre (Figure S17a) was applied through the piezoelectric actuator. The laser beam focused at the tip of the short edge measured an RMS displacement of 19 ± 2 nm, whereas the second laser beam positioned at the tip of the tall edge measured 33 ± 5 nm (Figure S17c and d). The difference in amplitude between the two signals resulted mostly from the fact that for the same rotation, larger displacements are observed at the tip of the longer stereocilia. In agreement with the previous result, the associated power spectra demonstrated distinct peaks at the distortion-product frequencies for the two sides of the hair bundle.

Because a flexible glass probe cannot completely control the displacement of a hair bundle, we used a stiff glass pipette filled with perilymph to which suction could be applied to attach it firmly to a stereocilium or to the kinocilium. Such a configuration suppressed all movements that could be induced by internal forces or nonlinear properties of the hair bundle at the stimulated edge. Before attaching the pipette, a free, mechanically active hair bundle showed spontaneous oscillations at 5–10 Hz with an RMS amplitude of 36 ± 7 nm (Figure S18a). The associated power spectra demonstrated substantial power at low frequencies. After the stiff pipette had been attached near the tip of the hair bundle's tall edge, mechanical stimuli were applied at 90 Hz and 115 Hz. In the absence of suction, the coupling between the pipette's tip and the hair bundle was loose. The strength of the mechanical stimulus was adjusted such that the amplitude of the evoked movements did not
exceed that of the spontaneous oscillations and remained in the linear range of stimulation and recording. Stimulation generated robust distortion products that were clearly apparent in the spectra obtained from both edges of the hair bundle (Figure S18b). As expected, when the hair bundle was treated with 5 mM BAPTA to disrupt the tip links and abolish the nonlinearity associated with channel gating, the same mechanical stimulus evoked movements at only the primary frequencies (Figure S18c).

**Figure S18**: Control experiments demonstrating that the distortion products require intact tip links. (a) Time series and the associated PSDs for the two edges of a spontaneously oscillating hair bundle. (b) Time series and the PSDs for the same hair bundle stimulated at 90 Hz and 115 Hz demonstrate the robust distortion products. (c) These distortion products disappear after the hair bundle is treated with BAPTA.
Finally, in the key series of experiments described in the main text, tight coupling of the rigid pipette to the hair bundle’s kinociliary bulb was ensured by applying negative pressure in the pipette, constraining the movement of the tall edge of the hair bundle. Therefore, when stimulated at 90 Hz and 115 Hz, the tall edge displayed no distortion products. Nevertheless, stereocilia at the short edge demonstrated clear distortion products (Fig. 3a). Owing to the viscous and elastic coupling between the stereocilia, however, the distortion products’ amplitudes were approximately one-tenth those characteristic of the loose coupling to the pipette.

These experiments allowed us to accomplish two objectives. First, they clearly demonstrate the relative mode of motion between stereocilia, which is revealed by the application of a stimulus mimicking the physiological excitation at the kinocilium, and allow for its quantification. The relative mode can be assessed more accurately with this approach than by the measurement of a hair bundle’s thermal motion. Indeed, stimulation at particular frequencies allows one to characterize the relative mode with respect to these reference signals. Furthermore, when the tall edge is constrained, the motion at the short edge at the distortion frequencies represents the relative mode, which is directly apparent in the spectra without additional data processing (Fig. 3a, left). This experimental approach allowed us to avoid uncertainties usually associated with the analysis of thermal motions. Indeed, in that case the relative mode’s amplitude can be estimated only by subtracting the movements at one edge from those at the other edge, but this procedure is subject to large errors owing to the difference in the absolute amplitudes at the two edges related to geometrical factors. Moreover, the present approach is more appropriate for the analysis of very small relative motions because the periodic nature of the
signals allows one to reduce the background noise through averaging. As we had inferred previously from thermal motion measurements\(^1\), this relative mode is indeed very small, only a fraction of a nanometre between adjacent stereocilia (Fig. 3a and Figure S19; see details below).

Second, by using the amplitudes of the distortion products measured at the short edge while the tall edge was subjected sequentially to the two boundary conditions of stiff and loose coupling to the probe, we could verify the strength of the mechanical coupling between stereocilia and in particular the stiffness of the horizontal top connectors used in the finite-element model. We describe this procedure in the following paragraphs.

**Quantification of the nonlinearities**

To quantify the nonlinearities, we analyzed separately and identically the records for the two edges of a hair bundle from which they were acquired simultaneously. For every cell, 20 1-s-long measurements were aligned to the template

\[ A \sin(2\pi f_1 t) + B \sin(2\pi f_2 t) \]

such that all started at the same phase angle. Here \( f_1 = 90 \) Hz and \( f_2 = 115 \) Hz are the two excitation frequencies and the coefficients \( A \) and \( B \) are the particular amplitudes associated independently with each record. After the alignment, the traces were averaged to reduce the noise. The resulting sample was then divided in segments of 200 ms, which corresponded to the shortest time interval containing integer numbers of both stimulus periods. After removing the onset and offset transients and subtracting the constant component of the signal in time, we averaged again over the divisions. The final mean signal \( x_m(t) \) was then analyzed for its harmonic content. The amplitudes of the two primary frequencies
were reconstructed by fitting the function \( x_r(t) = A \sin(2\pi f_1 t) + B \sin(2\pi f_2 t) \) to \( x_m(t) \). This signal \( x_r(t) \) then served as a reference to compute an orthonormal series of vectors \( g_i(t) \) consisting of the power series of vectors \( x_r(t)^j \), such that each \( g_i(t) \) was a linear combination of the different \( x_r(t)^j \), with \( j \leq i \). The orthogonality between the different \( g_i(t) \) was ensured by the Gram-Schmidt algorithm. The projection of \( x_m(t) \) onto these basis vectors provided the coefficients of the expansion of the measured data as a power series of the undistorted input signal \( x_r(t) \):

\[
 x_m(t) = C_1 g_1(t) + C_2 g_2(t) + ... = \sum_{i=1}^{\infty} C_i g_i(t). \tag{7.1}
\]

This procedure assumed that the distorted part of the measured signal \( x_d(t) = x_m(t) - x_r(t) \) is solely a function of the reference signal \( x_r(t) \). Plotting this distortion against the reference signal \( x_r(t) \) confirmed this hypothesis (Figure S19). The coefficient \( C_1 \) characterizes the reference signal as \( x_r(t) = C_1 g_1(t) \), and the coefficients \( C_2, C_3, C_4, ... \) characterize the distortions.

Because the tall edge of the hair bundle was not moving at the distortion-product frequencies when constrained by the stiff pipette, we focus in the following on the response displacements of the opposite, short edge. Although some cells demonstrated prominent quadratic and cubic distortions (Figure S17), in other cells—presumably because of different operating points—the cubic distortion products were buried in the noise. Therefore, only the first coefficient \( C_2 \) was significant for most of the experimental data, in that fitting to higher-order polynomials did not increase significantly the quality of the fit (fitting and comparison procedures were performed with anova1 and multicompare functions in MATLAB).
Thus, the distortion $x_d(t)$ reduced under this approximation to a quadratic function of the reference signal $x_r(t)$:

$$x_d(t) = \frac{x_r(t)^2}{D_2}. \quad (7.2)$$

The coefficient $D_2$ has the units of length and quantifies the distortion.

**Figure S19:** The quadratic part of the distorted signal $x_d(t)$ as a function of the undistorted input $x_r(t)$. (a) Experimental data for the short edge of a hair bundle are shown in orange, and for the tall edge in blue. The fits are shown as the orange solid line for the short edge, and as the dashed blue line for the tall edge. The boundary condition corresponds to the loose coupling of the probe. (b) Same as (a) but with tight coupling to the probe as the boundary condition.

The amplitude of the distortions observed is between zero and five nanometres (Figure S19). Given the number of stereociliary rows in a hair bundle, typically five to ten, this observation implies that the characteristic variation of the gap between each pair of adjacent stereocilia is on the order of a fraction of a nanometre, which is comparable to the size of a water molecule.

**Matching the experimental results and the finite-element model**

To reproduce the experimental observations with our finite-element model, we used the fact that the distortion products originate from channel gating and assumed that
this nonlinear response is a function of bundle deflection only. In the finite-element model, we represent channel gating by a force exerted at both ends of a tip link in opposite directions along its length. This force acts on the pair of stereocilia to which the tip link is connected, and has the same phase and amplitude for all tip links. Because the distortion stems from tip-link mechanics and is a quadratic function of the reference signal \( x_r(t) \), we can make the ansatz that this tip-link force is proportional to the distortion \( x_d(t) \) and is therefore quadratic in \( x_r(t) \):

\[
F(t) = \frac{x_r(t)^2}{E_2},
\]

in which \( E_2 \) is an unknown coefficient. The linear finite-element model computes the ratio between the applied force along the tip links and the displacement at the short edge:

\[
k = \frac{F(t)}{x_d(t)}.
\]

Here the global stiffness coefficient \( k \) depends both on the tip-link and top-connector stiffness values used in the model and on the boundary conditions at the tall edge (loose or tight coupling to the probe). In the finite-element computation, we mimicked these two experimental situations by either leaving the tall edge free or by blocking the motion there completely. Using Eqs. (7.2), (7.3) and (7.4), we obtain the coefficient \( E_2 \) as:

\[
E_2 = \frac{D_2}{k}.
\]

Associated with the two experimental boundary conditions for which we obtain two fitting coefficients \( D_2^{\text{loose}} \) and \( D_2^{\text{tight}} \), we obtain two FEM-derived stiffness coefficients \( k^{\text{loose}} \) and \( k^{\text{tight}} \). As mentioned above, the tip-link force is assumed to be a function not
of the boundary condition, but solely of the linear bundle displacement. Thus, the coefficient $E_2$ in Eq. (7.3) is the same in both conditions, such that $E_2^{\text{loose}} = E_2^{\text{tight}}$. Using Eq. (7.5), we finally get as a consequence that the following ratio should be conserved between experimental and modelling data:

$$
\frac{D_2^{\text{loose}}}{D_2^{\text{tight}}} = \frac{k^{\text{loose}}}{k^{\text{tight}}}.
$$

(7.6)

For a given reference signal $x_r(t)$, when the tall edge is free to move at the distortion frequencies, tip links have to work mostly against the small interstereociliary sliding drag and the tip links’ stiffness to create a given distortion signal $x_d(t)$ at the hair bundle’s short edge; when the tall edge is constrained, the same tip links must additionally work against the larger interstereociliary squeezing drag and against the top connectors’ stiffness. Therefore, the corresponding $x_d(t)$ is smaller in the second case, which according to Eq. (7.2) corresponds to a larger $D_2$. Consequently, the ratio given above is smaller than unity (see also the main text).

For the two boundary conditions at the tall edge, we computed the response of the hair bundle to a force along all tip links with the same amplitude and phase. The model with a tip-link stiffness of $1 \text{ mN}\cdot\text{m}^{-1}$ and a top-connector stiffness of $20 \text{ mN}\cdot\text{m}^{-1}$ agreed well with the experimental data in that it reproduced the ratio of the two values for the coefficient $D_2$ as given by Eq. (7.6).
Section 8. Electron microscopy

Bullfrog sacculi to be used in transmission electron microscopy were dissected and treated with enzymes as described in Section 1 above, then fixed for 2–18 h at 4 °C in 200 mM glutaraldehyde, 400 mM formaldehyde, 1 mM CaCl₂, and 75 mM sodium cacodylate at pH 7.2. After a brief wash in the fixative-free solution, the tissue was postfixed for 1–2 h at 4 °C in 50 mM OsO₄, 5 mM potassium ferrocyanide, 1 mM CaCl₂, and 75 mM sodium cacodylate at pH 7.2. For preparation at a low ionic strength, the concentration of sodium cacodylate was lowered to 25 mM in all solutions, the Ca²⁺ concentration was reduced to 10 μM, and 100 mM D-mannitol was added.

Following a wash in distilled water, the specimens were progressively dehydrated at 4 °C through a graded series of ethanol concentrations and treated for 1 h at room temperature with 0.4 % uranyl acetate in 95 % ethanol. The tissue was dehydrated by immersion for 2 h in 100 % ethanol and for 2 h in propylene oxide at room temperature. Each specimen was impregnated with an epoxy-resin mixture (EMbed-812 resin, Electron Microscopy Sciences, Hatfield, PA, USA), transferred to a silicone embedding mold, and heated to 50 °C under vacuum for 48 h to harden the plastic.

Specimens were sectioned at a thickness of 70 nm with a diamond knife (Diatome Ultra 45°, Electron Microscopy Sciences) in an ultramicrotome (Ultracut-E, Reichert-Jung, Vienna, Austria). Serial sections were collected and stained for 2 min with 50 % saturated aqueous uranyl acetate in 50 % acetone and for 1.5 min with lead salts. Micrographs were acquired with a transmission electron microscope.
(Tecnai G2-12 Biotwin, FEI Co., Eindhoven, The Netherlands) equipped with a digital camera (XR60, Advanced Microscopy Techniques Corp., Danvers, MA, USA).

Specimens meant for scanning electron microscopy were dissected and subjected to primary and secondary fixation as described above except that potassium ferrocyanide was omitted. After a wash in distilled water, the sacculi were dehydrated through a graded series of ethanol concentrations, dehydrated for 2 h in 100 % ethanol, critical-point dried from liquid CO$_2$ (Autosamdri-815, Tousimis Research Corp., Rockville, MD, USA), sputter-coated with gold-palladium, and examined with a 30-µm aperture at an accelerating voltage of 5 kV in a microscope with a Schottky field-emission electron source (Zeiss-LEO Gemini 1550, Carl Zeiss NTS GmbH, Oberkochen, Germany).
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