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

$$F_{ext} = F_1\, e^{-i\omega_1 t} + F_1^*\, e^{i\omega_1 t} \qquad \textbf{[1]}$$

at frequency ω_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

$$X(t) = \sum_n X_n\, e^{-in\omega_1 t}\ . \qquad \textbf{[2]}$$

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, χ 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 ω_c of the oscillating instability. This result can be expressed as

$$\chi_0^{-1}(\omega) \cong a(\omega - \omega_c) + b(\Theta - \Theta_c)\,, \qquad \textbf{[3]}$$

where *a* and *b* are two complex coefficients, Θ is a control parameter that takes the critical value Θ_c when the system is exactly at the bifurcation point (1). This expression can be rewritten as:

$$\chi_0^{-1} \cong 2\,e^{i\alpha}\left(i\Lambda\left(\omega-\omega_0\right)+K\right), \qquad \textbf{[4]}$$

where $\omega = \omega_c - Re(b/a)(\Theta - \Theta_c)$, $\Lambda = |a|/2$, $K = -\Lambda\, Im(b/a)(\Theta - \Theta_c)$, 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 ω_0 (2):

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

This response function is characterized by the stiffness K and the friction coefficient Λ. The phase α 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 ω_0: $X(t) = \sum_n X_n e^{-in\omega_0 t}$. 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

$$X(t) = \sum_{nm} X_{nm} e^{-i\left(n\omega_1 t + m\omega_0 t\right)}\ . \qquad \textbf{[6]}$$

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 ω_1 approaches ω_0, the linear response diverges and

$$\chi(\omega) \sim \left(\omega-\omega_0\right)^{-1}\ . \qquad \textbf{[7]}$$

The linear response function $\chi_0(\omega) = \chi_0'(\omega) + \chi_0''(\omega)$, where χ_0' and χ_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 sacculus (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 χ 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. 6*A*), 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. 6*B*). 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 Δt characterizes the time step. The discrete dynamics then reads

$$X_{n+1} = X_n + \frac{\Delta t}{\lambda}\left(-\mathrm{K}_{gs}Y_n - \mathrm{K}_{sp}X_n + F_{ext,n} + \eta_n\right), \quad \textbf{[8]}$$

$$X_{a,n+1} = X_{a,n} + \frac{\Delta t}{\lambda_a}\left(\mathrm{K}_{gs}Y_n - \gamma\, N_a f\, p(C_n) + \eta_{a,n}\right), \quad \textbf{[9]}$$

$$C_{n+1} = C_n + \frac{\Delta t}{\tau}\left(-C_n + C_M P_0 + \delta c_n\right), \quad \textbf{[10]}$$

where $Y_n = X_n - X_{a,n} - DP_0$, $P_0 = \left(1 + A\, e^{-(X_n - X_{a,n})/\delta}\right)^{-1}$, and $F_{ext,n} = F_{ext}(n\Delta t)$. The random terms are given by

$$\eta_n = \left(k_B T\, \lambda/\Delta t\right)^{1/2} \xi_n\ , \quad \textbf{[11]}$$

$$\eta_{a,n} = \left(k_B T_a\, \lambda_a/\Delta t\right)^{1/2} \xi_{a,n}\ , \quad \textbf{[12]}$$

$$\delta c_n = C_M\left(\tau_c/N\Delta t\right)^{1/2} \xi_{c,n}/2\ , \quad \textbf{[13]}$$

where ξ_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 $\Delta t = 1.2\, 10^{-5}\, s$. We verified that the results were not significantly affected if instead of a Gaussian distribution of random variables ξ_n, a rectangular distribution with equal variance was used.

1. Camalet, S., Duke, T., Jülicher, F. & Prost, J. (2000) *Proc. Natl. Acad. Sci. USA* **97**, 3183-3188.

2. Martin, P., Hudspeth, A. J. & Jülicher, F. (2001) *Proc. Natl. Acad. Sci. USA* **98**, 14380-14385.

3. Pikowsky A., Rosemblum, M. & Kurths J. (2003) *Synchronization: A Universal Concept in Nonlinear Science* (Cambridge Univ. Press, Cambridge, U.K.).

4. Martin, P. & Hudspeth, A. J. (2001) *Proc. Natl. Acad. Sci. USA* **98**, 14386-14391.

5. Martin, P., Bozovic, D., Choe, Y. & Hudspeth, A. J. (2003) *J. Neurosci.* **23**, 4533-4548.

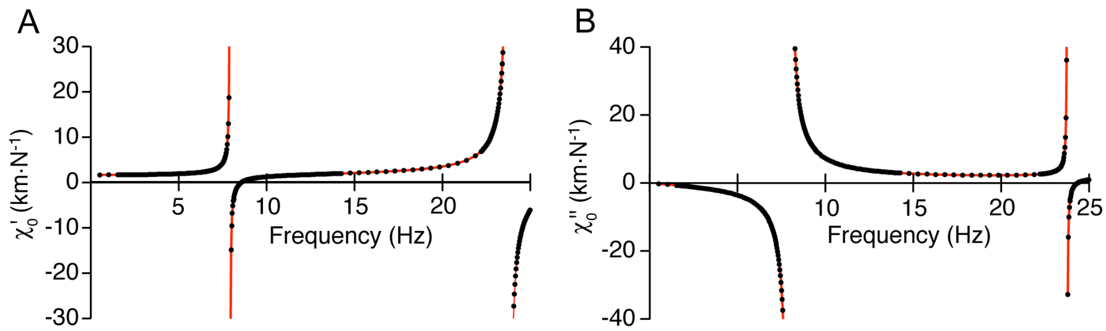

FIG. 5: (*A*) Real part χ_0' of the linear response function $\chi_0 = \chi_0' + i\chi_0''$ of an oscillating hair bundle in the absence of noise as a function of frequency. (*B*) Imaginary part χ_0'' of the same response function. The response function was obtained numerically for the model of spontaneous hair bundle oscillations defined by Eqns. **2-4** in the main manuscript. Parameters used are those given in Table 1 together with $S = 0.65$, $f_{\max} = 350\mathrm{pN}$ and no noise terms. For this choice of parameters the hair bundle oscillates spontaneously at $\nu_0 = 8.7\mathrm{Hz}$. Because here the open probability of transduction channels is 0.5, singularities are observed only for odd harmonics of ν_0.

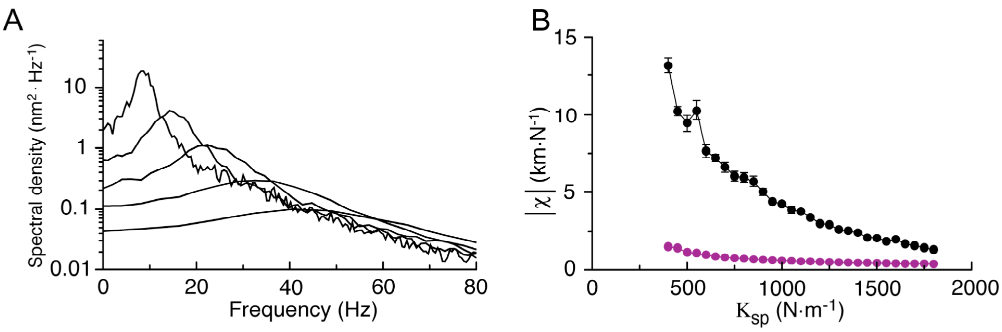

FIG. 6: Effect of mechanical load on hair-bundle oscillation. (*A*) The spectral density of spontaneous movements is displayed as a function of frequency for five values of the combined stiffness K_{SP} of the stereociliary pivots and of the load. When K_{SP} was raised from $600\mu\mathrm{N{\cdot}m}^{-1}$ to $1800\mu\mathrm{N{\cdot}m}^{-1}$ in $300\mu\mathrm{N{\cdot}s}^{-1}$ increments, the peak shifted towards regions of higher frequencies and widened. (*B*) Maximal (black symbols) and minimal (purple symbols) sensitivity $|\chi|$ to sinusoidal stimuli at the characteristic frequency as a function of the combined stiffness K_{SP} of stereociliary pivots and load. Maximal sensitivities occurred in response to small stimuli whereas intense stimuli resulted in minimal sensitivities. With parameter values listed in Table 1 of the main manuscript, the hair bundle was characterized by a maximum negative stiffness of $-1365\mu\mathrm{N{\cdot}m}^{-1}$ in its force-displacement relation.