

Mechanical amplification of stimuli by hair cells

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Unlike any other known sensory receptor, the hair cell uses positive feedback to augment the stimulus to which it responds. In the internal ears of many vertebrates, hair cells amplify the inputs to their mechanosensitive hair bundles. Outer hair cells of the mammalian cochlea display a unique form of somatal motility that may underlie their contribution to amplification. In other receptor organs, hair cells may effect amplification by hair-bundle movements driven by the activity of myosin or of transduction channels. Recent work has demonstrated the presence of several myosin isozymes in hair bundles, confirmed that bundles display myosin ATPase activity, and shown that the work performed by myosin molecules could account for one aspect of the amplificatory process.

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Current Opinion in Neurobiology 1997, 7:480–486

<http://biomednet.com/eleceref/0959438800700480>

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Abbreviations

dB decibels sound-pressure level
 SOAE spontaneous otoacoustic emission
 zJ zeptojoule (10^{-21} joule)

Introduction

The ear has a problem. Like other sensory receptors, hair cells in the ear must detect stimuli at the lowest possible intensity. Unlike most other receptors, though, hair cells confront a dearth of energy in threshold stimuli. For example, photoreceptors capture light, each quantum of which imparts a substantial amount of energy; a single green photon has an energy content of 400 zJ, 100-fold the average thermal noise level (kT) of 4 zJ. Olfactory neurons can potentially garner stimulus energy by binding their ligands tightly; the capture of an olfactant by an odorant receptor with a dissociation constant in the nanomolar range would impart as much as 80 zJ to the cell. Hair cells, by contrast, operate at their thresholds with far smaller energies—we probably hear sounds at intensities so low that they vanish into the internal ear's thermal noise. Psychophysical experiments confirm that humans can detect auditory and vestibular stimuli that provide each receptor cell with an energy near the thermal level [1,2]. Each hair cell thus has an energetic threshold only 1% that of a photoreceptor.

How can the ear reliably respond to stimuli at the level of thermal noise? Hair cells can, in principle, take

advantage of a favorable feature of most auditory stimuli, their repetitive, sinusoidal nature. Sound energy can be accumulated over several cycles of stimulation by making use of mechanical resonance. Just as small, periodic pushes enhance the oscillation of a child's swing, sinusoidal sound stimuli can gradually increase the motion of a hair cell's receptive organelle, the hair bundle. At the same time, the random effect of thermal noise on the bundle is averaged out over time. Increasing its responsiveness by signal averaging, the ear can thus purchase sensitivity at the price of temporal resolution.

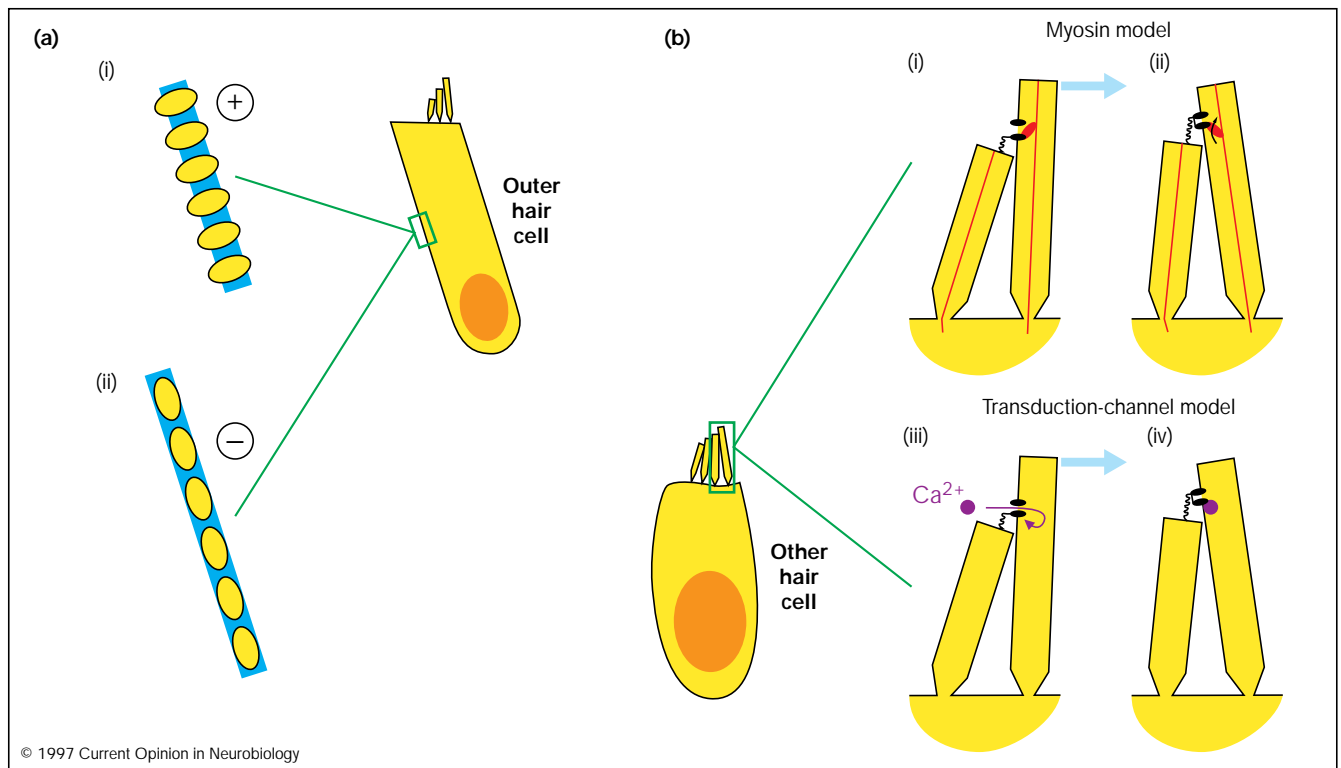
Although it seems an admirable strategy, mechanical resonance of hair bundles appears at first glance to be impossible. Each bundle is immersed in a fluid, endolymph, that has essentially the viscosity of water. For an object the size of a hair bundle and moving at a frequency characteristic of our auditory system, this viscosity is imposing. Oscillating in response to a stimulus frequency of 1 kHz, a 5 μ m hair bundle encounters a viscous regime similar to that of a tuning fork trying to vibrate under water. Viscosity steals energy from the moving bundle, damping its resonance and interfering with time-averaging of threshold auditory stimuli.

Hair cells have found a way—perhaps more than one—to overcome the effect of viscous damping. They have developed an active process, a motor for the mechanical amplification of low-intensity stimuli. By imparting mechanical energy to a hair bundle, this amplificatory process partly counters the loss of energy through viscous drag. This 'solution' evolved at least 380 million years ago, for it is employed by amphibians, reptiles, and birds, as well as by mammals. Fish ears too may well display mechanical amplification, which, like the structure of the hair cell, may prove to be a general feature of vertebrates. In this review, I discuss the evidence demonstrating the existence of an active process and examine recent contributions to the elucidation of its cellular mechanism.

Evidence for an amplificatory process

The active process of hair cells manifests itself in two ways. The first of these is the ear's technical performance, the extraordinary sensitivity and sharp frequency selectivity of the auditory periphery, whether measured as basilar-membrane motion, hair-cell responsiveness, or eighth nerve fiber activity. The ear can respond to hair-bundle stimuli less than 1 nm in peak-to-peak magnitude [3] and can discriminate stimulus frequencies that differ by a fraction of 1%. Modeling studies indicate that the ear's performance exceeds that possible for a passive system [4]—some form of amplification is essential.

Figure 1



Possible molecular mechanisms of mechanical amplification by hair cells. **(a)** In the mammalian cochlea, the plasma membranes of outer hair cells are replete with intramembrane proteins. (i) Depolarization causes the proteins to decrease their surface areas within the membrane, causing cellular shortening; (ii) hyperpolarization has the opposite effect. **(b)** For other hair cells, which lack the membrane specializations of outer hair cells, mechanical amplification is thought to be effected by either of two means. (i,ii) Myosin (small red oval) in stereocilia provides a possible means of amplifying hair-bundle motions. (i) In this model, displacement of a bundle in the positive direction triggers the contraction of myosin molecules associated with tip links, which are thought to represent the gating springs attached to transduction channels. (ii) Power strokes by the myosin molecules increase tip-link tension, moving the hair bundle back in the negative direction. (iii,iv) Another potential form of motility is based upon the mechano-electrical transduction channel. (iii) Positive bundle displacement opens this channel, allowing K^+ and some Ca^{2+} to enter the cell. The latter ion is hypothesized to bind near the channel's interior aspect, promoting channel reclosure. (iv) The resultant rise in tip-link tension jerks the bundle back in the negative direction.

The second, more surprising, and most compelling indication of the ear's amplificatory process is the existence of otoacoustic emissions [5]. The ears of many vertebrate species, including those of most humans, can emit sounds; these may be spontaneous otoacoustical emissions (SOAEs) or emissions evoked during or after acoustical stimulation. In humans and other species that have been examined, every ear has a unique and largely stable pattern of emission frequencies and amplitudes. The features of SOAEs are quite similar throughout the vertebrates [6].

Although the presence of SOAEs demonstrates that the internal ear is capable of active motility, it is not immediately obvious that these emissions are related to useful amplification of auditory inputs. The two phenomena are nonetheless connected. A SOAE is suppressed when a stimulus tone is presented at a nearby frequency. If an experimenter systematically examines the effects of stimulus frequency and amplitude on a particular emission, the result—an isosuppression tuning curve—closely resembles the ordinary tuning curve

defining the responsiveness to an acoustical stimulus. In both mammalian and nonmammalian ears [7,8], the process underlying SOAEs and that responsible for sharply tuned, highly sensitive hearing display similar sensitivities to stimuli. It is most probable that these processes are identical: the cochlear amplifier is also the source of SOAEs. Amplification is known to be highly nonlinear, such that threshold inputs are amplified about 100-fold as strongly as moderately loud sounds [9]. In a very quiet environment, the amplifier's gain apparently becomes so great that spontaneous emissions occur [4].

The molecular motor for amplification in outer hair cells

The molecular basis of mechanical amplification is uncertain. Although three possible mechanisms have been proposed, and evidence has been garnered in support of each, there is no receptor organ in which the mechanism is unequivocally known. Moreover, there may be more than one means by which amplification is effected: the strongest candidate to explain amplification in the

mammalian cochlea is unlikely to operate in the receptor organs of amphibians, reptiles, or birds.

The outer hair cell, a unique embellishment of the mammalian cochlea, is essentially an elongated cylinder with a hair bundle at its apical end and a nucleus near its base. Several lines of evidence indicate that the 12,000 outer hair cells in each human cochlea effect mechanical amplification. Even though most afferent innervation stems from the cochlea's 3,500 inner hair cells, stimulation of the copious efferent nerve supply to outer hair cells reduces the sensitivity of hearing [10]. It follows that the activity of outer hair cells sensitizes inner hair cells, probably by enhancing their mechanical input. Selective chemical damage to outer hair cells substantially reduces the cochlea's sensitivity [11], implying that the active process has been compromised. Finally, acoustical overstimulation, which is thought to transiently damage outer hair cells, temporarily diminishes basilar-membrane vibration [12].

The outer hair cell exhibits an unprecedented form of motility (Figure 1). Depolarization of the cell's plasma membrane causes the cylindrical soma to shorten; hyperpolarization leads to elongation [13,14]. These motions are associated with a specialization of the cell's lateral plasma membrane, which is endowed with several billion intrinsic molecules of an unknown protein [15,16]. Changes in membrane potential cause these molecules to contract or expand within the plane of the membrane, thus changing the hair cell's length. These movements, which are confined to the lateral cellular surfaces [17–19], are powered by the electrical field itself [20,21]: ATP hydrolysis does not directly participate in the phenomenon [22].

Although the outer hair cell's motor for somatal movements has evidently been located, there remain two important challenges to understanding how amplification occurs in the mammalian cochlea. The first is explaining how the active process can operate at the highest frequencies to which mammalian ears are responsive, which extend above 10 kHz in humans and perhaps ten times that high in whales and bats. Powered by membrane potential, somatal motility can occur over most of this range of frequencies under experimental conditions. A problem arises, however, in understanding the cells' operations *in vivo*. Because of the hair cell's membrane time constant, around 1 ms, the receptor potential displays little phasic response at frequencies in excess of 1 kHz; there is accordingly little change in voltage to drive mechanical oscillations [23]. Although the situation might be improved if motility were driven by extracellular electrical fields [24], the mechanism is of questionable efficacy [25].

The second uncertainty about cochlear amplification lies in understanding how active somatal movements of outer hair cells help to excite inner hair cells.

Although an explicit model of the process is lacking, outer hair cells may somehow accentuate the motion of the basilar membrane as a whole [13,14]. Because outer hair cell contractions cause movements within the organ of Corti [26], amplification may alternatively arise from such oscillations superimposed upon those of the basilar membrane itself [27,28].

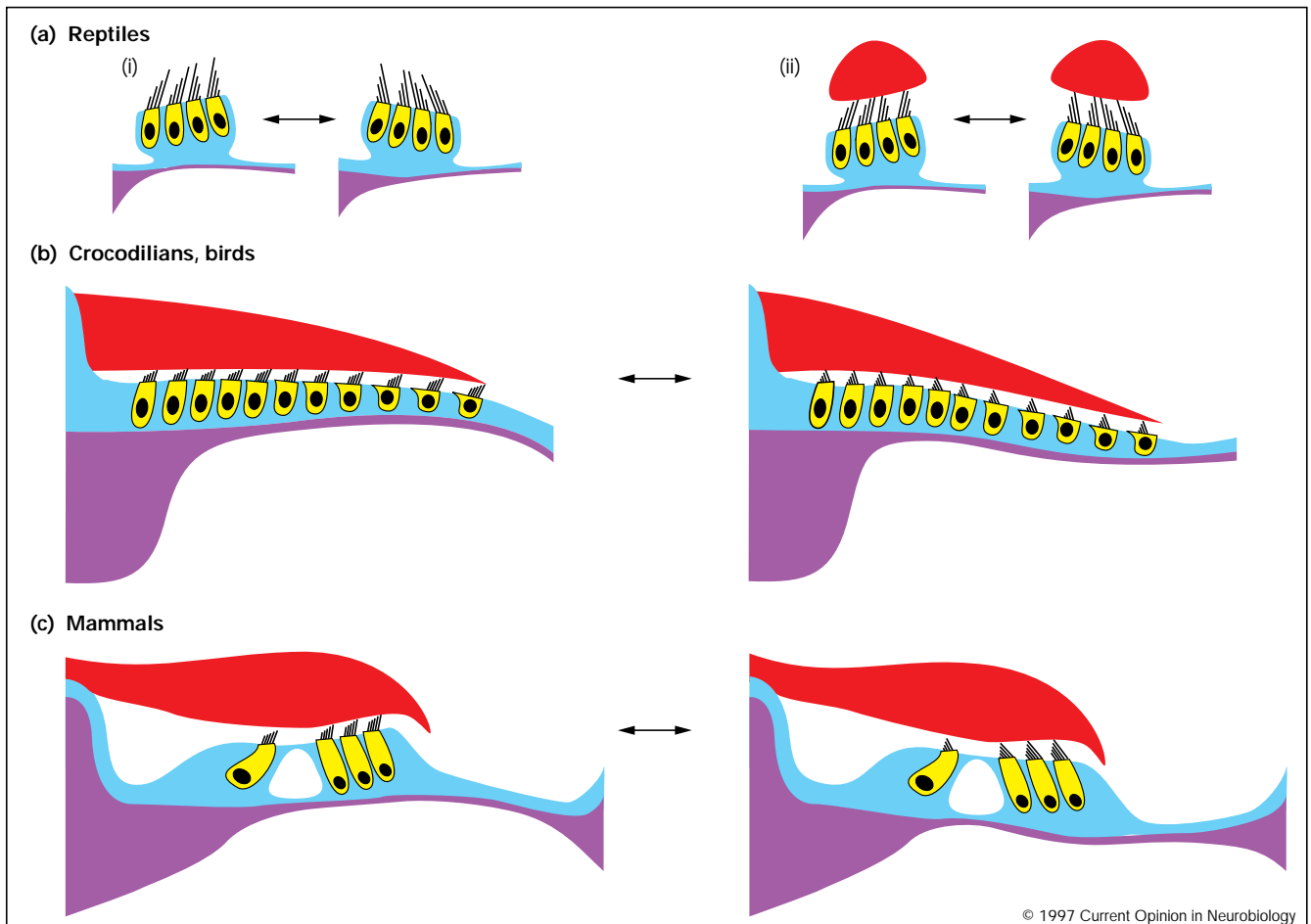
The molecular motor for amplification in nonmammalian hair cells

Because the ears of nonmammalian vertebrates lack outer hair cells, their active process must reside elsewhere. In many receptor organs, the hair cells are structurally uniform; it is therefore likely that all these cells contribute to mechanical amplification. A plausible location for the motor in such cells is the hair bundle, which may be both a receptor organelle and a motile structure. Indeed, when stimulated by a mechanical deflection, the hair bundle makes twitching or oscillatory movements [29–32]. This evoked motion, which can exceed 40 nm in magnitude, is graded in size with the amplitude of stimulation and is associated with sensitive mechano-electrical transduction [33]. In the presence of a physiological Ca^{2+} concentration, the hair bundle produces oscillatory movements at a frequency near the hair cell's characteristic frequency [33]; at a higher Ca^{2+} concentration, twitching is accelerated [34]. Spontaneous bundle motions can also occur [29,30,32,33].

Myosin is the first of two candidates to constitute the motor for rapid hair-bundle movements [30,35,36]. A hair cell adapts to sustained stimulation by resetting its position of mechanical sensitivity [37], an adjustment associated with mechanical changes within the bundle [30,38]. Adaptation's dependence on hydrolyzable nucleoside triphosphates and its sensitivity to phosphate analogs suggest the involvement of a mechanoenzyme [39,40[•]]; the presence of actin in stereociliary cores implicates myosin in particular [30,41]. Moreover, hair bundles both contain myosin and display myosin-based ATPase activity [42,43,44[•]]. Because it is concentrated near the insertions of tip links in the hair bundle, the myosin I β isozyme, in particular, is an attractive candidate to mediate adaptation.

In addition to subserving adaptation, hair-bundle myosin might produce active bundle motions and thus participate in mechanical amplification by making abrupt, concerted movements (Figure 1). Like the myosin molecules of insect flight muscle, myosin in hair bundles could be stress-activated; bundle movements initiated by sound would then be accentuated by the participation of myosin [36]. Most interestingly, the number of myosin molecules thought to be involved in adaptation could readily provide the energy dissipated by SOAEs [45[•]]. One observation speaks against this model: although myosin-based tightening of tip links should increase the open probability of transduction channels, bundle twitches are instead associated with channel closure [33].

Figure 2



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Structures responsible for frequency selectivity in the inner ears of various species. A cross-section of each receptor organ is illustrated at the two extremes of its oscillatory motion, whose amplitude exaggerates by a thousand times that in a threshold response. In each instance, hair cells are shown in yellow; the remainder of the sensory epithelium and contiguous epithelium is in light blue, the basilar membrane and related connective tissue in mauve, and the accessory structure, when present, in red. **(a)** Reptiles. (i) In the simplest case, found in the cochleæ of certain lizards, hair cells with free-standing hair bundles lie in an epithelial ridge, or basilar papilla, resting upon the elastic basilar membrane. As sound pressure drives the membrane up-and-down, the basilar papilla rocks side-to-side; hydrodynamic drag on the hair bundles then deflects and stimulates them. (ii) In other reptiles, the hair bundles insert into a gelatinous mass, either a compact sallet or a more extended tectorial membrane. Sound moves the basilar membrane and rocks the basilar papilla, causing each ensemble of hair bundles to oscillate in synchrony; the bundles' mechanical properties and the mass of the overlying structure determine the resonant frequency. **(b)** In the more complex cochleæ of crocodylians and birds, the basilar papilla is so flattened as to make its lateral oscillation unlikely; the papilla and tectorial membrane evidently move up-and-down like a pair of hinged flaps. **(c)** The mammalian cochlea represents a further elaboration of this arrangement. Here the characteristic oscillatory frequency is largely determined by the local mechanical properties of the basilar membrane, organ of Corti, and tectorial membrane. Somatal contraction of the three rows of outer hair cells is thought to amplify mechanical inputs by accentuating the basilar membrane's motion. The hair bundles of outer hair cells, which are tightly connected to the tectorial membrane and graded in length along the cochlea, may contribute to tuning and to amplification. The bundles of the single row of inner hair cells are free-standing; these cells sense shearing movements of the endolymph beneath the tectorial membrane, but probably contribute little to tuning or to generation of otoacoustic emissions.

The second proposed mechanism for active hair-bundle movement, which does not suffer from the problem noted above, involves the mechanical activity of the transduction channels themselves. Because the gating springs attached to these channels contribute much of a hair bundle's stiffness [30,38], channel gating should influence the bundle's mechanical properties. Such an effect is observed: the opening and closing of transduction

channels causes a decrease in bundle stiffness, termed the gating compliance [31,46,47].

If channel gating can affect a hair bundle's steady-state stiffness, it is apparent that transient changes in channel open probability could underlie active movements of the bundle [31] (Figure 1). A plausible trigger for these motions is Ca^{2+} , whose extracellular concentration strongly

influences the timecourse of bundle twitches [33,34]. When a transduction channel opens under the influence of mechanical stimulation, K^+ carries most of the current. Even though the extracellular Ca^{2+} concentration around the hair bundle is only 30–250 μM , the channel's high Ca^{2+} permeability [48–50] permits a significant amount of Ca^{2+} to enter a stereocilium as well [51]. The binding of Ca^{2+} to a site associated with the transduction channel's cytoplasmic surface may increase the channel's probability of reclosure, for intracellular dialysis of hair cells with Ca^{2+} buffers blocks rapid channel reclosure following stimulation [52,53].

In addition to regulating channel closure, Ca^{2+} could actually power the amplifier. In the model presented above [31,34], Ca^{2+} binds to the channel-closing site when the ion is present at a high concentration immediately inside the open pore. Soon after channel closure, however, the local Ca^{2+} concentration falls substantially as a result of ionic diffusion and extrusion. As a result, the Ca^{2+} concentration at the time of dissociation is much lower. This difference could, in principle, be harnessed to perform mechanical work: in the optimal case, a cycle of operation by each channel could contribute an amount of work, W , given by

$$W = kT \ln \left(\frac{[Ca^{2+}]_{\text{binding}}}{[Ca^{2+}]_{\text{release}}} \right),$$

in which k is the Boltzmann constant and T the temperature; the subscripts denote the Ca^{2+} concentrations during Ca^{2+} binding, shortly after channel opening, and during Ca^{2+} release, well after channel reclosure. For plausible values of these Ca^{2+} concentrations (100 μM and 0.1 μM , respectively), a cycle would yield around 30 zJ of work per channel. A bundle that includes about 100 transduction elements [31,54] could then readily provide the energy needed to account for twitches [33]. More importantly, such a machine could overcome the viscous energy loss sustained by a hair bundle in the human ear when responding to a high-frequency stimulus: even at a frequency of 15 kHz, there would be power enough to drive a bundle with a realistic drag coefficient [31,55] through ± 8 nm, a movement corresponding to a moderately loud, 50 dB stimulus [9,56].

The basis of frequency selectivity

In addition to a motor capable of performing mechanical work, the ear's amplificatory process requires a means of sharply tuning each hair cell's sensitivity to a narrow range of stimulus frequencies [57]. This might be accomplished by individually setting each cell's mechanical amplifier to the appropriate frequency, for example, by adjusting the rate constants of one or more critical reaction steps. Although this arrangement may in fact be implemented, its drawback is obvious: the values of relevant parameters

of the reaction would need to be individually specified for each hair cell in an array of thousands.

Another strategy for achieving sharp frequency selectivity and highly sensitive hearing would separate the tuning and amplificatory steps of the process. In this instance, the mechanical properties of each oscillatory unit would largely set the frequency to which that unit is tuned (Figure 2). In some receptor organs, tuning would be accomplished either by individual hair bundles [58] or by hair bundles and an associated tectorial mass [59•]; in other receptor organs, frequency selectivity would depend upon the mechanical properties of the basilar membrane, sensory epithelium, and tectorial membrane. In each case, the active process would pump energy into the system at a phase appropriate to achieve net positive feedback. The amplifier would then act like the escapement of a pendulum clock: the oscillation frequency of this instrument is determined by the pendulum's natural frequency, while the device overcomes friction (including viscous drag) through the escapement's exertions.

Directions for future research

A detailed understanding of the ear's active process requires forging of a chain of evidence that extends from observation of amplificatory phenomena to delineation of the underlying mechanisms at the cellular and eventually the molecular level. At present, the results stem from numerous methodological approaches on a variety of experimental preparations. Although the data are compelling in their support of an active process, there is no instance in which amplification is understood in sufficient detail to permit a quantitative description of the process.

One requirement for future work is more detailed measurement of the physical properties of the internal ear's structures. For example, because of differences in experimental technique and in the integrity of the preparations, estimates of the stiffness of the mammalian basilar membrane differ by over an order of magnitude [60–62]. If the membrane is relatively compliant, the hair bundles of the organ of Corti constitute a substantial part of the elastic reactance observed when the basilar membrane moves. This interpretation would imply that a large fraction of the stimulus energy is directed to the hair bundles [63], thus accounting for the ear's remarkable efficiency in energy transduction. Under the same circumstances, the mechanical nonlinearity of hair bundles would explain the ear's generation of audible difference tones [64]. Most importantly, hair bundles could participate in frequency tuning through their stiffness and in amplification through their active movement. If the basilar membrane's stiffness lies near the greatest estimate, however, other explanations must be sought for these phenomena.

Precise measurements are also required to produce a quantitative understanding of the role of outer hair cell contractility in frequency tuning. Modeling of this process

requires that the elastic forces attributable to the outer hair cell's stiffness [65], as well as the cell's active force production [66], be comparable to the forces involved in basilar-membrane movement. It is also necessary to compare the length of the basilar membrane affected by an acoustical stimulus with the spatial extent of amplification [67]. The data in hand are broadly consistent with a role for somatal motion; still better measurements are required to permit quantitative modeling of the amplificatory process.

The last important goal of future research in this area is confirmation of the mechanism of hair-bundle motility. This task is complicated by the complex role of Ca^{2+} ; a possible participant in motility powered by transduction channels, Ca^{2+} also regulates adaptation [52] and thus affects the hair bundle's capacity to produce active movements [33]. The lack of specific reagents to interfere with known isozymes of hair-bundle myosin [44*] also poses a challenge. Finally, it would be valuable to examine the role of bundle motility in mammalian preparations as well. Because of the remarkable similarity of auditory sensitivity, frequency selectivity, and otoacoustic emissions throughout the vertebrates, the possibility remains that hair-bundle motility underlies amplification in all hair cells.

Acknowledgements

The author thanks M Magnasco for productive discussions; EA Lumpkin, RE Marquis, and CE Stewart kindly commented on the manuscript. The original work cited in this review was supported by National Institutes of Health grants DC00241 and DC00317. The author is an Investigator of Howard Hughes Medical Institute.

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