Integrating the active process of hair cells with cochlear function

A. J. Hudspeth

Abstract | Uniquely among human senses, hearing is not simply a passive response to stimulation. Our auditory system is instead enhanced by an active process in cochlear hair cells that amplifies acoustic signals several hundred-fold, sharpens frequency selectivity and broadens the ear's dynamic range. Active motility of the mechanoreceptive hair bundles underlies the active process in amphibians and some reptiles; in mammals, this mechanism operates in conjunction with prestin-based somatic motility. Both individual hair bundles and the cochlea as a whole operate near a dynamical instability, the Hopf bifurcation, which accounts for the cardinal features of the active process.

Bifurcation
An abrupt, qualitative change in the character of a dynamical system in response to a continuous change in the value of a particular variable, the control parameter.

Howard Hughes Medical Institute and Laboratory of Sensory Neuroscience, The Rockefeller University, 1230 York Avenue, New York, New York 10065, USA.
e-mail: hudspaj@rockefeller.edu
doi:10.1038/nrn3786
Published online
6 August 2014

Although hearing is so central to our daily lives that we rarely consider its effectiveness, the performance of the human ear is remarkable in several ways. Our auditory sensitivity is so great that we can detect signals that vibrate the eardrum by about a picometre[1]. Indeed, we can hear sounds down to the level at which they are drowned in the din of thermally agitated water molecules in the cochlea[2]. Although our hearing encompasses frequencies from 20 Hz to 20 kHz, we can distinguish the sources of sounds and the nuances of speech because our frequency resolution extends to one-thirtieth of the interval between successive keys on a piano[3,4]. From the faintest audible sounds to the roar of jet engines, our hearing displays a dynamic range of an astonishing trillion-fold in acoustic power[5]. These qualities emerge because the ear does not simply receive and interpret sound but instead expends metabolic energy to enhance its mechanical inputs. The cardinal features of human hearing stem from this phenomenon, which is termed the active process.

Beginning in the 1980s, investigators began to assimilate several unusual and seemingly unrelated properties of hearing into the concept of an active process with four key characteristics[6–8]. First, a normal ear amplifies its inputs by several hundred-fold. This feature is most apparent when the cochlea is damaged: the loss of amplification renders the victim hard of hearing, with a sensitivity less than 1% of normal. The active process next enhances frequency selectivity, the capacity to discriminate between similar tones. The degraded tuning of a damaged ear explains why a hearing aid so often proves to be unsatisfactory: although the electronic device augments the signals reaching an ear, it cannot compensate for the loss of frequency resolution that is key to the discrimination of speech sounds. The third property of the active process, compressive nonlinearity, implies that the gamut of sound intensities is not represented linearly in the range of responses. Telescoping a million-fold variation in the amplitude of sound into only a hundred-fold range in the ear's response enables the ear to encode an enormously broad range of sound intensities. Last, in a very quiet environment, a normal human cochlea can produce spontaneous otoacoustic emissions, which are tones broadcast from the ear. As epiphenomena of the active process, these signals indicate that amplification by the ear, like that by a public-address system, can become so great that positive feedback evokes oscillations.

Research during the past decade has clarified the cellular basis of the active process, which stems from the interplay between two unusual forms of subcellular motility. These investigations have additionally revealed that the four cardinal aspects of the active process reflect the operation of hair cells near a dynamical instability, the Hopf bifurcation. This article reviews the operation of the cochlea and its constituent hair cells, and then discusses the forms of motility that underlie the active process. The final sections consider the evidence for the operation of the ear's components near a Hopf bifurcation and the consequences of that arrangement.

Transduction in the mammalian cochlea

The receptor for sound in the human ear is the organ of Corti, a strip of sensory epithelium running some 33 mm along the spiral cochlea[9,10]. The key components of the

organ of Corti are hair cells, the sensory receptors of the inner ear. Cochlear hair cells are of two types, each of which has distinct functions in the transduction process[11]. Each of the 3,500 inner hair cells, which are arranged in a single row, forms synapses with an average of ten afferent nerve terminals. The associated axons in turn carry auditory information into the cochlear nucleus of the brainstem. Although the 12,000 outer hair cells in three rows also make sparse contacts on afferent fibres, their principal role is mechanical: outer hair cells mediate the cochlear active process (FIG. 1).

The travelling wave. In the auditory organ of a fish or amphibian, the hair cells occupy an epithelium that is firmly fixed to connective tissue or bone and receive stimuli through movements of the tectorial or otolithic membrane that surmounts the hair bundles. Although this membrane may provide some resolution of stimuli of differing frequencies, tuning is largely accomplished by alternative strategies such as electrical resonance based on the activity of ion channels[12]. In the cochlea of an amniote, by contrast, the substrate beneath the hair cells has been liberated from its attachments to produce a basilar membrane that is capable of mechanical resonance. The elongation of this membrane in mammals has eventuated in a still more complex phenomenon, the cochlear travelling wave, the basis of our extraordinary frequency discrimination.

When a mammalian cochlea receives a pure-tone acoustic stimulus, pressure differences between its liquid-filled compartments set the basilar membrane into oscillation at the frequency of stimulation. The membrane does not move as a unit; instead, successive waves propagate from the base of the cochlea towards its apex (FIG. 1d). At any instant, the elastic membrane bears about three complete cycles of oscillation.

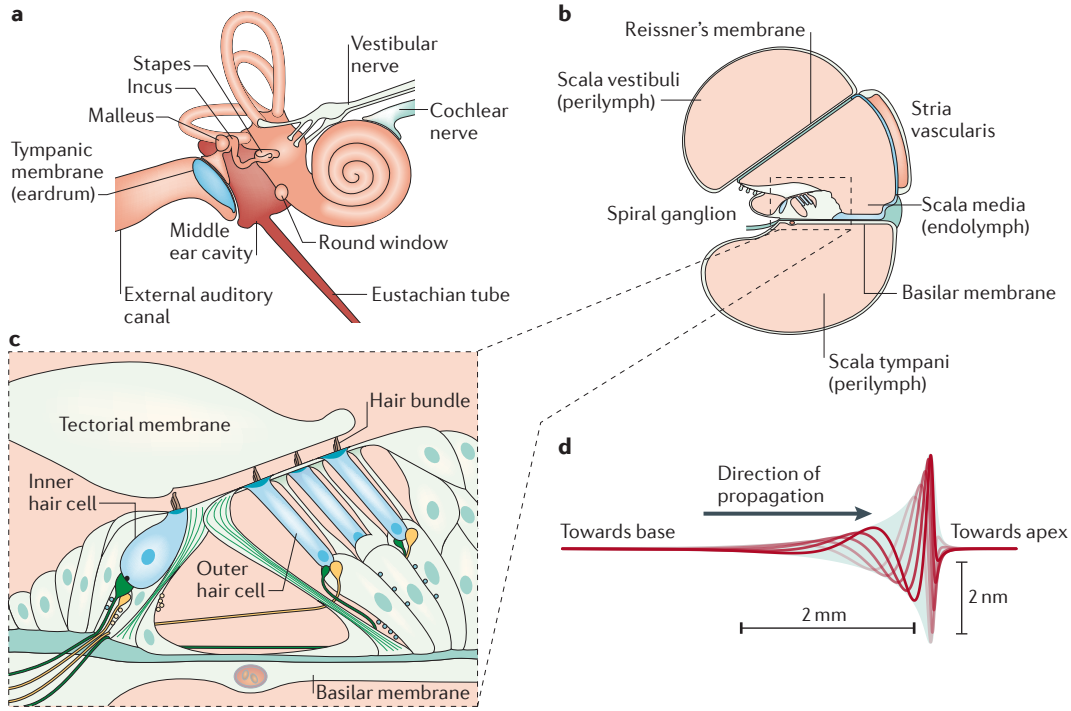


Figure 1 | **The cochlea and organ of Corti.** **a** | The cochlea is a part of the internal ear, which also includes the vestibular apparatus and lies in the temporal bone of the skull. Sounds impinge upon the eardrum, whose vibrations are communicated through the three miniscule bones of the middle ear to initiate oscillatory changes in the pressure within the coiled cochlea. **b** | As shown in a cross-section, the cochlea comprises three liquid-filled compartments, the scalae, which are separated by two elastic partitions: Reissner's membrane and the basilar membrane. The scala vestibuli and scala tympani contain perilymph, an ordinary extracellular fluid similar to that surrounding most neurons. The scala media contains K^+-rich endolymph, which is secreted by cells of the stria vascularis and which also endows the scala media with a potential of about +80 mV with respect to other extracellular compartments. The somata of the afferent neurons that innervate hair cells lie in the spiral ganglion. **c** | Upon the collagenous basilar membrane rests the organ of Corti, a strip of epithelial cells that have highly varied structures. The human cochlea includes a single row of inner hair cells, which detect and transmit most of the afferent information to the brain. The three rows of outer hair cells have a motor function, implementing the active process that enhances hearing. The tectorial membrane is a gelatinous strip that is attached at its bottom surface to the tips of the longest stereocilia in the hair bundles of outer hair cells. Movement of the membrane relative to the bundles accordingly deflects the outer hair cell bundles. The hair bundles of inner hair cells are instead deflected by motion of the liquid beneath the tectorial membrane. **d** | When the cochlea is excited by sound, the back-and-forth motion of the stapes produces alternate increases and decreases in the pressure of the liquid at the base of the scala vestibuli. The pressure difference across the basilar membrane elicits a series of travelling waves that progress along the membrane at a speed of some metres per second, far below the velocity of sound in water.

Transduction
In sensory neuroscience, the term refers to the representation of a physical stimulus — for example, light, sound, acceleration, touch and chemicals — as electrical activity in an appropriate receptor cell.

Hair bundles
Mechanically sensitive organelles of a hair cell, each consisting of an upright cluster of cylindrical stereocilia that extend from the cell's apical surface.

Basilar membrane
A flat strip of connective tissue that spirals along the mammalian cochlea and supports the organ of Corti, which is the receptor for acoustic stimuli.

Travelling wave
A mechanical disturbance that propagates along the basilar membrane from the base towards the apex of the cochlea in response to acoustic stimulation.

The remarkable feature of a cochlear travelling wave is the relation of its magnitude to its position along the basilar membrane. As each wave progresses, it grows in amplitude, decreases in wavelength — and then abruptly vanishes. This comes about because the physical properties of the basilar membrane vary smoothly along its length: near the base, the membrane is relatively narrow, light and taut, whereas towards the apex it grows broad, heavy and flaccid. A travelling wave of a given frequency therefore encounters a continuously varying medium as it advances. As a result, the wave progressively grows in magnitude but decreases in wavelength until it reaches the characteristic place for the relevant frequency. Most of the energy associated with sound of any particular frequency is deposited at a specific position along the basilar membrane, beyond which propagation ceases and the travelling wave collapses.

Because the travelling wave associated with each stimulus frequency excites only a restricted coterie of hair cells and afferent nerve fibres, the basilar membrane acts as a real-time frequency analyser that decomposes a complex sound into superimposed travelling waves that peak at positions determined by the constituent frequencies. High frequencies, up to 20 kHz in the human, are represented at the cochlear base; low frequencies, down to 20 Hz, excite the apex. The intervening characteristic frequencies increase exponentially with the distance from the cochlear apex towards the base. This arrangement underlies our capacity to resolve tones that differ in frequency by only 0.2%[4,13].

The cochlear active process. The movements of the basilar membrane, hair bundles and other oscillating components of the ear are opposed by hydrodynamic drag, which imposes a force proportional to the speed of motion. Viscosity is therefore the enemy of hearing in that the mechanical signals derived from a sound tend to dissipate, with the effect most severe at high frequencies. The active process probably evolved to counter the effects of this viscous dissipation.

In the mammalian cochlea, the active process of outer hair cells amplifies the vibration of each increment of the basilar membrane, contributing energy that sustains a travelling wave[14]. As the wave progresses, the activity of successive outer hair cells counters the dissipative effect of viscosity. Although this effect is weak basal to the position at which the wave peaks, the amplification is cumulative[15,16]: the active process may ultimately enhance oscillation by more than a thousand-fold. This performance requires a delicate adjustment of the power supplied by the active process; if too little power were provided the wave would decline, whereas an excess would lead to instability. In fact, the spontaneous otoacoustic emissions observed in very quiet circumstances probably reflect the delivery of a power level that is incompatible with stability. It is not understood how the cochlear active process is normally adjusted to achieve an effective but stable degree of amplification[17].

The active process of the mammalian cochlea has two components. First, the mechanically sensitive organelles of hair cells, their hair bundles, can produce mechanical forces that augment the basilar membrane's motion. Active hair-bundle motility, which is discussed in more detail below, constitutes the entire active process of amphibians and some reptiles. The preponderance of the mammalian active process resides in a second mechanism termed somatic motility or electromotility[18,19], the characteristics and molecular basis of which have been reviewed extensively[20–23].

The somatic motility of outer hair cells in mammals and of some hair cells in birds[24] is powered by the transmembrane electrical field rather than by a high-energy chemical substrate such as ATP. Whether caused by mechanical stimulation of the hair bundle *in vivo* or by electrical stimulation with a micropipette *in vitro*, depolarization causes an outer hair cell to shorten and hyperpolarization evokes an elongation[18,19]. For an unrestrained, enzymatically isolated hair cell, the change in length can be as great as 5%. When lodged in the organ of Corti, an outer hair cell is attached at its apex and base to the adjacent supporting cells that restrain its length changes. An electrically stimulated cell can nonetheless exert forces that are thought to accentuate the basilar membrane's motion.

The membrane of an outer hair cell is endowed with millions of copies of prestin, a protein that displays piezoelectricity[25]. Prestin is a tetramer of 80 kDa monomers that undergo a rearrangement in their shape and packing in response to changes in membrane potential, thus changing the area of a cell's lateral membrane and hence the cell's length[26–28]. It remains controversial as to whether prestin's voltage sensor consists of amino-acid residues in the protein or associated anions such as bicarbonate[29]. When a hair cell's membrane is depolarized, a structural change in prestin causes it to occupy less area in the lateral plasmalemma; as a consequence, the cell shortens. Hyperpolarization has the opposite effect: that is, it increases prestin's membrane area and elongates the cell. Somatic motility can operate *in vitro* at frequencies exceeding 100 kHz and thus may function *in vivo* at the upper limits of mammalian hearing[30,31].

The interaction between active hair-bundle motility and somatic motility remains an active area of research. In confirmation of the importance of somatic motility for the active process, mutations in the gene encoding prestin spare mechanoelectrical transduction but drastically reduce auditory sensitivity and frequency selectivity[32–34]. At the same time, because the hair bundles of outer hair cells constitute a considerable mechanical load on the basilar membrane[35,36] and are capable of active movements[37,38], their activity is expected to influence the travelling wave. Indeed, when only active hair-bundle motility operates in a segment of the mammalian cochlea, amplification persists but is substantially diminished[16,39]. Pharmacological perturbation of bundle motility also affects the travelling wave[40]. Modelling studies confirm that the two motile processes can collude in cochlear amplification[41–43]: hair-bundle motility confers properly timed amplification of the transduction current that drives somatic motility, which in turn provides the brawn necessary for power gain[44–46].

Piezoelectricity
The phenomenon whereby application of a mechanical force to a substance produces an electrical potential difference across that substance, or vice versa, such as the application of changes in voltage to the piezoelectric protein prestin, which causes it to undergo a conformational change that results in an elongation or contraction of the cell.

Mechanoelectrical transduction

Hair cells, the sensory receptors of the auditory and vestibular systems, are the best-understood mechanoreceptors in eukaryotes. These cells are specialized to detect stimuli of amplitudes well below a nanometre and frequencies far above a kilohertz. Commencing with the first recordings of electrical responses from hair cells subjected to controlled mechanical stimulation[47], experiments of growing sophistication have now largely characterized the transduction process in these cells. In deference to the several earlier reviews of this subject[44,48–51], the present description is relatively brief.

Whether the relevant input is sound in the auditory system, acceleration in the vestibular system or water motion in the lateral-line system, transduction commences when a stimulus deflects the hair cell's elegant mechanoreceptive organelle, the hair bundle. Fundamentally a strain gauge, the hair bundle uses an incompletely characterized group of proteins to transduce a stimulus force into an electrical response, the receptor potential, which is a change in the voltage across the cell's membrane. The receptor potential in turn modulates the release of the neurotransmitter glutamate from synapses at the hair cell's base and thus transmits the response to the CNS (FIG. 2).

Mechanoelectrical transduction. Investigations of hair cells *in vitro* have revealed the mechanism of mechanoelectrical transduction, which is the representation of a mechanical stimulus as a receptor potential or graded change in the transmembrane voltage. For most hair cells bathed in physiologically appropriate media — K^+-rich endolymph on the apical surface and Na^+-rich perilymph on the basal aspect — physiological experiments show that about 30% of the transduction channels are open in the undisturbed bundle[52,53]. The open probability approaches 50% for unstimulated mammalian outer hair cells[54–57]. The resting potentials of hair cells are therefore determined in part by the continuous influx of cations through the transduction channels[58]. Mechanical force applied near the top of the clustered stereocilia deflects the top of the bundle in a plane parallel to the epithelial surface, changing the open probability of transduction channels and eliciting a receptor potential. The electrical response is generally graded with the amplitude of stimulation, but some hair cells produce action potentials, especially during development[47,59–62]. The relation of open probability to bundle deflection is sigmoidal and remarkably narrow, with deflections as small as a few tens of nanometres leading to a shift between 10% and 90% in channel opening[63–65].

The latency of transduction by a hair cell lies in the range of microseconds, which indicates that hair-bundle deflection acts directly to open and close mechanically sensitive channels[52,66]. Although considerable evidence supports the hypothesis that deflection of a hair bundle is communicated to the transduction channels through elastic elements termed gating springs, the identity of these hypothetical structures remains controversial. The initial candidate was the tip link, a fine molecular strand 105–170 nm in length that connects the distal tip of each stereocilium to the side of the tallest adjacent process[67–69].

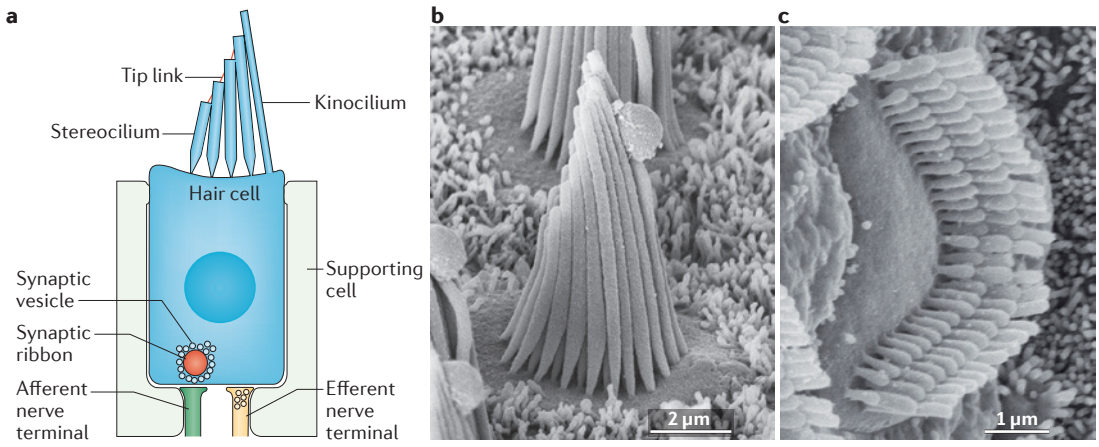


Figure 2 | **The hair cell and hair bundle.** **a** | A schematic drawing depicts a cylindrical hair cell surrounded by supporting cells. A ribbon synapse at the base of the cell releases glutamate, which excites the afferent nerve terminal and sends information into the brain. Neural centres in the brainstem can reduce the hair cell's sensitivity by activating an efferent nerve terminal that releases acetylcholine. The mechanoreceptive hair bundle consists of 10–300 cylindrical processes, the stereocilia, that project as a compact cluster from the cell's apical surface. As hypertrophic derivatives of microvilli, the stereocilia are filled with parallel actin filaments that are held in register by crosslinks of plasmin, fimbrin, espin and other proteins. The stereocilia vary systematically in length across each hair bundle so that the structure is bevelled like the tip of a hypodermic needle. Tip links interconnect successive stereocilia along the bundle's axis of mirror symmetry. A single true cilium, the kinocilium, stands at the bundle's tall edge. During development, this structure has a role in organizing and orienting the bundle. **b** | In a lateral view, a scanning electron micrograph shows an individual hair bundle from the frog's sacculus, an organ that detects gravity and ground-borne vibration. About 8 µm in height, the bundle comprises a single kinocilium with a bulbous swelling at its tip and about 60 cylindrical stereocilia with tapered basal insertions. **c** | A top view shows the specialized hair bundle of an outer hair cell from the bat's cochlea, which is V-shaped and has only three ranks of stereocilia, the shortest of which is 0.5 µm in height.

A tip link is a dimer of dimers: the upper two-thirds embrace two parallel molecules of cadherin 23, whereas the lower one-third comprises a parallel pair of protocadherin 15 molecules[70–73]. These atypical cadherins interact through side-by-side overlap of their distal, amino-terminal cadherin repeats[74] rather than through the interdigitating tryptophan residues that are characteristic of conventional cadherins. Numerous mutations that destabilize the interaction domains lead to deafness[75]. Because the structure of cadherin domains depends on the binding of Ca^{2+}, chelation of that ion rapidly severs tip links and abolishes mechanoelectrical transduction[76]. The links can regenerate within a few hours[77,78], as they are also thought to do following the moderate acoustical damage that elicits a temporary threshold shift.

Despite the evidence associating the tip link with transduction, the structure's mechanical properties do not accord with those expected of a gating spring. Analysis of channel gating suggests that an individual gating spring has a stiffness on the order of 500 $\mu N \cdot m^{-1}$, a value typical of flexible filamentous proteins[79]. However, electron microscopy suggests that tip links are rigid[68], and the constituent cadherin repeats are generally difficult to deform[74]. Molecular-dynamical simulations suggest that a tip link's stiffness approaches 50 $mN \cdot m^{-1}$, about a hundred times the value of the physiologically defined gating spring[74]. To clarify the nature of the gating spring, investigators are now pursuing two possibilities. First, the elasticity associated with each tip link may reside at its insertions rather than along its length[80–82]. An alternative prospect is that the tip link's stiffness has been overestimated owing to crystallization of the Ca^{2+}-stabilized cadherin domains at a Ca^{2+} concentration much greater than the 20 μM that prevails in mammalian endolymph[83,84]. Ca^{2+} ions affect the stiffness of gating springs[85], which additionally display complex viscoelastic properties[86].

Movement of a hair bundle's top within its plane of bilateral symmetry, as occurs when a bundle is stimulated by sound, causes the largest electrical response; orthogonal stimulation is without effect[87]. Displacement towards the bundle's tall edge, which is defined as a positive stimulus, opens transduction channels and causes depolarization. Deflection in the opposite direction closes channels and evokes hyperpolarization. Several lines of evidence indicate that the transduction channels lie at the top of a hair bundle[88–90]. Although early evidence suggested that the channels are located at both ends of tip links[91], more recent physiological data indicate that they are asymmetrically located at the links' lower insertions[63,92] (FIG. 3).

The mechanotransduction channel. The identity of the hair cell's mechanoelectrical transduction channel — the elusive hearin molecule[93] — remains uncertain. Because there are at most a few hundred active transduction channels per cell[92,94], and inasmuch as ears ordinarily contain but a few thousand hair cells, the channel has been inaccessible to conventional biochemical approaches. The absence of high-affinity blockers and of a physical signature such as an optical absorption spectrum has compounded the problem. Even state-of-the art mass spectrometry of proteins purified from hair bundles has not yielded clear candidates[95]. Although genetic approaches have identified mechanosensitive channels in bacteria, plants, *Cænorhabditis elegans* and *Drosophila melanogaster*, none of these proteins is known to be expressed in the hair cells of vertebrates[96–103].

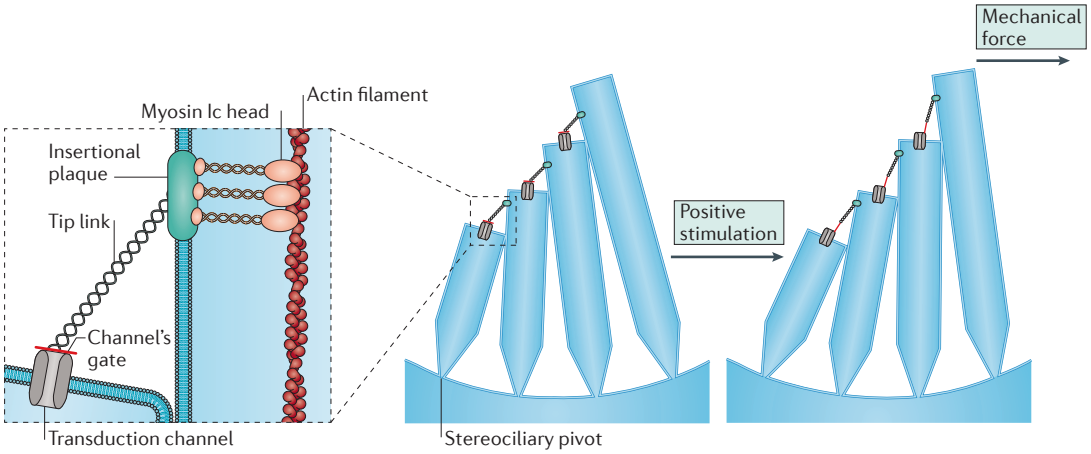


Figure 3 | **Gating of transduction channels.** Each transduction element of a hair cell, which is located at the top of the hair bundle, includes a tip link that interconnects adjacent stereocilia (left diagram). The lower end of the link is thought to be connected to two mechanically sensitive channels. The upper end terminates at the insertional plaque, the site of the myosin Ic molecules involved in adaptation of the transduction process. When a mechanical stimulus deflects a hair bundle in the positive direction (right diagram) towards its longest stereocilia and kinocilium, the stereocilia pivot at their basal insertions but remain in contact with one another near their tips. The resultant shearing motion between contiguous stereocilia increases the tension in the tip link that extends from the top of each short stereocilium to the insertional plaque on the side of the tallest adjacent stereocilium. This enhanced tension increases the probability that the channels at the link's lower insertion will open. Ionic current, carried predominantly by K^+ but including Ca^{2+}, then enters the stereocilium, depolarizes the hair cell and triggers the release of glutamate at the ribbon synapses on the cell's basolateral surface.

Analysis of heritable deafness in humans suggests that the transduction channel consists, at least in part, of the transmembrane channel-like protein 1 (TMC1) and TMC2 (REFS 104–106). Physiological experiments demonstrate an absence of normal mechanotransduction in the hair cells of knockout mice lacking functional TMC proteins. The two paralogues display distinct spatial and temporal patterns of expression that correlate with physiological differences in channel properties[107]. Moreover, selective expression of one or the other of the two genes in mouse hair cells results in distinct Ca^{2+} permeabilities and single-channel conductances of transduction channels[108–110]. These results suggest that, either directly or indirectly, TMC subunits influence the conductance pathway.

The picture is clouded, however, by recordings obtained from mice in which *Tmc1* and *Tmc2* have been mutated at different positions[110]. Mechanical stimulation of hair bundles from these animals evokes currents with an unexpected property: depolarization results from hair-bundle deflection in the negative rather than the positive direction. Developing hair bundles from the zebrafish also display reversed mechanosensitivity[111], as do bundles from the mouse's cochlea after tip-link scission or in mutants lacking tip links[110,112,113].

What is the source of reversed mechanosensitivity? Upon the cleavage of tip links, protocadherin 15 alone forms short inter-stereociliary connections[78]. Although these structures might engender anomalous responses by activating channels in a distinct way, they evidently take hours to form, whereas reversed responses can emerge within seconds[113]. A second potential explanation of reversed responsiveness is that other members of the TMC family of channels[114] are recruited in the absence of TMC1 and TMC2 and are gated in an unconventional manner, for example by membrane stretch. A final possibility is that TMC subunits are not the core constituents of the transduction channels but are essential for directing them to stereociliary tips, for conveying mechanical signals to them or for modulating their conductances. The manifestations of reversed mechanosensitivity reported to date involve immature hair cells. Hearing requires at least two additional membrane-spanning proteins that might represent components of the transduction apparatus[115–120]. The piezos — widely distributed, mechanically sensitive proteins that are responsible for some forms of touch responsiveness[121–123] — are also plausible candidates for the core constituents of transduction channels.

Gating compliance. If a hair cell's transduction channels are gated directly by the force applied to the hair bundle, the opening and closing of the channels should reciprocally influence the bundle's mechanical properties. More specifically, a hair bundle should become softer over the range of positions in which channels gate[124]. The demonstration of this decrease in hair-bundle stiffness, termed the gating compliance, provides evidence in support of the gating-spring theory[125]. The gating compliance can become so great that it outweighs the stiffness of a hair bundle's other components, whereupon the bundle's total stiffness becomes negative[126]. This situation is peculiar: a hair bundle displaying negative stiffness does not oppose an applied stimulus force but instead augments the stimulus by contributing additional force in the same direction. Negative stiffness, which has been demonstrated experimentally[37,63,127], leads to mechanical instability: a hair bundle cannot reside stably near the region of negative stiffness because a thermal deflection in either direction drives the bundle into a region of positive stiffness. This instability underlies the hair bundle's ability to function as an amplifier and oscillator (FIG. 4).

The magnitude of the estimated gating compliance is disquieting. Geometrical considerations dictate that the spatial separation of the two linear domains of the displacement–force relation should be approximately d/γ, in which d is the swing of the channel's gate. The geometrical gain factor γ relates the sliding of stereociliary tips, and thus gating-spring extension, to hair-bundle displacement. For the frog's hair bundles[63,127], in which $\gamma \approx 0.14$, the gating non-linearity encompasses 35–75 nm, which implies a gating distance of about 8 nm. This value seems too great to represent an intramolecular rearrangement of an ion channel. Perhaps channel opening rapidly triggers a second, larger movement in some associated protein that is sensitive to tip-link tension.

Adaptation

Like most other sensory receptors, hair cells possess an adaptation mechanism by means of which they can disregard continuous or slowly changing stimuli while retaining sensitivity to transient stimuli of potentially greater behavioural relevance. The direct mechanical gating of transduction channels in hair cells fosters the rapid responsiveness that permits hearing in some mammals at frequencies of 100 kHz or even greater[66]. However, because auditory transduction does not proceed through a biochemical signalling cascade such as those characteristic of vision, smell and taste, a novel mechanism is required to effect adaptation to sustained stimulation.

Slow adaptation. If a hair bundle is experimentally displaced from its resting position, the locus of mechanical sensitivity migrates towards its new position with a time constant of a few tens of milliseconds[128]. This process, termed slow adaptation, reduces the electrical response of the hair bundle, thus precluding saturation and ensuring that the bundle remains sensitive to further stimuli. Adaptation is incomplete: the hair bundle typically shifts its sensitivity by 80% of the distance through which it has been displaced[129]. As a consequence, a hair cell can provide both a tonic electrical output that represents the time-averaged position of the hair bundle and a phasic response that reports transient deviations from that position.

Adaptation is accompanied by mechanical signals that suggest a physical rearrangement of components within the hair bundle[79]. When responding to a positive force, a bundle initially moves a distance that is inversely proportional to its stiffness, but then the bundle sags farther in the same direction. Moreover, as the position of mechanosensitivity migrates, the relation of force to displacement

Gating compliance
A decrease in hair-bundle stiffness owing to the gating of transduction channels.

Adaptation
Resetting of the sensitivity in a sensory system. This involves an adjustment of the range of hair-bundle displacements over which a hair cell's electrical response varies.

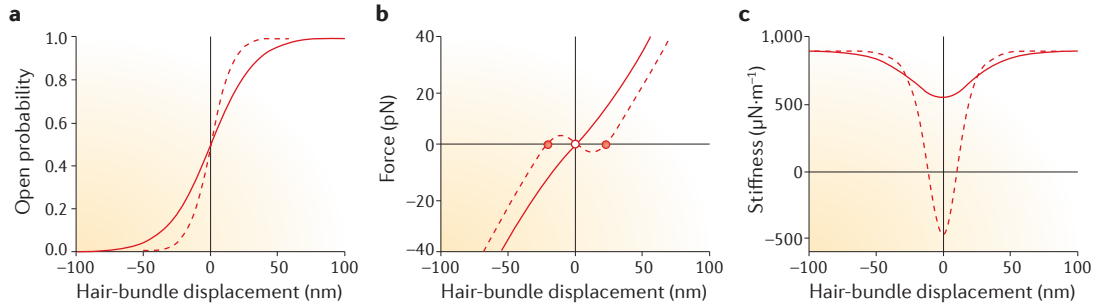


Figure 4 | **Non-linearity associated with channel gating.** **a** | The relation between hair-bundle displacement and the open probability of mechanotransduction channels is described by a sigmoidal curve. When the value of the gating force that characterizes the sensitivity of the channels is small (continuous line), the relation is shallower than for large values (dashed line). The lateral offset of the curve depends on the extracellular Ca^{2+} concentration: higher concentrations close more channels at rest and shift this and the two subsequent relations to the right. **b** | The relation between hair-bundle displacement and applied force (in piconewtons) is a steadily ascending curve for low values of the gating force (continuous line). A large gating force (dashed line), however, introduces a region of negative slope. In the absence of adaptation or external force, a hair bundle can reside at either of the two stable fixed points (solid red dots). A third fixed point (red circle) is unstable, for bombardment by thermally excited water molecules drives the bundle to the stable point either at the right or at the left. **c** | The relation between displacement and hair-bundle stiffness displays gating compliance, a decrease in the bundle's stiffness over the range of positions in which channels are opening and closing. Although the reduction in stiffness is modest for a low gating force (continuous line), a high gating force results in negative stiffness (dashed line).

relocates as well. In other words, the apparatus that senses mechanical stimulation is reset during adaptation to accord with the hair bundle's new position (FIG. 5).

Adaptation is thought to occur as the upper ends of tip links slide along the stereociliary shafts to which they are attached[79,130]. During a protracted positive stimulation, each tip link is tensed and the associated channels are more likely to open. As the insertional plaque that anchors the link's upper insertion slides downwards during continued stimulation, however, the tension decreases and most of the channels reclose. Adaptation in the positive direction depends on the downward force owing to tip-link tension, which is responsible for pulling the insertional plaque downwards. At least in the hair bundles of frogs and those of mammalian vestibular hair cells[129,131,132], but apparently not those of mammalian cochlear hair cells[133], the Ca^{2+} that enters through transduction channels promotes this slippage.

When the bundle is instead subjected to a negative force that closes the channels that are open at rest, adaptation requires a restoration of tip-link tension to foster channel reopening. In this instance, work must be done to raise the insertional plaque despite the residual tension in the link. Myosin Ic is thought to mediate this process. The pharmacological sensitivity of adaptation accords with the expectation for a myosin-based process[134,135], and myosins of class I have several properties that would be favourable for a role in adaptation[136,137]. Biochemical analysis and light-microscopic immunohistochemistry demonstrate active myosin Ic in hair bundles[138,139]; electron-microscopic studies suggest that the protein is concentrated at insertional plaques[140,141]. Most importantly, site-directed mutagenesis of myosin Ic confers an altered substrate specificity upon the adaptation motor[142].

The hair bundles of mammalian outer hair cells, which serve primarily a motor rather than a sensory function, show only limited slow adaptation[133]. In these cells, myosin VIIa, which is expressed at the insertional plaques, is necessary to produce sufficient tension in tip links to open some transduction channels at rest[143,144]. Because myosin VIIa is also expressed in other hair cells, including vestibular cells, it may have a general role, such as constituting the extent spring thought to restrict the range of adaptation[135]. Consistent with that hypothesis, insertional plaques do not migrate extensively after the tip links have been severed[144].

Fast adaptation. A critical but poorly understood behaviour of hair bundles is fast adaptation. When a hair bundle is abruptly displaced in the positive direction, as would occur during high-frequency acoustic stimulation, a large fraction of the transduction channels that open initially proceed to reclose within a millisecond or less[79]. In amphibian and reptilian hair bundles[145], but arguably not those of mammalian outer hair cells[133,146], the rate and extent of this response depend on the extracellular Ca^{2+} concentration. Associated with fast adaptation is a mechanical phenomenon termed the twitch: after initially moving in the direction of stimulation, the hair bundle jerks back in the opposite direction by as much as 20 nm and occasionally even more[147,148]. This movement occurs for only a limited range of positive stimulus amplitudes, up to approximately 100 nm for anuran hair bundles.

The importance of fast adaptation lies in its possible involvement in the mechanical amplification of high-frequency signals[37,44,149]. Because of its ability to produce rapid forces *in vitro* and potentially to perform mechanical work, fast adaptation seems likely to be a component of the active process in the mammalian cochlea. It remains uncertain, however, exactly how fast adaptation operates in the intact cochlea. Accordingly, an important requirement for future research is the development of an experimental preparation in which

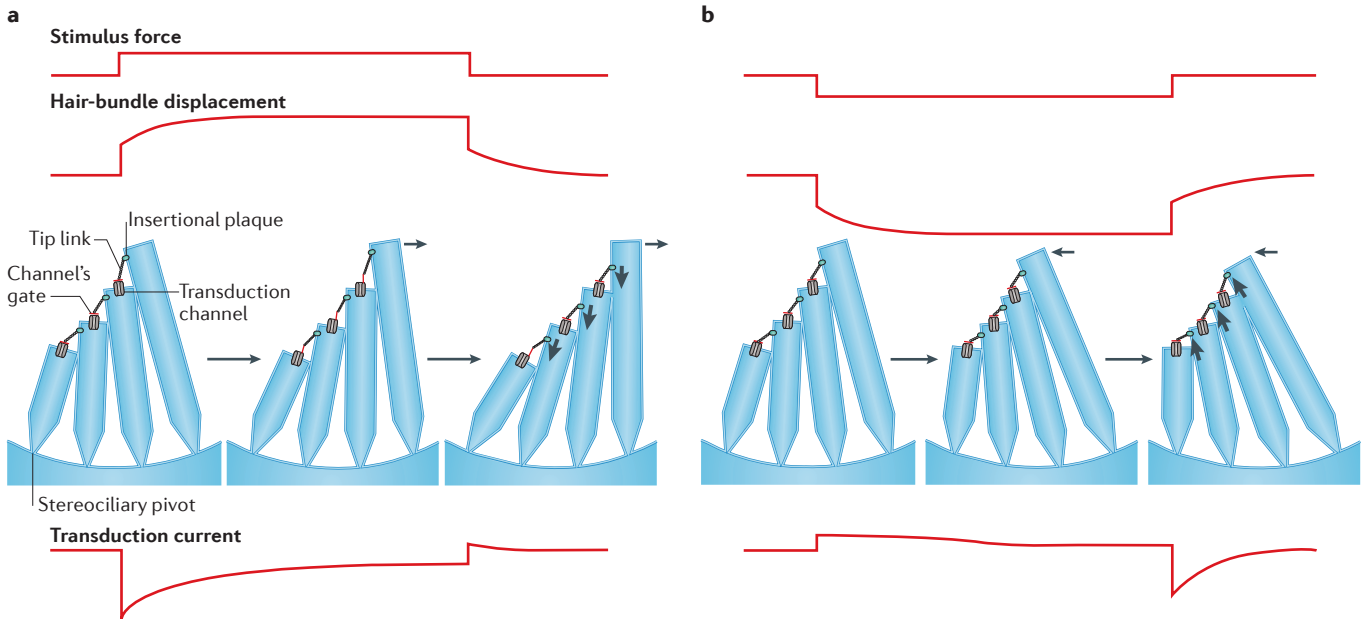


Figure 5 | **Slow adaptation.** **a** | When stimulated by a positive force, a hair bundle first undergoes a rapid displacement, then progresses farther in the same direction until the stimulus concludes. At the same time, the inward (negative) transduction current rapidly reaches a peak, then declines to a constant level with a similar time course. The inferred mechanism of adaptation is schematized in the three diagrams of hair bundles. Displacement initially extends the gating springs, here represented by tip links, and opens the channels. As the upper ends of the tip links descend, which begins to occur as soon as the hair bundle is displaced, the gating springs relax and some channels reclose. The relaxation explains the growing displacement of the bundle during adaptation, whereas the channel reclosure accords with the reduction in transduction current. **b** | During adaptation to a negative stimulus, the hair bundle's displacement record is inverted and the transduction current reaches zero just after the onset of stimulation. Myosin Ic motors in the insertional plaques at the upper ends of the tip links then ascend, restoring tension to the gating springs. As noted in the displacement record, this tension pulls the hair bundle farther in the negative direction. The increased tension enables some channels to reopen; moreover, when the stimulus force abates and the bundle returns towards its resting position, the gating springs bear an excess of tension that provokes a brief burst of channel opening.

the mechanical contributions of hair bundles can be examined in a cochlear preparation that sustains normal travelling waves.

There are at least four possible explanations for fast adaptation. First, the phenomenon might simply represent the viscoelastic relaxation of some element in series with the transduction channels[79,86,133]. Because this mechanism is passive, it could not supply energy useful for amplifying mechanical inputs. A second possibility is that fast adaptation reflects a hair bundle's traversal of its region of negative stiffness, again a passive phenomenon[150]. In this instance, repriming of the bundle for successive movements would be mediated by myosin-based slow adaptation, a possibility supported by site-directed mutagenesis in the murine vestibular system[151]. The ear's capacity to amplify its mechanical inputs evidently extends to many tens of kilohertz[152], however, an ability that seemingly challenges the kinetic capability of any myosin.

A third possibility is that myosin mediates fast adaptation by operating at greater frequencies than has been appreciated. Analysis of the power emitted during spontaneous otoacoustic emission suggests that only a few of the numerous myosin molecules at an insertional plaque need to conduct power strokes during each cycle of oscillation[153,154]. If each molecule were active only every few tens of cycles, the oscillation frequency could substantially exceed the rate of myosin activity measured *in vitro*. Insect flight muscle, in which myosin molecules remain associated with actin filaments between successive power strokes, can vibrate at frequencies reaching 1 kHz or more[155]. A hair bundle might use a similar strategy to reduce the time required for the rate-limiting rebinding of myosin with actin, in which case the activity of myosin might explain fast adaptation. Flight muscle operates as part of a mechanically resonant system, which a hair bundle can also constitute when loaded with a suitable mass[46,156].

A final possibility is that fast adaptation is driven by an energy source other than ATP, the stereospecific binding of which might be a rate-limiting step. The Ca^{2+} that enters as a component of the transduction current[157,158], for example, might bind to the mechanically gated channel itself or to an associated protein and thus directly provoke reclosure[125,159]. As the Ca^{2+} concentration within each stereocilium varied cyclically, the ion's binding energy would then power amplification or oscillation. A system based on this premise could plausibly operate at frequencies of 10 kHz or more[149]. A related conjecture is that Ca^{2+} binds to a relaxation or release element, a hypothetical component in series with the tip link[81,151,160]. Altering the stiffness or length of this element on a cycle-by-cycle basis could yield a parametric amplifier

or oscillator. The experimental signature expected of this mechanism is an asymmetry in the rate or extent of viscoelastic relaxation of a hair bundle driven in the positive or negative direction under normal ionic conditions. Although this effect has not been observed in bundles of the mammalian cochlea[133], the difficulties involved in stimulating these complexly shaped bundles (FIG. 3) complicate the interpretation of the experiments[78].

Active hair-bundle motility

Hair bundles display a range of transient and prolonged mechanical activities. Because of the limited accessibility of hair cells in the intact inner ear, these signals are generally investigated *in vitro*. Hair cells are usually left in an epithelial sheet, but occasional studies use enzymatically isolated cells[159]. Although recording from the apical surfaces of hair cells is frustrating owing to the difficulty in forming tight seals with electrodes[94,161], the basolateral membranes — when accessible to electrodes — are readily amenable to recording.

Techniques of mechanical recording. To control and measure the motion of a hair bundle, an investigator attaches atop the bundle the tip of a flexible glass fibre up to 100 μm in length and 1 μm in diameter. Because the stiffness of such a fibre, 100–2,000 $\mu N \cdot m^{-1}$, is comparable with that of the hair bundle under investigation, changes in the bundle's mechanical properties can bend the fibre. To measure the bundle's motion and the fibre's flexion, a compound microscope projects a magnified image of the fibre's tip onto a photomicrometer. Moving the proximal end of the glass fibre with a piezoelectrical actuator enables an experimenter to deliver displacement or force stimuli to the hair bundle.

Measurements made by these means are limited by three factors[147]. First, the inertia of the piezoelectrical stimulator and fibre restricts the rate at which stimuli rise to tens or hundreds of microseconds. Next, the viscous drag resulting from rapid movement of the stimulus fibre and hair bundle through water imposes a similar time constant. These considerations together limit the frequency response of the system to a few kilohertz. Last, within that bandwidth, vibration of the experimental apparatus and noise in the illumination system typically restrict the spatial resolution to approximately 1 nm. Dedicated solid-state mechanosensors may overcome some of these limitations[162].

Spontaneous oscillation of hair bundles. In addition to displaying mechanical responses to stimulation, the hair bundles of many species exhibit spontaneous movements that reflect their capacity to participate in the active process[160,163]. Although the oscillations described in amphibian and reptilian hair bundles have been confined to frequencies of only a few tens of hertz, modelling indicates that oscillations could plausibly occur into the kilohertz range[149]. When freed from accessory structures such as tectorial or otolithic membranes, bundles often undergo slow oscillations of relatively great magnitude. In some instances, these movements are relaxation oscillations, with distinct fast and slow phases[127,160]; in other cases, complex motions with several distinct timescales ensue[164]. Although the movements vary widely in frequency, amplitude and waveform, a simple dynamical model suggests that these differences largely reflect the effects of mechanical loading on the bundles[156]. Increasing the stiffness of a glass fibre attached to a bundle, for example, leads to smaller oscillations at a higher frequency and ultimately suppresses oscillation altogether[160,165].

Spontaneous hair-bundle oscillations emerge from the combination of negative hair-bundle stiffness and adaptation. The positions of the insertional plaques atop the tip links represent a compromise between downward slipping, which is promoted by tension in the links, and upward climbing, which is mediated by adaptation motors. If a hair bundle exhibits a positive stiffness at all positions, the system can attain a steady state at which the motors stall and the transduction channels exhibit a stable open probability.

The situation changes, however, if a hair bundle displays negative stiffness. Suppose that such a bundle starts with a small open probability for its transduction channels and consequently a low concentration of Ca^{2+} in the stereocilia. Acting through the ascent of myosin Ic motors, adaptation begins to increase the open probability towards the steady-state set point. This activity has the effect of progressively shifting the displacement–force relation to the left and downwards. At some point, however, the region of positive slope vanishes abruptly and the hair bundle must leap to a new fixed point on the curve's opposite limb. Now the opposite situation occurs: the open probability is quite high and the intracellular Ca^{2+} concentration is increased, so the insertional plaque begins to descend. When mechanical stability again vanishes, the hair bundle jerks back in the negative direction. Continually unable to achieve a steady state, the bundle progresses back-and-forth in a limit-cycle oscillation[127,160] (FIG. 6).

The physiological importance of hair-bundle oscillation lies not in spontaneous movement, which is an epiphenomenon of the active process, but in the entrainment of oscillations by external stimuli. If even a small force of the appropriate frequency is applied to an active hair bundle, the spontaneous oscillations readily adopt the phase of the stimulus[166,167]. Moreover, the amplitude of the response may exceed that of the stimulus, providing amplification with a power gain[166]. Thermal noise, the bombardment of a hair bundle by water molecules, restricts amplification by an individual hair bundle to about tenfold. When hair bundles are coupled through a tectorial or otolithic membrane, however, the effects of noise average out, which suppresses noise and boosts the gain to one hundred or more[168,169].

It is probable that unprovoked hair-bundle oscillations underlie the spontaneous otoacoustic emissions of non-mammalian tetrapods. Such an emission requires the delivery of power by the cellular oscillators at the source of the hair bundles. Although this rate of energy expenditure is not great[154], it represents the mechanical output of at least several hair bundles — probably dozens to hundreds — whose movements must somehow

Limit-cycle oscillation
A stable pattern of oscillation in a non-linear dynamical system to which the system will return even if started in a different configuration.

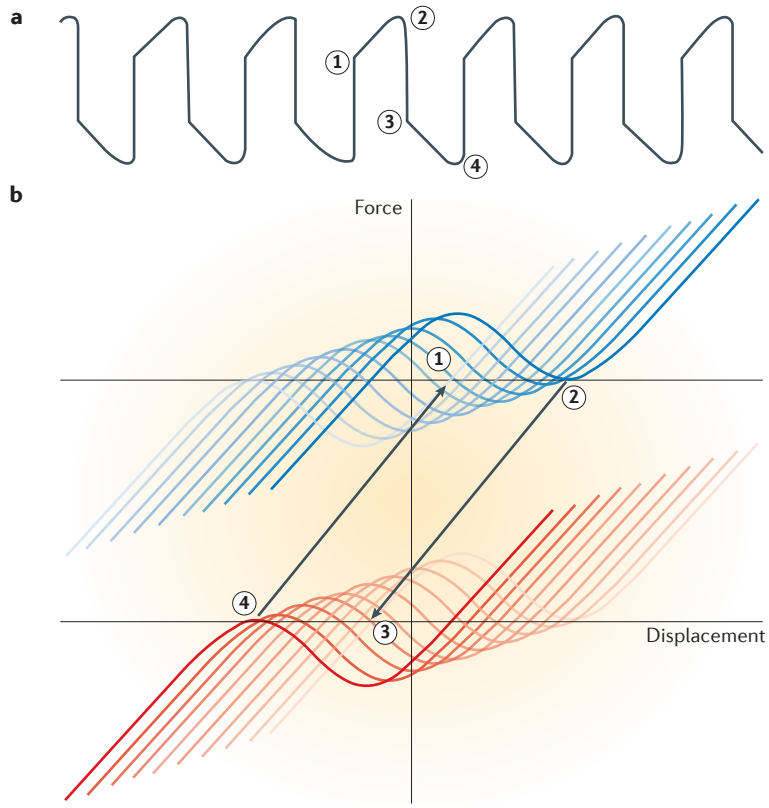


Figure 6 | **Hair-bundle oscillation.** **a** | The spontaneous limit-cycle oscillation of a hair bundle *in vitro* can be simulated with a computational model. When the simulation includes an otolithic or tectorial membrane, a bundle oscillates at a greater frequency, with a smaller amplitude and in a more sinusoidal manner. The numbers correspond to positions in the following panel. **b** | The temporal evolution of the hair bundle's displacement–force relation reveals the mechanism of oscillation. Commencing when the transduction channels display a very high open probability (step 1), adaptation relaxes tip links, closes channels and causes the relation to move to the right and upwards. When the stable fixed point along the abscissa vanishes (step 2), however, the hair bundle must leap to a new fixed point (step 3), which here has been vertically offset for clarity. Now the open probability is very low, so the myosin Ic motors at the insertional plaques tighten the tip links, thus reopening some channels and shifting the displacement–force relation to the left and downward. Before the system can achieve a steady state, the stable fixed point again vanishes (step 4), the bundle jumps in the positive direction and the cycle repeats.

be synchronized. Modelling of the reptilian cochlea confirms that the non-linearity inherent in hair bundles readily accomplishes synchronization if adjacent bundles are coupled through elastic connections or by fluid viscosity[170,171]. The same mechanism may well operate in the mammalian cochlea. Another interesting possibility is that a spontaneous otoacoustic emission arises when an irregularity in the cochlear partition imposes a focal change in mechanical impedance that reflects a forward-travelling wave. The reverse wave is then partially transmitted through the middle ear, yielding an emitted sound, and partly reflected from the oval window, producing a standing wave on the basilar membrane. In this model, numerous hair cells are entrained to oscillate at a common frequency, providing energy to maintain the oscillation and thus constituting the excitable medium in a laser-like phenomenon[172].

The dynamical basis of the active process

Remarkably enough, the four characteristics of the hair cell's active process can be unified as consequences of a simple phenomenon, the Hopf bifurcation[17,149,173,174]. Near a Hopf bifurcation, but on its stable side, a system acts as an amplifier of its inputs. Tuned to a specific frequency of stimulation, it displays compressive non-linearity and in particular evinces a power-law behaviour with a characteristic exponent of one-third. The dynamical system exhibits greater gain, sharper tuning and more profound compression as it approaches the bifurcation. And upon crossing the bifurcation, the system becomes unstable and begins to oscillate spontaneously. Because the active process of hair cells operates near a critical point — namely, the Hopf bifurcation — it is termed a critical oscillator[17] (BOX 1).

Evidence for a Hopf bifurcation in the active process. Each of the four generic features of the active process finds a correlate in the behaviour of individual hair bundles. When driven by a sinusoidal force, such as that resulting from an acoustic stimulus, a hair bundle can increase the magnitude of the mechanical signal. Moreover, this enhancement is associated with power gain, the benchmark of true amplification[166]. Next, each hair bundle's amplification is greatest at a specific frequency termed the characteristic frequency; stimulation by frequencies markedly higher or lower than this characteristic frequency evokes only a passive response[175,176]. The third characteristic of the active process, compressive non-linearity, is exemplified by the dependence of a hair bundle's response on the stimulus force. Plotted in logarithmic coordinates, this relation follows a power law with a slope near one-third[175]. In other words, the response grows in proportion to the cube root of the stimulus, a seemingly perplexing relationship. Last, an unstimulated hair bundle can nonetheless oscillate vigorously, a probable correlate of spontaneous otoacoustic emissions[160,163].

Most of the evidence implicating a Hopf bifurcation in the behaviour of hair cells stems from investigations of amphibian and reptilian mo del systems, in which it is possible to manipulate individual hair bundles and observe their responses. Nevertheless, the following eight seemingly unconnected observations from psychophysics and cochlear physiology can be explained by the proposal that hair cells of the mammalian cochlea also operate near a Hopf bifurcation[50].

Human hearing displays a striking compressive non-linearity: twelve orders of input power are condensed into two or three orders of magnitude in sensation. This characteristic, which allows us to appreciate a soloist whose instrument produces an acoustic signal that is only one hundredth that of the accompanying orchestra, is reflected in the use of a logarithmic decibel scale for the measurement of loudness. The dependence of basilar-membrane vibration on the level of stimulation is also profoundly non-linear for stimulation near the characteristic frequency. In some species, the sensitivity follows a power-law relation with a slope of one-third[177,178], the exponent expected for a system situated at a Hopf bifurcation[173]. The responsiveness of individual hair bundles fits the same compressive power law[175].

Box 1 | **The Hopf bifurcation**

In a dynamical system subject to a Hopf bifurcation, the behaviour of a complex variable Z is governed by the relation:

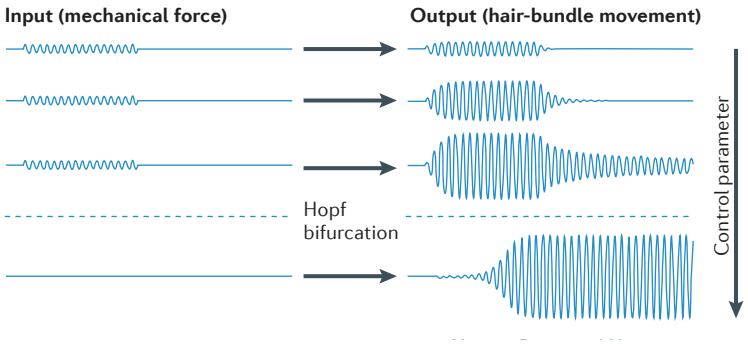
$$\dot{Z} = (\mu - i\omega_0)\, Z - |Z|^2 Z \qquad (1)$$

Here μ is termed the control parameter and ω_0 is the characteristic frequency at which oscillation occurs near the bifurcation. In the instance of a hair cell, $Z = X + iY$ might represent the hair bundle's deflection X and some internal variable Y.

For $\mu < 0$, the system is stable: without stimulation and thermal noise there is no oscillation. For $\mu > 0$, the system displays a limit-cycle oscillation of a constant amplitude and angular frequency, a behaviour that may underlie spontaneous otoacoustic emissions. If the system resides at the bifurcation and is stimulated with a force F at its characteristic frequency, the response of the active system displays a compressive power-law dependence on the stimulus force: $|X_{ACTIVE}| \propto |F|^{1/3}$. This behaviour contrasts with that of a passive system, for which the response is linearly related to the input: $|X| \propto |F|$. The amplification or gain G afforded by operation near the bifurcation is thus:

$$G = \frac{|X_{ACTIVE}|}{|X_{PASSIVE}|} \propto \frac{1}{|F|^{2/3}} \qquad (2)$$

This relation implies that, as the stimulus force grows progressively smaller, the gain of the active system becomes ever greater. A critical amplifier thus exhibits the desirable feature of enhancing the weakest stimuli most and amplifying the strongest stimuli least.



The sensitivity of a system may be controlled by adjusting its proximity to the Hopf bifurcation. When the relevant control parameter lies far from the critical value, the system is essentially linear in its responsiveness. The non-linearity increases and the gain rises as the system approaches the bifurcation, beyond which the system enters into spontaneous oscillation.

When a normal listener attends to a pair of nearby frequencies, a higher tone f_2 and a lower one f_1, he or she hears a range of additional tones at non-harmonic frequencies. Discovered in the eighteenth century by the violinist Giuseppe Tartini[179], these distortion products play important parts in certain musical compositions by György Ligeti, Karlheinz Stockhausen and other composers[180]. Although quadratic distortion is common in many mechanical systems, the most prominent distortion product is cubic in nature and corresponds to $2f_1 - f_2$: that is, twice the lower frequency minus the higher one. Interferometric measurements of basilar-membrane motion confirm that distortion products are generated by non-linearities in the cochlea[181–183]. Moreover, mechanical measurements disclose these signals in the movements of individual hair bundles[184,185]. The predominantly cubic responsiveness of a system operating near a Hopf bifurcation accounts for these distortion products.

Distortion products
Oscillations at specific frequencies produced within a hearing organ by the non-linear properties of hair bundles exposed to acoustic stimuli at other frequencies. They are also called combination tones or phantom tones and are used in the medical diagnosis of hearing deficits.

Trained observers listening to pairs of simultaneous tones can identify a family of distortion products of progressively higher order and lower amplitude[186,187]. These include not only $2f_1 - f_2$ but also $3f_1 - 2f_2$, $4f_1 - 3f_2$, and others of the form $(n+1)f_1 - nf_2$. These higher-order signals are also apparent on the cochlea's basilar membrane, which bears a complex array of superimposed vibrations whose amplitudes decline with increasing separation from the stimulus tones[181,182]. A hair bundle subjected to two frequencies of stimulation also displays numerous higher-order distortion products; moreover, their amplitudes decline as a function of frequency with the exponential pattern expected for a system operating at a Hopf bifurcation[185].

As the stimuli that engender a distortion product become stronger, the perceived loudness of the distortion product increases in parallel with the strength of stimulation[186]. This behaviour is atypical of saturating non-linearities, which ordinarily grow at a rate dependent on the order of the non-linearity; for example, a cubic distortion product might be expected to rise with the cube of sound intensity. In fact, the unexpected psychophysical observation that distortion products increase in proportion to stimuli led to the concept of an 'essential non-linearity', a distortion intrinsic to the mechanism of the ear's operation[186]. Hair bundles too display mechanical distortion products that grow at the same rate as their response to stimulation[185]. A Hopf bifurcation explains these phenomena: both the response of a dynamical system and the associated distortion products grow with the cube root of the stimulus strength near such an instability.

Simultaneous exposure to a pair of closely spaced frequencies renders a listener less sensitive to each input. At the level of the cochlea, this two-tone suppression appears as diminished vibration of the basilar membrane in response to one tone when a second tone at a nearby frequency is presented[181]. When stimulated simultaneously at two frequencies, an individual hair bundle displays a comparable phenomenon[185]. This behaviour is expected for a system operating near a Hopf bifurcation: adding stimulation at a second frequency increases the hair bundle's motion, reducing amplification by the active process and thus diminishing sensitivity to the original tone[188,189].

When an animal responds to sounds near threshold loudness, the activity of its auditory nerve fibres does not rise appreciably as the sound intensity grows. Instead, the background firing rate persists but the action potentials become progressively more synchronized with the stimulus[190,191]. Individual hair bundles behave similarly: a growing stimulus increasingly entrains a spontaneously oscillating hair bundle well before the amplitude of movement rises[175]. This behaviour is expected for a dynamical system operating near a Hopf bifurcation, which is highly susceptible to phase entrainment[192].

Spontaneous otoacoustic emissions emerge from at least 70% of normal human ears in an ultraquiet environment[193–195], and the ears of rare individuals continue to emit audible tones even under normal circumstances. Similar emissions occur from the ears of other

mammals, reptiles including birds, and amphibians; in many instances, the signals are stronger and more regularly arranged than those from humans[196,197]. When maintained in physiological saline solutions, hair bundles routinely display spontaneous oscillations that could power such emissions[160,163]. Spontaneous oscillation is inevitable on the unstable side of a Hopf bifurcation, the condition under which a dynamical system enters into limit-cycle oscillation[17,149].

For a passive resonator, as the basilar membrane was originally thought to be, the characteristic frequency at each position is proportional to the square root of the ratio of stiffness to mass. Owing to this square-root dependence, the frequency range of human hearing — three orders of magnitude — would in a passive system require a million-fold variation in the ratio. However, the tuning of individual hair bundles is sharpened by the active process[175]. The frequency of a resonator operating near a Hopf bifurcation is directly proportional to the stiffness[46], so a far smaller variation in the value of that parameter can explain the range of hearing.

The eight characteristics described above, which link the operation of the cochlea to the physiological properties of hair cells, are all consistent with the cells' operation in the vicinity of a Hopf bifurcation. Rather than a model dependent on a particular physiological mechanism, this bifurcation is a phenomenon resembling a phase transition. It is analogous to the freezing of a liquid: irrespective of the particular substance involved and its specific freezing point, the transition between the liquid and solid states is a generic phenomenon with universal characteristics. The generic features of a Hopf bifurcation not only betray the presence of this dynamical instability but also suggest how it benefits the hearing process.

Impact of the Hopf bifurcation on hearing. A Hopf bifurcation exhibits a feature that renders it particularly appropriate for the analysis of auditory signals: it is the simplest form of bifurcation — local, codimension-1 and with only two variables — for which a dynamical system remains tuned to a nearly constant frequency as it approaches and traverses the bifurcation[198]. As a consequence, a given hair cell can adjust its sensitivity, tuning and compression without altering its characteristic frequency. When operating at a distance from the bifurcation, the hair bundle is relatively insensitive to stimulation, shows broad tuning around the characteristic frequency and displays little compressive non-linearity. As the bundle nears the bifurcation, it grows progressively more sensitive, better tuned to a specific frequency and increasingly compressive. And upon crossing the bifurcation, the hair bundle becomes unstable and begins to oscillate at or near the same frequency. These features are highly desirable for the auditory nervous system, in which information about each stimulus frequency ascends through a hierarchy of tonotopically ordered neural relays[199]. If a given hair cell were to markedly change its characteristic frequency under various conditions, the meaning of neural activity in all the subsequent neurons would likewise be corrupted.

Despite its several advantages, a Hopf bifurcation does introduce at least two important problems. First, as amplification grows stronger near the bifurcation, the rise time of responses also increases. Because this property can slow the reaction to behaviourally important sounds, temporal resolution is compromised in favour of sensitivity. The other problematic issue is stability. Unless its control parameters are precisely regulated, a system operating near a Hopf bifurcation can veer into a regime of relative insensitivity or a state of spontaneous oscillation, either of which degrades hearing. Although some pharmacological treatments are known to affect the state of the active process[56,145,160], an important challenge for further research is the identification of the mechanism by which hair cells regulate their excitability[17].

Although the discussion above primarily relates to the so-called supercritical Hopf bifurcation, in which oscillations grow gradually in amplitude as a system progresses into the unstable regime, other related bifurcations may also occur. For example, under some conditions hair bundles leap from quiescence to large-amplitude oscillations, the hallmark of a subcritical Hopf bifurcation[150,200]. Still more complex patterns, such as runs of oscillation punctuated by periods of inactivity, reflect bifurcations of a higher order[164]. Analytical modelling has delineated the behaviours expected when a hair bundle is subjected to particular combinations of force, elastic load and other parameters[156,201]. By systematically exploring this state space, researchers can delineate in greater detail the bifurcation landscape in which hair cells operate.

A Hopf bifurcation can arise in various dynamical systems. The manifestations of a Hopf bifurcation in cochlear physiology might reflect the properties of active hair-bundle motility, of somatic motility or conceivably of some other process altogether. In view of the incremental and opportunistic nature of evolution, however, it is most probable that a Hopf bifurcation originated early in vertebrate evolution as a feature of active hair-bundle motility and was exploited for its utility in mechanoelectrical transduction[44]. The subsequent development of piezoelectrically competent prestin provided the motive force necessary to amplify the oscillations of the entire basilar membrane, a substantially greater load than that shouldered by hair bundles in basal clades. From this point of view, the Hopf bifurcation in the mammalian cochlea is an emergent property of the combined system and does not inhere in either motile process alone.

Conclusion

Natural selection acts through random mutation to optimize the fitness of each organism, despite the fact that the interactions of its organs, tissues, cells and proteins are numerous and complex. Satisfying the requirements of natural selection doubtlessly requires many compromises with non-ideal behaviours, so the processes of life can rarely be codified by straightforward mathematical rules. Natural selection has nevertheless led to a simple dynamical phenomenon that endows our

sense of hearing with unique and valuable properties. The hair cells of the inner ear are not passive detectors of the mechanical energy provided by sound; instead, they use an active process that improves their performance in several ways. The effectiveness of this active process rests on its operation under particular conditions, specifically in the vicinity of a Hopf bifurcation. Evolution has evidently seized upon the many advantages of this instability — and accepted the associated drawbacks — to optimize the ear's performance.

1. Dalhoff, E., Turcanu, D., Zenner, H.-P. & Gummer, A. W. Distortion product otoacoustic emissions measured as vibration on the eardrum of human subjects. *Proc. Natl Acad. Sci. USA* **104**, 1546–1551 (2007).
2. Harris, G. G. Brownian motion in the cochlear partition. *J. Acoust. Soc. Am.* **44**, 176–186 (1968).
3. Sek, A. & Moore, B. C. Frequency discrimination as a function of frequency, measured in several ways. *J. Acoust. Soc. Am.* **97**, 2479–2486 (1995).
4. Reichenbach, T. & Hudspeth, A. J. Discrimination of low-frequency tones employs temporal fine structure. *PLoS ONE* **7**, e45579 (2012).
5. Yost, W. A. & Killion, M. C. in *Encyclopedia of Acoustics* Vol.3, Ch. 123 (ed. Crocker, M. J.) 1545–1554 (Wiley-Interscience, 1997).
6. Davis, H. An active process in cochlear mechanics. *Hear. Res.* **9**, 79–90 (1983).
7. Manley, G. A. Cochlear mechanisms from a phylogenetic viewpoint. *Proc. Natl Acad. Sci. USA* **97**, 11736–11743 (2000).
8. Manley, G. A. Evidence for an active process and a cochlear amplifier in nonmammals. *J. Neurophysiol.* **86**, 541–549 (2001).
9. Pickles, J. O. *An Introduction to the Physiology of Hearing* 4th edn (Emerald Group Publishing, 2012).
10. Hudspeth, A. J. in *Principles of Neural Science* 5th edn (eds Kandel, E. R., Schwartz, J. H., Jessel, T. M., Siegelbaum, S. A. & Hudspeth, A. J.) 654–681(McGraw-Hill Medical, 2013).
11. Retzius, G. *Das Gehörorgan der Wirbelthiere. II. Das Gehörorgan der Reptilien, der Vögel und der Säugethiere* 354 (Samson & Wallin, 1884).
12. Fettiplace, R. & Fuchs, P. A. Mechanisms of hair cell tuning. *Annu. Rev. Physiol.* **61**, 809–834 (1999).
13. Spiegel, M. F. Performance on frequency-discrimination tasks by musicians and nonmusicians. *J. Acoust. Soc. Am.* **76**, 1690 (1984).
14. De Boer, E. No sharpening? A challenge for cochlear mechanics. *J. Acoust. Soc. Am.* **73**, 567–573 (1983).
15. Reichenbach, T. & Hudspeth, A. J. Dual contribution to amplification in the mammalian inner ear. *Phys. Rev. Lett.* **105**, 118102 (2010).
16. Fisher, J. A. N., Nin, F., Reichenbach, T., Uthaiah, R. C. & Hudspeth, A. J. The spatial pattern of cochlear amplification. *Neuron* **76**, 989–997 (2012).
17. Camalet, S., Duke, T., Jülicher, F. & Prost, J. Auditory sensitivity provided by self-tuned critical oscillations of hair cells. *Proc. Natl Acad. Sci. USA* **97**, 3183–3188 (2000).
18. Brownell, W. E., Bader, C. R., Bertrand, D. & de Ribaupierre, Y. Evoked mechanical responses of isolated cochlear outer hair cells. *Science* **227**, 194–196 (1985).
19. Ashmore, J. F. A fast motile response in guinea-pig outer hair cells: the cellular basis of the cochlear amplifier. *J. Physiol.* **388**, 323–347 (1987).
20. Brownell, W. E., Spector, A. A., Raphael, R. M. & Popel, A. S. Micro- and nanomechanics of the cochlear outer hair cell. *Annu. Rev. Biomed. Eng.* **3**, 169–194 (2001).
21. Dallos, P., Zheng, J. & Cheatham, M. A. Prestin and the cochlear amplifier. *J. Physiol.* **576**, 37–42 (2006).
22. Dallos, P. Cochlear amplification, outer hair cells and prestin. *Curr. Opin. Neurobiol.* **18**, 370–376 (2008).
23. Ashmore, J. Cochlear outer hair cell motility. *Physiol. Rev.* **88**, 173–210 (2008).
24. Beurg, M., Tan, X. & Fettiplace, R. A prestin motor in chicken auditory hair cells: active force generation in a nonmammalian species. *Neuron* **79**, 69–81 (2013).
25. Zheng, J. *et al.* Prestin is the motor protein of cochlear outer hair cells. *Nature* **405**, 149–155 (2000).
26. Zheng, J. *et al.* Analysis of the oligomeric structure of the motor protein prestin. *J. Biol. Chem.* **281**, 19916–19924 (2006).
27. Wang, X., Yang, S., Jia, S. & He, D. Z. Z. Prestin forms oligomer with four mechanically independent subunits. *Brain Res.* **1333**, 28–35 (2010).
28. Hallworth, R. & Nichols, M. G. Prestin in HEK cells is an obligate tetramer. *J. Neurophysiol.* **107**, 5–11 (2012).
29. He, D. Z. Z., Lovas, S., Ai, Y., Li, Y. & Beisel, K. W. Prestin at year 14: progress and prospect. *Hear. Res.* http://dx.doi.org/10.1016/j.heares.2013.12.002 (2013).
30. Scherer, M. P. & Gummer, A. W. Vibration pattern of the organ of Corti up to 50 kHz: evidence for resonant electromechanical force. *Proc. Natl Acad. Sci. USA* **101**, 17652–17657 (2004).
31. Frank, G., Hemmert, W. & Gummer, A. W. Limiting dynamics of high-frequency electromechanical transduction of outer hair cells. *Proc. Natl Acad. Sci. USA* **96**, 4420–4425 (1999).
32. Liberman, M. C. *et al.* Prestin is required for electromotility of the outer hair cell and for the cochlear amplifier. *Nature* **419**, 300–304 (2002).
33. Cheatham, M. A., Huynh, K. H., Gao, J., Zuo, J. & Dallos, P. Cochlear function in prestin knockout mice. *J. Physiol.* **560**, 821–830 (2004).
34. Dallos, P. *et al.* Prestin-based outer hair cell motility is necessary for mammalian cochlear amplification. *Neuron* **58**, 333–339 (2008).
35. Liberman, M. C., Zuo, J. & Guinan, J. J. Jr. Otoacoustic emissions without somatic motility: can stereocilia mechanics drive the mammalian cochlea? *J. Acoust. Soc. Am.* **116**, 1649–1655 (2004).
36. Chan, D. K. & Hudspeth, A. J. Mechanical responses of the organ of corti to acoustic and electrical stimulation *in vitro*. *Biophys. J.* **89**, 4382–4395 (2005).
37. Kennedy, H. J., Crawford, A. C. & Fettiplace, R. Force generation by mammalian hair bundles supports a role in cochlear amplification. *Nature* **433**, 880–883 (2005).
38. Kennedy, H. J., Evans, M. G., Crawford, A. C. & Fettiplace, R. Depolarization of cochlear outer hair cells evokes active hair bundle motion by two mechanisms. *J. Neurosci.* **26**, 2757–2766 (2006).
39. Chan, D. K. & Hudspeth, A. J. Ca^{2+} current-driven nonlinear amplification by the mammalian cochlea *in vitro*. *Nature Neurosci.* **8**, 149–155 (2005).
40. Nin, F., Reichenbach, T., Fisher, J. A. N. & Hudspeth, A. J. Contribution of active hair-bundle motility to nonlinear amplification in the mammalian cochlea. *Proc. Natl Acad. Sci. USA* **109**, 21076–21080 (2012).
41. Markin, V. S. & Hudspeth, A. J. Modeling the active process of the cochlea: phase relations, amplification, and spontaneous oscillation. *Biophys. J.* **69**, 138–147 (1995).
42. Ó Maoiléidigh, D. & Jülicher, F. The interplay between active hair bundle motility and electromotility in the cochlea. *J. Acoust. Soc. Am.* **128**, 1175–1190 (2010).
43. Meaud, J. & Grosh, K. Coupling active hair bundle mechanics, fast adaptation, and somatic motility in a cochlear model. *Biophys. J.* **100**, 2576–2585 (2011).
44. Hudspeth, A. J. Making an effort to listen: mechanical amplification in the ear. *Neuron* **59**, 530–545 (2008).
45. Peng, A. W. & Ricci, A. J. Somatic motility and hair bundle mechanics, are both necessary for cochlear amplification? *Hear. Res.* **273**, 109–122 (2011).
46. Ó Maoiléidigh, D. & Hudspeth, A. J. Effects of cochlear loading on the motility of active outer hair cells. *Proc. Natl Acad. Sci. USA* **110**, 5474–5479 (2013).
47. Hudspeth, A. J. & Corey, D. P. Sensitivity, polarity, and conductance change in the response of vertebrate hair cells to controlled mechanical stimuli. *Proc. Natl Acad. Sci. USA* **74**, 2407–2411 (1977).
48. Fettiplace, R. & Hackney, C. M. The sensory and motor roles of auditory hair cells. *Nature Rev. Neurosci.* **7**, 19–29 (2006).
49. Gillespie, P. G. & Müller, U. Mechanotransduction by hair cells: models, molecules, and mechanisms. *Cell* **139**, 33–44 (2009).
50. Hudspeth, A. J., Jülicher, F. & Martin, P. A critique of the critical cochlea: Hopf—a bifurcation—is better than none. *J. Neurophysiol.* **104**, 1219–1229 (2010).
51. Richardson, G. P., de Monvel, J. B. & Petit, C. How the genetics of deafness illuminates auditory physiology. *Annu. Rev. Physiol.* **73**, 311–334 (2011).
52. Corey, D. P. & Hudspeth, A. J. Kinetics of the receptor current in bullfrog saccular hair cells. *J. Neurosci.* **3**, 962–976 (1983).
53. Crawford, A. C., Evans, M. G. & Fettiplace, R. The actions of calcium on the mechano-electrical transducer current of turtle hair cells. *J. Physiol.* **434**, 369–398 (1991).
54. Patuzzi, R. & Rajan, R. Does electrical stimulation of the crossed olivo-cochlear bundle produce movement of the organ of Corti? *Hear. Res.* **45**, 15–32 (1990).
55. Kirk, D. L., Moleirinho, A. & Patuzzi, R. B. Microphonic and DPOAE measurements suggest a micromechanical mechanism for the 'bounce' phenomenon following low-frequency tones. *Hear. Res.* **112**, 69–86 (1997).
56. Bobbin, R. P. & Salt, A. N. ATP-γ-S shifts the operating point of outer hair cell transduction towards scala tympani. *Hear. Res.* **205**, 35–43 (2005).
57. Legan, P. K. *et al.* A targeted deletion in α-tectorin reveals that the tectorial membrane is required for the gain and timing of cochlear feedback. *Neuron* **28**, 273–285 (2000).
58. Farris, H. E., Wells, G. B. & Ricci, A. J. Steady-state adaptation of mechanotransduction modulates the resting potential of auditory hair cells, providing an assay for endolymph $[Ca^{2+}]$. *J. Neurosci.* **26**, 12526–12536 (2006).
59. Evans, M. G. & Fuchs, P. A. Tetrodotoxin-sensitive, voltage-dependent sodium currents in hair cells from the alligator cochlea. *Biophys. J.* **52**, 649–652 (1987).
60. Marcotti, W., Johnson, S. L., Rusch, A. & Kros, C. J. Sodium and calcium currents shape action potentials in immature mouse inner hair cells. *J. Physiol.* **552**, 743–761 (2003).
61. Rutherford, M. A. & Roberts, W. M. Spikes and membrane potential oscillations in hair cells generate periodic afferent activity in the frog sacculus. *J. Neurosci.* **9**, 10025–10037 (2009).
62. Tritsch, N. X. *et al.* Calcium action potentials in hair cells pattern auditory neuron activity before hearing onset. *Nature Neurosci.* **13**, 1050–1052 (2010).
63. Martin, P., Mehta, A. D. & Hudspeth, A. J. Negative hair-bundle stiffness betrays a mechanism for mechanical amplification by the hair cell. *Proc. Natl Acad. Sci. USA* **97**, 12026–12031 (2000).
64. He, D. Z. Z., Jia, S. & Dallos, P. Mechanoelectrical transduction of adult outer hair cells studied in a gerbil hemicochlea. *Nature* **429**, 766–770 (2004).
65. Johnson, S. L., Beurg, M., Marcotti, W. & Fettiplace, R. Prestin-driven cochlear amplification is not limited by the outer hair cell membrane time constant. *Neuron* **70**, 1143–1154 (2011).
66. Corey, D. P. & Hudspeth, A. J. Response latency of vertebrate hair cells. *Biophys. J.* **26**, 499–506 (1979).
67. Pickles, J. O., Comis, S. D. & Osborne, M. P. Cross-links between stereocilia in the guinea pig organ of Corti, and their possible relation to sensory transduction. *Hear. Res.* **15**, 103–112 (1984).
68. Kachar, B., Parakkal, M., Kurc, M., Zhao, Y. & Gillespie, P. G. High-resolution structure of hair-cell tip links. *Proc. Natl Acad. Sci. USA* **97**, 13336–13341 (2000).
69. Auer, M. *et al.* Three-dimensional architecture of hair-bundle linkages revealed by electron-microscopic tomography. *J. Assoc. Res. Otolaryngol.* **9**, 215–224 (2008).
70. Siemens, J. *et al.* Cadherin 23 is a component of the tip link in hair-cell stereocilia. *Nature* **428**, 950–955 (2004).
71. Söllner, C. *et al.* Mutations in cadherin 23 affect tip links in zebrafish sensory hair cells. *Nature* **428**, 955–959 (2004).
72. Ahmed, Z. M. *et al.* The tip-link antigen, a protein associated with the transduction complex of sensory hair cells, is protocadherin-15. *J. Neurosci.* **26**, 7022–7034 (2006).
73. Kazmierczak, P. *et al.* Cadherin 23 and protocadherin 15 interact to form tip-link filaments in sensory hair cells. *Nature* **449**, 87–91 (2007).

74. Sotomayor, M., Weihofen, W. A., Gaudet, R. & Corey, D. P. Structural determinants of cadherin-23 function in hearing and deafness. *Neuron* **66**, 85–100 (2010).
75. Sotomayor, M., Weihofen, W. A., Gaudet, R. & Corey, D. P. Structure of a force-conveying cadherin bond essential for inner-ear mechanotransduction. *Nature* **492**, 128–132 (2012).
76. Assad, J. A., Shepherd, G. M. & Corey, D. P. Tip-link integrity and mechanical transduction in vertebrate hair cells. *Neuron* **7**, 985–994 (1991).
77. Zhao, Y., Yamoah, E. N. & Gillespie, P. G. Regeneration of broken tip links and restoration of mechanical transduction in hair cells. *Proc. Natl Acad. Sci. USA* **93**, 15469–15474 (1996).
78. Indzhykulian, A. A. *et al.* Molecular remodeling of tip links underlies mechanosensory regeneration in auditory hair cells. *PLoS Biol.* **11**, e1001583 (2013).
79. Howard, J. & Hudspeth, A. J. Mechanical relaxation of the hair bundle mediates adaptation in mechanoelectrical transduction by the bullfrog's saccular hair cell. *Proc. Natl Acad. Sci. USA* **84**, 3064–3068 (1987).
80. Howard, J. & Spudich, J. A. Is the lever arm of myosin a molecular elastic element? *Proc. Natl Acad. Sci. USA* **93**, 4462–4464 (1996).
81. Bozovic, D. & Hudspeth, A. J. Hair-bundle movements elicited by transepithelial electrical stimulation of hair cells in the sacculus of the bullfrog. *Proc. Natl Acad. Sci. USA* **100**, 958–963 (2003).
82. Powers, R. J. *et al.* Stereocilia membrane deformation: implications for the gating spring and mechanotransduction channel. *Biophys. J.* **102**, 201–210 (2012).
83. Bosher, S. K. & Warren, R. L. Very low calcium content of cochlear endolymph, an extracellular fluid. *Nature* **273**, 377–378 (1978).
84. Ikeda, K., Kusakari, J., Takasaka, T. & Saito, Y. The Ca^{2+} activity of cochlear endolymph of the guinea pig and the effect of inhibitors. *Hear. Res.* **26**, 117–125 (1987).
85. Marquis, R. E. & Hudspeth, A. J. Effects of extracellular Ca^{2+} concentration on hair-bundle stiffness and gating-spring integrity in hair cells. *Proc. Natl Acad. Sci. USA* **94**, 11923–11928 (1997).
86. Kozlov, A. S., Andor-Ardó, D. & Hudspeth, A. J. Anomalous Brownian motion discloses viscoelasticity in the ear's mechanoelectrical-transduction apparatus. *Proc. Natl Acad. Sci. USA* **109**, 2896–2901 (2012).
87. Shotwell, S. L., Jacobs, R. & Hudspeth, A. J. Directional sensitivity of individual vertebrate hair cells to controlled deflection of their hair bundles. *Ann. NY Acad. Sci.* **374**, 1–10 (1981).
88. Hudspeth, A. J. Extracellular current flow and the site of transduction by vertebrate hair cells. *J. Neurosci.* **2**, 1–10 (1982).
89. Lumpkin, E. A. & Hudspeth, A. J. Detection of Ca^{2+} entry through mechanosensitive channels localizes the site of mechanoelectrical transduction in hair cells. *Proc. Natl Acad. Sci. USA* **92**, 10297–10301 (1995).
90. Jaramillo, F. & Hudspeth, A. J. Localization of the hair cell's transduction channels at the hair bundle's top by iontophoretic application of a channel blocker. *Neuron* **7**, 409–420 (1991).
91. Denk, W., Holt, J. R., Shepherd, G. M. & Corey, D. P. Calcium imaging of single stereocilia in hair cells: localization of transduction channels at both ends of tip links. *Neuron* **15**, 1311–1321 (1995).
92. Beurg, M., Fettiplace, R., Nam, J.-H. & Ricci, A. J. Localization of inner hair cell mechanotransducer channels using high-speed calcium imaging. *Nature Neurosci.* **12**, 553–558 (2009).
93. Hudspeth, A. J. Transduction and tuning by vertebrate hair cells. *Trends Neurosci.* **6**, 366–369 (1983).
94. Holton, T. & Hudspeth, A. J. The transduction channel of hair cells from the bull-frog characterized by noise analysis. *J. Physiol.* **375**, 195–227 (1986).
95. Shin, J.-B. *et al.* Molecular architecture of the chick vestibular hair bundle. *Nature Neurosci.* **16**, 365–374 (2013).
96. Christensen, A. P. & Corey, D. P. TRP channels in mechanosensation: direct or indirect activation? *Nature Rev. Neurosci.* **8**, 510–521 (2007).
97. Chalfie, M. Neurosensory mechanotransduction. *Nature Rev. Mol. Cell Biol.* **10**, 44–52 (2009).
98. Arnadóttir, J. & Chalfie, M. Eukaryotic mechanosensitive channels. *Annu. Rev. Biophys.* **39**, 111–137 (2010).
99. Martinac, B. Bacterial mechanosensitive channels as a paradigm for mechanosensory transduction. *Cell. Physiol. Biochem.* **28**, 1051–1060 (2011).
100. Marshall, K. L. & Lumpkin, E. A. The molecular basis of mechanosensory transduction. *Adv. Exp. Med. Biol.* **739**, 142–155 (2012).
101. Sukharev, S. & Sachs, F. Molecular force transduction by ion channels: diversity and unifying principles. *J. Cell Sci.* **125**, 3075–3083 (2012).
102. Delmas, P. & Coste, B. Mechano-gated ion channels in sensory systems. *Cell* **155**, 278–284 (2013).
103. Wilson, M. E., Maksaev, G. & Haswell, E. S. MscS-like mechanosensitive channels in plants and microbes. *Biochemistry* **52**, 5708–5722 (2013).
104. Kurima, K. *et al.* Dominant and recessive deafness caused by mutations of a novel gene, *TMC1*, required for cochlear hair-cell function. *Nature Genet.* **30**, 277–284 (2002).
105. Labay, V., Weichert, R. M., Makishima, T. & Griffith, A. J. Topology of transmembrane channel-like gene 1 protein. *Biochemistry* **49**, 8592–8598 (2010).
106. Holt, J. R., Pan, B., Koussa, M. A. & Asai, Y. TMC function in hair cell transduction. *Hear. Res.* http://dx.doi.org/10.1016/j.heares.2014.01.001 (2014).
107. Kawashima, Y. *et al.* Mechanotransduction in mouse inner ear hair cells requires transmembrane channel-like genes. *J. Clin. Invest.* **121**, 4796–4809 (2011).
108. Kim, K. X. & Fettiplace, R. Developmental changes in the cochlear hair cell mechanotransducer channel and their regulation by transmembrane channel-like proteins. *J. Gen. Physiol.* **141**, 141–148 (2013).
109. Pan, B. *et al.* TMC1 and TMC2 are components of the mechanotransduction channel in hair cells of the mammalian inner ear. *Neuron* **79**, 504–515 (2013).
110. Kim, K. X. *et al.* The role of transmembrane channel-like proteins in the operation of hair cell mechanotransducer channels. *J. Gen. Physiol.* **142**, 493–505 (2013).
111. Kindt, K. S., Finch, G. & Nicolson, T. Kinocilia mediate mechanosensitivity in developing zebrafish hair cells. *Dev. Cell* **23**, 329–341 (2012).
112. Alagramam, K. N. *et al.* Mutations in protocadherin 15 and cadherin 23 affect tip links and mechanotransduction in mammalian sensory hair cells. *PLoS ONE* **6**, e19183 (2011).
113. Marcotti, W. *et al.* Transduction without tip links in cochlear hair cells is mediated by ion channels with permeation properties distinct from those of the mechano-electrical transducer channel. *J. Neurosci.* **34**, 5505–5514 (2014).
114. Keresztes, G., Mutai, H. & Heller, S. *TMC* and *EVER* genes belong to a larger novel family, the TMC gene family encoding transmembrane proteins. *BMC Genomics* **4**, 24 (2003).
115. Mitchem, K. L. *et al.* Mutation of the novel gene *Tmie* results in sensory cell defects in the inner ear of spinner, a mouse model of human hearing loss DFNB6. *Hum. Mol. Genet.* **11**, 1887–1898 (2002).
116. Naz, S. *et al.* Mutations in a novel gene, *TMIE*, are associated with hearing loss linked to the *DFNB6* locus. *Am. J. Hum. Genet.* **71**, 632–636 (2002).
117. Gleason, M. R. *et al.* The transmembrane inner ear (Tmie) protein is essential for normal hearing and balance in the zebrafish. *Proc. Natl Acad. Sci. USA* **106**, 21347–21352 (2009).
118. Longo-Guess, C. M. *et al.* A missense mutation in the previously undescribed gene *Tmhs* underlies deafness in hurry-scurry (*hscy*) mice. *Proc. Natl Acad. Sci. USA* **102**, 7894–7899 (2005).
119. Shabbir, M. I. *et al.* Mutations of human *TMHS* cause recessively inherited non-syndromic hearing loss. *J. Med. Genet.* **43**, 634–640 (2006).
120. Xiong, W. *et al.* TMHS is an integral component of the mechanotransduction machinery of cochlear hair cells. *Cell* **151**, 1283–1295 (2012).
121. Coste, B. *et al.* Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. *Science* **330**, 55–60 (2010).
122. Coste, B. *et al.* Piezo proteins are pore-forming subunits of mechanically activated channels. *Nature* **483**, 176–181 (2012).
123. Woo, S.-H. *et al.* Piezo2 is required for Merkel-cell mechanotransduction. *Nature* **509**, 622–626 (2014).
124. Howard, J., Roberts, W. M. & Hudspeth, A. J. Mechanoelectrical transduction by hair cells. *Annu. Rev. Biophys. Biophys. Chem.* **17**, 99–124 (1988).
125. Howard, J. & Hudspeth, A. J. Compliance of the hair bundle associated with gating of mechanoelectrical transduction channels in the bullfrog's saccular hair cell. *Neuron* **1**, 189–199 (1988).
126. Denk, W., Keolian, R. M. & Webb, W. W. Mechanical response of frog saccular hair bundles to the aminoglycoside block of mechanoelectrical transduction. *J. Neurophysiol.* **68**, 927–932 (1992).
127. Le Goff, L., Bozovic, D. & Hudspeth, A. J. Adaptive shift in the domain of negative stiffness during spontaneous oscillation by hair bundles from the internal ear. *Proc. Natl Acad. Sci. USA* **102**, 16996–17001 (2005).
128. Eatock, R. A., Corey, D. P. & Hudspeth, A. J. Adaptation of mechanoelectrical transduction in hair cells of the bullfrog's sacculus. *J. Neurosci.* **7**, 2821–2836 (1987).
129. Hacohen, N., Assad, J. A., Smith, W. J. & Corey, D. P. Regulation of tension on hair-cell transduction channels: displacement and calcium dependence. *J. Neurosci.* **9**, 3988–3997 (1989).
130. Assad, J. A. & Corey, D. P. An active motor model for adaptation by vertebrate hair cells. *J. Neurosci.* **12**, 3291–3309 (1992).
131. Assad, J. A., Hacohen, N. & Corey, D. P. Voltage dependence of adaptation and active bundle movement in bullfrog saccular hair cells. *Proc. Natl Acad. Sci. USA* **86**, 2918–2922 (1989).
132. Holt, J. R., Corey, D. P. & Eatock, R. A. Mechanoelectrical transduction and adaptation in hair cells of the mouse utricle, a low-frequency vestibular organ. *J. Neurosci.* **17**, 8739–8748 (1997).
133. Peng, A. W., Effertz, T. & Ricci, A. J. Adaptation of mammalian auditory hair cell mechanotransduction is independent of calcium entry. *Neuron* **80**, 960–972 (2013).
134. Gillespie, P. G. & Hudspeth, A. J. Adenine nucleoside diphosphates block adaptation of mechanoelectrical transduction in hair cells. *Proc. Natl Acad. Sci. USA* **90**, 2710–2714 (1993).
135. Yamoah, E. N. & Gillespie, P. G. Phosphate analogs block adaptation in hair cells by inhibiting adaptation-motor force production. *Neuron* **17**, 523–533 (1996).
136. Batters, C. *et al.* Myo1c is designed for the adaptation response in the inner ear. *EMBO J.* **23**, 1433–1440 (2004).
137. Batters, C., Wallace, M. I., Coluccio, L. M. & Molloy, J. E. A model of stereocilia adaptation based on single molecule mechanical studies of myosin I. *Phil. Trans. R. Soc. Lond. B* **359**, 1895–1905 (2004).
138. Gillespie, P. G., Wagner, M. C. & Hudspeth, A. J. Identification of a 120 kd hair-bundle myosin located near stereociliary tips. *Neuron* **11**, 581–594 (1993).
139. Schneider, M. E. *et al.* A new compartment at stereocilia tips defined by spatial and temporal patterns of myosin IIIa expression. *J. Neurosci.* **26**, 10243–10252 (2006).
140. Garcīa, J. A., Yee, A. G., Gillespie, P. G. & Corey, D. P. Localization of myosin-Iβ near both ends of tip links in frog saccular hair cells. *J. Neurosci.* **18**, 8637–8647 (1998).
141. Steyger, P. S., Gillespie, P. G. & Baird, R. A. Myosin Iβ is located at tip link anchors in vestibular hair bundles. *J. Neurosci.* **18**, 4603–4615 (1998).
142. Holt, J. R. *et al.* A chemical-genetic strategy implicates myosin-1c in adaptation by hair cells. *Cell* **108**, 371–381 (2002).
143. Kros, C. J. *et al.* Reduced climbing and increased slipping adaptation in cochlear hair cells of mice with Myo7a mutations. *Nature Neurosci.* **5**, 41–47 (2002).
144. Grati, M. & Kachar, B. Myosin VIIa and sans localization at stereocilia upper tip-link density implicates these Usher syndrome proteins in mechanotransduction. *Proc. Natl Acad. Sci. USA* **108**, 11476–11481 (2011).
145. Ricci, A. J. & Fettiplace, R. The effects of calcium buffering and cyclic AMP on mechano-electrical transduction in turtle auditory hair cells. *J. Physiol.* **501**, 111–124 (1997).
146. Kennedy, H. J., Evans, M. G., Crawford, A. C. & Fettiplace, R. Fast adaptation of mechanoelectrical transducer channels in mammalian cochlear hair cells. *Nature Neurosci.* **6**, 832–836 (2003).
147. Benser, M. E., Marquis, R. E. & Hudspeth, A. J. Rapid, active hair bundle movements in hair cells from the bullfrog's sacculus. *J. Neurosci.* **16**, 5629–5643 (1996).
148. Ricci, A. J., Crawford, A. C. & Fettiplace, R. Active hair bundle motion linked to fast transducer adaptation in auditory hair cells. *J. Neurosci.* **20**, 7131–7142 (2000).
149. Choe, Y., Magnasco, M. O. & Hudspeth, A. J. A model for amplification of hair-bundle motion by cyclical binding of Ca^{2+} to mechanoelectrical-transduction channels. *Proc. Natl Acad. Sci. USA* **95**, 15321–15326 (1998).
150. Tinevez, J.-Y., Jülicher, F. & Martin, P. Unifying the various incarnations of active hair-bundle motility by the vertebrate hair cell. *Biophys. J.* **93**, 4053–4067 (2007).

151. Stauffer, E. A. *et al.* Fast adaptation in vestibular hair cells requires myosin-1c activity. *Neuron* **47**, 541–553 (2005).
152. Kössl, M. Otoacoustic emissions from the cochlea of the 'constant frequency' bats, *Pteronotus parnellii* and *Rhinolophus rouxi*. *Hear. Res.* **72**, 59–72 (1994).
153. Hudspeth, A. J. & Gillespie, P. G. Pulling springs to tune transduction: adaptation by hair cells. *Neuron* **12**, 1–9 (1994).
154. Manley, G. A. & Gallo, L. Otoacoustic emissions, hair cells, and myosin motors. *J. Acoust. Soc. Am.* **102**, 1049–1055 (1997).
155. Pringle, J. W. The contractile mechanism of insect fibrillar muscle. *Prog. Biophys. Mol. Biol.* **17**, 1–60 (1967).
156. Ó Maoiléidigh, D., Nicola, E. M. & Hudspeth, A. J. The diverse effects of mechanical loading on active hair bundles. *Proc. Natl Acad. Sci. USA* **109**, 1943–1948 (2012).
157. Lumpkin, E. A., Marquis, R. E. & Hudspeth, A. J. The selectivity of the hair cell's mechanoelectrical-transduction channel promotes Ca^{2+} flux at low Ca^{2+} concentrations. *Proc. Natl Acad. Sci. USA* **94**, 10997–11002 (1997).
158. Ricci, A. J. & Fettiplace, R. Calcium permeation of the turtle hair cell mechanotransducer channel and its relation to the composition of endolymph. *J. Physiol.* **506**, 159–173 (1998).
159. Cheung, E. L. M. & Corey, D. P. Ca^{2+} changes the force sensitivity of the hair-cell transduction channel. *Biophys. J.* **90**, 124–139 (2006).
160. Martin, P., Bozovic, D., Choe, Y. & Hudspeth, A. J. Spontaneous oscillation by hair bundles of the bullfrog's sacculus. *J. Neurosci.* **23**, 4533–4548 (2003).
161. Kroese, A. B., Das, A. & Hudspeth, A. J. Blockage of the transduction channels of hair cells in the bullfrog's sacculus by aminoglycoside antibiotics. *Hear. Res.* **37**, 203–217 (1989).
162. Doll, J. C., Peng, A. W., Ricci, A. J. & Pruitt, B. L. Faster than the speed of hearing: nanomechanical force probes enable the electromechanical observation of cochlear hair cells. *Nano Lett.* **12**, 6107–6111 (2012).
163. Crawford, A. C. & Fettiplace, R. The mechanical properties of ciliary bundles of turtle cochlear hair cells. *J. Physiol.* **364**, 359–379 (1985).
164. Roongthumskul, Y., Fredrickson-Hemsing, L., Kao, A. & Bozovic, D. Multiple-timescale dynamics underlying spontaneous oscillations of saccular hair bundles. *Biophys. J.* **101**, 603–610 (2011).
165. Strimbu, C. E., Fredrickson-Hemsing, L. & Bozovic, D. Coupling and elastic loading affect the active response by the inner ear hair cell bundles. *PLoS ONE* **7**, e33862 (2012).
166. Martin, P. & Hudspeth, A. J. Active hair-bundle movements can amplify a hair cell's response to oscillatory mechanical stimuli. *Proc. Natl Acad. Sci. USA* **96**, 14306–14311 (1999).
167. Fredrickson-Hemsing, L., Ji, S., Bruinsma, R. & Bozovic, D. Mode-locking dynamics of hair cells of the inner ear. *Phys. Rev. E Stat. Nonlin. Soft Matter Phys.* **86**, 021915 (2012).
168. Dierkes, K., Lindner, B. & Jülicher, F. Enhancement of sensitivity gain and frequency tuning by coupling of active hair bundles. *Proc. Natl Acad. Sci. USA* **105**, 18669–18674 (2008).
169. Barral, J., Dierkes, K., Lindner, B., Jülicher, F. & Martin, P. Coupling a sensory hair-cell bundle to cyber clones enhances nonlinear amplification. *Proc. Natl Acad. Sci. USA* **107**, 8079–8084 (2010).
170. Vilfan, A. & Duke, T. Frequency clustering in spontaneous otoacoustic emissions from a lizard's ear. *Biophys. J.* **95**, 4622–4630 (2008).
171. Gelfand, M., Piro, O., Magnasco, M. O. & Hudspeth, A. J. Interactions between hair cells shape spontaneous otoacoustic emissions in a model of the tokay gecko's cochlea. *PLoS ONE* **5**, e11116 (2010).
172. Shera, C. A. Mammalian spontaneous otoacoustic emissions are amplitude-stabilized cochlear standing waves. *J. Acoust. Soc. Am.* **114**, 244–262 (2003).
173. Eguíluz, V. M., Ospeck, M., Choe, Y., Hudspeth, A. J. & Magnasco, M. O. Essential nonlinearities in hearing. *Phys. Rev. Lett.* **84**, 5232–5235 (2000).
174. Kern, A. & Stoop, R. Essential role of couplings between hearing nonlinearities. *Phys. Rev. Lett.* **91**, 128101 (2003).
175. Martin, P. & Hudspeth, A. J. Compressive nonlinearity in the hair bundle's active response to mechanical stimulation. *Proc. Natl Acad. Sci. USA* **98**, 14386–14391 (2001).
176. Martin, P., Hudspeth, A. J. & Jülicher, F. Comparison of a hair bundle's spontaneous oscillations with its response to mechanical stimulation reveals the underlying active process. *Proc. Natl Acad. Sci. USA* **98**, 14380–14385 (2001).
177. Overstreet, E. H. I. I. I., Temchin, A. N. & Ruggero, M. A. Basilar membrane vibrations near the round window of the gerbil cochlea. *J. Assoc. Res. Otolaryngol.* **3**, 351–361 (2002).
178. Ruggero, M. A., Rich, N. C., Recio, A., Narayan, S. S. & Robles, L. Basilar-membrane responses to tones at the base of the chinchilla cochlea. *J. Acoust. Soc. Am.* **101**, 2151–2163 (1997).
179. Walker, D. P. *Studies in Musical Science in the Late Renaissance* (Warburg Institute, 1978).
180. Campbell, M. & Greated, C. *A Musician's Guide to Acoustics* (Oxford Univ. Press, 2002).
181. Robles, L., Ruggero, M. A. & Rich, N. C. Two-tone distortion in the basilar membrane of the cochlea. *Nature* **349**, 413–414 (1991).
182. Robles, L., Ruggero, M. A. & Rich, N. C. Two-tone distortion on the basilar membrane of the chinchilla cochlea. *J. Neurophysiol.* **77**, 2385–2399 (1997).
183. Kozlov, A. S., Risler, T., Hinterwirth, A. J. & Hudspeth, A. J. Relative stereociliary motion in a hair bundle opposes amplification at distortion frequencies. *J. Physiol.* **590**, 301–308 (2012).
184. Jaramillo, F., Markin, V. S. & Hudspeth, A. J. Auditory illusions and the single hair cell. *Nature* **364**, 527–529 (1993).
185. Barral, J. & Martin, P. Phantom tones and suppressive masking by active nonlinear oscillation of the hair-cell bundle. *Proc. Natl Acad. Sci. USA* **109**, E1344–E1351 (2012).
186. Goldstein, J. L. Auditory nonlinearity. *J. Acoust. Soc. Am.* **41**, 676–689 (1967).
187. Smoorenburg, G. F. Audibility region of combination tones. *J. Acoust. Soc. Am.* **52**, 603 (1972).
188. Jülicher, F., Andor, D. & Duke, T. Physical basis of two-tone interference in hearing. *Proc. Natl Acad. Sci. USA* **98**, 9080–9085 (2001).
189. Stoop, R. & Kern, A. Two-tone suppression and combination tone generation as computations performed by the Hopf cochlea. *Phys. Rev. Lett.* **93**, 268103 (2004).
190. Anderson, D. J., Rose, J. E., Hind, J. E. & Brugge, J. F. Temporal position of discharges in single auditory nerve fibers within the cycle of a sine-wave stimulus: frequency and intensity effects. *J. Acoust. Soc. Am.* **49** (Suppl 2), 1131 + (1971).
191. Köppl, C. Phase locking to high frequencies in the auditory nerve and cochlear nucleus magnocellularis of the barn owl, *Tyto alba*. *J. Neurosci.* **17**, 3312–3321 (1997).
192. Izhikevich, E. M. Neural excitability, spiking and bursting. *Int. J. Bifurc. Chaos* **10**, 1171–1266 (2000).
193. Kemp, D. T. The evoked cochlear mechanical response and the auditory microstructure - evidence for a new element in cochlear mechanics. *Scand. Audiol. Suppl.* 35–47 (1979).
194. Talmadge, C. L., Long, G. R., Murphy, W. J. & Tubis, A. New off-line method for detecting spontaneous otoacoustic emissions in human subjects. *Hear. Res.* **71**, 170–182 (1993).
195. Penner, M. J. & Zhang, T. Prevalence of spontaneous otoacoustic emissions in adults revisited. *Hear. Res.* **103**, 28–34 (1997).
196. Köppl, C. & Manley, G. A. Spontaneous otoacoustic emissions in the bobtail lizard. I: general characteristics. *Hear. Res.* **71**, 157–169 (1993).
197. Manley, G. A. Spontaneous otoacoustic emissions from free-standing stereovillar bundles of ten species of lizard with small papillae. *Hear. Res.* **212**, 33–47 (2006).
198. Izhikevich, E. M. *Dynamical Systems in Neuroscience: The Geometry of Excitability and Bursting* (MIT Press, 2010).
199. Oertel, D. & Doupe, A. J. in *Principles of Neural Science* 5th edn Ch. 31 (eds Kandel, E. R., Schwartz, J. H., Jessel, T. M., Siegelbaum, S. A. & Hudspeth, A. J.) 682–711 (McGraw-Hill Medical, 2013).
200. Fredrickson-Hemsing, L., Strimbu, C. E., Roongthumskul, Y. & Bozovic, D. Dynamics of freely oscillating and coupled hair cell bundles under mechanical deflection. *Biophys. J.* **102**, 1785–1792 (2012).
201. Nadrowski, B., Martin, P. & Jülicher, F. Active hair-bundle motility harnesses noise to operate near an optimum of mechanosensitivity. *Proc. Natl Acad. Sci. USA* **101**, 12195–12200 (2004).

Acknowledgements
An investigator of Howard Hughes Medical Institute, the author thanks T. Reichenbach for the programme used to generate figure 1d and the members of his research group for comments on the manuscript.

Competing interests statement
The author declares no competing interests.