Supporting Text

Supporting Methods

Preparation and Measurement. ***Stimulus signal.*** Displacement amplitudes were of the order of 1 nm, so that a linear relationship between stimulus and response could be safely assumed; this was also verified experimentally (second and third harmonics were >30 dB below the fundamental). Therefore, a multitone signal could be safely used as the command voltage. It contained 81 frequency components between 480 Hz and 67 kHz, with equal amplitude but random phase, uniformly distributed on the interval $[0, 2\pi]$. Frequency spacing was almost logarithmic with a ratio of ≈ 1.07 between adjacent frequencies. To reduce harmonic distortion products in the measured velocity, care was taken that no frequency was within 1% of the first four harmonics of a lower frequency. The maximum (total) amplitude of the multitone stimulus was 6-10 V.

Estimation of maximum transmembrane potential change. The transmembrane voltage U_{tm} was estimated from the extracellular voltage gradient along the cell by assuming the cell to be a voltage divider with a ratio of 1:1 between its apical and basal halves. The extracellular current through the chamber per spectral point was 1-2 mA, depending on frequency. The electrical impedance of the chamber was 70 Ω. Therefore, the voltage drop between the electrodes was 70-140 mV per spectral point. The distance between the electrodes was 3.9 mm, so that the voltage gradient in the chamber was 18-36 μV/μm. Therefore, for an outer hair cell (OHC) of length L_{OHC} (in units of μm), we have $U_{tm} = (9\text{-}18)L_{OHC}$, in units of μV. The influence of the electrical impedance of the reticular lamina (RL) on the current path was neglected in this calculation, because (*i*) the organ was small compared with the cross-sectional area through which the current passed (1 cm^2), and (*ii*) current could flow around the RL. Moreover, we had no means of monitoring the extracellular potential within the organ of Corti up to the high experimental frequencies. However, the true voltage gradient in the chamber can only be smaller than our estimate, so that the estimate provides an upper bound.

Velocity measurement. Velocity was measured with a laser Doppler vibrometer (LDV, OFV-302, wavelength 633 nm, power 1 mW) equipped with a demodulator (OFV-3000, bandwidth 100 kHz), both from Polytech (Waldbronn, Germany). The laser beam was focused on the object by coupling it into the optical path of an upright microscope (Axioskop 2FS, Zeiss) via a beam splitter (AHF Analysentechnik, Tübingen, Germany), which was highly reflective only above 590 nm but transparent for shorter wavelengths. The microscope objective was a water-immersion objective with ×40, numerical aperture 0.8, and working distance 3.61 mm (Zeiss). The laser spot had approximately a Gaussian profile, with full-width at $1/e^2$ of maximum power of 0.63 μm (quantified with a knife-edge method). The velocity spectrum was corrected for the measured transfer function of the LDV. Phase is positive for motion toward the microscope objective. The noise floor, expressed as displacement but measured as velocity, decreased from 100 pm at 480 Hz to 1 pm at 67 kHz (effective averaging time, 25 s).

Channel blocking. Pharmacological block of channels was used to test for nonspecific electrically induced motion of the organ of Corti and also to identify current pathways. The volume of the (streamlined) experimental chamber was ≈10 ml. Typically, 30–50 ml was used to exchange the medium, which required ≈3 min. Washout was with fresh Hanks' balanced salt solution (HBSS). All solutions were at the same temperature, osmolarity, and pH, as given in the text.

Supporting Results: Displacement

Channel Blocking. ***Estimation of the electrically induced force.*** The main assumption is that the motor component of the electromechanical force produced by the OHCs is negligible during the measurement of the point impedance of the organ of Corti. Algebraically, the external force, F, applied to the organ of Corti can be written as $F = Zv + f$, where Z is the impedance of the organ of Corti (OHCs included), v is the velocity, and f is the motor component of the electromechanical force produced by the OHCs. For an organ stiffness of 150 mN/m measured in ref. 1, and displacement amplitudes of 1 nm, we have $F > 150$ pN. To estimate f, consider the corresponding impedance measurements on an isolated OHC fixed at is basal pole and externally loaded by a cantilever at its apical pole. To calculate the axial force ΔF produced by an OHC for an elongation ΔL, we use the piezoelectric, two-port model presented by Dong *et al.* (2), with the input terminated by the resistance, R, of the basal cell membrane. Using standard two-port theory, one can readily show that

$$\frac{\Delta F}{\Delta L} = \frac{1}{c_{22}}\left[\frac{1 + j\varpi\tau}{1 + j\varpi\tau\,(1-k^2)}\right] \qquad [\mathbf{1}]$$

where ω is the radial frequency, $j = \sqrt{-1}$, $k^2 = c_{12}c_{21}/c_{11}c_{22}$, with c_{11} the (total) membrane capacitance, c_{22} the (total) axial compliance of the cell, $c_{12} = c_{21}$ is the piezoelectric coefficient of the cell, and $\tau = Rc_{11}$ is the electrical time constant of the cell membrane. The parameter k is called the coupling coefficient of the cell (3). We assume that all of these parameters are frequency independent. One can assert that the parameter k^2 is approximately independent of cell length because for cells isolated from base to apex of the cochlea it has been shown experimentally that: (*i*) the specific cell capacitance, C_s, is independent of cell length (4), (*ii*) the force per unit strain, K_s, is independent of cell length (5, 6), (*iii*) the elongation per unit change of transmembrane potential for zero electromechanical force is directly proportional to cell length (5), and (*iv*) $c_{12} = c_{21}$ as required for piezoelectricity (2). For a cell length of 50 μm, we have c_{21} = 20 nm/mV (2), c_{11} = 31 pF using a cell radius of 5 μm, and $C_s = 2$ μF/cm^2 (4), and c_{22} = 135 m/N using $K_s = 370$ nN (2). These values give $1 - k^2 = 0.904$ or $k = 0.31$. [This value agrees with the maximum value of k derived for a two-state piezoelectric area model by Iwasa (3)]. Because $\omega\tau >> 1$ (4), and $1 - k^2$ is close to unity, we can safely assert that the bracketed term in Eq. **1** can be approximated as $1 + k^2$ for all frequencies used in our experiments. Consequently, the motor component of the electromechanical force is $f_M = \Delta L\, k^2/c_{22}$. For $\Delta L = 1$ nm and $c_{22} = 135$ m/N, we have $f_M \approx 0.7$ pN. [Notice that for low frequencies ($\omega\tau << 1$), the bracketed term in Eq. **1** is approximately unity, so that f_M asymptotes to zero.] Because the measurement region was extremely localized on the reticular lamina (preloading <1 μm), we maintain that the total motor force, f, derives

from no more than five OHCs; namely, from the OHC under the cantilever tip and its four closest neighbors; that is, $f \approx 5f_M$. In other words, for all stimulus frequencies and recording locations, f is unlikely to be more than about 4 pN, which is much smaller than the external force of 150 pN acting on the organ of Corti and can, therefore, be neglected. (q.e.d.).

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