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The Cellular Basis of Hearing: The Biophysics of Hair Cells

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Summary. A crucial event in the hearing process is the transduction of mechanical stimuli into electrical signals by hair cells, the sensory receptors of the internal ear. Stimulation results in the rapid opening of ionic channels in the mechanically sensitive organelles of these cells, their hair bundles. These transduction channels, which are nonselectively permeable, are directly excited by hair-bundle displacement. Hair cells are selectively responsive to particular frequencies of stimulation, both due to the mechanical properties of their hair bundles and because of an ensemble of ionic channels that constitute an electrical resonator.

With over a million essential moving parts, the auditory receptor organ, or cochlea, is the most complex mechanical apparatus in the human body. There has been for decades a good understanding of how sounds are conducted to the inner ear, and there are excellent descriptions of the signals that move from the cochlea to the brain along the eighth cranial nerve. The daunting complexity of the cochlea, however, has delayed our understanding of how this pea-sized organ produces electrical signals in response to acoustical stimulation. Within the last decade, the availability of tools for the study of the ear's receptor cells in vitro has made possible a detailed biophysical description of how hearing occurs at a cellular level. At the same time, experimental data from intact animals have revealed a new level of complexity in the cochlea's operation.

The hair cell is the receptor cell of the auditory, vestibular, and related sensory systems. It performs an essential duty of these systems, transduction, the rendering of sensory inputs into electrical signals. The hair cell carries out this task with subtlety, making use of a variety of mechanical, hydrodynamic, and electrical strategies to measure signals with great sensitivity and remarkable frequency discrimination.

Hair Cells

Hair cells are epithelial cells that originate not from the neural tube that forms the brain but from the surface ectoderm of the vertebrate embryo. The cells maintain their epithelial character in the mature organs of the ear (Fig. 1); they are constituents of a sheet of cells, joined to one another by tight and intermediate junctions and resting upon a basal lamina. Although they lack axons and dendrites, the characteristic processes of neurons, hair cells make synapses onto afferent nerve fibers of the eighth cranial nerve and also receive efferent synaptic contacts from axons originating in the brainstem.

The unique structural feature of the hair cell is the hair bundle, an elegant assemblage of microscopic processes protruding from the cell's top or apical surface (Figs. 1 and 2). Each of these processes, which are termed stereocilia, consists of a straight rod of fasciculated actin filaments surrounded by a tube of membrane. Because the microfilaments are extensively cross-bridged (*1*), each stereocilium behaves as a rigid rod. When mechanically disturbed, it remains relatively straight along its length but pivots about a flexible basal insertion (*2*).

Hair bundles occur in a variety of sizes and shapes (*3*); the functional significance of this diversity of form will be considered below. They range in length from 0.8 μm in the bat's cochlea to about 50 μm in certain vestibular organs. Their constituent processes, termed stereocilia, number from 30 in the cochleas of some lizards to more than 300 in the chick's ear; stereociliary diameters range from 0.1 μm in the cochleas of mammals to 1 μm in those of lizards. Certain features of the hair bundle are universal, however (*4*); stereocilia uniformly lie in a hexagonal array and always increase in length from one edge of the hair bundle to that opposite.

Despite the variety of stimuli to which hair cells respond in various organs, including sound in the cochlea, linear acceleration and ground-borne vibration in the utriculus and sacculus, angular acceleration in the semicircular canals, and water motion in lateral-line organs, every hair cell is evidently sensitive to the same proximal stimulus. The hair cell is a mechanoreceptor; it produces an electrical signal, or receptor potential, in response to mechanical stimulation of its hair bundle.

Hair bundles are deflected in two distinct manners. In many organs, the bundles protrude unencumbered into the surrounding fluid. When the fluid moves in response to sound, the bundles are bent by the force of viscous drag, thereby initiating a response. Under these circumstances, the physical properties of the hair bundles are of paramount importance in determining which stimuli are effective. The freestanding hair bundles of lizards (Fig. 2D) exemplify this structural pattern; the inner hair cells of the mammalian cochlea (Fig. 2C) are probably stimulated in a similiar manner. In other organs, each hair bundle is ligated at or near the distal tip of its tall edge to an extracellular accessory structure.

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Stimuli are then initially transmitted to the accessory structure—a tectorial membrane, otolithic membrane, or cupula—that in turn deflects the attached hair bundles. Although the hair bundles may exert a substantial influence on the activities of such organs, the properties of the accessory structures undoubtedly play important roles in determining the organs' sensitivities. Hair bundles of the sacculus (Figs. 1A and 2, A and B) and the outer hair cells of the mammalian cochlea typify this arrangement.

Because naturally occurring sounds are generally not pure tones, auditory receptor organs not only must detect acoustical stimuli but also must analyze them into constituent frequencies as a first step in the stimulus-recognition process. Accordingly, hair cells in many organs are tuned; that is, they are selective for particular frequencies of mechanical stimulation. The restricted responsiveness of hair cells to specific frequencies also serves a second purpose; by limiting its sensitivity to a narrow band of frequencies, a cell can reject noise components outside this range. Frequency tuning is therefore not only a means of analyzing complex signals but also a strategy for noise reduction.

Before considering how hair cells translate mechanical inputs into electrical outputs, I shall describe how stimuli reach the cells and how hair bundles influence cellular responses. I shall consider stimulation and frequency tuning in organs representative of both the freestanding and the assisted forms of stimulation, the primitive cochlea of the lizard and the complex mammalian cochlea.

Mechanical Tuning at the Cellular Level

The observation that various organs of the inner ear possess a wide variety of hair bundles, but that each bundle in a given part of an organ tends to be stereotyped in form, suggests that variations in hair-bundle morphology are of some functional significance. The role of hair bundles in determining a cell's responsiveness is most clearly illustrated by hair cells with freestanding bundles that do not contact any accessory structure. The activity of such hair cells must be dominated by the physical properties of the hair bundles: their dimensions, especially their lengths, their masses, and their hydrodynamic drag in the surrounding fluid.

Within the cochleas of many lizards, hair bundles vary severalfold in length in a systematic manner. In the alligator lizard, for example, the bundles are shortest at the basal end of the organ; they progressively grow longer toward the organ's center until they are nearly threefold as long as at the base (*5*). Electrophysiological investigations indicate that the frequencies to which hair cells are most sensitive, their characteristic frequencies, vary inversely with the lengths of the hair bundles; cells with short bundles are tuned to frequencies near 4 kHz, whereas cells with the longest bundles are tuned to frequencies near 1 kHz (*6*).

When the lizard's cochlea is stimulated at various frequencies and observed microscopically under stroboscopic illumination, it is apparent that hair bundles of different heights do not move equivalently (*7*). Low-frequency stimuli extensively deflect the long, massive bundles at the organ's center, while the short, comparatively stiff bundles remain relatively undisturbed (Fig. 3, B and C). High-frequency stimuli, conversely, bend the short hair bundles to the exclusion of long ones. Although the hydrodynamic behavior of a hair bundle is quite complex, mathematical models suggest that the observed tuning may be accounted for by the bundle's physical properties (*8*).

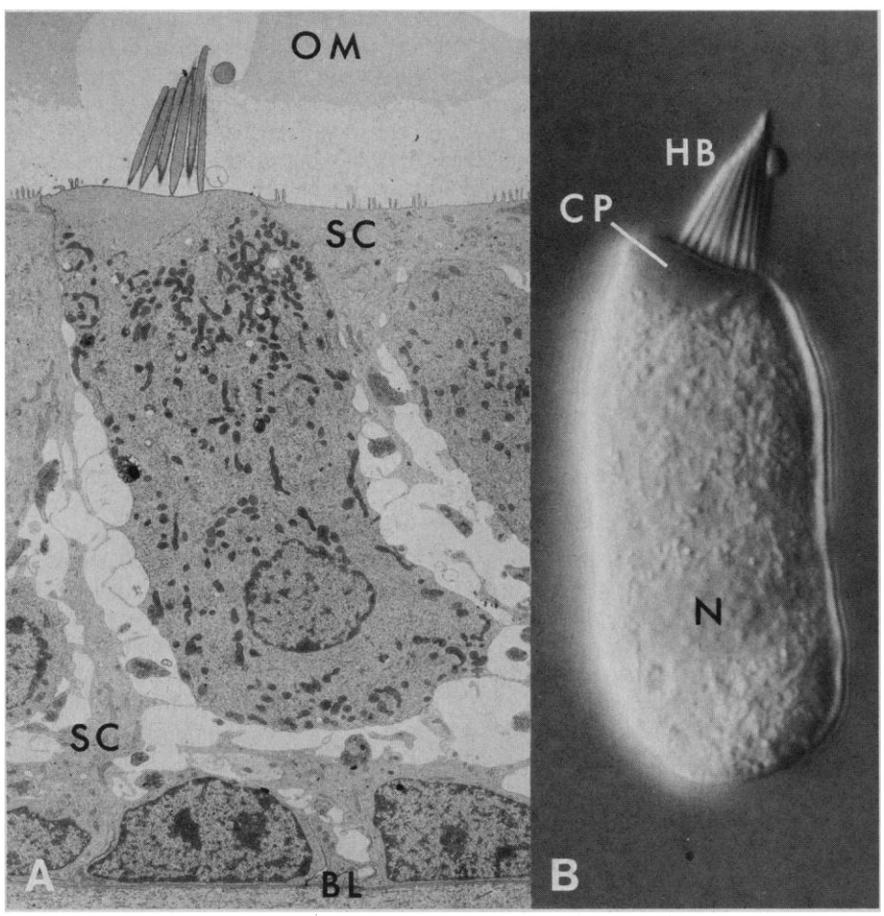

Fig. 1. (A) A transmission electron micrograph of a typical hair cell in its normal environment. The hair cells and adjacent supporting cells (SC) from the bullfrog's sacculus form an epithelial sheet resting upon the basal lamina (BL). The hair bundle extends from the apical surface of the hair cell to contact the otolithic membrane (OM), an accessory structure that conveys mechanical stimuli to the bundle. (B) A light micrograph of a living hair cell enzymatically isolated from the sacculus. The cell body is cylindrical; afferent and efferent synaptic contacts occur basal to the nucleus (N). The hair bundle (HB) protrudes from the fibrous cuticular plate (CP) at the cell's apex (magnification ×2300; corrected for shrinkage during histological processing).

Mechanical Tuning in the Mammalian Cochlea

While the structure of the mammal's cochlea is extraordinarily complex, this organ operates in a manner that is fundamentally simple. Sound, which consists of a pattern of pressure changes at the eardrum, is mechanically conducted through the chain of bones within the middle ear. The last of these three bones, the stapes, is mounted like a piston in contact with fluid within the cochlea. As the stapes moves back and forth in re-

sponse to stimulation, it transmits pressure changes into the cochlear fluids.

Although it superficially resembles a snail and derives its name from the Greek word for that mollusk, the cochlea actually consists of three fluid-filled chambers. These tubes are separated from one another by two elastic partitions and are helically coiled, one atop another, about a common axis. When the stapes compresses the fluid within one chamber, the basilar membrane, one of the partitions between cochlear chambers, is deflected. Even when stimulated with as simple a sound as a pure tone, the basilar membrane moves in a complex manner. Because the dimensions and mechanical properties of the membrane vary from its base to its apex, it does not act like a homogeneous string on a plucked musical instrument. Instead, the basilar membrane develops a traveling wave in a region along its length that depends upon the stimulus frequency: low frequencies, down to 20 Hz in humans, excite motions near the apex of the cochlea, whereas high frequencies, up to 20 kHz in humans, deflect the basal parts of the partition.

On the human basilar membrane rests the organ of Corti, a 34-mm-long helical structure containing, among other cell types, some 16,000 hair cells in four parallel rows (Fig. 3D). Each hair cell has about 100 stereocilia, so there are over a million of these receptive organelles in an ear. Because each frequency moves a specific zone of the basilar membrane with the greatest amplitude, it follows that any given tone influences a particular group of hair cells most strongly. One of the cochlea's principal virtues ensues from this arrangement: the basilar membrane functions as a spectral analyzer, decomposing a complex sound—the human voice, for example—into its pure tonal constituents. It is left for the hair cells that receive information about a particular tonal input somehow to transduce the mechanical motions of the basilar membrane into electrical signals that are suitable for analysis by the nervous system.

When driven up and down by acoustical stimuli, the basilar membrane carries with it hair cells of the organ of Corti. The distal ends of the hair bundles of the outer hair cells are attached to the tectorial membrane, a gelatinous shelf of protein that spirals along the entire extent of the organ of Corti. As each hair cell moves, a shear develops between its top surface and the lower surface of the tectorial membrane; this shear bends the hair bundles that extend across the intervening space (Fig. 3E).

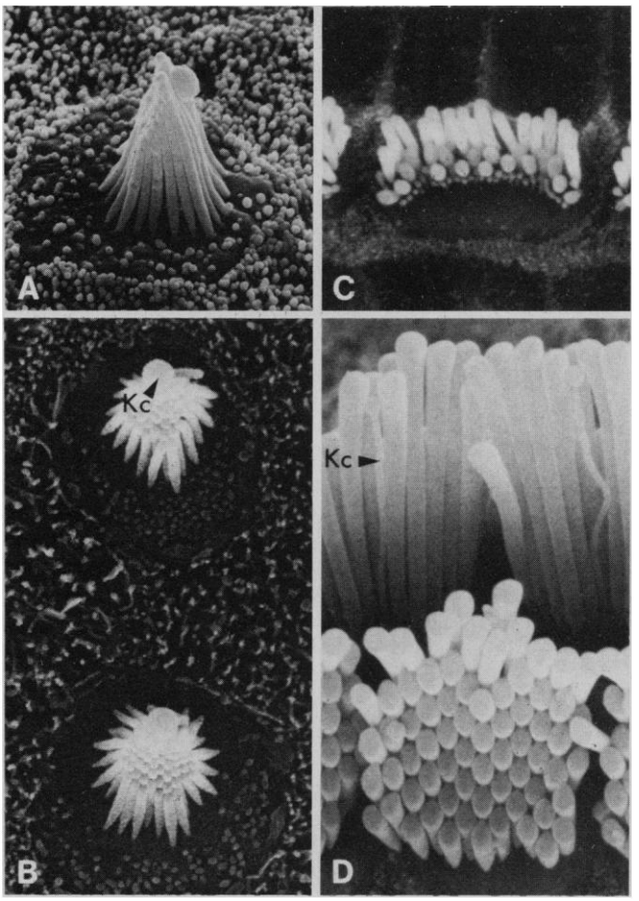

Fig. 2. A variety of hair bundles as demonstrated by scanning electron microscopy. (A) The general structural features of the hair bundle in a lateral view of a bundle from the bullfrog's sacculus. The bundle comprises about 60 hypertrophied microvilli, called stereocilia, whose lengths increase progressively from the bundle's left to its right edge. A single true cilium, the kinocilium, occurs at the tall edge of the bundle; its distal tip, which is normally attached to the otolithic membrane that provides mechanical input to the bundle, has a bulbous swelling. (B) A top view of two hair cells from the same organ, revealing the regular packing of the stereocilia and the eccentric location of the kinocilium (Kc). (C) The bundle of an inner hair cell from the cat. Here the stereocilia form a palisade rather than a compact cluster. (D) Top view of a hair bundle from the fence lizard (*Scelóporus jarrovi*) with very large stereocilia in a compact hexagonal array. Two other bundles, seen *en face* in the background, display kinocilia (Kc) at their long edges. The four micrographs are shown at the same magnification (×4300) to illustrate the morphological diversity of hair bundles from various organs. The largest stereocilia of the lizard's cells are over 30 μm long and 0.9 μm in diameter, whereas the cat's hair bundle is just 2 μm tall and its smallest stereocilia measure only 0.1 μm across.

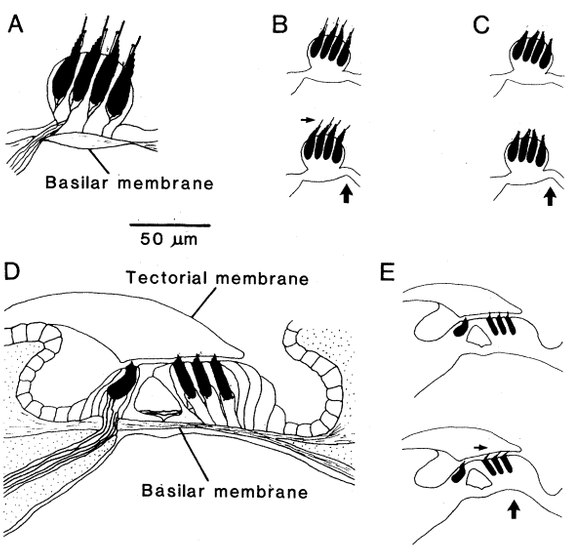

Fig. 3. Structures and operations of representative cochleas. Hair cells are shown solid black. (A) A schematic cross-section through the cochlea of the alligator lizard (*Gerrhonotus multicarinatus*), which contains the organ's longest freestanding hair bundles. (B) When the lizard's ear is stimulated by low-frequency sound, differences in fluid pressure across the basilar membrane cause it to move vertically. As the hair cells upon the basilar membrane sway laterally, the inertia and hydrodynamic resistance of the hair bundles cause them to be bent; this deflection in turn excites the hair cells. (C) Because of their lesser inertia and drag, the short hair bundles at the organ's opposite end do not respond to low-frequency stimulation. (D) The organ of Corti of the cat, shown schematically. The organ possesses four rows of hair cells whose hair bundles are surmounted by a gelatinous tectorial membrane. (E) When sound pressure deviates the basilar membrane, the pivoting of the organ of Corti causes a shear between it and the tectorial membrane. The hair bundles of the three rows of outer hair cells are inserted into the tectorial membrane and are deflected directly by this shear. The bundles of the inner hair cells are probably not attached to the tectorial membrane, but it is thought that they are bent by fluid moving between the tectorial membrane and the underlying hair cells.

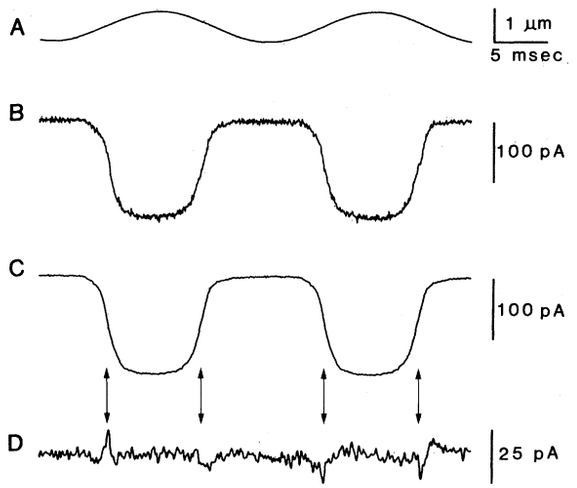

Fig. 4. Transduction current and the associated noise. When the hair bundle's distal tip is moved back and forth sinusoidally (A), a voltage-clamp amplifier measures the flow of transduction current [in picoamperes (pA)] into the cell (B). Positive stimuli [upward deflections in (A)] open transduction channels, allowing an inward flow of positive ions [downward deflections in (B)]. The trace in (B) is noisy; the level of noise, which is in excess of that due to instrumentation, reflects the random gating of transduction channels. When the responses to 136 repetitions of the same stimulus are averaged (C), the transduction-associated noise largely vanishes. Subtraction of the mean record in (C) from an individual trace such as that in (B) demonstrates, at a higher gain, the noise due to the random opening and closing of transduction channels (D). The noise is minimal when most channels are closed and the mean current is most positive and also when most channels are open and the mean current is most negative. The greatest noise occurs when half the channels are open (arrows). Analysis of the data from this cell indicates the presence of 160 transduction channels, each with a conductance of 12 pS at 10°C.

Having seen what stimuli reach representative hair cells, we may formulate a number of specific questions about the operation of this cell type. How does the hair cell convert a mechanical input into an electrical output? How can the ear reliably measure mechanical stimuli whose average amplitudes at threshold approximate the diameter of a hydrogen atom? How can one explain a sensory system that can measure events that occur 20 times more frequently than nerve fibers can signal? How does a hair cell contribute to the frequency selectivity of the internal ear?

Mechanoelectrical Transduction

Although it has been possible to learn a considerable amount about the operation of hair cells in the mammalian cochlea, a more nearly complete picture of the transduction process has emerged from in vitro investigations of hair cells from lower vertebrates. One valuable experimental preparation is that of the bullfrog's sacculus, an organ containing large hair cells of typical structure and great hardiness during physiological recording. I shall discuss below some of the details about transduction that have emerged from study of the sacculus and then assimilate them in a general model for transduction by vertebrate hair cells.

When a saccular hair cell is stimulated by deflecting the tip of its hair bundle with a glass probe, the membrane potential deviates from its resting value of approximately −60 mV. Stimuli directed toward the tall edge of the hair bundle—the edge at which there also occurs a single true cilium, the kinocilium—produce depolarizations of up to 20 mV. Motion in the opposite direction elicits hyperpolarizations about a quarter as large (*9*).

If the bundle is deflected at right angles to its plane of morphological symmetry, the membrane potential does not change at all (*10*). The hair cell's responsiveness is highly directional. It can decompose an arbitrarily directed stimulus to its hair bundle into two components and respond in a graded fashion to the component along its axis of symmetry while disregarding the perpendicular component altogether.

As is the case in neurons, the electrical signals in the hair cell originate from the flow of ionic currents across the membrane. This may be demonstrated by voltage-clamping a hair cell so that its transmembrane current is measured directly (Fig. 4, A and B). When the hair bundle is moved in the positive direction (toward its taller edge) the membrane conductance increases; that is, the membrane becomes more permeable to positively charged ions (*9*). This behavior suggests the existence of transduction channels, transmembrane pores through which ions can pass when the hair bundle is appropriately stimulated.

It is possible to use electrophysiological techniques to estimate how many transduction channels occur in each hair cell. The basis of this measurement is shown in Fig. 4. When a cell's hair bundle is moved back and forth sinusoidally with a stimulating probe, transduction current flows into the cell in a cyclical pattern. Because each of the transduction channels in the membrane operates independently of the others, however, each cycle of the response is not exactly the same as any other. Each time the stimulus is repeated, a slightly different pattern of activation occurs, resulting in fluctuations (noise) in the response. This noise may be quantified and analyzed on the basis of particular models for transduction. If each channel has the same ionic conductance when open and zero conductance when closed, the variance, or square of the measured noise, is related parabolically to the mean transduction current (*11*). Measurement of both the average transduction current and the noise from trace to trace leads to estimates for the number and conductance of the individual channels (*12*).

In the frog's sacculus, there are only about 280 transduction channels per hair cell, fewer than half a dozen for each stereocilium (*13*). This makes the isolation and biochemical characterization of transduction channels a rather bleak prospect, for there seem to be a millionfold fewer transduction molecules per hair cell than there are, for example, rhodopsin molecules in each rod photoreceptor.

Despite the paucity of transduction channels, it is possible to obtain some information about their chemical nature by examining their ability to "catalyze" the movement of ions across the membrane. Voltage-clamp measurements after substitution of various ions in the fluid bathing the hair bundle indicate which ions can traverse the transduction channel. The cation normally present in the highest concentration in the fluid, K^+, readily passes throught the channel; so do the other alkali ions: Li^+, Na^+, Rb^+, and Cs^+. Divalent cations, such as Ca^{2+}, Sr^{2+}, and Ba^{2+}, are still more permeant (*14*, *15*). Even small organic cations, such as choline, tetramethylammonium, and tris(hydroxymethyl)aminomethane, can carry transduction current. The fact that such ionic species can traverse the transduction channel, while slightly larger ones cannot, suggests that the bore of the channel is about 0.7 nm (*14*). The channel's preference for cations over anions indicates that the amino-acid residues exposed on its inner surface bear negative charges. Finally, the relatively poor discrimination among cations implies that there is not a close fit between the ions and the groups lining the channel. The ions are probably moving in hydrated form through a relatively

large pore. Variance measurements indicate that the hair cell's transduction channel has a conductance of 17 picosiemens (pS) at room temperature (*13*), a value in line with the pore's meager ionic selectivity and hydrated nature.

Because the chemical identity of the transduction channel is not yet known, there is at present no way of localizing the channel by biochemical means, for example through the use of antibodies. The flow of ionic current into transduction channels, however, provides a means of inferring with moderate spatial resolution where they occur. As ionic current streams toward the transduction channels, its flow across the resistance afforded by the saline solution around the hair bundle produces minute potential changes. Although these variations in voltage are less than 15 μV in amplitude, they can be detected and their spatial distribution mapped with suitable electrodes (*16*). Rather surprisingly, the strongest electrical signal, that indicative of the site of transduction, does not occur at the bases of stereocilia, where they bend during mechanical stimulation. Instead, the maximum response is found at the top of the hair bundle, a result suggesting that the transduction channels occur at or near the distal tips of the stereocilia.

A striking feature of the transduction process of vertebrate hair cells is its rapidity. Humans can detect stimuli at frequencies as great as 20 kHz, and bats and whales can hear sounds of five- to tenfold higher frequency. Channel opening in hair cells is so rapid that it is difficult to resolve at room temperature. At an experimental temperature of 28°C, channels begin to carry current within a few microseconds of the application of a stimulus (*17*). The response, moreover, becomes faster as the amplitude of stimulation increases; saturating stimuli evoke responses that peak within 100 μsec. The dependence of response kinetics on stimulus amplitude is intriguing, for such behavior suggests that mechanical stimuli somehow affect the rate constants for the reactions of channel opening and closing.

A Model for Mechanoelectrical Transduction

How are the hair cell's transduction channels gated; that is, how are their opening and closing controlled by mechanical stimuli? The localization of transduction to the hair bundle's top, together with transduction's being too rapid to permit the intervention of second messengers, suggests that some interaction among the stereocilia initiates the response. The kinetic evidence led to the suggestion that displacement of the stereocilia exerts tensile force upon a linkage connected to each transduction channel (*17*, *18*). Since that model was put forward, anatomical experiments have supported it by demonstrating a morphological candidate for the hypothetical linkage. When hair cells are appropriately prepared for electron microscopy, the tip of each stereocilium displays a fine filament linking it to the flank of the adjacent longer stereocilium along the hair bundle's axis of symmetry (*19*). This filament is admirably positioned to report shear between adjacent stereocilia. Its alignment along the cell's axis of symmetry also explains the directional sensitivity of transduction in hair cells. Although the kinetic model does not depend on a particular site for the transduction linkage, it is tempting to believe that the linkage has now been observed.

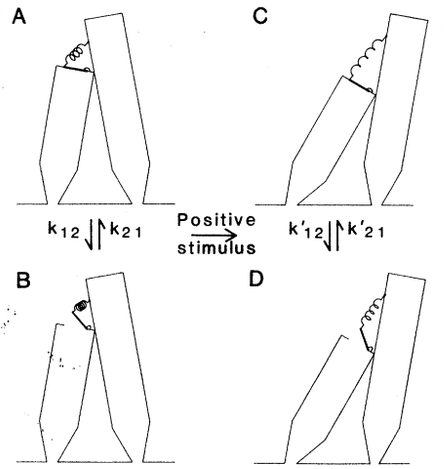

Fig. 5. A model for mechanoelectrical transduction by hair cells. At any instant, each transduction channel at a stereocilium's tip may be either closed (A) or open (B). The relative values of the rate constants for channel opening and closing, k_{12} and k_{21}, respectively, determine the fraction of the transduction channels open in the undisturbed steady state. When the hair bundle is deflected with a positive stimulus (C and D), the values of the rate constants are altered; the opening rate constant (k'_{12}) is larger and the closing rate constant (k'_{21}) smaller than the original values. The new steady-state transduction current is therefore greater, and the cell is depolarized. Pushing the hair bundle in the opposite direction has a contrary effect on the rate constants, culminating in a hyperpolarizing response.

The essential features of the model are shown in Fig. 5. At or near the tip of each stereocilium lie one to a few transduction channels, each equipped with a gate that regulates the flow of ionic current into the cell. When the hair bundle is in its resting position, each gate swings back and forth between its closed and open configurations (Fig. 5, A and B, respectively) under the influence of a constant thermal buffeting by surrounding molecules. Depending on the strength of its intrinsic "door-closing" spring, each channel will, on average, spend a certain fraction of its time open in the absence of stimulation; experiments indicate that this fraction is about 20 percent.

When a stimulus pushes the tip of the hair bundle in the positive direction, the stereocilia in successive ranks slide along one another. The transduction linkage connecting each channel to the adjacent stereocilium is therefore presumably stretched. A given channel continues to fluctuate between its closed and open states (Fig. 5, C and D, respectively). Now, however, the additional force exerted upon the channel's gate by the elongated transduction linkage causes the channel to spend more of its time open. As a result, a larger average current flows through each channel, and the cell is depolarized.

Although somewhat abstract, the model includes the requisite properties for a description of transduction by hair cells. Because the channels rattle between the open and closed states with little in the way of an intervening energy barrier, they are capable of responding to an imposed stimulus with the great rapidity characteristic of auditory transduction. The kinetic data indicate that the interstate barrier is 0.8 kcal/mol in the bullfrog (*17*); in the absence of stimulation, a channel opens and closes over a thousand times a second. Because the response is probabilistic, it is capable, when averaged over a sufficiently long period, of providing information about stimuli of the atomic dimensions associated with the auditory threshold. The model is in good quantitative agreement with some experimental measurements (*20*). The steady-state probability of a channel's being open, for example, nicely fits the predicted Boltzmann relationship (*13*). Although detailed experiments remain to be done on single cells, the model also qualitatively predicts the hair cell's response kinetics (*17*).

Absolute Sensitivity of the Transduction Process

The primary function of any sensory receptor cell is the detection of some particular form of stimulus energy. It is of considerable importance to the organism that a receptor be capable of reli-

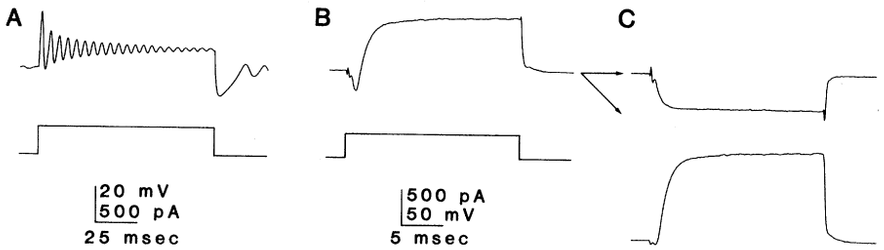

Fig. 6. Electrical resonance and its ionic basis in the bullfrog's saccular hair cell. When a single cell is stimulated by the intracellular injection of current (A, lower trace), the membrane potential displays a damped, sinusoidal resonance at a frequency of 203 Hz (A, upper trace). This is the frequency at which the cell would be most sensitive upon mechanical stimulation of its hair bundle. When the same cell is depolarized from −85 mV to −40 mV under voltage-clamp conditions (B, lower trace), the ionic current that flows across its membrane is biphasic (B, upper trace); there is an early, inward component (downward deflection) followed, after a delay, by a larger, outward component (upward deflection). If the same cell is exposed to 10 m*M* tetraethylammonium ion, the late component is blocked, unmasking a voltage-sensitive Ca^{2+} current that persists throughout the depolarizing stimulus (C, upper trace). Subtraction of the Ca^{2+} current from the total current isolates the Ca^{2+}-sensitive K^+ current (C, lower trace).

ably detecting very low levels of stimulation. It is desirable, in fact, for a receptor's sensitivity to be limited only by physical constraints on the stimulus rather than by inefficiency or noise in the receptor itself. Rod photoreceptors, for example, are able to detect, with a modest error rate, the capture of individual photons. These detectors could not be much more efficient. Their absolute sensitivity is limited by the quantum nature of light. How well does a hair cell perform in this regard? In the absence of a stimulus of quantum nature, what limits the sensitivity of this receptor?

The rod photoreceptor provides a revealing comparison with the hair cell in regard to absolute sensitivity. The initial step in phototransduction, the light-induced isomerization of retinal, proceeds through an activated intermediate whose free energy exceeds that of the starting material by about 30 kcal/mol. A photon imparts a free-energy change of about 57 kcal/mol upon absorption, an energy so much greater than that of the activated intermediate that isomerization is highly efficient. Of equal importance, however, is the relationship between isomerization energy and thermal energy. At room temperature, the average thermal energy imparted to each degree of freedom in a rhodopsin molecule is only 0.3 kcal/mol. This is so much less than the energy of the activated complex that spontaneous isomerizations are very rare; in agreement with calculations, they occur every millenium per molecule. For a photoreceptor, even one with over a billion rhodopsin molecules, spontaneous signals are therefore rather infrequent, occurring only once a minute (*21*).

The hair cell is less fortunate, for the stimulus energies with which this receptor cell must reckon are far smaller. When one estimates the energy imparted to the auditory or the vestibular system at the human behavioral threshold, in fact, it appears that the energy supplied to each hair cell is of the same order of magnitude as that of thermal motion (*22*). Although the point has not been demonstrated experimentally at the cellular level, it appears that the sensitivity limit in hair cells is set by Brownian motion—we may be able to hear everything "louder" than the molecular motions within the ear (*23*).

As a result of the nature of acoustical stimuli, the hair cell is probably able to do somewhat better than one might think possible at detecting faint signals. Most natural sounds have durations that are many times as long as the periods of their constituent sinusoidal frequencies. Because a hair cell attending to a particular frequency generally receives many cycles of the stimulus, it can in effect average its input. Random noise, in this case due to the transduction of Brownian motion, has a diminishing effect as a hair cell averages its input over an increasing period of time. It was suggested recently that the limit on auditory sensitivity is actually set by quantum indeterminancy (*24*); it remains to be seen whether this provocative notion can be substantiated.

The photoreceptor, then, has evolved for the reliable detection of a single, brief, high-energy input in a low-noise environment. The hair cell, by contrast, specializes in the measurement of prolonged input of low energy compared with the background noise.

Electrical Tuning

The traveling-wave phenomenon doubtlessly constitutes the principal means by which the mammalian cochlea discriminates among stimulus frequencies, whereas the mechanical properties of free-standing hair bundles dominate the responsiveness of some simpler cochleas. These mechanical strategies, however, are not the only means by which tuning may be accomplished. In the internal ears of some lower vertebrates, substantial tuning occurs after mechanoelectrical transduction has taken place, through the action of an electrical resonance in the hair cell's membrane.

When a hair cell of the turtle's cochlea or the frog's sacculus is stimulated acoustically or mechanically, it responds best at some particular frequency, its characteristic frequency (*25, 26*). If the cell is instead stimulated by injection of a constant-current pulse across its membrane, the membrane potential does not settle exponentially, as is the case with most neurons and other cells, but instead manifests a damped, sinusoidal oscillation at the cell's characteristic frequency (Fig. 6A).

How does this membrane resonance arise? My research group has investigated the problem in hair cells of the bullfrog's sacculus, an organ dedicated to the detection of ground-borne vibration at frequencies below 300 Hz (*27*). Hair cells from this preparation may be enzymatically isolated so that whole-cell recordings may be made from them with gigaohm-seal microelectrodes (*26*). When a voltage-clamped cell is depolarized, the resulting transmembrane current is biphasic (Fig. 6B). The initial component represents the flow of positive current into the cell, and the larger, later component indicates the delayed flow of positive charge out of the cell. Substitution of various ions in the experimental solution demonstrates that the early, inward current is carried by Ca^{2+} (Fig. 6C). The second, delayed component of the response is carried by K^+. Unlike the Ca^{2+} channels, or for that matter the K^+ channels associated with the action potential in nerve fibers, the K^+ channels involved in resonant tuning are relatively insensitive to membrane potential. The gating of the K^+ channels is instead primarily influenced by the intracellular concentration of Ca^{2+}. Calcium-sensitive K^+ channels have been demonstrated in a variety of neuronal cell types, where they characteristically serve to modulate electrical excitability (*28*). In the hair cell, these channels have been pressed into service in a more rapid and more complex process.

The way electrical resonance occurs

can be readily visualized in qualitative terms (Fig. 7). Stimulation of the hair bundle depolarizes the cell directly, by opening transduction channels, and through the regenerative activation of Ca^{2+} channels. The accumulation of Ca^{2+} intracellularly, however, opens Ca^{2+}-sensitive K^+ channels that act to repolarize the membrane. The resonance arises from the interplay of inward Ca^{2+} currents and outward K^+ currents. The process somewhat resembles the production of action potentials by the sequential activation of Na^+ and K^+ channels, with the striking difference that an action potential is of a stereotyped amplitude, whereas the resonance in hair cells is graded with the intensity of the signal that initiates it.

At least in hair cells of the frog, our understanding of the electrical resonance is rather complete. The various ionic currents may be modeled on the basis of their behavior during voltage-clamp experiments on whole cells or on excised patches of membrane. Solutions of the resultant equations (*29*) closely resemble actual experimental records (*30*). A tantalizing problem remains, however: how do individual hair cells become tuned to the proper frequencies? During each cell's development, it somehow acquires an electrical resonance that makes the cell most sensitive at a particular frequency of stimulation. One can imagine many ways in which this could come about: by regulation of the number of Ca^{2+} or K^+ channels, by adjustment of the kinetics of channel gating, by changes in membrane capacitance, by regulation of the cytoplasmic Ca^{2+}-buffering capacity, or by control of the rate at which intracellular Ca^{2+} is removed. There are as yet insufficient recordings, however, to reveal which of these or other possible parameters sets the tuning frequency. Still further in the future lies an understanding how this tuning is established, whether it is wholly programmed or is adjusted on the basis of experience.

Conclusion

Despite the considerable recent growth in our understanding of the auditory system, its performance continues to exceed our ability to account for it, even taking into account the possibilities of mechanical and electrical tuning at the cellular level. It appeared for a time that the remarkable performance of the cochlea could be explained by positing a "second filter" interposed between initial frequency analysis on the basilar membrane and transduction by hair cells. More refined measurements, however, recently demonstrated that sharp frequency discrimination with high sensitivity occurs equally in basilar-membrane motion (*31*) and in hair-cell receptor potentials (*32*). This implies that the mechanical performance of the basilar membrane somehow exceeds the predictions from simple hydrodynamic modeling. Models can be made to agree reasonably well with the data, however, by incorporating into them negative damping of the basilar membrane (*33*)—by assuming, in other words, that under some circumstances the organ of Corti can supply energy to the basilar membrane's motion rather than passively dissipate it.

During the period when the sharp frequency tuning of basilar-membrane motion was being measured, other unexpected evidence pointed toward the existence of an active mechanical process in the cochlea. When Kemp (*34*) recorded the pressure near the eardrum with sensitive instrumentation, he found that presenting a click stimulus to some human ears evoked the transitory emission of one or more bursts of pure tones. This evoked auditory emission is not simply an acoustical echo: the tones emerge much later than anticipated for the passive conduction of sound through the cochlea and back to the recording apparatus. Both the frequency tuning of hair cells and the mechanical impedance of the ear are affected by stimulating the efferent nerve supply to the cochlea (*35*). Because the efferent fibers largely terminate on outer hair cells, these cells are presently considered the most likely site of mechanical changes and of emissions. Even more striking manifestations of mechanical activity within the cochlea are ears that spontaneously emit pure tones (*36*). The existence of an animal whose ear produces 59-dB sound (*37*) is a potent argument that cochlear mechanical activity is more than a modeler's abstraction.

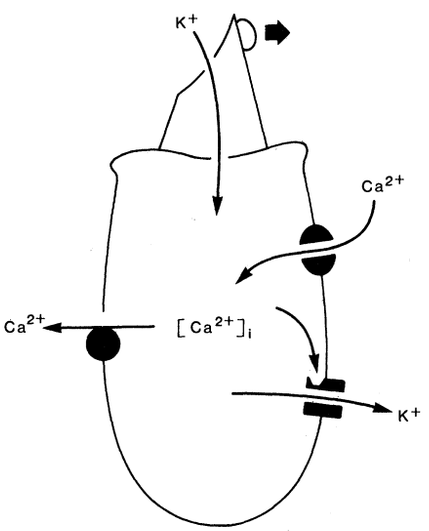

Fig. 7. A model for electrical resonance in hair cells. When the hair bundle is deflected, transduction channels open and positive ions, largely K^+ in vivo, enter the cell. The depolarization evoked by this transduction current activates voltage-sensitive Ca^{2+} channels. As Ca^{2+} ions flow into the cell, they augment the depolarization. At the same time, however, the influx of Ca^{2+} raises the intracellular concentration of this ion, $[Ca^{2+}]_i$, especially the local concentration just beneath the surface membrane. The high Ca^{2+} concentration brings into play the Ca^{2+}-sensitive K^+ channels. As K^+ exits through these pores, it begins to repolarize the membrane, thereby diminishing the activation of Ca^{2+} channels. The fluid bathing the apical surface of a hair cell characteristically has a much higher K^+ concentration than that contacting the basolateral cellular surface. As a consequence, K^+ can both enter and leave the cell passively. By the time the membrane potential is somewhat more negative than its steady-state value, the intracellular Ca^{2+} concentration is reduced by the sequestering of the ion within organelles and by its extrusion through ion pumps. As the Ca^{2+}-sensitive K^+ channels close, the cell returns to approximately its initial condition, and another cycle of the electrical resonance commences.

How can the evidence for active mechanical processes in the cochlea be reconciled with the cochlea's high sensitivity and sharp frequency selectivity? The widespread belief at present is that outer hair cells are capable of some form of motility and that, when appropriately stimulated, they move in a manner that amplifies the incoming mechanical stimulus. According to this model, the basilar membrane's motion is unexpectedly sensitive and frequency-selective because it is governed not only by passive hydrodynamics but also by an active contribution from the organ of Corti (*38*). This mechanical activity would, of course, supply the negative damping of cochlea models. The suggested process, a form of positive feedback, might be mediated through electrical resonance (*39*). Excessive gain in such feedback presumably produces the oscillations of evoked and spontaneous auditory emissions.

A central goal of current auditory research is the elucidation of the cellular and molecular bases for the active process in the organ of Corti. If the present models are correct, the contribution of this active process must be made on a cycle-by-cycle basis. In other words, the activity must occur every few microseconds or tens of microseconds to facilitate high-frequency hearing. Two active processes have been observed in vitro. Outer hair cells from the organ of Corti are

capable of reversible contractions, but to date these have been demonstrated to occur on a time scale of seconds to minutes, not of microseconds (*40*). The hair bundles of the turtle's cochlea display oscillatory motions in phase with their electrical resonance; as yet, however, the observations extend to a frequency of only 171 Hz (*41*). Because the mechanism of motility is unknown in both instances, it remains to be seen whether either type of movement can account for sharp, high-frequency tuning and, therefore, whether a process that explains cochlear activity is now in hand.

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12. By the two-conductance-state model, the mean current through N transduction channels is:
$$\bar{I} = N\gamma V_m p_o$$
in which p_o is the probability of any channel's being open. γ is the single-channel conductance, and V_m is the membrane potential. The variance in the transduction current is:
$$\sigma^2 = N\gamma^2 V_m^2 p_o(1 - p_o)$$
Eliminating p_o from these formulas yields:
$$\sigma^2 = \gamma V_m \bar{I} - \bar{I}^2/N$$
Fitting experimental data to this parabolic relation allows estimation of N and γ.
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20. The rate constants for channel opening and closing are:
$$k_{12} = (kT/h)e^{-[G_{12} + \delta(G_1 - Zx)]/RT}$$
$$k_{21} = (kT/h)e^{-[G_{12} - (1 - \delta)(G_1 - Zx)]/RT}$$
G_{12} is the height of the energy barrier between the closed and open states and G_1 is their intrinsic free-energy difference, represented in Fig. 5 by the "door-closing" spring on the transduction channel's gate. When the hair bundle's tip is deflected by a distance x, the transduction linkage is stretched by a fraction δ of this displacement; the resultant increment in the free-energy barrier between the channel's two states is proportional to the sensitivity Z, which is approximately 6 kcal/mol-μm. The other parameters have their usual thermodynamic meanings: k and h are, respectively, Boltzmann's and Planck's constants, R the ideal-gas constant, and T the absolute temperature. When the hair bundle is abruptly displaced from one position to another, the transduction current relaxes from one steady-state value to another with a time constant τ, given by:
$$\tau = \frac{1}{k_{12} + k_{21}}$$
In the steady-state condition, the rates of channel opening and closing are equal; the probability p_o that a given channel is open at any instant, or the fraction of the channels that are open, is:
$$p_o = \frac{1}{1 + (k_{21}/k_{12})} = \frac{1}{1 + e^{(G_1 - Zx)/RT}}$$
In agreement with experimental results (*13*), plotting p_o against x produces a symmetrical, saturating, sigmoidal curve.
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29. The Ca^{2+} current fits a third-order exponential relaxation:
$$i_{Ca^{2+}} = (m^3 \cdot \bar{g}_{Ca^{2+}})(V_m - E_{Ca^{2+}})$$
in which $\bar{g}_{Ca^{2+}}$ is the maximal conductance of the Ca^{2+} channels, $E_{Ca^{2+}}$ the associated reversal potential, and m an activation parameter whose rate of change depends exponentially upon the membrane potential, V_m. It is thought that the Ca^{2+} accumulates in a buffered, submembrane volume from which it is removed by a first-order process:
$$d[Ca^{2+}]_i/dt = \alpha i_{Ca^{2+}} - \beta[Ca^{2+}]_i$$
in which α and β are constants. The simplest model that explains the kinetic behavior and the sensitivity of the K^+ channel to Ca^{2+} includes three closed states for the channels, corresponding to the binding of zero, one, or two Ca^{2+} ions, and two open states, corresponding to the binding of two or three ions (*42*). The solution of the differential equations for Ca^{2+}- and K^+-channel gating, together with the simultaneous equations for leakage and capacitive currents, reconstructs the observed electrical resonance.
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