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Hair bundles: nano-mechanosensors in the inner ear

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Abstract

Hair cells of the inner ear detect mechanical stimuli by deflections of the hair bundle, which open tension-gated transduction channels in the cell membrane to admit cations from the surrounding fluid. Recent experiments have shown that the hair bundle has an active response and is not just a passive elastic structure. Indeed, spontaneous oscillations of the bundle have been observed in the absence of a stimulus. We have proposed the general concept of 'self-tuned criticality' to explain why such oscillations occur, and how they help the ear to hear. According to this idea, when working normally each hair cell is maintained at the threshold of an oscillatory instability. Poised on the verge of vibrating at a characteristic frequency, a hair bundle is especially responsive to weak periodic stimuli at that frequency. The cellular basis of the activity and the tuning mechanism remain to be established. We have put forward two alternative models. In the first, oscillations are generated by molecular motors within the hair bundle and self-regulation is accomplished by a feedback involving calcium ions which enter through the transduction channels. In the second, oscillations are generated by the interaction of calcium ions with the transduction channels, and tuning to the critical point is accomplished by a set of myosin-1C motors which are attached to the channels. In both cases, the combination of motor protein activity and calcium dynamics provides an active amplifier which enables the ear to detect faint sounds.

1. Introduction

The performance of our senses is remarkable, and none more so than hearing. The faintest sounds that we can hear impart no more energy, per cycle, than the thermal motion [1]. At the same time, the ear can cope with loud noises that carry more than 12 orders of magnitude more energy. And, of course, it can analyse frequency: two tones that differ by a few per cent can be resolved. Just how this astonishing feat is achieved has been a puzzle for over a century, but recent research is beginning to penetrate the mystery.

The key to the ear's acuity is an active system of sound detection [2]. The ear is powered. That this was likely to be the case was first realized by Gold [3], more than 50 years ago. He pointed to a problem with the theory of hearing propounded by Helmholtz [4], who had argued that the ear uses a set of inertial resonators to capture the energy of sound waves. Given that the cochlea is filled with fluid, and the likely size of the resonators is a few microns at most, the strong damping precludes a sharp resonant response. Gold proposed that the ear must work like a regenerative radio receiver, and add in energy at the very frequency it is trying to detect. It was clear to him that such a mechanism must be very delicate, however, as it would require a positive feedback of exactly the right magnitude to cancel the damping. Any less and the ear would be insensitive; any more and it would ring spontaneously.

It was not until the 1970s that the idea of an active cochlea began to be taken seriously. At that time, the first successful measurements on a living ear revealed it to respond far more sensitively than the dead cochlea [5, 6]. Subsequently it was discovered that the ear can spontaneously emit sounds [7]. A microphone placed in the ear canal usually records a faint hum, but occasionally shrill whistles can be detected as well. Clearly, something within the cochlea is oscillating. In this section, we shall explore how active oscillators can help the ear to hear and discuss whether motor proteins might be an important component of the active system.

2. Hair bundles

A hair bundle is an appendage measuring a few microns high that sticks up above the surface of every hair cell [8] (see figure 1). It consists of a number of stereocilia (each composed of a bundle of actin filaments) which slope up against each other to form a pyramidal structure. Each stereocilium is connected to its neighbour by a fine filament, called a 'tip link'. Shear flow in the cochlea fluid causes the whole bundle to deflect, with each stereocilium pivoting at its base, so that the tip links get stretched. Each tip link connects directly to a tension-gated transduction channel in the cell membrane, which admits potassium ions. So the deflection leads to a change in the ionic current that, in turn, alters the cell potential. The hair bundle is therefore a transducer which provides a very direct conversion of a mechanical stimulus to an electrical signal.

In the 1980s it was discovered that hair bundles can oscillate spontaneously [9]. This behaviour had been very difficult to reproduce, however, until recently when it became possible to control the extracellular ionic concentrations, so that they resemble the conditions *in vivo* [10]. In this situation, oscillations occur quite readily. It appears that calcium ions, which are present at low concentration in the fluid surrounding the bundle, and which are also admitted by the transduction channel, are crucial to the active process.

Hair bundles also contain molecular motors. Attached to the transduction channel are a number of myosin-1C motors. They are believed to play a role in adapting the hair bundle to varying cellular conditions, by maintaining the proper tension in the tip links [11]. But they might also be more directly involved in generating bundle oscillations. The hair bundles of non-mammalian vertebrates also incorporate a 'kinocilium', which has the same architecture as an axoneme. The dynein motors within this structure might also play an active part in the hair bundle response.

3. Active amplification

There is considerable evidence, then, that hair bundles contain a force-generating dynamical system that can generate spontaneous oscillations at a characteristic frequency ω_C. In general,

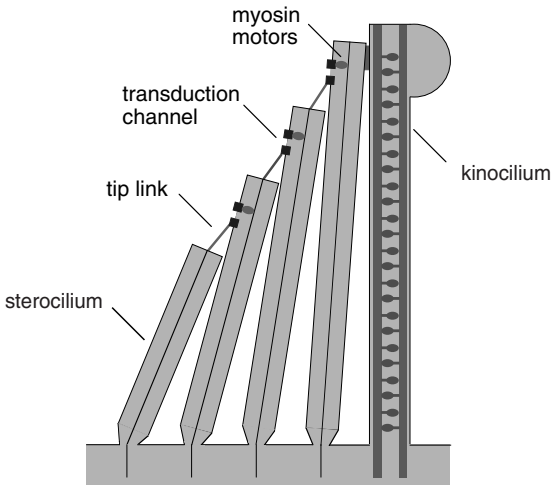

Figure 1. Hair bundle of a non-mammalian vertebrate.

the behaviour of such a nonlinear dynamical system may be described by a control parameter C. Above a critical value, $C > C_c$, the system is stable; for $C < C_c$ it oscillates spontaneously. At the critical point $C = C_c$, which is a Hopf bifurcation, the system has remarkable response properties. Because the behaviour close to critical points is generic, we can calculate these properties without a detailed knowledge of the physical process that gives rise to the dynamical instability [12, 13].

If a stimulus force $f(t)$ is applied at a frequency ω, the hair bundle displacement $x(t)$ has a principal component at that same frequency. So writing the complex Fourier coefficients at frequency ω as $\tilde{f}$ and $\tilde{x}$, the response may be expressed as a systematic expansion:

$$\tilde{f} = \mathcal{A}\tilde{x} + \mathcal{B}|\tilde{x}|^2\tilde{x} + \cdots, \tag{1}$$

where $\mathcal{A}(\omega, C)$ and $\mathcal{B}(\omega, C)$ are two complex functions. For a system that undergoes a Hopf bifurcation, the first nonlinear term is cubic. The bifurcation point is characterized by the fact that $\mathcal{A}$ vanishes for the critical frequency, $\mathcal{A}(\omega_c, C_c) = 0$.

Suppose that the system is poised right at the critical point, $C = C_c$. Then the response to a stimulus at the critical frequency, $\omega = \omega_C$, has amplitude

$$|\tilde{x}| \approx \frac{|\tilde{f}|^{1/3}}{|\mathcal{B}|^{1/3}}. \tag{2}$$

This is a highly compressive response, which boosts weak signals much more than strong signals. Indeed, the gain

$$r = \frac{|\tilde{x}|}{|\tilde{f}|} \sim \frac{1}{|\tilde{f}|^{2/3}} \tag{3}$$

becomes arbitrarily large for small forces. The critical Hopf oscillator acts as a nonlinear amplifier.

If the stimulus frequency differs from the critical frequency, the linear term in equation (1) is non-zero and can be expressed to first order as $\mathcal{A}(\omega, C_c) \approx A_1(\omega - \omega_c)$. When this term exceeds the cubic term in equation (1), active amplification is lost and the response becomes linear:

$$|\tilde{x}| \approx \frac{|\tilde{f}|}{|(\omega - \omega_c)A|}. \tag{4}$$

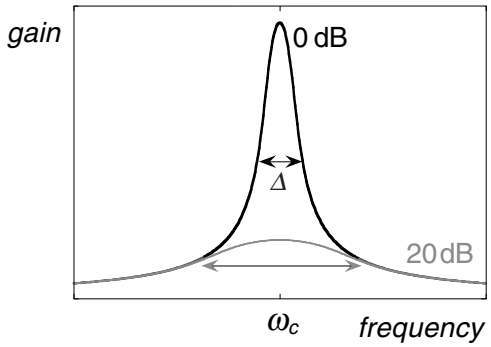

Figure 2. Hopf resonance. The gain and sharpness of response are much greater for a weak stimulus (black) than for a stimulus of ten times greater amplitude (grey).

The bandwidth of active amplification Δ therefore depends on the level of the stimulus:

$$\Delta \approx \frac{|\mathcal{B}|^{1/3}}{|A_1|}|\tilde{f}|^{2/3}. \tag{5}$$

The resonant response of a critical Hopf oscillator is summarized in figure 2. The active system acts as a sharply tuned high-gain amplifier for weak stimuli and as a low-gain filter for strong stimuli. These main features are displayed by the response of the basilar membrane in the mammalian cochlea [14].

4. Self-tuned criticality

The Hopf resonance is perfectly suited to the ear's needs. It permits frequency discrimination; it boosts faint sounds; and the strongly compressive response provides a huge dynamic range—the 12 orders of magnitude of sound energy that we can comfortably hear give rise to hair bundle displacements that vary by only a factor of 100. To profit from the nonlinear amplification, however, each oscillator has to be very close to its critical point. Clearly some kind of regulation mechanism is required to ensure that this is the case.

A feedback mechanism that links the response of the system to the control parameter can permit the system to operate *automatically* close to the bifurcation point, whatever its characteristic frequency [12] (figure 3). Suppose that some mechanism causes the control parameter to decrease as long as the system does not oscillate. After some time, critical conditions will be reached and spontaneous oscillations will ensue. The onset of oscillations triggers an increase of the control parameter which tends to restore stability. Hence the system converges to an operating point close to the bifurcation point. The following simple feedback, which changes C in response to deflections x, illustrates the general idea:

$$\frac{1}{C}\partial_t C = \frac{1}{\tau}\left(\frac{x^2}{\delta^2} - 1\right), \tag{6}$$

where δ is a small amplitude. If no external force is applied, this feedback, after a relaxation time τ, tunes the control parameter to a value C_δ (just less than C_c) for which spontaneous oscillations with $|\tilde{x}| \approx \delta$ occur. These small-amplitude oscillations are referred to as *self-tuned critical oscillations* (STCO). Maintained on the threshold of vibrations by this control mechanism, a hair bundle is exquisitely sensitive to perturbation by periodic stimuli at its characteristic frequency.

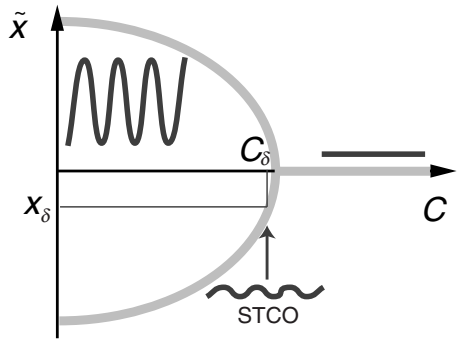

Figure 3. The working point of the self-tuned system is just on the oscillating side of the bifurcation, yielding STCO.

5. Motor-driven oscillations controlled by calcium

What is the physical basis of the dynamical system that generates the oscillations? Because motor proteins are specialized to produce motility, they are an obvious candidate. Recently, a simple physical mechanism has been proposed which allows motor proteins operating in collections to generate spontaneous oscillations by traversing a Hopf bifurcation [15]. Typically, motors move along cytoskeletal filaments and elastic elements oppose this motion. In this case, two possibilities exist: the system either reaches a stable balance between opposing forces, or it oscillates around the balanced state. Two timescales characterize this behaviour: the mechanical relaxation time $\tau_{mech} = \zeta/K$, where ζ is the friction coefficient and K is the spring constant; and the biochemical cycle time τ_{chem} of the motor. The system oscillates if the spring constant does not exceed an upper limit $K_{max} \approx k_0 N$, where k_0 is the crossbridge elasticity of a motor and N is the total number of motors, and the characteristic frequency is given by the geometric mean of the above two timescales:

$$\omega_c \approx \left(\frac{1}{\tau_{mech}\tau_{chem}}\right)^{1/2}. \tag{7}$$

The maximal frequency, obtained when $K = K_{max}$, can be significantly higher than the ATP hydrolysis rate $\alpha = 1/\tau_{chem}$.

One possible location of the motors is within the kinocilium [12], which is a true cilium containing a cylindrical arrangement of microtubule doublets and dynein motors (figure 4). A simple two-dimensional model can be used to discuss the main physical properties of a vibrating cilium near a Hopf bifurcation [16, 17]. In this model, motors induce the bending of a pair of elastic filaments separated by a distance a (corresponding to the distance between neighbouring microtubule doublets in the axoneme). An isolated kinocilium of length L and bending rigidity κ, fixed at the basal end but free at its tip, will vibrate in a wave-like mode with wavelength $\Lambda \approx 4L$. The effective spring constant experienced by the motors as they move along the microtubule doublets is $K \approx \kappa/(La^2)$ and the effective friction coefficient, due to the drag of the kinocilium, is $\zeta \approx \eta L^3/a^2$, where η is the viscosity of the surrounding fluid. Thus, the frequency of a vibrating cilium at the bifurcation point is given by

$$\omega_c \approx \left(\frac{\kappa\alpha}{\eta}\right)^{1/2}\frac{1}{L^2}. \tag{8}$$

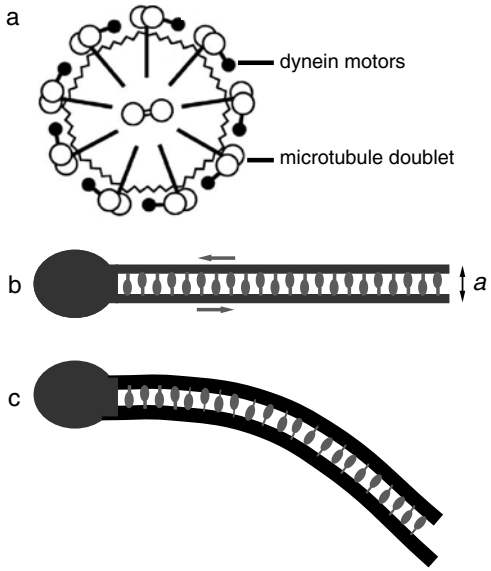

Figure 4. (a) Cross-section through a kinocilium. (b) Model of a kinocilium. (c) Bending caused when the lower filament slides forwards relative to the upper filament.

Using $\alpha \approx 10^2$ s^{-1} and $\kappa \approx 4 \times 10^{-22}$ N m^2 (the bending rigidity of 20 microtubules), the frequency range between 10 Hz and 1 kHz can be spanned by changing the length of the kinocilium between 10 and 1 μm.

How could self-tuning to the critical point be realized in this system? One possibility is that the influx of calcium ions through the transduction channel down-regulates the motor activity (figure 5(a)) [12]. In this case, the Ca^{2+} concentration plays the role of the control parameter C. Assuming that ion pumps in the cell membrane constantly pump Ca^{2+} out of the cell, C obeys the dynamical equation

$$\partial_t C = -\frac{C}{\tau_{ion}} + J P_o(x), \tag{9}$$

where τ_{ion} is the ionic relaxation time and J is the Ca^{2+} current through an open transduction channel. The probability, $P_o(x)$, that a channel is open depends on the hair bundle displacement, as shown in figure 5(b). This is the typical sigmoidal relation, of the form

$$P_o(x) = \frac{1}{1 + A\mathrm{e}^{-x/\delta}} \tag{10}$$

that is expected if the channel makes rapid stochastic transitions between an open and a closed state, gated by the tension in the tip link. The coefficient A is large, so that when the bundle is still, there is only a slight probability that the channel is open. However, owing to the curvature of $P_o(x)$ at $x = 0$, the mean probability of the channel being open increases if the bundle becomes unstable and starts to oscillate. Equation (9) then implies that C rises. If the Ca^{2+} ions down-regulate the motors, for example by decreasing the rate Ω at which they detach, the system moves back towards the quiescent regime. This feedback control is a robust way of generating STCO, as illustrated in figure 5(c).

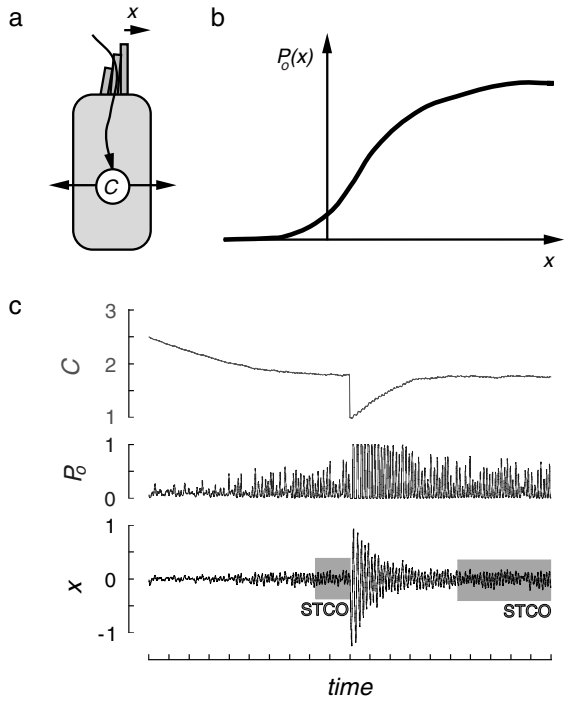

Figure 5. Self-tuning mechanism. (a) Calcium flux through the hair cell. (b) Channel open probability $P_o(x)$. (c) The bundle settles down to STCO after a relaxation time τ_{ion}, whether the Ca^{2+} concentration C is initially too high or too low (reproduced from [12]).

6. Channel compliance and relaxation oscillations

An alternative mechanism by which oscillations might be generated is suggested by recent micromanipulation experiments on hair bundles [18]. When the tip of a bundle is abruptly displaced by a small amount, the bundle reacts by generating a force in the opposite direction. Indeed, the instantaneous force–displacement relation of the bundle (i.e. the relation obtained before adaptation processes mediated by motors or calcium have an affect) displays a region of negative slope (figure 6(a)).

One potential explanation of this behaviour is *channel compliance* [18, 19]. Suppose that the transduction channel has a lever arm, similar to that of a myosin molecule, which amplifies the small movements associated with the opening and closing of the channel (figure 6(b)). Then, when the hair bundle is pushed a distance x in the positive direction, adjacent stereocilia are sheared by $y = \gamma x$, where γ is a geometric factor that depends on the bundle height. The increased tension T_{tl} in the tip links causes the channels to open. Indeed, assuming that the channel kinetics is rapid enough for there to be an equilibrium between open and closed states, the open probability P_o is

$$P_o = \frac{1}{1 + A\mathrm{e}^{-K_{tl}dy/kT}}, \tag{11}$$

where K_{tl} is the elastic constant of the tip link, d is the swing of the lever arm and A is a dimensionless constant whose value depends on the free energy difference of the two channel states. The associated movement of the lever arm diminishes the tension in the tip links, so that

$$T_{tl} = K_{tl}(y - dP_o). \tag{12}$$

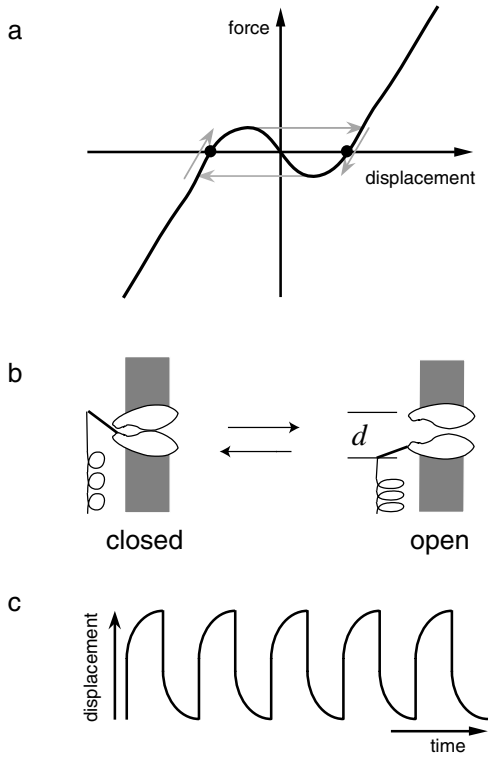

Figure 6. (a) Two-state transduction channel with a lever arm. (b) The force–displacement relation has a region of instability owing to the channel compliance. (c) The action of the myosin-1C motors can push the system around the arrowed curve, generating relaxation oscillations.

The total reactive force of the bundle has contributions from both the tip links and the deformation of the stereocilia pivots:

$$F = N(\gamma T_{tl} + K_{sp}x), \tag{13}$$

where N is the total number of stereocilia and K_{sp} is the effective spring constant due to a single sterocilium. The force–displacement relation resulting from equations (11)–(13) has a region of negative slope if

$$K_{tl}d^2 > 4kT. \tag{14}$$

In this situation, there is a range of applied forces for which the bundle is *bistable*. If the position of the adaptation motors is fixed, the hair bundle will settle at one or another of the stable positions. However, this state of affairs can be upset if calcium down-regulates the motors, as suggested above. Suppose, for example, that the bundle is at the fixed point with the higher value of x, for which there is a high probability that the transduction channels are open. The Ca^{2+} ions entering through the channel bind to the motors, causing a fraction of them to detach; the diminishing force exerted by the motors causes the tension in the tip links to fall and the bundle to move backwards. As indicated in figure 6(a), the fixed point vanishes at a critical value of the motor force and the system then abruptly jumps to the other fixed point. At this lower value of x, the channels are mostly closed. The resulting drop in calcium concentration augments the number of bound motors, increasing the tension in the tip links

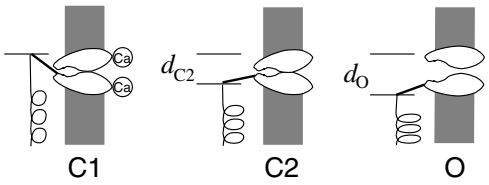

Figure 7. Three-state channel, in which one of the closed states is stabilized by calcium.

until the lower fixed point becomes unstable, whereupon the hair bundle jumps back to its initial position.

This dynamics, whereby the myosin-1C motors shift the bundle from one stable fixed point to another, results in relaxation oscillations with a characteristic form, shown in figure 6(c). Oscillations of this type have been observed in hair bundles dissected from frogs [10, 18]. It is not clear, however, whether this mechanism forms the basis of the active amplifier. There is no obvious way that the system could be regulated to the critical point at which the oscillations have vanishing amplitude.

7. Channel-driven oscillations controlled by motors

There is a second adaptation process that modifies the transduction current, which is much faster than the mechanism mediated by the myosin-1C motors [11, 20]. It depends on the concentration of calcium ions outside the cell and is believed to be caused by Ca^{2+} ions binding to the transduction channels and favouring their closure. Thus, a more appropriate model of the channel might incorporate three states, as shown in figure 7, in which one of the closed states is stabilized by the binding of Ca^{2+}. The dynamics of calcium, which enters through the transduction channels and is continually pumped out of the cell, as described by equation (9), provides a fast feedback. By shifting the channel states and modifying the contribution of the channel compliance, it modifies the force–displacement relation. If the feedback is strong enough, the region of negative stiffness is eliminated and the system has a single fixed point [21]. However, this fixed point may be either stable or unstable and, in the latter case, the bundle executes limit cycle oscillations. Thus the interaction of the calcium with the channel is able to generate a Hopf bifurcation. With their long lever arms, the channels effectively act like molecular motors, driven by calcium rather than by ATP.

The characteristic frequency of oscillation depends on two timescales:

$$\omega_c \approx \left(\frac{1}{\tau_{mech}\tau_{ion}}\right)^{1/2}, \tag{15}$$

where τ_{ion} is the relaxation time of the calcium concentration and τ_{mech} is the viscous relaxation time of the bundle. It scales as

$$\omega_c \sim \left(\frac{N}{L^3\tau_{ion}}\right)^{1/2}, \tag{16}$$

and thus depends strongly on the architecture of the bundle.

Crucially, the stability of the fixed point is determined by the location of the myosin-1C motors. If these motors generate a large force, the hair bundle oscillates; if they generate a small force, the bundle is quiescent. This suggests that the function of the myosin-1C motors is to automatically tune the system to the critical point via a feedback mechanism [21]. It is known, for example, that molecular motors are regulated by calcium. Suppose that Ca^{2+} ions

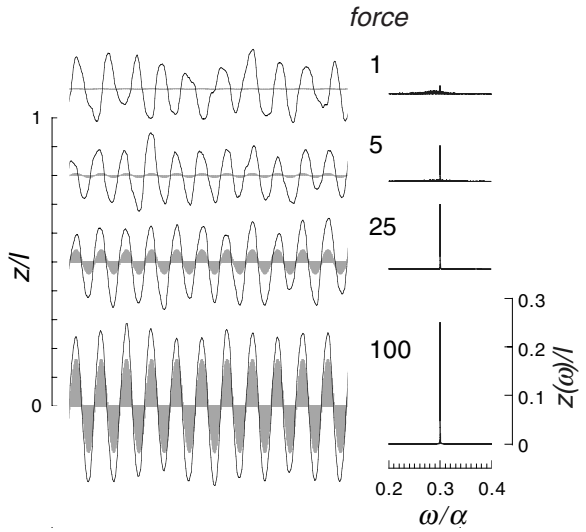

Figure 8. Detection in the presence of noise. Response to sinusoidal forces of different magnitude (left) and Fourier transform of the response (right). The response of an equivalent passive system in the absence of noise is indicated in grey (reproduced from [12]).

binding to the myosin-1C motors influence them to detach from the actin filaments within the stereocilia. Then when the bundle oscillates spontaneously, the increased level of Ca^{2+} entering through the transduction channels will cause the total force exerted by the motors to decline and thereby tend to shut off the oscillation. Conversely, the low concentration of Ca^{2+} in a quiescent bundle will cause more motors to bind and the increase in force will tend to set the bundle into motion. Such a motor-mediated feedback mechanism could therefore maintain the system at the critical point.

Thus, the two adaptation mechanisms that have been identified for hair cells can together generate STCO. Calcium acting on the channels creates a dynamical instability and the molecular motors, acting on a slower timescale, adjust the system to the immediate vicinity of the critical point.

8. Hearing at the noise limit

Hair bundles are subject to noise from a number of sources. In addition to the Brownian forces of the molecules in the surrounding fluid, the stochastic nature of the force-generating system adds further randomness to the system. How can the bundle detect a weak signal in the presence of this noise?

As a consequence of the stochasticity, the STCO are irregular, as illustrated in figure 5(c). The response of a self-tuned hair bundle to a sinusoidal force with a frequency approximately equal to the bundle's characteristic frequency is illustrated in figure 8. For weak stimuli, the amplitude of the oscillation does *not* increase with the amplitude of the applied force; this is because the small response to the stimulus is masked by the noisy, spontaneous motion. Instead, the *phase* of the hair-bundle oscillation becomes more regular; as it does so, a peak emerges from the Fourier spectrum at the driving frequency. The height of the peak grows as the cube root of the stimulus amplitude, following the generic behaviour at a Hopf bifurcation specified by equation (2).

This predicted response of a noisy Hopf oscillator [12] has recently been observed in micro-manipulation experiments on frog hair bundles [22]. It suggests how a hair bundle can achieve its remarkable sensitivity to weak stimuli [12]. By profiting from the periodicity of a sinusoidal input, and measuring phase-locking rather than the amplitude of response, the mechanosensor can detect forces considerably weaker than those exerted by a single molecular motor (if the bundle were a simple, passive structure, its response to such forces would be smaller than its Brownian motion). An important implication of this detection mechanism is that the signal must be encoded by the interval between spikes elicited in the auditory nerve. Paradoxically, the stochastic noise serves a useful purpose. It ensures that the STCO of the hair bundle are incoherent, so that the pattern of spontaneous firing in the nerve is irregular. Against this background, the regular response to a periodic stimulus can easily be detected. Another beneficial feature of noise arises from the fact that weak stimuli do not increase the amplitude of oscillation above the spontaneous amplitude. Thus the Ca^{2+} concentration remains constant, the hair bundle stays in the critical regime and active amplification can be sustained indefinitely.

The active system of detection, in which motor protein dynamics and calcium dynamics both play a key role, explains how hair bundles can detect sound waves whose energy per cycle is similar to that of the thermal noise.

Acknowledgments

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