Hair-Bundle Friction from Transduction Channels' Gating Forces

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Abstract. Hearing starts when sound-evoked mechanical vibrations of the hair-cell bundle activate mechanosensitive ion channels, giving birth to an electrical signal. As for any mechanical system, friction impedes movements of the hair bundle and thus constrains the sensitivity and frequency selectivity of auditory transduction. We have shown recently that the opening and closing of the transduction channels produce internal frictional forces that can dominate viscous drag on the micrometer-sized hair bundle and thus provide a major source of damping [2]. We develop here a physical theory of passive hair-bundle mechanics that explains the origin of channel friction. We show that channel friction can be understood quantitatively by coupling the dynamics of the conformational change associated with channel gating to tip-link tension. As a result, varying channel properties affects friction, with faster channels producing smaller friction. The analysis emphasizes the dual role of transduction channels' gating forces, which affect both hair-bundle stiffness and drag. Friction originating from gating of ion channels is a general concept that is relevant to all mechanosensitive channels.

INTRODUCTION

The acute sensitivity and sharp frequency selectivity of auditory detection rely on efficient transmission of the energy derived from the acoustic stimulus to the apparatus that mediates mechano-electrical transduction in the hair-cell bundle. Yet, at least three sources of friction threaten to dissipate the energy of the vibrating hair bundle and thus pose a fundamental challenge to hearing. First, viscous drag by the surrounding fluid provides a minimum source of damping [4, 7]. Second, viscoelasticity of the oblique tip links that interconnect neighboring stereocilia in the bundle, or of proteins in series with these links, may result in additional dissipation during hair-bundle deflections [6]. Third, an intrinsic source of friction - called "channel friction" in the following - is related to thermal fluctuations of the transduction channels between their open and closed states [10]. The fluctuation-dissipation theorem dictates that this source of mechanical noise be related to frictional forces on the hair bundle.

We have recently combined a dynamic force assay with pharmacological tools to experimentally decipher the relative contributions of viscous drag, tip-link viscoelasticity and channel friction to hair-bundle friction [2]. Friction was characterized by analyzing hysteresis in the force-displacement relation of single hair-cell bundles from the bullfrog's sacculus in response to periodic triangular stimuli. Disrupting the tip links afforded a means to characterize viscous drag on the hair-bundle structure by decoupling the transduction apparatus from bundle motion. In this case, frictional forces did not depend on bundle position and increased in proportion to bundle velocity with the hydrodynamic friction coefficient $\lambda_H = 86 \pm 29$ nN·s·m^{-1} (mean ± SD; n = 10). With intact tip links and at bundle velocities high enough to outrun adaptation, friction instead peaked within the range of positions that spanned the regions of gating compliance of the force-displacement cycle and displayed a sublinear growth with velocity. By using a channel blocker to test the implication of transduction channels' gating, we observed a collapse of the friction peak and thus unveiled the contribution of channel friction. We found that channel friction can dominate viscous drag. In this paper (see also SI Appendix in [2]), we develop a description of passive hair-bundle mechanics to explain the physical origin of channel friction.

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GATING SHIFT EVOKED BY TRIANGULAR BUNDLE MOVEMENTS

Consider a periodic stimulus that imposes a symmetric triangular waveform of hair-bundle motion $X(t)$ as a function of time, with $X_{MIN} \leq X \leq X_{MAX}$ and $X_{MIN} = -X_{MAX}$. On each ramp, the bundle velocity is thus constant with $V^+(X) = -V^-(X) = V$. Here and in the following, the superscripts denote the directionality of bundle motion. By convention, a positive movement increases tip-link tension and, in turn, the open probability of the transduction channels. The transduction apparatus of the hair bundle is composed of N transduction channels that operate in parallel and that are each mechanically connected to an elastic gating spring. A transduction channel is described by two states, open or closed, and assumes an open probability P_o. Within the framework of the gating-spring model of mechanoelectrical transduction [3, 8], the energy difference $\Delta G(X) = -Z \times (X - X_0)$ between the open and the closed states is linearly related to bundle position X. In this relation, the parameter Z represents the reduction in gating-spring tension - the gating force - upon channel opening and X_0 is the position at which the two states have the same energy. We assume here that there is no adaptation and thus that X_0 is constant. At thermal equilibrium, the channels' open probability $P_o = P_\infty$ is related to bundle position by the sigmoidal function

$$P_\infty = 1/[1 + \exp(-(X - X_0)/\delta)], \tag{1}$$

where $\delta = (k_B T)/Z$ is a lengthscale that characterizes mechanosensitivity of the transduction apparatus and $k_B T$ is the thermal energy. Without loss of generality, we set $X_0 = 0$ and thus $P_\infty(0) = 1/2$.

When the bundle is deflected, the open probability changes. However, because channel equilibration is not instantaneous [3, 11], the open probability does not equal its equilibrium value ($P_o \neq P_\infty$) . Using first-order kinetics, channel activation can be described by the relaxation process

$$\tau_c \frac{dP_o}{dt} = P_\infty - P_o, \tag{2}$$

in which the activation time $\tau_c = 1/(k_{co} + k_{oc})$ is the inverse sum of the transition rate k_{co} between the closed and open states of the channel and the rate k_{oc} for the reverse transition (Fig. 1A). Because the height of the energy barrier between the two states depends on the energy difference $\Delta G(X)$, the time τ_c varies with bundle position X [3]. We use $\tau_c(X) = \tau / \cosh(X/(2\delta))$, where τ represents the activation time for small step deflections near an open probability of ½ (see SI Appendix in [2]).

For a ramp of positive motion, we write $X = X_{MIN} + Vt$. In the limit of large displacements ($X_{MIN} \to -\infty$), the integration of Eq. (2) yields:

$$P_o^+(X) = \frac{1}{V\tau} \int_{-\infty}^{X} P_\infty(u) \times \mathrm{M}(X, u)\ du, \tag{3}$$

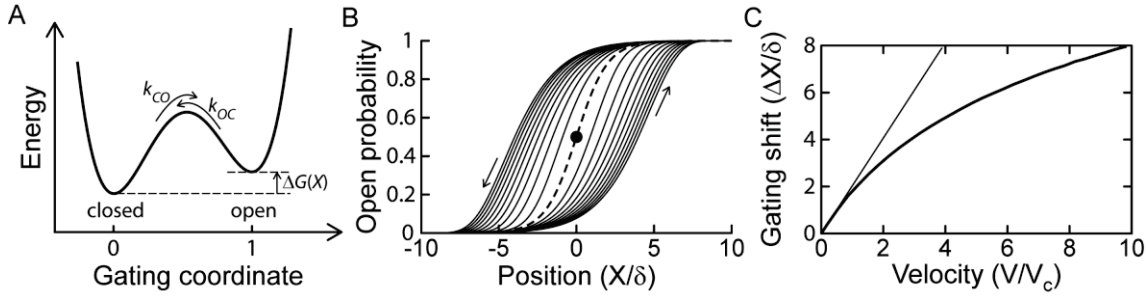

FIGURE 1. Hysteresis from finite activation kinetics of the transduction channels. (A) Schematic energy landscape of a transduction channel with two states (open and closed). The channel switches between its two states with rate constants k_{CO} and k_{OC}. The energy difference $\Delta G(X)$ between the two states depends on bundle position X. (B) Open probability of the transduction channels as a function of normalized hair-bundle position when the bundle is subjected to a series of symmetric triangular waveforms of motion. Bundle velocity varies from V_c to $10 \times V_c$ in 9 steps, where $V_c = \delta/\tau$ is a characteristic velocity. The dashed curve corresponds to the steady-state relation $P_\infty(X)$. The hysteretic cycles (black) display inversion symmetry with respect to the point marked by a disk and get broader for increasing bundle velocities. As a result, the larger the velocity, the further the bundle has to move in each direction before the channels can gate. The bundle displays a clockwise circulation around the cycles (arrows). (C) Normalized gating shift –defined as the horizontal width of a hysteretic cycle at $P_o = 0.5$– as a function of normalized bundle velocity. The tangent going through the origin (thin line) has a slope of 2.

in which we have introduced the memory kernel

$$\mathrm{M}(X,u) = \cosh(u/(2\delta)) \times \exp\left\{-2\frac{\delta}{V\tau}\left[\sinh(X/(2\delta)) - \sinh(u/(2\delta))\right]\right\}. \quad (4)$$

Note that that at low velocity ($V < V_c = \delta/\tau$) and for small deflections $|X| \ll 2\delta$, the memory kernel in Eq. (3) can be replaced by the more tractable form: $\mathrm{M}(X,u) \cong \exp(-(X-u)/(V\tau))$. On the way back, the position varies as $X = -X_{MIN} - Vt$. We find that the open probability obeys the inversion-symmetry relation $P_o^-(X) = 1 - P_o^+(-X)$. If the channels were able to equilibrate instantaneously ($\tau = 0$), their open probability would be given by the steady-state value (Eq. 1) and thus would not depend on the directionality of bundle motion: $P_o^+(X) = P_o^-(X) = P_\infty(X)$. However, because channel activation takes time (Eq. 2), the channels' open probability depends on the history of bundle motion, i.e. on past positions (Eq. 3-4). As a result,

$$P_o^+(X) < P_\infty(X) < P_o^-(X). \quad (5)$$

The relation between open probability and position displays hysteresis. It takes larger displacements $X_{0.5}^{\pm}$, in the positive and negative directions, to open and close half the channels, respectively, than for quasi-static deflections (Fig. 1B). The width of the hysteretic cycle $P_o(X)$ at $P_o = 0.5$ defines the "gating shift". At low velocities ($V < V_c = \delta/\tau$), the gating shift $\Delta X \cong 2\tau \times V$ increases in proportion to velocity with a slope that provides twice the characteristic activation time τ (Fig. 1C).

MECHANICAL HYSTERESIS FROM DELAYED GATING FORCES

The direct mechanical coupling between the transduction channels and the gating springs imposes a reciprocal relation between the channels' open probability and gating-spring tension [5, 9]. Ignoring viscous drag and adaptation, the relation between the external force F and the hair-bundle position X can be written as

$$F(X) = K_\infty X - NZ \times (P_o(X) - 0.5) + F_0, \quad (6)$$

where $F_0 = F(0)$ at steady state. For large deflections that saturates transduction ($P_o \cong 0$ or 1), the hair bundle behaves as a Hookean spring of stiffness K_∞ that corresponds to the combined stiffness of the gating springs and stereociliary pivots. Within the narrow region of deflections that elicit a significant change of the channels' open probability P_o, gating forces of magnitude Z reduce the slope $dF/dX = K_\infty - NZ\, dP_o/dX$ of the force-displacement relation and thus effectively soften the hair bundle. Gating compliance is maximal at the position where the slope of the probability-displacement relation is the highest, which happens near $P_o = 0.5$ (Fig. 1B).

When the hair bundle is subjected to triangle stimulation, the channels' open probability at any given position is smaller on the positive half cycle than that on the negative half cycle (Eq. (5), Fig. 1B). As a result, $F^+(X) > F^-(X)$ and the force-displacement relation displays hysteresis (Fig. 2A). Hysteresis is the consequence of the gating shift between the positions $X_{0.5}^{\pm}$ where half the channels are open (Fig. 1C) or, equivalently, between the positions of maximal gating compliance (Fig. 2B). Thus, although there is no explicit source of dissipation, the hair bundle experiences friction.

The external force $F^{\pm} = F_r \pm F_d^{\pm}$ may be written as a combination of a reactive force F_r and of a dissipative force F_d. Note that the reactive force does not depend on the directionality of bundle motion $F_r(X) = F_r^+(X) = F_r^-(X)$. At any given position, the reactive force can be obtained by allowing the transduction channels to reach thermal equilibrium at this position, i.e. by imposing $P_o = P_\infty$ in the force-displacement relation (Eq. 6):

$$F_r(X) = K_\infty X - NZ \times (P_\infty(X) - 0.5) + F_0. \quad (7)$$

The dissipative component of the external force —the frictional force— is then given by:

$$F_d^{\pm}(X) = \pm NZ\left(P_\infty(X) - P_o^{\pm}(X)\right). \quad (8)$$

Equation (8) clarifies that friction is here the direct consequence of the finite activation kinetics of the transduction channels, for this property imposes that the open probability $P_o^{\pm}$ be different than its steady-state value P_∞ (Eq. 2). On each half cycle of triangular motion, the dissipative force depends on position and peaks at the position $\left(\leq X_{0.5}^{\pm}\right)$ where the slope of the force-displacement relation matches hair-bundle stiffness at steady state (Fig. 2B-C).

To estimate the true frictional force $F_d^{\pm} = \pm(F^{\pm} - F_r)$, it is necessary to measure both the external force and its reactive component as a function of bundle position. In the case of the hair-cell bundles that we studied experimentally [2], the relation $F_r(X)$ contains an unstable region of negative stiffness that is difficult to measure accurately [9]. We can nevertheless characterize friction by measuring the half-height $\Phi(X) = [F^+(X) - F^-(X)]/2$

of the hysteretic force-displacement relation [2]. Because $F_r^+(X) = F_r^-(X)$, the force $\Phi(X) = [F_d^+(X) + F_d^-(X)]/2$ represents the arithmetic mean of the absolute frictional force on the positive half cycle and that on the negative half cycle, at the same position *X*. Using Eq. (6), the force Φ can be written as

$$\Phi(X) = (NZ/2)[P_o^-(X) - P_o^+(X)]. \tag{9}$$

Combining Eq. (9) with Eq. (3) yields a bell-shaped relation between the mean frictional force Φ and bundle position *X*, with a maximum at the position where half the transduction channels are open at steady state, here $X = 0$ (dotted line in Fig. 2C). At the peak ($X = 0$), the force Φ increases with bundle velocity as:

$$\Phi(X = 0, \quad V) = -(NZ/2)\left[\frac{2}{V\tau}\int_{-\infty}^{0} P_\infty(u)\ \times \mathrm{M}(X = 0, V, u)\ du - 1\right]. \tag{10}$$

The relation between channel friction and velocity is thus nonlinear. The channel frictional force displays a sublinear growth (Fig. 2D) that eventually saturates at the maximum value $\Phi_{max} = NZ/2$ in the limit of large velocities ($V \gg V_c$). At low velocities ($V \ll V_c = \delta/\tau$), we obtain the linear regime of friction

$$\Phi(X = 0, V) \cong \lambda_C V, \tag{11}$$

with the friction coefficient

$$\lambda_C = NZ^2\tau/(4k_BT). \tag{12}$$

With standard parameter values for hair bundles from the bullfrog's sacculus ($N = 50$, $Z = 0.8$ pN and $\tau = 0.5$ ms), we find $\lambda_C = 1$ μN·s·m^{-1} ≅ 10×λ_H, where λ_H.is the hydrodynamic friction coefficient of the hair bundle. Our analysis thus explains why gating of the transduction channels produces frictional forces that can be strong enough to dominate viscous drag on the hair-bundle structure [2].

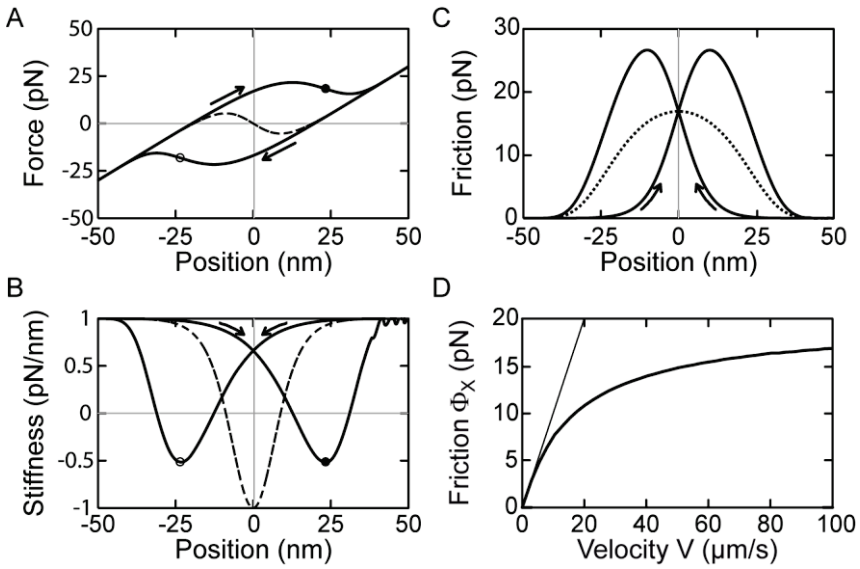

FIGURE 2. Hair-bundle stiffness and friction. (A) External force *F* as a function of hair-bundle position *X*. At steady-state (dashed line), the external force is purely reactive: $F(X) = F_r(X)$. When the hair-bundle follows a triangular waveform of motion, a dissipative force —channel friction— adds to the reactive component of the external force, resulting in a hysteretic cycle. The positive and negative half cycles $F^{\pm}(X)$ are indicated by arrows. (B) Hair-bundle stiffness, defined as the derivative of the relations shown in A, is plotted as a function of bundle position. Gating of the transduction channels soften the hair bundle, a phenomenon called gating compliance. Stiffness is minimal at positions $X_{0.5}^{\pm}$ (marked by a disk and a circle) where nearly, but not precisely, half the channels are open. (C) Dissipative component of the external force $F_d^{\pm} = \pm(F^{\pm} - F_r)$ as a function of bundle position (continuous lines). The relation between the mean frictional force $\Phi(X) = [F_d^+(X) + F_d^-(X)]/2$ and bundle position is shown as a dotted line. In (A)-(C), arrows indicate the directionality of bundle motion. (D) Force Φ at X = 0, noted Φ_X, as a function of bundle velocity *V* when dissipation comes only from transduction channels' gating. At large velocities, the frictional force saturates at $\Phi_{\max} = 20$ pN. The thin line represents the initial slope $\lambda_C = 1$ μN·s·m^{-1} of the relation Φ_X(V). Velocity and amplitude of triangular motion in (A)-(C): $V = 100$ μm·s^{-1} and $A = 100$ nm. Other parameters: $K_\infty = 1$ pN/nm, $N = 50$, $Z = 0.8$ pN, $\tau = 0.5$ ms, $k_BT = 4$ zJ.

DISCUSSION

Mechanosensitivity of the hair-cell bundle relies on strong coupling between the gating dynamics of the transduction channels and tip-link tension. Changes in tip-link tension affect the open probability of the transduction channels. Reciprocally, channel gating must impinge on tip-link tension and thus produce force on the hair bundle. The phenomenon of gating compliance has long been recognized as the elastic correlate of channel gating forces [5, 12]. Channel gating can reduce hair-bundle stiffness by a maximal amount

$$\Delta K_C = NZ^2/(4k_BT), \tag{13}$$

which is proportional to the number N of transduction channels and to the square of the gating force Z (Eq. 7). When the bundle is continuously set in motion by a triangular stimulus, we showed here that channel gating forces not only affect hair-bundle stiffness (Fig. 2B) but also hair-bundle friction (Fig. 2C). At low bundle velocity, channel friction can be linked to gating compliance by combining Eqs. (13) and (12):

$$\lambda_C = \Delta K_C \times \tau. \tag{14}$$

This relation demonstrates that gating forces serve a dual role in passive hair-bundle mechanics.

Gating compliance and channel friction are both intimately related to the function of the hair-cell bundle as a mechanoreceptor. These properties are relevant to any system where a conformational change of a protein is influenced by force. In the case of the hair-cell bundle, gating forces are large enough to provide a major contribution to hair-bundle friction. The hair bundle is thus not optimized to keep friction at the minimum level imposed by viscous drag on the hair-bundle structure. However, large gating forces also promote negative stiffness of the hair bundle (when $\Delta K_C > K_\infty$). This mechanical instability has been shown to be instrumental in an active process that can power spontaneous hair-bundle oscillations, negate friction, and in turn amplify weak sinusoidal stimuli [1]. Transduction channels' gating forces may thus be both a prominent source of friction and part of the solution of the challenge posed by friction to hair-bundle mechanosensitivity.

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COMMENTS AND DISCUSSION

Joseph Santos-Sacchi: Interesting. I note that prestin also displays memory and we find that delays imposed by kinetic transitions may be underlying. We have also considered temperature effects in our studies. My question is whether temperature could have differential effects on the three sources of friction you discuss. First, because you have different molecular elements contributing to the sources (each could have different sensitivities), and second because gating in a variety of ionic channels displays an enormous range in Arrhenius activation energies. Thus the gating itself (of the molecularly undefined MET channel) may have evolved different temperature sensitivity apart from the accessory apparatus. What might be the implications of temperature differences between mammals and lower vertebrates on your view of functional effects of the different sources?

Pascal Martin [reply to Joseph Santos-Sacchi]: I would expect temperature to affect channel friction more severely than viscous drag or tip-link viscoelasticity. Temperature effects on the activation kinetics of the transduction channels have been characterized in lower vertebrates (see Fettiplace (1989) Pflugers Archiv 458:1115-1123 for review). With hair cells from the bullfrog's sacculus or the turtle's cochlea, an increase by 10°C results in a twofold reduction of the channel activation time ($Q_{10} \approx 2$). Channel friction is expected to drop by the same amount (Eq. (12) in the manuscript), channel, whereas viscous drag ought to be reduced by only 20-30% (assuming that it varies similarly to water viscosity). Thus, the larger body temperature of a mammal, with respect to that a cold-blooded animal like the frog, may lower the dominance of channel friction over other sources of dissipation, allowing for faster hair-bundle movements and higher frequencies of auditory detection. Further experiments are required to assess channel friction in mammals.