Cochlear outer hair cells (OHCs) are among the fastest known biological motors and are essential for high-frequency hearing in mammals. It is commonly hypothesized that OHCs amplify vibrations in the cochlea through cycle-by-cycle changes in length, but recent data suggest OHCs are low-pass filtered and unable to follow high-frequency signals. The fact that OHCs are required for high-frequency hearing but appear to be throttled by slow electromotility is the “OHC speed paradox.” The present report resolves this paradox and reveals origins of ultrafast OHC function and power output in the context of the cochlear load. Results demonstrate that the speed of electromotility reflects how fast the cell can extend against the load, and does not reflect the intrinsic speed of the motor element itself or the nearly instantaneous speed at which the coulomb force is transmitted. OHC power output at auditory frequencies is revealed by emergence of an imaginary nonlinear capacitance reflecting the phase of electrical charge displacement required for the motor to overcome the viscous cochlear load.

The cochlea endows mammals with the ability to hear sounds over a frequency range far surpassing the capability of other vertebrate classes. Superior performance has primary origins in the function of outer hair cells (OHCs), which are uniquely electromotile and respond to a change in voltage with change in length (1). OHCs are ultrafast under some conditions, capable of generating forces at frequencies exceeding 80 kHz (2). The motor mechanism requires expression of the protein prestin in the lateral wall membrane (3), which imparts OHCs with properties similar to piezoelectric materials where the electric field generates a coulomb force that drives charge displacement and concomitant mechanical strain on a cycle-by-cycle basis (4, 5). The idea of cycle-by-cycle amplification at auditory frequencies has been challenged by recent experimental evidence that OHC membranes exhibit low-pass characteristics (6, 7). Precisely how OHCs circumvent low-pass characteristics and provide power to the cochlea at high auditory frequencies is the primary subject of the present report.

OHCs sense sound through mechano-electrical transduction (MET) channels that open cycle-by-cycle in response to sound-induced displacement of their apical stereocilia (8). The MET current entering the cell is modulated at auditory frequencies and drives changes in intracellular voltage. Like all cells, OHC membranes have electrical capacitance, which reduces the voltage modulation as the sound frequency is increased above the membrane RC corner frequency (RC: resistance times capacitance). The RC corner is unusually high in OHCs owing to a standing K⁺ conductance in the membrane (9). Ultrafast K⁺ channel gating might also play a role in extending the effective RC (10). Evidence that OHCs can modulate voltage at auditory frequencies is compelling, but whether or not the motor mechanism can be driven by voltage cycle-by-cycle is less clear. Direct experimental measurement of electrical charge displacement and motility in OHCs and membrane patches suggests prestin-dependent electromotility is too slow to support cycle-by-cycle amplification (6, 11–13).

The present report is focused on high-frequency power output of OHCs and applies a thermodynamic approach to examine whole-cell function. Results demonstrate the OHC speed paradox arises in part from the misleading nature of conventional capacitance recordings and the relationship between charge displacement and OHC power output under load. The paradox is resolved by accounting for the reversible interplay between charge displacement, voltage, stress, and temperature using first principles set forth by Maxwell, Seebeck, Currie, and Newton. Results explain high-frequency force generation in isolated OHCs (2), low-pass nonlinear capacitance (NLC) in membrane patches (13), and OHC power output in the cochlea across the frequency bandwidth of hearing. Fundamental mechanisms are revealed through examination of load-dependent electrical charge displacement in the piezoelectric membrane complex. The same principles are shown to explain the origins of infrared laser-induced charge displacement in hair cells, neurons, and model membranes (14, 15).

### Results

#### Capacitance Susceptibility

Isolated OHCs exhibit a signature voltage-dependent capacitance reflecting reversible electromechanical charge displacement in the membrane. Examining the origin of NLC provides insight into how OHCs function in the cochlea. OHC membranes are complex inhomogeneous mixtures of lipids, proteins, and charged macromolecules, bordered on each side by ionic double layers and membrane-associated macromolecules. From an experimental point of view, it is generally impossible to directly control or measure the nanoscale distribution of charge associated with the membrane, but straightforward to experimentally control the total voltage drop across the membrane $V$, the temperature $\Theta$, and the stress $T_i$ ($i = 1, 2, 3$). For small perturbations about the resting state ($V_0, \Theta_0, T_0$), the chain rule of calculus provides the electrical displacement current $ID$ across the membrane in terms of the charge $Q$:

$$Q = \frac{\partial V}{\partial t} + \frac{\partial \Theta}{\partial t} + \frac{\partial T_i}{\partial t}$$

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The capacitance voltage susceptibility is \( C_E = \partial Q / \partial V \) (electric Maxwell effect, farad, coulomb-volt\(^{-1}\)), capacitance temperature susceptibility is \( C_T = \partial Q / \partial T \) (thermal Seebeck effect, coulomb-kelvin\(^{-1}\)), and the capacitance stress susceptibility is \( C_{PI} = \partial Q / \partial \sigma \) (piezoelectric Curie effect, coulomb-meter\(^{-2}\)newton\(^{-1}\)). Einstein’s summation convention applies for repeated indices. Capacitance susceptibilities describe the charge displacement driven by small perturbations in each of the three thermodynamic state variables; they are thermodynamically independent but related to each other by reciprocity, e.g., \( \partial^2 Q / \partial V \partial T = \partial^2 Q / \partial T \partial V \) requires \( \partial C_E / \partial T = \partial C_C / \partial V \). The term “susceptibility” is used here to distinguish values from idealized capacitor theory (16). Measurements of cell membrane capacitance often assume \( dQ / dV = C_m (\partial C/V/\partial t) \) and report \( C_m \) as “electrical capacitance,” but this approach can be misleading for piezo- or thermo-electric membranes because stress and/or temperature can change with voltage, coupling multiple terms in Eq. 1. The role of OHCs in the cochlea is to convert electrical power into mechanical power, requiring OHC membranes to invoke capacitance voltage susceptibility \( C_E \) and capacitance stress susceptibility \( C_{PI} \) at the same time. Results in the present report demonstrate how these two terms interact to enable OHC power output at auditory frequencies.

The capacitance susceptibilities in Eq. 1 arise from first principles of thermodynamics, and can be described agnostic to the specific molecular origins. All results in the present report are based on general thermo-piezoelectric materials where the electro-mechanical properties are determined from derivatives of the Gibbs free energy. The standard second order theory is used to describe thermo-electromechanics of nonexcitable membrane domains (17, 18) and a nonlinear extension is used to describe thermo-piezo-electromechanics of excitable domains (SI Appendix, A). The two domains are configured in parallel electrically and in series mechanically.

### Capacitance Voltage Susceptibility in OHCs

The capacitance voltage susceptibility \( C_E \) in OHCs arises from the addition of voltage-driven charge displacement in the passive membrane domains (linear capacitance: \( C_E^{0} \)), plus voltage-driven charge displacement in piezoelectric membrane domains [NLC: \( C_E^{NL} = C_E^{NL}(\xi) \)].

Holding stress and temperature constant, the total capacitance voltage susceptibility is as follows:

\[
C_E = C_E^{0} + C_E^{NL}(\xi). \tag{2}
\]

The standard electrostriction form \( C_E^{0} \approx C_E^{0}(1 + a_2(V + y)^2) \) is used for the passive domain (19) (SI Appendix, Eq. C4 and Fig. S1), where \( C_E^0 = A^e \epsilon / h^2 \), \( \epsilon \) is the electrical permittivity, \( A^e \) is the area of the passive domain, and \( h^2 \) is the thickness. A small voltage dependence arises from the electrostriction parameter \( a_2 \) and spontaneous polarization \( y \). The increased linear capacitance present in OHCs at hyperpolarized voltages is not included in the present analysis. The piezoelectric capacitance susceptibility in Eq. 2 arising from the motor domains \( C_E^{NL}(\xi) \) is highly nonlinear (SI Appendix, Eq. A6). \( C_E^{NL} \) is the peak NLC voltage susceptibility occurring at voltage \( V^{NL} \) (at resting temperature and stress) arising from the piezoelectric coefficients, the motor domain compliance tensor, and the area of the motor domain. The nonlinearity \( f(\xi) \) describes strain-dependent saturation of the piezoelectric charge displacement as a function of thermodynamic state of the membrane \((V_0, T_0, \Theta_0)\). Saturation arises from prestin extending from its fully contracted configuration to its fully extended configuration, and hence is directly dependent on strain in the motor domain.

Dependence on strain makes \( C_E^{NL} \) dependent on all three state variables: voltage, force, and temperature. Specifically, the argument of \( f \) is proportional to strain and written \( \xi = (V - V^{NL} + \beta_F F + \beta_\Theta \Theta) / \lambda^2 \), where \( \beta_\Theta \) is the temperature sensitivity and \( \beta_F \) is an axial force sensitivity. (Note: the force term \( \beta_F F \) is a simplified one-dimensional version of \( \beta_F T_i \) and, by Laplace’s law at low frequencies, can alternatively be written in terms of intracellular pressure \( \beta_P \) and load.) The charge sensitivity is \( \lambda = k_B \Theta / \text{ ze} \), where \( \Theta \) is absolute temperature, \( k_B \) is Boltzmann’s constant, \( z \) is the maximum charge movement between saturated extended and contracted states. In the present report, \( f(\xi) \) is approximated using the first derivative of the Langevin function, so \( f(\xi) = 3f_0((1/\xi^2) - \text{Cosh}(\xi^2)) \), where the temperature scaling factor is \( f_0 = \Theta / \Theta_0 \). A Langevin function is used here with the recognition that prestin conformational changes likely involve multiple transition states (20), resulting in broader tails in the voltage-displacement curve than would be predicted by a simple two-state Boltzmann, but use of an alternative functional form does not change conclusions of the present report related to the OHC speed paradox.

To establish confidence in the thermo-piezoelectric description, the capacitance voltage susceptibility \( C_E \) from Eq. 2 is compared to data from isolated OHCs in Fig. 1 (model parameters are listed in Table 1). Unlike most cells, piezoelectric capacitance voltage susceptibility \( C_E^{NL} \) introduces a strong voltage dependence in OHCs that can double the capacitance at voltage \( V^{NL} \). Theoretical predictions (solid curves) are compared to data from Kakehata and Santos-Sacchi (21) at two different intracellular pressures in Fig. 1 A and B and to data from Santos-Sacchi and Huang (22) at three different temperatures in Fig. 1 C and D. It should be noted that the data in Fig. 1 C and D (22) are shifted relative to OHCs in the cochlea where \( V^{NL} \) is closer to the cell resting potential of \(-40 \sim -50 \text{ mV} \) (9). An increase in intracellular pressure shifts the nonlinear piezoelectric capacitance to the right without a detectable change in \( f_0 \) or \( \lambda \), while an increase in temperature shifts the NLC to the right while increasing both \( f_0 \) and \( \lambda \). All curves in Fig. 1 C use the same value of \( C_E^{0} \), and the shift in magnitude and voltage dependence arises naturally from temperature dependence of \( f(\xi) \), not from any change in constitutive parameters.

### Capacitance Stress Susceptibility in OHCs

The capacitance stress susceptibility arises from the piezoelectric domains and determines the charge displacement for small perturbations in membrane stress \( C_T (\partial T_i / \partial t) \), axial force \( \partial F / \partial \sigma \), or intracellular pressure \( \partial P / \partial \sigma \) (SI Appendix, A). To facilitate comparison to experimental data, the capacitance pressure susceptibility \( C_P = \partial Q / \partial P \) is as follows:

\[
C_P = C_E^{NL} \beta_P(\xi), \tag{3}
\]

and is shown as a function of voltage in Fig. 1E. \( C_P \rightarrow 0 \) at highly hyperpolarized and depolarized voltages, and peaks at \( V^{NL} \). The significance of \( C_P \) is that it determines the electrical displacement current evoked by a change in intracellular pressure, \( I_{DP} = C_P (\partial P / \partial t) \) [which can be converted to displacement current induced by a change in axial force \( I_{DF} = C_P (\partial F / \partial \sigma) \) or membrane stress \( I_{DT} = C_T (\partial T_i / \partial t) \)]. Under dynamic load, the stress-induced charge displacement interacts with the voltage-induced change displacement on a cycle-by-cycle basis. This interaction provides feedback, where the active piezoelectric element responds to both the load and voltage.

### Capacitance Temperature Susceptibility

The capacitance temperature susceptibility \( C_T \) arises from both the passive and piezoelectric domains, and determines the charge displacement for small perturbations in membrane temperature. In OHCs,
To first order, the contribution from the passive membrane is 
\[ C_{\Theta} \approx C_0 + C_p \beta_p f(\xi). \]  

This term arises from the spontaneous polarization arising from the ionic conditions, and \( c_1 \) is the “thermostriction” coefficient arising primarily from thinning of the membrane that occurs with increases in temperature. thermostriction, derived here from thermo-piezoelectricity, is the thermal analog to electrostriction in lipid bilayers (19) and explains the origins of capacitive currents induced by infrared laser pulses in passive membranes (14) (SI Appendix, Fig. S2). 

The contribution from the piezoelectric domains \( C_{pk} \) is closely related to the capacitance voltage susceptibility and is found by taking the partial derivative of the charge displacement with respect to temperature, 

Fig. 1F plots the capacitance temperature susceptibility \( C_{\Theta} \) for OHCs. The dashed line in Fig. 1F is the contribution of the passive membrane \( C_0 \), while the solid curve is the total capacitive temperature susceptibility including the contribution of piezoelectricity (Eq. 4). It is important to note that \( C_{\Theta} \) cannot be completely determined by temperature-dependent changes in electrical capacitance susceptibility alone. This is most clearly illustrated by the fact that \( C_{\Theta} \) in Fig. 1F is negative for all voltages below the spontaneous polarization and hence the heat-pulse–evoked current is always inward and excitatory. The change in electrical capacitance reverses sign, which would imply a change in the direction of the capacitive current in models based simply on variable capacitance (15). This distinction is illustrated for an OHC in Fig. 1G where \( \Delta C_{\Theta} \) is shown as a function of time in response to an infrared laser heat pulse (Inset, i) at different holding potentials (black curves). Results are for a 1 °C increase in temperature occurring in 500 μs followed by relaxation to resting temperature over ~1 s (Inset, red) (parameters for all figures are listed in Table 1).

**Speed and Load Dependence of OHC Charge Displacement.** The OHC motor residing in the membrane always operates against a mechanical load, arising from the cell itself and the external
environment. As a result, OHCs invoke capacitance voltage susceptibility and stress susceptibility at the same time, with the combination of the two providing the total electrical charge displacement and mechanical strain in the membrane. To examine how the load influences OHC function high frequencies, constitutive equations for the passive membrane and the piezoelectric domains were combined as a mixture composite and subjected to a mechanical load imposed by the cell itself and the external environment. Equations were simplified for small perturbations in voltage and axial force, and converted to the frequency domain (Methods and SI Appendix, A and B).

To examine intrinsic speed of the motor element, the cell was clamped to a fixed length (strain = 0) and excited by sinusoidal voltage clamp. Although the whole-cell strain was zero in the simulations, the

### Table 1. Parameters

| Symbol | Value (SI units) | Description | Present estimation method | Data source |
|--------|-----------------|-------------|--------------------------|-------------|
| \( \alpha_2 \) | 0.13 (V\(^{-2}\)) | Electrostriction coefficient | See SI Appendix, Fig. S1 | Based on refs. 43 and 44 |
| \( c_1 \) | 0.0036 (°C\(^{-1}\), V\(^{-1}\)) | Thermoelectric coefficient | See SI Appendix, Fig. S2 | Based on ref. 14 |
| \( C^L_e \) | Variable (F) | Linear electrical capacitance susceptibility. OHC size dependent (~1 \( \mu \)F-cm-2) | Curve fit Eq. 2 to low-frequency NLC data (Fig. 1A) | E.g., refs. 21, 45, and 46 |
| \( C^P_e \) | Variable (F) | Peak piezoelectric electrical capacitance susceptibility. Prestin expression dependent (nominal 1.1 C) | Curve fit Eq. 2 to low-frequency NLC data (Fig. 1A) | E.g., refs. 21 and 46 |
| \( l_c \) | Variable (m) | Hair cell length. Cochlear place dependent | Set by cochlear place | SI Appendix and Fig. 3A, based on ref. 41 |
| \( n \) | 0.7 (–) | Fractional derivative governing relaxation spectrum | From power law frequency roll-off of the real NLC | Data from Fig. 2 and ref. 24 |
| \( V^{pk} \) | –0.047 (V) | Voltage of peak NLC | Curve fit Eq. 2 to low-frequency NLC data (Fig. 1A) | E.g., refs. 21 and 46 |
| \( \beta_p \) | –0.054 (V\(^{-1}\)-kPa\(^{-1}\)) | Pressure sensitivity | Curve fit Eq. 2 and 3 to low-frequency NLC data (Fig. 1A and B) | Data from ref. 21 |
| \( \beta_o \) | –0.0012 (V\(^{-1}\)-C\(^{-1}\)) | Temperature sensitivity | Curve Eqs. 2 and 4 to the low-frequency NLC (Fig. 1C and D) | Data from ref. 22 |
| \( \delta f \) | –0.118 (V\(^{-1}\)) | Effective OHC piezoelectric strain coefficient times f in microchannel experiments | Fit Eq. 5, with \( 0 < f < 1 \) treated as unknown, and compliance known (Fig. 2B) | Data from Fig. 4 and in ref. 2 |
| \( \delta f \) | –0.412 (V\(^{-1}\)) | Whole-cell OHC piezoelectric strain coefficient \( V^{pk} \) (note: \( \delta f = 0 \) for \( f = 0 \)) | Fit Eq. 5. to low-frequency OHC strain under zero load | Based on refs. 47 and 48 |
| \( \kappa^L \) | \( 3.5 \times 10^7 \) (N\(^{-1}\)) | Low-frequency OHC compliance, strain per Newton at \( V^{pk} \) | Low-frequency whole-cell compliance converted to strain per Newton | Based on refs. 2 and 49 |
| \( (\kappa^L + \kappa^P)/\kappa^L \) | 1 (–) | External load compliance \( \kappa^L \) for an isolated cell | By definition | Based on power efficiency, e.g., ref. 30 |
| \( (\kappa^L + \kappa^P)/\kappa^L \) | 2 (–) | Load compliance \( \kappa^L \) in the cochlea comes from the internal OHC stiffness and the external load stiffness | Stiffnesses matched | Based on power efficiency, e.g., ref. 30 |
| \( \kappa^P/\kappa^L \) | 0.8 (–) | Ratio of compliance of the piezoelectric domain \( \kappa^P \) to the whole-cell \( \kappa^L \) | From frequency roll-off of real NLC and magnitude of the imaginary NLC relative to the real NLC | Based on refs. 13 and 27 |
| \( \lambda \) | 0.032 (V) | Voltage sensitivity | Curve fit Eq. 2 to low-frequency NLC data (Fig. 1A) | Data from refs. 21 and 46 |
| \( \tau^P \) | \( 2 \times 10^{-7} \) (s) | Relaxation time constant of piezoelectric domain | Lack of corner in Bode force up to 80 kHz | Based on Fig. 4 from ref. 2 |
| \( \tau^C \) | \( 2 \times 10^{-7} \) (s) | Relaxation time constant of composite | Lack of corner in Bode force up to 80 kHz | Based on Fig. 4 from ref. 2 |
| \( \tau_{RC} \) | Variable (s) | Electrical time constant of the OHC. OHC size and location dependent | From cochlear map | SI Appendix and Fig. 3B, based on refs. 9 and 42 |
| \( \omega_n \) | Variable (s\(^{-1}\)) | Natural frequency of the isolated OHC based on cell length | Frequency where OHC disp. phase is ~0 (µ-chamber) | SI Appendix and Fig. 3C, based on Fig. 2 from ref. 2 |
| \( \omega_n \) | Variable (s\(^{-1}\)) | Natural frequency of the cochlear load at the tonotopic place | Defined by cochlear place principle | SI Appendix and Fig. 3 A and B and Figs. 41 and 42 |
| \( \omega_c \) | \( \omega_n/2 \) (s\(^{-1}\)) | Viscous corner frequency of the OHC in media based on cell size. (damping coefficient ~1) | Curve fit Bode plots in µ-chamber configuration | From Fig. 2 and ref. 2 |
| \( \omega_c \) | \( 1.4 \omega_n \) (s\(^{-1}\)) | Damping corner frequency of the combined OHC and cochlear load. (damping coefficient ~0.36) | Underdamped based on passive cochlear tuning | E.g., refs. 50 and 51 |
motor domain was allowed to extend into the passive domain based on their respective viscoelastic properties (Fig. 2A and SI Appendix, Eqs. B1–B5). The force $B$ required to prevent the OHC from changing length in response to voltage $V$ is (tildes denote the frequency domain):

$$F_V(\omega) = \frac{B}{V} \delta \approx \frac{\delta \omega}{\kappa}.$$  \[5\]

where the composite piezoelectric coefficient is $\delta = \delta f(\xi)/(1 + j\omega \tau)$ and the composite compliance is $\kappa = \kappa f(\xi)/(1 + j\omega \tau)$. The material parameter $\delta$ is the composite piezoelectric strain coefficient at $\xi = 0$ ($f(\xi) = 1$). Time constants $\tau$ and $\tau'$ govern the intrinsic speed(s) of piezoelectric strain extension into the passive domain under zero whole-cell strain. Elegant experiments by Frank et al. (2) measured $F_V(\omega)$ by inserting the basal pole of OHCs into a large pipette ($\mu$-chamber) to control the extracellular voltage acting on the basal region of the cell, and measuring the force generated in the frequency domain using an atomic-force microscope. Experiments were conducted under nearly constant cell length, with results revealing a flat gain and phase of $F_V(\omega)$ relative to the $\mu$-chamber voltage up to at least 80 kHz. The measured force did not depend on the length of the cell extending outside of the $\mu$-chamber, consistent with Eq. 5. Although the precise intracellular voltage was not known in the Frank et al. experiments [i.e., $f(\xi)$ and transmembrane $V$ not known], a very broad frequency response was clearly demonstrated.

The whole-cell displacement was examined to determine how the viscoelastic properties of the external load and the OHC itself limit speed of electromotility. The displacement $D$ in response to sinusoidal voltage clamp is as follows:

$$D_V = \frac{D}{V} = \frac{l_i \delta_i}{H_i}.$$  \[6\]

where $l_i$ is the length of cell and $H_i$ is the nondimensional mechanical impedance of the total mechanical load. Three specific loads were considered: 1) OHC in isolation where $H_i$ arises from intrinsic properties of the cell itself plus the fluid media, 2) a membrane patch where $H_i$ arises from intrinsic properties of the patch and fluid, and 3) OHC in the cochlea where $H_i$ arises from the cell plus the extracellular cochlear load. In all three cases, the load was modeled as a spring-mass damper system. Specifically, $H_i = ((\kappa' + \kappa^2)/\kappa^2)(1 - (\omega/\omega_0)^2) + \rho_0^2(\omega/\omega_0)^2$, where $(\kappa' + \kappa^2)/\kappa^2$ is the ratio of the total compliance divided by the compliance of the load, $\omega_0$ is the undamped natural frequency of the load, and $\omega_0$ is the damping corner frequency (nondimensional damping coefficient $\zeta \approx \omega_0/2\omega_n$ for $n = 1$). The fractional derivative $\mathbf{n}$ models the relaxation spectrum arising from the frequency-dependent viscous properties (SI Appendix, Eq. B5).

Parameters are provided in Table 1 for all loading conditions. For an isolated OHC, the stiffness arises from the cell itself $[(\kappa' + \kappa^2)/\kappa^2 = 1, \kappa^2 \rightarrow \infty]$, while mass and viscosity arise from the OHC plus the extracellular media. Due to the high viscosity and low mass, isolated OHCs do not show resonance or tuning in their displacement evoked by voltage. Lack of displacement tuning is demonstrated in Fig. 2C, which shows OHC voltage-evoked displacement data from Frank et al. (2) in the $\mu$-chamber configuration. Like Fig. 2B, the precise amplitude of the transmembrane voltage was not measured in the experiments, but the frequency response is still revealing. Experimental data (symbols) are compared to Eq. 6 (solid curves) for two different cell lengths extending outside the $\mu$-chamber. Model parameters (Table 1) are the same.
for all curves in Fig. 2A–D, with the exception of length outside the chamber in Fig. 2C (black, blue). Although the force generated under zero strain is independent of frequency (Fig. 2B), the displacement under zero force begins to roll off as the frequency is increased (Fig. 2C). The roll-off arises from intrinsic viscosity and mass of the cell. The frequency with a displacement phase of $-\pi/2$ defines the intrinsic natural frequency $\omega_N$ of the unloaded cell (Fig. 2C, vertical dashed line) where mass and stiffness cancel and OHC power output is dissipated by the intrinsic viscous load (see SI Appendix and Fig. 3 for isolated OHC $\omega_N$ based on cell length). Although the displacement shows no frequency tuning, the power output to the viscous load does.

Electromechanical behavior of the OHC, including power output, can be determined from whole-cell capacitance recordings. When the OHC is under load, charge displacement arises from both the capacitance voltage susceptibility and the capacitance stress susceptibility. Under voltage-clamp conditions in the frequency domain, the two terms provide the total electrical displacement current as $I_D = i\omega C_m^\epsilon V^\epsilon$, where the complex-valued NLC is as follows (SI Appendix, Eq. 6b):

$$C_m^\epsilon = C_m^p(f(\xi)H_C), \quad [7a]$$

$$H_C = 1 \left( \frac{\omega^2}{k^2} \right) \left( H_L - 1 \right), \quad [7b]$$

$C_m^\epsilon$ is the complex-valued analog to the real-valued NLC commonly discussed in the literature for OHCs. For consistency with earlier reports, $\text{Re}(C_m^\epsilon)$ is termed the real NLC ($\text{Re}$ NLC), and $\text{Im}(C_m^\epsilon)$ is termed the imaginary NLC ($\text{Im}$ NLC). Nonlinearity appears through $f(\xi)$, while load dependence arises from $H_L$. The nondimensional ratio $\frac{\omega^2}{k^2}$ in Eq. 7 plays an important role and is the compliance of the piezoelectric domain divided by the compliance of the whole cell. If the piezoelectric domain had zero compliance, it would not deform under load and Eq. 7 would predict zero frequency dependence of $\text{Re}$ NLC, which is known not to be the case (13). The fact that $C_m^\epsilon$ is frequency dependent means the piezoelectric domain is compliant, and the magnitude of compliance can be estimated from the frequency dependence of NLC.

The NLC described by Eq. 7 is reversible and no net charge is lost, yet the piezoelectric capacitance has an imaginary component that leads to what would be interpreted experimentally as an electrical conduction current. Frequency-domain measurements of whole-cell admittance include a load-dependent effect of piezoelectric charge displacement in both the real and imaginary components. Ignoring this effect can lead to incorrect conclusions about OHC function based on the basis of admittance measurements.

The conventional NLC measured experimentally corresponds to the real part of the complex-valued capacitance in Eq. 7 $\text{Re}(C_m^\epsilon)$, which is the solid black curve in Fig. 2D for an OHC in the $\mu$-chamber configuration (Fig. 2C, black). The imaginary part $\text{Im}(C_m^\epsilon)$ for the same cell is the blue dashed curve. Three major conclusions can be drawn from Eq. 7 and results in Fig. 2D. First, $\text{Re}(C_m^\epsilon)$ begins to roll off at a corner frequency $\omega_c$, which in the $\mu$-chamber experiments is aligned with roll-off in whole-cell displacement (Fig. 2 C and D). Second, the roll-off simply reflects the intrinsic load imposed by the media and the cell itself and does not occur if the cell is held at zero strain (Fig. 2B). Third, $\text{Im}(C_m^\epsilon)$ becomes negative as frequency is increased, and peaks at a frequency well above the capacitive corner frequency $\omega_c > > \omega_c$. In isolated cells, the frequency $\omega_c$ arises from the intrinsic natural frequency $\omega_N$ of the cell itself (Fig. 2C). The influence of artificially changing the intrinsic natural frequency of the cell itself is illustrated in Fig. 2E. The frequency shift arises from the intrinsic load $H_L$ in Eq. 7—the load shifts the corner frequency, but does not reflect the intrinsic speed of the motor element itself.

$$PWR = \frac{1}{2} \omega \text{Im}(\hat{C}_m^\epsilon) \hat{V}^2. \quad [8]$$

Appearance of frequency $\omega$ in Eq. 8 pushes the maximum power output frequency even higher, above the peak $\text{Im}(C_m^\epsilon)$ frequency.
The peak power output always occurs at a frequency well above the conventional $Re(C_m^r)$ corner frequency $\omega_c$ and corresponds to the frequency $\omega_P$ when the piezoelectric admittance of the electrical admittance is peak.

Complex-valued capacitance and frequency-dependent power output are illustrated in Fig. 3 for two voltage-clamp recording conditions: ideal whole-cell voltage clamp of a 54-μm-length OHC (Fig. 3A and B), and ideal voltage clamp of an excised membrane macropatch (Fig. 3C and D). Fig. 3D shows the real and imaginary components of the NLC and real admittance as functions of whole-cell holding potential for six different frequencies (0.1 to 100 kHz), while Fig. 3B shows the NLC and admittance as functions of frequency at four different voltages.

Results for an isolated cell subject to intrinsic mass, stiffness, and viscosity arising from the cell itself and the fluid media load. The magnitude of $Re(C_m^r)$ begins rolling off immediately with frequency, while the magnitude of $Im(C_m^p)$ builds up (with no change in voltage dependence if $n$ is held constant with voltage). Frequency dependence under whole-cell voltage clamp is most clearly shown in Fig. 3B. Of course, current voltage-clamp technology has a limited frequency bandwidth, but it is still useful to examine what would be expected based on Eqs. 7 and 8 for an isolated cell. The key point is that the imaginary NLC builds up reaching a peak negative value at a frequency $\omega_P$. The maximum power output is determined by the real part of the piezoelectric admittance (bottom panel) and peaks at frequency $\omega_P > > \omega_c$. This occurs because $Re(C_m^r)$ reflects the piezoelectric charge displacement working against reversible elasticity of the cell and the load, while $Im(C_m^p)$ reflects the piezoelectric charge displacement working against the dissipative viscous load.

The NLC of an excised macropatch of membrane (Fig. 3C and D) is predicted to follow trends similar to the whole cell (Fig. 3A and B), but reduced in magnitude and shifted in frequency because of size and mechanical constraints on the patch. Results in Fig. 3D are the most revealing, and directly compare experimental real NLC from Santos-Sacchi and Tan (red dashed curve) (24) to Eq. 7. $Re(C_m^p)$ measured experimentally exhibits a power-law frequency dependence (red dashed), captured in the model by the broad relaxation spectrum (fractional derivative $n = 0.7$). The imaginary component was not reported, but present results suggest $Im(C_m^p)$ peaks near 30 kHz at $-10$ IF. Most importantly, peak power output is predicted to occur near 50 kHz in the macropatch configuration, a frequency where the real NLC is almost zero. Hence, the corner frequency of $Re(C_m^p)$ understimates the best power output frequency $\omega_P$ by more than an order of magnitude both in the whole-cell and macropatch configurations. Simulations in Fig. 3 assumed the patch did not induce static stress (i.e., $f(\xi) = 1$) and the relaxation spectrum was constant ($n = 0.7$).

To explore how OHCs function in the cochlea, cells were loaded with a spring-mass-damper system to simulate the tonotopic cochlear load. The natural frequency of the loaded system and the length of the cell were set by a model tonotopic map with OHC length and tonotopic location in the cochlea. OHC length, linear capacitance, and membrane conductance were set by the specific location in the cochlea. The length of the cell was set by a model tonotopic map with OHC length and tonotopic location in the cochlea.
simulations (e.g., Fig. 4C, black dashed). Power output shown in Fig. 4D supports the hypothesis that individual OHCs contribute power to cochlear amplification primarily at frequencies near their location in the tonotopic cochlea.

Discussion

The present report is focused primarily on resolving the OHC speed paradox, a paradox most clearly exemplified by disparity between the ultrafast cycle-by-cycle isometric force generated by OHCs (2) vs. the slow low-pass-filtered characteristics of electrical charge displacement in OHC membranes (13). The paradox is resolved using first principles to show how the piezoelectric behavior of OHCs explains both results. High-frequency experimental results of Frank et al. (2) are reproduced in Fig. 2A and B, and low-frequency roll-off of NLC reported by Santos-Sacchi and Tan (13) are reproduced in Fig. 3D using exactly the same physics. Three major factors were taken into account to resolve the paradox and describe how OHCs function at high frequencies.

The first factor involves interpretation of OHC NLC. The problem with the traditional approach in OHCs is that voltage induces load-dependent stress and strain, and the strain alters the charge displacement. Therefore, the capacitance recorded using conventional methods changes with conditions of the experiment. To describe the charge displacement in the frequency domain requires a load-dependent complex-valued NLC $\tilde{C}_m$ (Eq. 7). The traditional approach is adequate for low frequencies where the coulomb force is resisted by an elastic load, but fails when the force is resisted by viscous or inertial loads, which is always the case at high auditory frequencies. Viscous drag shifts the phase by $-90^\circ$ and introduces a negative-valued imaginary NLC $\text{Im}(\tilde{C}_m)$, which appears in electrical admittance measurements as a frequency- and voltage-dependent, positive, real-valued admittance. $\text{Im}(\tilde{C}_m)$ reflects a reversible charge displacement but, as described previously, can be incorrectly interpreted as a conduction current based on traditional interpretation of electrical admittance (27).

The second factor involves the relationship between charge displacement and power output of the OHC. In the frequency domain, the imaginary NLC times frequency $-\omega \text{Im}(\tilde{C}_m)$ is proportional to the power delivered to mechanical load (Eq. 9). If $\text{Im}(\tilde{C}_m) = 0$, the OHC power output is zero. Although the real component of NLC $\text{Re}(\tilde{C}_m)$ is revealing because it reflects a component of charge displacement, it is not a measure of power output or function of the OHC as a motor. The OHC peak power output frequency $\omega_P$ arising from the imaginary NLC is above the corner frequency of the real NLC by more than an order of magnitude, demonstrating why $\omega_P$ is a poor indicator of the frequency response or speed of OHCs. Given the thermodynamic origin of complex-valued NLC, this finding likely applies to all conditions: isolated OHCs, membrane patches, and OHCs in the cochlea (Figs. 3 and 4).

The third factor involves how OHCs are loaded in the cochlea vs. loaded in experiments. Experiments in the dish, especially at low frequencies, often result in very small $\text{Im}(\tilde{C}_m)$ because the OHC is working against an elastic load that does not absorb significant power. In the cochlea, OHCs work against a mechanical load consisting of elasticity, viscosity, and mass. Each location along the tonotopic map has a characteristic best frequency where the elastic force nearly balances the inertial force and the load becomes dominated by viscous drag. Present results indicate OHC power output is just before the traveling wave peak (28), with OHCs basal to the peak contributing to amplification (29) but at lower levels (Fig. 3E).

For efficient operation in the cochlea, OHCs must be sufficiently short to operate below their own intrinsic natural frequency, but sufficiently long to generate the required velocity. The relationship between power output and velocity (for frequencies near $\omega_P$) is illustrated schematically in Fig. 4D as the load changes from high drag (zero velocity, maximum force) to low drag (maximum velocity, zero force). Similar to skeletal muscle (30), OHC power output is maximized between the two extreme loading conditions. These two factors likely combine with electrical factors and channel expression to determine optimum OHC length as a function of best frequency in the cochlea.

The present report demonstrates how OHCs deliver cycle-by-cycle power to the cochlear amplifier at high frequencies well above the corner frequency defined by the real NLC. The analysis is agnostic to the specific molecules responsible for piezoelectricity but places constraints on what is thermodynamically feasible. It is known that OHC electromotility requires expression of the transmembrane protein *prestin*, a member of the SL26 family of anion transporters (3, 31, 32). There is strong evidence that $\text{CT}^-$ is essential and is electrostatically bound in the central core region of the protein (33–35). In the absence of $\text{CT}^-$, piezoelectric NLC is lost in OHCs but can be restored by inserting a charged residue near the putative $\text{CT}^-$ binding site in the core domain (36). These data support the hypothesis that the charge responsible for the piezoelectric coulomb force in $\omega$ OHCs is likely to be electrostatically bound $\text{CT}^-$ located in the prestin core. A force-driven conformational change in prestin could underlie piezoelectric behavior, but the present analysis is thermodynamic in nature and cannot distinguish between molecular mechanisms involving a single transition, “N” intermediate transition states, continuous transitions, or other hypothetical mechanisms that may involve interplay between charge, lipid, and protein. Differences on the molecular scale are subtle on the thermodynamic scale. For example, replacing the high-dimensional Langevin nonlinearity $f$ with a two-state Boltzmann function (37) or a multistate model (20) introduces a small change in the shape of the nonlinear voltage distribution but does not change any conclusions of the present report. The direct coupling between piezoelectric charge displacement and strain in OHCs (6) contrasts voltage-gated ion channels where the gating charge displacement precedes conformational changes responsible for channel open probability (38). Hence, the term charge displacement is used here to avoid confusion with the term gating charge, which is traditionally associated with displacement of specific residues preceding a protein-scale conformational change.

The present analysis further implies the piezoelectric coulomb force is always present within the membrane electric field, and that voltage-dependence arises from the saturating compliance of the piezoelectric element rather than charge shielding or charge movement outside the electric field (*SI Appendix*, Eqs. A5–A7). Consistent with this, force generation is ultrafast, reflecting the instantaneous coulomb force, while the speed of charge displacement is slower reflecting the speed of deformation against the intrinsic and external load.

The present analysis uses a simple piezoelectric model to demonstrate the importance of the load on OHC motor function, how the complex-valued NLC is related to power output by the cell, and why OHC power output is highest at frequencies well above the real NLC corner. All results were driven by voltage-clamp commands, which differs from the cochlea where OHCs are driven by MET currents and mechanical forces. Power tuning curves in Fig. 3 D and E partially account for the OHC electrical corner frequency by driving the cell with a low-pass-filtered voltage, but no attempt was made to address the influence of MET kinetics (39), ion channel gating and expression (9, 10), prestin expression (31), hair bundle electromotility (40), inhomogeneous expression and deformation, or mechanical forces associated with the traveling wave. The present OHC model is minimalistic, and reduces a complex cell with inhomogeneous expression and properties into a single lumped element, yet is sufficient to resolve the OHC speed paradox.

Methods

Electro-mechanical behavior, including capacitance susceptibility, has origins in the Gibbs free energy of the membrane complex. In the present analysis, therno-electromechanical behavior is examined within a control volume encompassing the entire membrane complex (see *SI Appendix* for compete
derivation). The control volume includes the inhomogeneous lipid bilayer, membrane-associated structural proteins, and charged coupled proteins including prestin but the approach is agnostic to the specific molecular arrangement and mechanisms. Under plane-stress thermodynamic equilibrium conditions, the Gibb’s free energy relates small changes in the mechanical stress $\sigma$ and strain $\epsilon$ to small changes in temperature $\theta$ and transverse electric field $(17, 18)$. Key constitutive parameters are as follows: compliance tensor $\chi$, piezoelectric coefficients $\delta$, thermal expansion coefficients $\alpha$, electrostriction coefficients $\rho$, electrical permittivities $\varepsilon$, and pyroelectric coefficients $\rho_j$. The OHC membrane was modeled a mixture of a piezoelectric material (for $\epsilon'$ and $\delta'$) occupying area fraction $\varphi$ and a passive material ($\varepsilon'$ and $\delta'$) occupying area fraction $\varphi$ and a passive material ($\varepsilon'$ and $\delta'$) occupying area fraction $\varphi$ and a passive material ($\varepsilon'$ and $\delta'$) occupying area fraction $\varphi$ and a passive material ($\varepsilon'$ and $\delta'$) occupying area fraction $\varphi$ and a passive material ($\varepsilon'$ and $\delta'$) occupying area fraction $\varphi$ and a passive material ($\varepsilon'$ and $\delta'$) occupying area fraction $\varphi$ and a passive material ($\varepsilon'$ and $\delta'$) occupying area fraction $\varphi$ and a passive material ($\varepsilon'$ and $\delta'$) occupying area fraction $\varphi$ and a passive material ($\varepsilon'$ and $\delta'$). A single time constant for each domain was used to model the speed of deformation under a step change in load. Constitutive parameters for the two materials combine to determine the effective piezoelectric coefficient and compliance of the composite. The general equations were simplified for a thin membrane subject to a transverse electric field. Equations were further simplified to a discrete lumped parameter model assuming axisymmetric, isotropic, isochoric, whole-cell deformations. Model parameters were determined from previously published experimental data primarily from guinea pig OHCs as detailed in Table 1. Model parameters were estimated. 

To estimate power output under cochlear load, the frequency-dependent load in the cochlea was simulated using a spring-mass-damper system with the natural frequency $\omega_0$ corresponding to the place principle in the cochlea. The load was slightly underdamped, $\omega_0 = 1.3\omega_1$, rad $^{-1}$. For simulations in Fig. 4 C and D, RC OHC size (length, membrane area, linear capacitance) and the passive RC corner frequency (conductance) were set according to a model place principle to illustrate how OHCs of different length deliver power to the cochlear amplifier (SI Appendix, Fig. 53). OHC lengths and intrinsic natural frequency are based on the guinea pig frequency map (41), while electrical passive electrical is based on gerbil (9, 42). Frequency domain simulations in the present study were done using identical piezoelectric material parameters at $V_{th}$ for all OHCs ($\varphi$, $\sigma'$, and $\delta'$), changing only length and loading conditions.

Data Availability. All data are from previously published reports as cited in Table 1. Parameter curve fitting and figures were generated using the software IgorPro (WaveMetrics). All study data are included in the article and SI Appendix.

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