Magnetic actuation of hair cells

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The bullfrog sacculus contains mechanically sensitive hair cells whose stereociliary bundles oscillate spontaneously when decoupled from the overlying membrane. Steady-state offsets on the resting position of a hair bundle can suppress or modulate this native motility. To probe the dynamics of spontaneous oscillation in the proximity of the critical point, we describe here a method for mechanical actuation that avoids loading the bundles or contributing to the viscous drag. Magnetite beads were attached to the tips of the stereocilia, and a magnetic probe was used to impose deflections. This technique allowed us to observe the transition from multi-mode to single-mode state in freely oscillating bundles, as well as the crossover from the oscillatory to the quiescent state. © 2011 American Institute of Physics. [doi:10.1063/1.3659299]

Auditory and vestibular systems can detect mechanical motion as small as a few Å.¹ This detection is performed by hair cells, named in reference to the bundles of stereocilia protruding from the cell bodies. Deflection of a hair bundle tenses the filamentous connections between its stereocilia, directly gating the opening of mechanically sensitive ion channels. Hair cells were shown to actively amplify imposed stimuli in a frequency-selective manner, with the gain and sharpness of tuning decreasing with increasing stimulus levels.² As they oscillate in an aqueous environment, they require an energy-consuming process to maintain oscillation against viscous dissipation.³

One of the manifestations of the active process,⁴ spontaneous limit-cycle oscillations have been observed in hair bundles *in vitro*.⁵ They arise from an interplay between two processes:⁶ mechano-electrical transduction that causes the ion channels to flicker between open and closed states, and an adaptation process^{7,8} that adjusts the position of the channels along the length of the stereocilia to maintain optimal tension in the tip links. Theoretical models have proposed that a bifurcation^{9–11} separates two dynamic states of the system, a limit cycle and a stable fixed point. We demonstrated¹² that a steady-state offset imposed on the resting position of the stereociliary bundle constitutes a potential control parameter that can induce a transition from the oscillatory to the quiescent state.

Mechanical stimulation of oscillatory hair bundles has previously⁶ been delivered with glass rods attached to the tips of the stereocilia, which inevitably imposes viscoelastic loading on the system. As we observed that attachment of a probe strongly affects hair cell dynamics and qualitatively changes the temporal profile of spontaneous oscillations,¹³ we pursued a method that eludes this external loading. Here, we present a mechanical manipulation technique that allows us to probe the dynamics of freely oscillating hair bundles. Paramagnetic micro-particles whose mass and drag coeffi-

cients were small with respect to the bundle were attached to the stereocilia (Fig. 1(a)), and a magnetic probe was placed at various distances from the bundle to impose offsets on its resting position.

We used *in vitro* preparations of the sacculus from the bullfrog (*Rana catesbeiana*). These planar preparations contain on the order of a thousand hair cells embedded in the supporting tissue. The sensory epithelia were exposed to artificial solutions that mimic the chemical environments under native conditions.⁶ Hair bundles were imaged and tracked as described previously,¹⁵ with a noise floor of ~3–5 nm. Ferromagnetic magnetite (Fe₃O₄) particles (Thermo Scientific) of 1 μm diameter were modified with carboxyl groups and conjugated to Concanavalin-a, a lectin that binds to glycosides, and then attached to the hair bundles. 2-(N-morpholino)ethanesulfonic acid (MES, 25 mM), bead stock (1% solids), Concanavalin-a (1 g/l), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC, 2.5 g/l) were used in the conjugation. A tool-steel probe with a 15 μm diameter tip was coated with polycaprolactone to reduce oxidation and eliminate any potential toxicity to the tissue and magnetized by 4 neodymium-iron-boron (Nd₂Fe₁₄B) permanent magnets. A motorized micromanipulator was used to apply a slow ramp (1.3 μm/s) to the position of the magnetic probe, bringing it into closer proximity to a selected bundle, increasing thus the magnetic force exerted on the Fe₃O₄ bead (Fig. 1(b)). Direction of movement towards the row of tallest stereocilia, corresponding to the preferential opening of transduction channels,¹ is defined to be positive. No hydrodynamic stimulation was observed at these ramp speeds: bundles without attached particles were not deflected by the approaching probe.

Figure 1(c) shows the effects of mechanical deflection imposed on hair bundles via magnetic actuation. Spontaneous oscillations show a drastic reduction of the original frequency with increasing deflection and were eventually fully suppressed by the imposed offset. Variation was observed among the cells, with some also displaying a reduction in the amplitude of innate oscillation as the system transitioned into quiescence. It

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FIG. 1. Bundle deflection via magnetic manipulation. (a) Scanning electron micrograph of a hair bundle with a ferrimagnetic bead attached. Particles were seen to preferentially attach to the tallest row, on the kinociliary side of the bundle. (b) First and last frame of a record during which a ramp was applied over 30 s to the position of the magnetic probe. (c) Traces of hair bundle motion obtained from a single cell under slow displacement ramps. Forces up to ~ 1 nN were applied, with the tip approached to ~ 10 μm of the beads. The scale bars denote 1 μm for (a) and 5 μm for (b); arrowheads indicate attached beads in all the images. The traces have been off-set for clarity.

was consistently noted that larger deflections were required to suppress the oscillations in the positive direction.

In a prior study, we imposed offsets on bundle positions with elastic mechanical probes¹² and observed the transition from the oscillatory behavior to the quiescent state. Our theoretical model indicated that the system can exhibit signatures of an infinite-period bifurcation, a supercritical Hopf bifurcation, or an admixture of the two. The dynamics observed near the critical point with deflections induced via magnetic actuation corroborate the prior findings and confirm that these complex bifurcations likewise arise in freely oscillating bundles.

Recordings taken over hundreds of cells consistently indicated that only a fraction of hair bundles sustain single-mode limit-cycle oscillations, as displayed in Figure 1(c). The majority of freely oscillating bundles exhibit complex temporal characteristics (Fig. 2(a)), with quiescent intervals interspersed with oscillatory behavior. As attachment of an elastic fiber¹³ eliminates or greatly modifies the occurrence of multi-mode oscillations, we used magnetic actuation to examine the transition from multi- to single-mode oscillation. Figure 2(b) shows the effects of increasing mechanical deflections on the temporal profile of freely oscillating hair bundles exhibiting the typical multi-mode character. Cross-over to the single-mode oscillation was observed both in the positive and negative directions. Note the different transitions observed in the two cases: deflections in the positive direction eliminate the low frequency component of the oscillation, while deflections in the negative direction favor the low frequency component, leading to a spike-like profile.

In a recent publication,¹³ we proposed that a variable-stiffness element exists inside the stereocilia, whose properties are modulated by calcium. The dynamics of calcium

attachment and detachment from the element were assumed to be slow compared to other timescales of bundle mechanics. With the inclusion of this slow manifold into our system of differential equations, the numerical model robustly reproduced the multi-mode character of experimentally observed oscillations. To simulate the effects of a slow ramp imposed on the resting position of a freely oscillating hair bundle, we included offsets into our numerical model,¹⁴ with parameters chosen to yield a multi-mode oscillation under unperturbed conditions. As shown in Figure 3, the model captured well the transition from multi- to single-mode oscillation seen in the experiments. Our results therefore indicate that offset deflection constitutes a potential control parameter that determines the dynamic state of a hair cell.

The transition from multi-mode to single-mode oscillation and from the oscillatory to the quiescent state was achieved here via externally imposed offsets. *In vivo*, stereociliary bundles are typically tethered to membranes or other overlying structures and hence may be pre-tensed. Any cellular mechanisms that would modulate an internal stiffness element, such as the proposed variable gating spring,¹³ would then allow the hair bundle to adjust its resting position and thus control its dynamic state.

In summary, we introduced a technique of actuating mechanically sensitive cells by attaching ferrimagnetic particles and manipulating them with permanent-magnet probes. Magnetic manipulation provides a potentially versatile tool for probing cell dynamics,^{16–19} as it avoids many adverse effects of mechanical or optical techniques—it readily penetrates biological tissue and does not heat or otherwise damage the cells. We applied the technique to explore hair bundle dynamics under effectively load-free conditions. We demonstrate that mechanical deflection of the position of the stereociliary bundle

FIG. 2. Transition from multi-mode to single-mode oscillation. The top trace shows spontaneous oscillations of a hair bundle with an attached magnetic bead. Multi-mode oscillation was not affected by the adhesion of the particles. Displacement ramps were imposed on bundles in the positive (middle trace) and negative (bottom trace) directions, which led to abolishing of the multi-mode character of the oscillation. Records shown were obtained from three different cells.

FIG. 3. Numerical simulation of the effects of slow ramp offsets on the oscillatory profile of a bundle. (Top trace) Simulation of spontaneous oscillations with zero offset. The model parameters¹⁴ are chosen to yield a multi-mode oscillation. The bottom two traces show the transition from the multi-mode to single-mode oscillation due to a positive ramp (middle trace), and a negative ramp (bottom trace). The external force term is assumed to be linearly dependent on time to simulate a slow ramp in the offset position of the bundle.

can suppress its spontaneous motility or induce a crossover from multi-mode to single-mode oscillation. Steady-state offset therefore constitutes a potential control parameter that determines the dynamic state of the hair cell.

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¹⁴See supplementary material at <http://dx.doi.org/10.1063/1.3659299> for the numerical model of active hair bundle motility.

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