Active hair-bundle motility harnesses noise to operate near an optimum of mechanosensitivity

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The ear relies on nonlinear amplification to enhance its sensitivity and frequency selectivity to oscillatory mechanical stimuli. It has been suggested that this active process results from the operation of dynamical systems that operate in the vicinity of an oscillatory instability, a Hopf bifurcation. In the bullfrog’s sacculus, a hair cell can display spontaneous oscillations of its mechanosensory hair bundle. The behavior of an oscillatory hair bundle resembles that of a critical oscillator. We present here a theoretical description of the effects of intrinsic noise on active hair-bundle motility. An oscillatory instability can result from the interplay between a region of negative stiffness in the bundle’s force-displacement relation and the Ca2+-regulated activity of molecular motors.

We calculate a state diagram that describes the possible dynamical states of the hair bundle in the absence of fluctuations. Taking into account thermal fluctuations, the stochastic nature of transduction channels’ gating, and of the forces generated by molecular motors, we discuss conditions that yield a response function and spontaneous noisy movements of the hair bundle in quantitative agreement with previously published experiments. We find that the magnitude of the fluctuations resulting from the active processes that mediate mechanical amplification remains just below that of thermal fluctuations. Fluctuations destroy the phase coherence of spontaneous oscillations and restrict the bundle’s sensitivity as well as frequency selectivity to small oscillatory stimuli. We show, however, that a hair bundle studied experimentally operates near an optimum of mechanosensitivity in our state diagram.

The mechanosensory hair cells of the vertebrate ear amplify their inputs to enhance sensitivity and frequency selectivity to weak oscillatory stimuli (reviewed in refs. 1–3). Although the cellular mechanisms that mediate this active process have remained elusive, in vitro (4–6) as well as in vivo (7) experiments have revealed that the mechanosensory organelle of the hair cell, the hair bundle, can generate active oscillatory movements that might underlie frequency-selective amplification. Four essential properties define active hair-bundle motility. First, a hair bundle can oscillate spontaneously (ref. 8 and references therein). Second, the response to weak oscillatory mechanical stimuli can be amplified (4, 6). Third, amplification is frequency selective, the greatest at stimulus frequencies near the bundle’s characteristic frequency of oscillation (4, 6). Finally, the interplay between negative hair-bundle stiffness and the Ca2+-dependent activity of the adaptation motors can generate oscillations (8, 19). This third mechanism provides the most convincing description of the hair-bundle oscillations observed in the bullfrog’s sacculus.

Although the observed hair-bundle movements are noisy and lose phase coherence after approximately one cycle of oscillation (5), previous theoretical studies neglected the effects of fluctuations on active hair-bundle motility. Noise blurs the distinction between active oscillations and fluctuations and thus conceals the bifurcation between oscillatory and nonoscillatory states. Here, we show that only by taking fluctuations into account, we can provide a quantitative theoretical description of a hair bundle’s responsiveness to oscillatory stimuli.

Active Hair-Bundle Mechanics

At steady state, an external force $F_{\text{ext}}$ applied at a hair bundle’s top is balanced by the elastic restoring forces provided by the parallel combination of gating springs and stereociliary pivots: $K_pY + K_oX = F_{\text{ext}}$. Here, $K_p$ characterizes the combined stiffness of the gating springs, $K_o$ that of the pivots, $X$ is the position of the hair-bundle tip along the axis of the applied force, and $\gamma = \ell / \gamma$ accounts for the extension $\ell$ of the gating springs projected on the $X$ axis. The geometric coefficient $\gamma$ relates the shearing motion between contiguous stereocilia to movement at the top of the bundle (20). We assume that the $N$ stereocilia of the hair bundle move as a unit and that the gating springs operate in parallel.

Channel opening yields a decrease of the gating-spring extension by an amount $d$, a phenomenon called gating compliance (14). At the bundle’s top, this gating swing corresponds to a displacement $D = d / \gamma$. As a consequence, the average extension of the gating springs can be expressed as $Y = X - X_a - DP_o$, where $P_o$ denotes the open probability of the transduction channels and $X_a$ the value of $Y$ for $X = 0$ if all channels are closed. In a two-state model for channel gating (21), the open probability can be written as

$$P_o = \frac{1}{1 + A e^{-k(Y-X_a)/\delta}} \quad \text{(1)}$$

where $A = \exp[[\Delta G + (K_pD^2)/(2N)]/k_BT]$ accounts for the intrinsic energy difference $\Delta G$ between the open and the closed states of a transduction channel and $\delta = Nk_BT/(K_pD)$. Here, $k_B$ is the Boltzmann constant and $T$ denotes the temperature.

The dynamic behavior of the hair bundle can be described by the following three coupled equations:

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Eq. 2 describes the dynamics of the hair-bundle position $X$. The hair bundle is subject to friction, characterized by the coefficient $\lambda$, as well as to the elastic forces $-K_{p}X$ and $-K_{p}Y$ and the external force $F_{ext}$. Active hair-bundle movements result from forces exerted by a collection of $N_{a}$ molecular motors within the hair bundle. By adjusting the gating-spring extension, these motors mediate mechanical adaptation to sustained stimuli (reviewed in ref. 13). The variable $X_{a}$ can be interpreted as the position of the motor collection. Eq. 3 describes the mechanics and the dynamics of these motors by a linear force-velocity relation of the form $\lambda_{a}dx/dt = -F_{t} + F_{mot}$, where $\lambda_{a}$ characterizes the slope of the force-velocity relation. In the hair bundle, the motors experience an elastic force $F_{mot} = K_{1}Y$. At stall, these motors produce an average force $F_{0} = N_{a}gfp$ that is proportional to the force $f$ generated by a single motor and to the probability $p$ that a motor is bound to an actin filament. Active force production by the motors corresponds to motors climbing up the stereocilia, i.e., $dx_{a}/dt < 0$, which tends to increase the extension of the gating springs and to open transduction channels. In Eq. 3, we neglect the fact that adaptation is incomplete. Incompletely adapted could be described by introducing an additional elastic element (22). Because mechanical adaptation is regulated by $Ca^{2+}$ (ref. 23; reviewed in ref. 24), we assume that the probability for an adaptation motor to be bound to actin $p(C)$ depends on the intracellular $Ca^{2+}$ concentration $C$ at the motor site. Eq. 4 describes the dynamics of $C$, which relaxes with a time constant $\tau$. The steady-state $Ca^{2+}$ concentration maintained by the cell if the transduction channels are closed is denoted $C_{0}$ and the concentration $C_{M} \gg C_{0}$ is the maximal $Ca^{2+}$ concentration at the location of the adaptation motors. It is expected to be proportional to the $Ca^{2+}$ concentration in the endolymph (8). In the following, we ignore nonlinearities in $p(C)$ and truncate a general expansion to linear order: $p(C) = p_{0} + p_{1}C$. We define the strength of the calcium feedback on the motor force $F_{0}$ by the dimensionless parameter $S = -C_{M}p_{1}/p_{0}$. As detailed in the next section, we have added noise terms $\eta$, $\eta_{a}$, and $\delta C$ to account for fluctuations that result from several types of stochastic processes.

State Diagram in the Absence of Noise

To explore the dynamic behaviors of the system described by Eqs. 2–4, we first ignore the effects of fluctuations and assume $F_{ext} = 0$. Steady states satisfy $dx_{a}/dt = 0, dx_{s}/dt = 0$, and $dC/dt = 0$. Linear stability analysis of these steady states reveals conditions for stability as well as for oscillating instabilities that lead to spontaneous oscillations by a Hopf bifurcation (25). Because calcium dynamics at the motor site is expected to be much faster than the hair-bundle oscillations observed in the bullfrog’s sacculus (26), we determined the state diagram for $\tau = 0$ (Fig. 1). The state diagram is a function of two parameters: the maximal force $f_{max} = N_{a}p_{0}$ produced by adaptation motors along their axis of movement, and the dimensionless feedback strength $S$ of the $Ca^{2+}$ regulation. We assumed that increased $Ca^{2+}$ levels at the motor site reduce active force generation by the motors ($p_{1} < 0$).

The state diagram exhibits different regimes (Fig. 1). If the force $f_{max}$ is small, the motors are not strong enough to pull transduction channels open. In this case, the system is monostable with most of the channels closed. Increasing $f_{max}$ leads to channel opening. For intermediate forces and weak $Ca^{2+}$ feedback, the system is bistable, i.e., open and closed channels coexist. For large $Ca^{2+}$ feedbacks, however, the motors cannot sustain the forces required to maintain the channels open. In this case, the system is again monostable with most channels closed. Spontaneous oscillations occur in a region of both intermediate forces and feedback strengths. The boundary between a stable state and an oscillatory state corresponds to a Hopf bifurcation. This bifurcation is subcritical near the bistable region (broken line in Fig. 1) but becomes supercritical at larger motor forces (solid line in Fig. 1). Note that there is no oscillation in the absence of $Ca^{2+}$ feedback, i.e., for $S = 0$.

The behavior of the system in the presence of an external force can be described by response functions. If the calculated linear response functions of the noiseless system in an oscillatory state display sharp singularities that are not observed experimentally (see Fig. 5, which is published as supporting information on the PNAS web site).

Fluctuations and Noise

Spontaneous hair-bundle oscillations are noisy (5). Noise terms $\eta$, $\eta_{a}$, and $\delta C$ in Eqs. 2–4 formally take into account the effects of various sources of fluctuations that destroy the phase coherence of hair-bundle movements. The stochastic forces $\eta$ and $\eta_{a}$ act on $X$ and $X_{a}$, respectively. The consequences of these forces have been analyzed for nonoscillating hair bundles (27). The fluctuations $\delta C$ of the $Ca^{2+}$ concentration in the stereocilia result from stochastic transitions between open and closed states of the transduction channels (28). Noise terms are zero on average. Their strengths are characterized by autocorrelation functions $\langle \eta(t)\eta(0) \rangle$, $\langle \eta_{a}(t)\eta_{a}(0) \rangle$, and $\langle \delta C(t)\delta C(0) \rangle$, respectively. We as-
The transduction channels dominates friction and opening respectively, to and from actin filaments generates friction with a coefficient \( \lambda_0 = \gamma N \delta k \tau \). Here, \( p = \tau_1/\tau_2 + \tau_2 \) is the probability for a motor to be attached to an actin filament, where \( \tau_1 \) and \( \tau_2 \) denote the lifetimes of the attached and detached states, respectively, and \( k \) is the stiffness of a single motor. By using \( P = 0.05 \), \( k = 500 \mu N/m \), and \( \tau = 10 \) ms, we estimate \( \lambda_0 = 1.5 \times 10^{-5} \text{NNs}^{-1} \cdot \text{m}^{-1} \). The strength of the thermal contribution \( \eta_0 \) to \( \eta_2 \) is \( \eta_2(\eta_2(\eta_2)) = 2k_B T \lambda \delta(t) \).

We now turn to the more interesting situation of active motors \((f \neq 0)\). Measurements of the initial adaptation rate as a function of the magnitude of step stimuli \((23)\) imply that \( \lambda_0 = 1.3 \times 10^{-5} \text{NNs}^{-1} \cdot \text{m}^{-1} \), which is in agreement with the value of the friction coefficient \( \lambda_0 \) estimated above for deactivated motors. We thus neglect the effect of motor activity on motor friction. However, the stochastic activity of motors generates an active contribution \( \eta_m \) to \( \eta_2 \) with

\[
\langle \eta_m(\eta_2)(\eta_2(\eta_2)) \rangle = N_a \gamma^p (1 - p)^2 e^{-|t|/\tau}.
\]

Here, we have assumed that the \( N_a \) motors fluctuate independently and that relevant time scales for a hair-bundle oscillation are longer than \( \tau \), where \( \tau_2 = (1/\tau_1 + 1/\tau_2)^{-1} = \tau \) is the characteristic time of force production by the motors. This noise strength can be described by introducing an effective temperature \( T_\text{eff} \) defined by

\[
\langle \eta_m(\eta_2)(\eta_2(\eta_2)) \rangle = 2k_B T \lambda \delta(t) \delta(T_\text{eff}/T_\text{eff} \lambda \delta(t) \delta(T_\text{eff}/T_\text{eff})).
\]

In this case, \( T_\text{eff} \) is approximated by

\[
(\delta(T_\text{eff})/\delta(T_\text{eff})) = C _B N \eta e^{-|t|/\tau_2}.
\]

We can assess the significance of these fluctuations in the limit where \( C \) relaxes instantaneously. The system is in turn reduced to Eqs. 2 and 3, with \( C = C_B + C_B p + \delta C \). The fluctuations of the \( Ca^{2+} \) concentration can then be written as \( (\delta(T_\text{eff})/\delta(T_\text{eff})) = (\delta(T_\text{eff})/\delta(T_\text{eff})) \). Because the probability \( p \) of motor binding to actin filaments depends on the \( Ca^{2+} \) concentration, fluctuations of \( C \) result in the generation of fluctuating forces \( \eta_2 \) by the motors. We estimate this random force \( \eta_2 = \gamma N \delta p_2/\delta C \). For times large compared with \( \tau_2 \), we find

\[
\langle \eta_m(\eta_2)(\eta_2(\eta_2)) \rangle = 2N \gamma^p (1 - p)^2 (e^{-t/\tau} - e^{-t/\tau_2}).
\]

Introducing an effective temperature \( T_\text{eff} \) with \( \langle \eta_m(\eta_2)(\eta_2(\eta_2)) \rangle = 2k_B T_\text{eff} \lambda \delta(t) \delta(T_\text{eff}/T_\text{eff}) \), we find \( T_\text{eff}/T_\text{eff} = (\gamma N \delta p_2)/(N k \delta k \lambda \delta(t)) = 0.13 \) for \( f_{\text{max}} = 350 \) pN, \( S = 0.65 \), \( P = 0.5 \) and parameter values from Table 1. This result suggests that fluctuations of the motor force evoked by \( Ca^{2+} \) fluctuations are below the unavoidable thermal fluctuations.

In our simulations of Eqs. 2-4, we used the noise strengths \( (\eta^p(\eta^p)(\eta^p)) = 2k_B T_\text{eff} \lambda \delta(t) \delta(T_\text{eff}/T_\text{eff}) \) and \( (\delta(T_\text{eff})/\delta(T_\text{eff})) = 2k_B N \eta e^{-|t|/\tau_2} \), with parameter values listed in Table 1. Note that it is not necessary to know the value of \( C_M \) if \( S \) and \( f_{\text{max}} \) are given.

### Spontaneous Movements and Response Function

Fluctuations destroy the phase coherence of spontaneous oscillations and conceal the bifurcations between the dynamic states displayed in the state diagram (Fig. 1). The response of a noisy oscillating system to a periodic force \( F_{\text{ext}} = F_{\text{ext}}e^{-t/\tau} + F_{\text{ext}}e^{t/\tau} \) thus behaves effectively as that of a stable system. The response function \( \chi(\omega) = X_1/F_{\text{ext}} \), where \( X_1 \) is the amplitude of the

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**Table 1. Parameter values for the simulations**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda )</td>
<td>Friction coefficient of hair bundle motors</td>
<td>2.8 ( \mu \text{NNs}^{-1} \cdot \text{m}^{-1} )</td>
<td>23</td>
</tr>
<tr>
<td>( \lambda_0 )</td>
<td>Friction coefficient of adaptation motors</td>
<td>10 ( \mu \text{NNs}^{-1} \cdot \text{m}^{-1} )</td>
<td></td>
</tr>
<tr>
<td>( K_{gs} )</td>
<td>Combined gating-spring stiffness</td>
<td>750 ( \mu \text{NNs}^{-1} \cdot \text{m}^{-1} )</td>
<td>19</td>
</tr>
<tr>
<td>( K_{sp} )</td>
<td>Combined stiffness of stereociliary pivots and load opening</td>
<td>600 ( \mu \text{NNs}^{-1} \cdot \text{m}^{-1} )</td>
<td>19</td>
</tr>
<tr>
<td>( d )</td>
<td>Gating-spring elongation on channel</td>
<td>8.7 nm</td>
<td>19</td>
</tr>
<tr>
<td>( \gamma )</td>
<td>Critical gain of stereociliary shear motion</td>
<td>0.14</td>
<td>20</td>
</tr>
<tr>
<td>( \tau )</td>
<td>Time constant of calcium feedback</td>
<td>0.1 ms</td>
<td></td>
</tr>
<tr>
<td>( \tau_c )</td>
<td>Dwell time of transduction channels</td>
<td>1 ms</td>
<td>30</td>
</tr>
<tr>
<td>( C_0 )</td>
<td>Intracellular ( Ca^{2+} ) concentration</td>
<td>0 mM</td>
<td></td>
</tr>
<tr>
<td>( N )</td>
<td>No. of stereocilia</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td>( N_a )</td>
<td>No. of motors in the hair bundle</td>
<td>3000</td>
<td>13</td>
</tr>
<tr>
<td>( \Delta G )</td>
<td>Intrinsic energy change on channel opening</td>
<td>10 ( k_B T )</td>
<td>38</td>
</tr>
<tr>
<td>( T_\text{eff} )</td>
<td>Effective temperature for Eq. 3</td>
<td>1.5 T</td>
<td></td>
</tr>
</tbody>
</table>

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phase-locked response, can be described for small stimulus amplitude by

\[ x_0(\omega) = \frac{1}{2} \left( e^{-i\omega_0} + e^{+i\omega_0} \right). \]

This response function is characterized by the stiffness \( K \) and the friction coefficient \( \Lambda \). The phase \( \alpha \) describes the phase lag of the bundle’s displacement with respect to the stimulus at the characteristic frequency \( \omega_0 \). As shown in Supporting Text, which is published as supporting information on the PNAS web site, this form of the linear response function is that of a noiseless system that operates in the vicinity of a Hopf bifurcation. In addition, the spectral density \( G(\omega) \) of hair-bundle displacements can be approximated by:

\[ G(\omega) = \frac{I}{K^2 + \Lambda^2(\omega - \omega_0)^2} + \frac{I}{K^2 + \Lambda^2(\omega + \omega_0)^2}, \]

where \( I \) is the noise strength (5). The parameters have been measured in the case of a spontaneously oscillating hair bundle from the bullfrog’s sacculus (5): \( K = 100 \mu \text{N.m}^{-1}, \lambda = 6.5 \mu \text{N.s.m}^{-1}, I = 0.14 \text{pN}^2 \text{s}^{-1} \), and \( \alpha = 0 \).

The response of the hair bundle to larger stimulus amplitudes reveals the existence of nonlinearities (6). The dominant nonlinearity is described by the generic expression for the sensitivity \( x^2 = x_0^2 + BX_0^2 \), which defines the nonlinear coefficient \( B \) (10). For the data published in ref. 6, \( |B| = 1.5 \times 10^{-2} \text{N.m}^{-1} \). In the nonlinear regime \( X_0 \sim F_1^{1/3} \), and the sensitivity decreases approximately according to the power law \( x \sim F_1^{2/3} \).

**Numerical Results**

We performed numerical simulations of Eqs. 2-4 (see Supporting Text). Most of the model parameters were fixed (Table 1). The only free parameters were the feedback strength \( S \) and the maximal motor force \( f_{\text{max}} \). In endolymph containing \( \text{Ca}^{2+} \) at a concentration of 250 \( \mu \text{M} \), the opening probability for which the adaptation motors reach steady state is between 0.25 and 0.5 (23, 31). Along a line of constant open probability \( P_o \) in the state diagram (Fig. 1), we determined the parameters in Eq. 10 as a function of \( f_{\text{max}} \) in the presence of noise. For \( P_o = 0.5 \), the characteristic frequency of spontaneous oscillations varied between a few Hertz and \( \approx 50 \text{ Hz} \) in the range \( f_{\text{max}} = 300-800 \text{ pN} \) within which a peak was detected in the spectral density of spontaneous movements. Because \( \alpha = 0 \) ensures that the linear response function has the same shape as that observed experimentally (5), we elected the value of the motor force \( f_{\text{max}} = 352 \text{ pN} \) at which this condition was satisfied for \( P_o = 0.5 \) (Fig. 1). At this operating point, the system displayed noisy spontaneous oscillations \( X(t) \) that were strikingly similar to the hair-bundle oscillations observed in the bullfrog’s sacculus (Fig. 2A). Their spectral density was peaked at a characteristic frequency \( \omega_0 = \omega_0/2\pi = 8.7 \text{ Hz} \) and had a width at half the maximal value of \( \delta \nu = 3.7 \text{ Hz} \), corresponding to a quality factor \( Q = n_0/\delta \nu = 2.4 \) (Fig. 2B).

We calculated the linear response function \( x_0 \) as a function of frequency (Fig. 3A and B) and found that it agreed quantitatively with the experimental observations (5). At the characteristic frequency of the spontaneous oscillations, the sensitivity \( |x| \) of the system to mechanical stimulation exhibited the three regimes observed experimentally (6) as a function of the stimulus amplitude \( F_1 \) (Fig. 3C): a linear regime of maximal sensitivity \( |x_0| = 8.5 \text{ kN}^{-1} \) at \( \omega = \omega_0 \) for small stimuli, a compressive nonlinearity for intermediate stimuli and a linear behavior of low sensitivity for large stimuli. The maximal sensitivity as well as the breadth of the nonlinear region were also in quantitative agreement with experiments. An important parameter that influenced the system’s maximal sensitivity was the stiffness of the load to which the hair bundle is coupled (see Fig. 6, which is published as supporting information on the PNAS web site). For \( f_{\text{max}} = 352 \text{ pN} \), power spectra of spontaneous oscillations and response functions were not significantly affected by varying \( P_o \) in the range 0.2–0.8. Thus, agreement between simulations and experiments did not qualify a particular value of \( P_o \).

Is the hair bundle’s operating point optimal? To address this issue, we determined, for different open probabilities of the transduction channels, how the sensitivity to small stimuli at resonance \( |x_0| \) varied with the maximal force \( f_{\text{max}} \) that the motors produce (Fig. 4A). We found that this sensitivity was at the global maximum \( |x_0| = 11.3 \text{ kN}^{-1} \) for \( P_o = 0.5 \) and \( f_{\text{max}} = 439 \text{ pN} \), near the center of the oscillatory region in the state diagram (Fig. 1). At this point, the spontaneous oscillation displayed a characteristic frequency of \( \nu_0 = 24.7 \text{ Hz} \) and a quality factor \( Q = 5.4 \) that was also near the global maximum (Fig. 4B). This point was clearly distinct from the operating point of the hair bundle (Fig. 1). There, the hair bundle was characterized by a sensitivity to small stimuli that was 25% lower than that at the optimum.

**Discussion**

The Importance of Fluctuations. We have presented a physical description of active hair-bundle motility that emphasizes the role played by fluctuations. The mechanical properties of oscillatory hair bundles can be described quantitatively only when fluctuations are taken into account. Fluctuations arise in part from Brownian motion of fluid molecules and from the stochastic gating of transduction channels. By consuming energy, the motors power frequency-selective amplification but also generate nonthermal fluctuations that add to the inevitable thermal fluctuations. We find, however, that the magnitude of fluctua-
uli as well as the range of stimulus magnitudes over which the ratio of the sensitivity at resonance to small stimuli
spite fluctuations, a single hair bundle amplifies its response to
34). As exemplified by our analysis, fluctuations restrict the
transduction channels is 0.5. In addition, variations of
Furthermore, the sensitivity is largest if the open probability of
near the center of the oscillatory region in the state diagram, thus
system oscillates (Fig. 4
A
happens inside the area of the state diagram where the noiseless
theoretical analysis demonstrates that significant amplification
cate that active hair-bundle motility provides a gain of
Sensitivity and Friction.
The value of the coefficient \( \lambda \) that characterizes friction of the hair bundle has a significant effect on the sensitivity \( |x_0| \) that the system can achieve. Decreasing this friction coefficient to the physical limit \( \lambda = \lambda_0 = 2 \times 10^{-7} \text{N s m}^{-1} \) given by hydrodynamic friction, indeed results in a threefold increase of sensitivity. This coefficient was estimated previously in experiments where the hair cells were immersed entirely in standard saline solution and did not, or very rarely, show spontaneous oscillations of their hair bundles (29). Because the \( \text{Ca}^{2+} \) concentration in standard saline is 16 times higher than in artificial endolymph, the feedback strength \( S = -C_M p_{\text{f}}/p_0 \) is probably much stronger in standard saline. A more efficient \( \text{Ca}^{2+} \) feedback could explain why the system operates in a monostable state with transduction channels mostly closed (Fig. 1). Under these circumstances, the experiments suggested that hydrodynamic friction alone accounted for the observed hair-

**Fig. 3.** Responsiveness to sinusoidal stimulation. (A) The real part \( x_0 \) of the linear response function \( x_0 \) displays a peak near the bundle's frequency of spontaneous oscillation, and is positive everywhere. (B) The imaginary part \( x_0 \) changes sign at a frequency near that of the bundle's spontaneous oscillation. The external sinusoidal force had an amplitude of 2 pN. From the fit (red lines) of the response function \( x_0 \) by Eq. 10, we found \( \alpha = 0, K = 74 \mu \text{N m}^{-1}, \Lambda = 4.3 \mu \text{N s m}^{-1} \), and \( v_0 = 2 m_v = 8.6 \text{ Hz} \), which is in quantitative agreement with the response function measured for an oscillatory hair bundle (S). (C) The sensitivity \( |y| \) displays a nonlinear compression in a regime of intermediate forces 3–30 pN when the magnitude of an external sinusoidal force is increased at the frequency of the spontaneous oscillations. This nonlinearity is consistent with a power law with an exponent of \(-2/3\) (red line). A fit to the relation \( x^{-1} = x_0^{-1} + B|x_0|^2 \) yielded the nonlinear coefficient \( B = 1.2 \times 10^{-11} \text{ N m}^{-1} \). For stimuli smaller than \( 1 \text{ pN} \), the sensitivity saturates at \( |y| = 8.5 \text{ km N}^{-1} \) (green line). For stimuli larger than \( 300 \text{ pN} \), the sensitivity approaches a constant, minimal value of \( 1 \text{ km N}^{-1} \) (green line). The parameters used in the simulation are the same as in Fig. 2.

<table>
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<th>Force (pN)</th>
<th>Sensitivity (km N(^{-1}))</th>
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<tr>
<td>0.1</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>100</td>
<td>50</td>
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</tbody>
</table>

**Fig. 4.** Sensitivity and spontaneous movements as a function of the hair bundle’s operating point. (A) Maximal (black symbols) and minimal (purple symbols) sensitivity \( |y| \) as a function of the maximal motor force \( F_{\text{max}} \) for \( P_s = 0.1 \) (•), 0.3 (▲), 0.4 (●), 0.5 (■), 0.6 (○), 0.7 (△), and 0.9 (□). The sensitivity displays a global maximum for \( F_{\text{max}} = 439 \text{ pN} \) and \( P_s = 0.5 \). Responses of maximal sensitivity \( |y| \) were obtained in response to small sinusoidal stimuli at the characteristic frequency of spontaneous bundle oscillation, whereas the minimal sensitivity was observed for intense stimuli (see Fig. 3). The ratio of maximal and minimal sensitivities determines the gain of the amplificatory mechanism. (B) Spectral density of spontaneous movements along the line \( P_s = 0.5 \) for the different values of \( F_{\text{max}} \) indicated in red.

<table>
<thead>
<tr>
<th>Force (pN)</th>
<th>Spectral Density (m² Hz(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>321 pN</td>
<td>0.1</td>
</tr>
<tr>
<td>329 pN</td>
<td>0.2</td>
</tr>
<tr>
<td>352 pN</td>
<td>0.3</td>
</tr>
<tr>
<td>439 pN</td>
<td>0.4</td>
</tr>
<tr>
<td>651 pN</td>
<td>0.5</td>
</tr>
<tr>
<td>833 pN</td>
<td>0.6</td>
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<tr>
<td>1043 pN</td>
<td>0.7</td>
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</tbody>
</table>

**Sensitivity and Friction.** The value of the coefficient \( \lambda \) that characterizes friction of the hair bundle has a significant effect on the sensitivity \( |x_0| \) that the system can achieve. Decreasing this friction coefficient to the physical limit \( \lambda = \lambda_0 = 2 \times 10^{-7} \text{N s m}^{-1} \) given by hydrodynamic friction, indeed results in a threefold increase of sensitivity. This coefficient was estimated previously in experiments where the hair cells were immersed entirely in standard saline solution and did not, or very rarely, show spontaneous oscillations of their hair bundles (29). Because the \( \text{Ca}^{2+} \) concentration in standard saline is 16 times higher than in artificial endolymph, the feedback strength \( S = -C_M p_{\text{f}}/p_0 \) is probably much stronger in standard saline. A more efficient \( \text{Ca}^{2+} \) feedback could explain why the system operates in a monostable state with transduction channels mostly closed (Fig. 1). Under these circumstances, the experiments suggested that hydrodynamic friction alone accounted for the observed hair-
bundle friction. For hair bundles immersed in artificial endolymph, our present analysis indicates instead that the contribution of channel clatter dominates friction \( \lambda = \lambda_c = 3 \times 10^{-6} \text{ N s m}^{-1} \). This apparent discrepancy can be explained by considering the Ca\(^{2+}\) dependence of the transduction channels’ open probability \( P_o \) and possibly of the channels’ gating swing \( d \). In standard saline, the average open probability is only \( P_o = 0.15 \) (23, 31). If in addition, the channel swing were reduced to \( d = 4 \) nm, as suggested by measurements of bundle stiffness in this ionic environment (14), the contribution of channel clatter to friction would be at the level of hydrodynamic friction (see Eq. 6). In addition, the dwell time \( \tau_c \) of transduction channels is also probably shorter in standard saline than it is in artificial endolymph (30, 31). Faster transduction channels would yield greater mechanosensitivity than that observed so far in the bullfrog’s saccule. A dwell time of \( \tau_c = 1 \text{ms} \) is probably fast enough for saccular hair cells to operate in a frequency range of 5–130 Hz, but auditory hair cells could have shorter dwell times, therefore reduced friction \( \lambda \) and increased mechanosensitivity. The calcium dependence of parameters such as \( P_o, d \), or \( \lambda_c \) can also account for the fast mechanical “twitches” that occur in standard saline when the hair bundle is deflected by abrupt step stimuli (14, 15, 17). Because these movements are beyond the scope of the present work, however, we have neglected these effects in our physical description.

**Operating Point.** We can only speculate about what determines the operating point of the hair bundle in the state diagram. It has been suggested that a general self-regulation mechanism adjusts a control parameter of the system to bring it close to a point of high sensitivity (10). An important issue is to identify possible parameters that the cell could regulate to optimize its behavior. Our work suggests that the feedback strength \( S \) and the maximal motor force \( f_{\text{max}} \) are natural candidates. If the hair cell could regulate both parameters, it could achieve high sensitivity by moving toward the point where the quality factor \( Q \) is the largest. For that purpose, the hair cell might take advantage of a filter (18), such as that provided by an electrical oscillator in the basolateral membrane of the hair cell (35, 36). The hair cell might also control one parameter, for instance, if the maximal motor force \( f_{\text{max}} \) were determined only by the intrinsic properties of the adaptation motors and the number of motors per stereocilium. By regulating \( S \) at fixed \( f_{\text{max}} \), the hair cell could nevertheless find an operating point of high sensitivity if the properties of the motors were such that \( f_{\text{max}} \) fell in an appropriate range. The hair cell could regulate the feedback strength \( S \) for example by controlling the intracellular level \( C_0 \) of Ca\(^{2+}\). Such a mechanism might also affect \( f_{\text{max}} \).

The ability of a single hair bundle to detect oscillatory stimuli by using critical oscillations is limited by fluctuations which conceal the critical point. This limitation could be overcome if an ensemble of hair cells with similar characteristic frequencies were mechanically coupled. Coupled noisy oscillators could approach the ideal case of a critical oscillator near a Hopf bifurcation. In an intact mammalian cochlea, the gain that characterizes amplification of basilar-membrane motion is up to 10\(^3\) (37), which can be compared to a gain of only \( \sim 10 \) for a single hair bundle in the bullfrog’s sacculus. This finding suggests that in the cochlea the effects of fluctuations of individual hair cells could be reduced by the cooperative action of many oscillatory cells, whether the oscillations are provided by active hair-bundle motility or by a different mechanism.

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FIG. 1: (A) Real part $\chi'_0$ of the linear response function $\chi_0 = \chi'_0 + i\chi''_0$ of an oscillating hair bundle in the absence of noise as a function of frequency. (B) Imaginary part $\chi''_0$ of the same response function. The response function was obtained numerically for the model of spontaneous hair bundle oscillations defined by Eqns. 2-4 in the main manuscript. Parameters used are those given in Table 1 together with $S = 0.65$, $f_{\text{max}} = 350pN$ and no noise terms. For this choice of parameters the hair bundle oscillates spontaneously at $\nu_0 = 8.7Hz$. Because here the open probability of transduction channels is 0.5, singularities are observed only for odd harmonics of $\nu_0$.

EFFECTS OF AN EXTERNAL LOAD ON NOISY OSCILLATIONS

Numerical simulations of spontaneous hair-bundle oscillations allow us to study the effects of fluctuations which result from thermal motion and also from non-thermal stochastic forces that are generated by motor molecules. Simulation results can be compared to experimental measurements of hair bundles’ response and autocorrelation functions [3, 4]. We find that taking fluctuations into account, the simple model discussed in the main manuscript can quantitatively account for experimental measurements.

In these in vitro experiments, the stiffness of the load to which the hair bundle is coupled influenced the bundle’s spontaneous oscillations [6]. There, this stiffness is that of an attached glass fiber, whereas in the ear it is given by the stiffness of an ancillary structure like the otolithic membrane for the sacculus. When in our simulations the stiffness of the load was increased, the oscillation got faster and of smaller magnitude (Fig. 2A), in agreement with previous experimental observations [6]. The spontaneous movements also became noisier, as revealed by a 70% reduction of the quality factor $Q$ when the combined stiffness of the load and the stereociliary pivots was raised from $600\mu\text{N} \cdot \text{s}^{-1}$ to $1800\mu\text{N} \cdot \text{s}^{-1}$. As a result, the sensitivity to small stimuli progressively declined as the stiffness of the load
Supporting Text

Frequency-Dependent Response Functions in the Absence of Noise

The mechanical behavior of a hair bundle can be characterized by its response to sinusoidal stimuli. The stimulus can be represented by the periodic force

\[ F_{\text{ext}} = F_1 e^{i\omega t} + F_1^* e^{-i\omega t} \]  

at frequency \( \omega_1 \) with amplitude \( F_1 \), where the star denotes the complex conjugate. The amplitude \( F_1 \) can be chosen to be a real number. External stimulation affects the amplitude of the frequency components of the hair-bundle displacements \( X(t) \). In the following, we discuss response functions of stable and oscillating states in the absence of noise.

Response of Stable States. Consider the case where the hair bundle is stable with \( X(t) = X_0 \) in the absence of a stimulus force. In the presence of the periodic stimulus, the bundle's deflection follows the stimulus at the same frequency and exhibits higher harmonics. The deflection can thus be written as

\[ X(t) = \sum_n X_n e^{-i\omega_1 t}. \]  

Here, the coefficients \( X_n \) are complex with \( X_n = X_{-n}^* \); they characterize the Fourier amplitudes of the frequency components of hair-bundle motion. We consider the component of hair-bundle motion at the frequency of stimulation; this response is characterized by the amplitude \( X_1 \). The sensitivity of the system at the stimulus frequency is defined as \( \chi(\omega = \omega_1) = X_1/F_1 \). In the limit of small \( F_1 \), \( \chi \) becomes the linear response function of the hair bundle: \( \chi_0 = \lim_{F_1 \to 0} \chi \). Exactly at a supercritical Hopf bifurcation, the inverse of the linear response function is zero at the characteristic frequency \( \omega_c \) of the oscillating instability. This result can be expressed as

\[ \chi_0^{-1}(\omega) \equiv a(\omega - \omega_c) + b(\Theta - \Theta_c), \]  

where \( a \) and \( b \) are two complex coefficients, \( \Theta \) is a control parameter that takes the critical value \( \Theta_c \) when the system is exactly at the bifurcation point (1). This expression can be rewritten as:
\[
\chi_0^{-1} \simeq 2 e^{i \alpha} \left( i \Lambda (\omega - \omega_0) + K \right), \quad [4]
\]

where \( \omega = \omega_c - \text{Re}(b/a)(\Theta - \Theta_c) \), \( \Lambda = \frac{|a|}{2} \), \( K = -\Lambda \text{Im}(b/a)(\Theta - \Theta_c) \), and \( e^{i \alpha} = -i a / |a| \).

Because \( \chi_0(\omega) = \chi_0^*(-\omega) \), the linear response function takes a simple general form for frequencies of stimulation close to the characteristic frequency \( \omega_0 \) (2):

\[
\chi_0(\omega) \simeq \frac{1}{2} \left( \frac{e^{-i \alpha}}{i \Lambda (\omega_0 - \omega) + K} + \frac{e^{+i \alpha}}{-i \Lambda (\omega_0 + \omega) + K} \right). \quad [5]
\]

This response function is characterized by the stiffness \( K \) and the friction coefficient \( \Lambda \).

The phase \( \alpha \) describes the phase lag of the bundle's displacement with respect to the stimulus at the characteristic frequency.

**Response of Oscillating States.** In the case of a spontaneously oscillating state, the response function can also be defined. In the absence of a stimulus force, the oscillatory state exhibits spontaneous periodic motion with angular frequency \( \omega_0 \): \( X(t) = \sum_n X_n e^{-i \omega_0 t} \). In the presence of the stimulus, because nonlinearities couple modes at the frequency of spontaneous oscillations to modes excited at the stimulus frequency, the displacement \( X(t) \) contains many Fourier components \( X_{nm} \). In Fourier representation, the displacement can be written as

\[
X(t) = \sum_{nm} X_{nm} e^{-i(n\omega_0 + m\omega) t}. \quad [6]
\]

The response at the frequency of stimulation is characterized by the amplitude \( X_{10} \). For \( \omega_1 \neq \omega_0 \), the sensitivity is \( \chi(\omega = \omega_1) = X_{10} / F_1 \). As \( \omega_1 \) approaches \( \omega_0 \), the linear response diverges and

\[
\chi(\omega) \sim (\omega - \omega_0)^{-1}. \quad [7]
\]

The linear response function \( \chi_0(\omega) = \chi_0'(\omega) + \chi_0''(\omega) \), where \( \chi_0' \) and \( \chi_0'' \) denote the real and imaginary part, respectively, exhibits a sharply localized, singular behavior at the oscillation frequency (Fig. 5). Such a response function differs qualitatively from those measured experimentally in the bullfrog's saccus (2). There, the linear response remains finite and is of significant magnitude over a relatively large range of frequencies. In addition, because the influence of fluctuations is ignored here, the response function \( \chi \) can exhibit discontinuities.
as a function of the forcing amplitude $F_1$; these discontinuities result from synchronization phenomena which are beyond the scope of this work (3).

**Effects of an External Load on Noisy Oscillations**

Numerical simulations of spontaneous hair-bundle oscillations allow us to study the effects of fluctuations that result from thermal motion and also from nonthermal stochastic forces that are generated by motor molecules. Simulation results can be compared with experimental measurements of hair bundles' response and autocorrelation functions (2, 4). We find that taking fluctuations into account, the simple model discussed in the main manuscript can quantitatively account for experimental measurements.

In these *in vitro* experiments, the stiffness of the load to which the hair bundle is coupled influenced the bundle's spontaneous oscillations (5). There, this stiffness is that of an attached glass fiber, whereas in the ear it is given by the stiffness of an ancillary structure like the otolithic membrane for the sacculus. When in our simulations the stiffness of the load was increased, the oscillation got faster and of smaller magnitude (Fig. 6A), in agreement with previous experimental observations (5). The spontaneous movements also became noisier, as revealed by a 70% reduction of the quality factor $Q$ when the combined stiffness of the load and the stereociliary pivots was raised from $600 \mu N \cdot s^{-1}$ to $1,800 \mu N \cdot s^{-1}$. As a result, the sensitivity to small stimuli progressively declined as the stiffness of the load was increased, reaching a low value at high stiffness similar to that obtained in response to intense stimuli (Fig. 6B). The load thus impeded the ability of an oscillatory hair bundle to amplify mechanical stimuli. Significant amplification by a single hair bundle was achieved only when the stiffness of the load remained smaller than the maximum negative stiffness that an oscillatory hair bundle manifests in its force-displacement relation. As suggested in the main manuscript, however, a load might *in vivo* also be beneficial: by mechanically coupling neighboring hair cells with similar characteristic frequencies, a load could reduce the limiting effects of fluctuations on mechanical amplification by such an ensemble of noisy oscillators. There could thus be a tradeoff between the impeding effect of a load on a single hair bundle
and the enhancement that the load might provide by enforcing the cooperative action of similar noisy oscillators.

**Numerical Simulations**

Numerical simulations were performed by discretizing in time the dynamic Eqs. 2-4 presented in the main text. The functions $X(t)$, $X_a(t)$, and $C(t)$ are represented by $X_n$, $X_{a,n}$, and $C_n$, where $t = n\Delta t$ and $\Delta t$ characterizes the time step. The discrete dynamics then reads

\[
X_{n+1} = X_n + \frac{\Delta t}{\lambda} \left(-K_{gs} Y_n - K_{sp} X_n + F_{ext,n} + \eta_n \right),
\]

\[
X_{a,n+1} = X_{a,n} + \frac{\Delta t}{\hat{\lambda}_a} \left(K_{gs} Y_n - \gamma N_{a,f} p(C_n) + \eta_{a,n} \right),
\]

\[
C_{n+1} = C_n + \frac{\Delta t}{\tau} \left(-C_n + C_M P_0 + \delta c_n \right),
\]

where $Y_n = X_n - X_{a,n} - D P_0$, $P_0 = \left(1 + A e^{-(X_n-X_{a,n})/\delta} \right)^{-1}$, and $F_{ext,n} = F_{ext}(n\Delta t)$. The random terms are given by

\[
\eta_n = (k_B T / \Delta t)^{1/2} \xi_n,
\]

\[
\eta_{a,n} = (k_B T_a / \hat{\lambda}_a / \Delta t)^{1/2} \xi_{a,n},
\]

\[
\delta c_n = C_M \left(\tau_c / N\Delta t\right)^{1/2} \xi_{c,n}/2,
\]

where $\xi_n$, $\xi_{a,n}$, and $\xi_{c,n}$ are uncorrelated Gaussian random numbers with zero mean and $\langle \xi_n^2 \rangle = \langle \xi_{a,n}^2 \rangle = \langle \xi_{c,n}^2 \rangle = 2$. In our simulations, we chose $\Delta t = 1.2 \times 10^{-5}$ s. We verified that the results were not significantly affected if instead of a Gaussian distribution of random variables $\xi_n$, a rectangular distribution with equal variance was used.


