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The remarkable cochlear amplifier

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ABSTRACT

This composite article is intended to give the experts in the field of cochlear mechanics an opportunity to voice their personal opinion on the one mechanism they believe dominates cochlear amplification in mammals. A collection of these ideas are presented here for the auditory community and others interested in the cochlear amplifier. Each expert has given their own personal view on the topic and at the end of their commentary they have suggested several experiments that would be required for the decisive mechanism underlying the cochlear amplifier. These experiments are presently lacking but if successfully performed would have an enormous impact on our understanding of the cochlear amplifier.

Introduction

by Barbara Canlon

Mechanoelectrical transduction in the mammalian cochlea occurs due to vibrations of the basilar membrane that cause the stereocilia of the outer hair cells to deflect resulting in the gating of mechanosensitive transducer channels. There is an active mechanical response that amplifies low-level and compresses high-level basilar membrane displacements. The amplification is frequency dependent and results in high auditory sensitivity and an extended dynamic range.

The idea of an active process in the cochlea was first proposed by Gold, 1948, and has been the focus of intense research for more many decades. In 1983 Hallowell Davis wrote, "We are in the midst of a major breakthrough in auditory physiology. Recent experiments force us, I believe, to accept a revolutionary new hypothesis concerning the action of the cochlea namely, that an active process increases the vibration of the basilar membrane (BM) by energy provided somehow in the organ of Corti". In his insightful paper he describes a cochlear model to include an active process and its underlying properties.

Numerous scientific reports have been aimed at characterizing the biophysical, biochemical and molecular properties of the active process. Two main mechanisms have been put forth to explain the mechanism underlying the cochlear amplifier. In brief, one is a voltage-dependent somatic motility resulting from the activity of the motor protein prestin in the lateral membrane of the outer hair cells. The other is dependent on hair-bundle motility driven by calcium currents. There is a continuum of articles being published

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regarding the role of stereocilia versus somatic motility as the mechanism for the active process and these publications often spark up intense discussions among the auditory community.

There are two main mechanisms discussed in these commentaries (somatic and stereocilia based active processes) and several authors are suggesting that mechanical amplification is driven by both somatic and stereocilia contributions. However, all authors are in agreement that further experimentation is needed to be fully convinced of the mechanistic basis of outer hair cell motility. There are still many basic questions that remain to be answered before the basis of the cochlear amplifier or amplifiers is fully understood. As mentioned in the commentaries, some basic experiments that are needed include determining the characteristics of amplification along the basilar membrane (high versus low frequencies); dissecting the contribution of somatic motility from hair-bundle motility via genetic modifications and finally targeted biophysical experiments to alter ion channels and protein levels in hair cell membranes and in stereocilia. Hopefully the suggested experiments will soon be tested by inquisitive scientists to help generate a full characterization of the cochlear amplifier. There is most probably no definitive experiment but a combination of studies that will help solved the many years of debate and controversy around the cochlear amplifier.

Cochlear amplification – Somatic or stereocilial forces? A first-person response

by Jonathan Ashmore

It is always said that experimental artefacts are the most convincing of results. From the moment that Brownell and colleagues in Geneva reported that when an outer hair cell was depolarised it shortened (Brownell et al., 1985), there was always a nagging doubt that this was an epiphenomenon – a consequence of doing the experiments in a particular way. The Geneva finding used Ake Flock's earlier (re-)discovery at the Karolinska Institute of how to produce good-looking isolated OHCs. Ian Russell and I even took some home-built equipment to Stockholm in late 1982 to measure isolated cell resting potentials. We always found that the electrical V–I curves were difficult to record at hyperpolarised potentials. As a good electrophysiologist, straight from working in the retina, I did not think to look down the microscope while recording data. Of course I know now, in retrospect, that the records were contaminated by the cell expanding off the microelectrode (Fig. 1). Reliable recordings of the cell biophysics required patch clamp techniques, but that came three years later. So why do I still think that OHC motility underlies the cochlear amplifier?

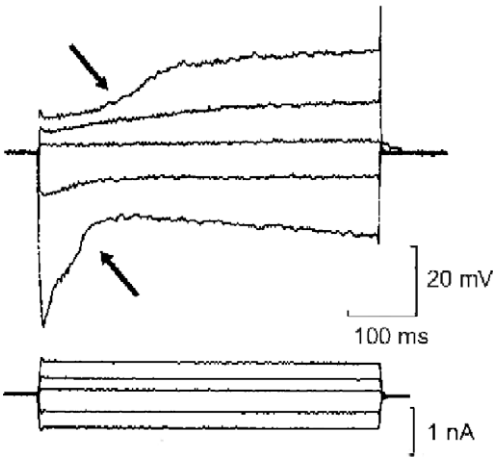

Fig. 1. Current-induced OHC movement. A microelectrode recording of potentials in an isolated guinea pig OHC during current injection, ca. 1983. Allowing for a low resting potential (ca. −30 mV), the voltage–current curves match data subsequently obtained by patch clamp recordings. The voltage distortions during current injection (arrowed) are almost certainly the result of the OHC changing length during the commands (Ashmore, unpublished).

It is a robust mechanism: OHC motility has been recorded in so many laboratories with so many different techniques that it is hard to believe any more that it is an artefact. The cells produce forces and motility is a robust phenomenon. This seems to me to be a necessary condition for its involvement in amplifying sound in the cochlea, or more specifically for injecting power into basilar membrane mechanics. The cochlea itself needs to be built with components which withstand some, if not all, the vicissitudes of life. I also like the idea that the sensor and the effector should be distinct and separated components of the cell. I do not think such arguments are foolproof, but experiments which point to hair-bundle forces are technically difficult to carry out. Although not ruled out for this reason, bundle forces appear to be much less robust.

It is fast: We now know that OHC length changes can be driven experimentally at acoustic speeds to over 70 kHz, (Frank et al., 1999). Bill Brownell and I managed to convince ourselves that OHCs could be driven faster than 1 kHz one December day in 1985 by using a photosensor and a huge, hardwired signal averager called a Biomac (serial number 5, since you asked, and whose 60 discrete component circuit boards I came to know intimately). But to relate these results to *in vivo* cochleas, it is necessary to argue around the 'RC-time constant problem' where any potential changes are filtered out by the membrane at acoustic frequencies. The host of ingenious resolutions of this problem, (including bundle forces), all involve some sort of cochlear modelling. I think that some of the most physiologically convincing (and most intuitively accessible) models which resolve the problem invoke larger transducer currents in basal cochlear OHCs to offset the membrane filter. Recent work with Pavel Mistrik also leads me to think that current flow along the cochlea, through the gap junctions, enhances the extracellular potentials with the correct phase so that the potentials driving prestin are further increased at high frequencies. In brief, there are cochlear models which seem to work.

It can be knocked out: Sydney Brenner once declared that if you delete a gene and something happens you have a party; if you delete a gene and nothing happens, you still have a party as it means that your gene is so important there is compensation. With prestin knocked out, auditory thresholds rise (Liberman et al., 2002); so there is a phenotype and you can have a party. The data is compelling, although there is still room for doubt as prestin may have other regulatory roles in the cell, for example, by controlling cell pH and metabolism (Ikeda et al., 1992). Mutated or absent prestins could easily distort other, non-motor, aspects of OHC physiology. There may be an opportunity for bundle mechanisms to steal in here, but the window is a small one.

What experiments might change my mind?

No effect of 'clean' prestin motor alterations: I would like to see more experiments to decouple transduction from the action of the prestin motor. There are some of these experiments under way, for example in a knockin mouse where the prestin voltage dependence is altered by a minimal peptide mutation (Dallos et al., 2008). It would also be good to design 'gain of function' mutations in prestin making a situation where the motor forces are enhanced. But what I would like to see most would be acute, reversible, experiments where the basilar membrane mechanics is measured during instantaneous inhibition of prestin – a 'caged salicylate', suddenly released, might be an attractive way to do this. And then to be surprised when nothing happened.

Convincing hair-bundle movements in the kilohertz range: I would like to see bundle force measurements carried out on mammalian hair bundles at frequencies over 5 kHz. For technical reasons, many of the arguments advanced so far for stereocilial forces are extrapolations from the data. To be convinced I would like to see measurements of the magnitude and the phase of real bundle forces from real mammalian cells. Moreover these need to be made from cells taken from different cochlear positions, for models predict that bundle forces should depend upon cochlear position before they contribute to the cochlear amplifier.

Top connectors of the hair-bundle are required for waveform distortion and suppression masking but not cochlear amplification

*by Paul Avan, Christine Petit**

Several major properties of sound perception rest upon the preprocessing of sound by the outer hair cells (OHC) in the mammalian inner ear, that is, one stage ahead of the mechanoelectrical transduction eventually achieved by inner hair cells (IHC). Those OHCs are the key element of a feedback loop whereby sound stimuli are mechanically amplified in a widely popular view (Davis, 1983; Gold, 1948). It is the most common explanation brought forward for explaining why the auditory system of mammals is sensitive enough to detect sound power levels hardly an order of magnitude above the thermal noise. Moreover, the fine tonotopy observed in the cochlea and reflected in the remarkable ability to discriminate two sounds with slightly different pitches, is also attributed to the regenerative amplifier with feedback, working through OHCs and that operates in a frequency-selective manner.

Natural sounds pose an additional challenge: several frequency components are presented simultaneously instead of sequentially. Spectral complexity increases in the presence of competing sound sources or background acoustic noise. In such cases, if applied indiscriminately to all spectral lines, gain would be inadequate because, acting equally on signal and noise, it would leave the latter swamp neural messages. Because the gain produced by OHCs is accompanied by filtering, but also because the nonlinearities it entails generate suppressive masking interactions, acoustic messages can be cleaned up.

The place of cochlear nonlinearities in the analysis of frequency mixtures deserves to be specifically examined. The concept of nonlinearity is very general, applying to any system whose response to two simultaneously presented signals is not the arithmetic sum of its responses to either signal when presented alone: instead, when mixed up, some components increase at the expense of others. Masking is a typically nonlinear psychophysical event defined by the fact that the loudness of one sound decreases or even vanishes when another sound interferes. Its cochlear correlate is suppressive masking whereby the mechanical or electrical response to a test tone decreases in the presence of a masking tone. This phenomenon, felt as a nuisance when it is the signal of interest that gets masked, globally turns as an advantage in that it allows the dominant frequency component at one place in the cochlea to become even more dominant by exerting a masking effect on competing, weaker signals. Therefore, suppressive masking can enhance contrasts.

There is now no doubt that cochlear mechanics is far from linear and it can express its nonlinearities in several ways. Besides suppressive masking, another example is that contrary to high-fidelity devices, OHC operation introduces conspicuous waveform distortions. These distortions are large enough to be heard although not being present in the initial sound stimulus (e.g., Tartini, 1754; Goldstein, 1967). In response to bitonal stimuli at frequencies f1 and f2, distortion of their waveforms generates combination tones at arithmetic combinations of f1 and f2 – hence the best known cubic difference tone at 2f1–f2, assuming f2 > f1. Not only does the cochlea produce audible sound distortion but it also reemits them as one category of otoacoustic emissions, namely distortion-product otoacoustic emissions (DPOAE) (Kim et al., 1980). Otoacoustic emissions have become a prominent tool for achieving neonatal hearing screening: when by being absent they signal OHC dysfunction and, according to the most popular interpretation, failure of the cochlear amplifier, inner hair cells also happen to be impaired in many cases, owing to the structural and functional kinship of the two types of sensory cells. Sensorineural deafness is then a likely diagnosis.

In summary, the currently accepted picture is that gain and filtering are two closely associated properties ensured by OHCs and that their way of operating induces strong waveform distortions coming out as non-invasively detectable DPOAEs. Last, the very mechanism that leads to instantaneous distortion of sound waveforms is likely strong enough to contribute to suppressive masking. This holistic view placing OHCs and their nonlinear behavior at the heart of the concept of cochlear amplifier and of many perceptive phenomena does not allow for the fact that the nonlinearities produced by OHCs do not share the same meaning and may thus have different structural or functional origins – e.g., the mechanotransduction channel for some of them, other molecules or substructures in the stereocilia bundle or cell body for others. Some types of nonlinearities in current use in electroacoustic amplifiers do not produce instantaneous waveform distortion, as is the case for compressive devices in hearing aids. Conversely, other types of nonlinearities do not need gain to generate waveform clipping.

Until now holistic models posited that at the core of OHC ability to produce gain, and the combination of filtering, and waveform distortion, and masking that comes with gain, is a common source, i.e., the intrinsic properties of the mechanotransduction channels.

A common explanation might be inherent to the mandatory nonlinearity associated with the thermodynamics of the mechanotransduction channel. This channel exists in at least two states, open and closed. Its opening probability relates to stereocilia deflection according to Boltzmann's law accounting for the different energies associated with the opened and closed states. Boltzmann's law is a sigmoid instead of a straight line, thus when stereocilia bundles are deflected by the sinusoidal pressure wave of a pure tone coming from outside, the current through mechanotransduction channels, proportional to the opening probability, exhibits a distorted waveform. The resulting mechanical feedback exerted through bi-directional transduction thus injects distortion into the initially sinusoidal sound wave. It was thought that waveform distortion, Tartini tones and DPOAEs were produced in this manner by OHCs. Simple mathematics then shows that waveform distortion generates suppressive masking (Engebretson and Eldredge, 1968).

This view of mechanotransduction channel properties as a central player in all aspects of sound pre-processing by OHCs suggested that OHCs ensured, in a remarkably parsimonious manner, a whole set of functions sharing a common origin. As a counterpart, failure of this intrinsic property of channels should also result in hearing impairment in relation to loss of cochlear amplification, and in the concomitant loss of all other beneficial aspects of cochlear pre-processing of sound.

A recent study of a mutant strain of mice in which the gene coding for stereocilin is inactivated has shown that the aforementioned holistic view seems not valid (Verpy et al., 2008). When these mutant mice are young enough (around 14–15 postnatal days, P14–15), their cochlear sensitivity is normal, as illustrated by the fact that across the whole frequency spectrum auditory brainstem evoked (ABR) and compound action potential (CAP) thresholds do not statistically differ in mutant mice and wild-type

littermates. Cochlear filtering is also normal in mutant mice, as indicated by the normal Q10s of their CAP masking tuning curves. Mechanoelectrical transduction currents derived from round-window measurements of cochlear microphonics are normal as well. These characteristics indicate the presence of a full supply of normally functioning mechanotransduction channels. Their thermodynamics thus obeys a normal Boltzmann law and the curve relating the transduction current to stereocilia deflection must be the same sigmoid as in normal ears. Yet in the absence of stereocilin, mice no longer distort waveforms, and for example their cochlear microphonics in response to loud tones remain sinusoidal up to 100 dB SPL. The electrical cochlear response to pure tones does not contain harmonics. Likewise, DPOAEs are totally absent. Furthermore, with even more significant perceptive consequences, when these mutant mice are exposed to a mixture of sounds, suppressive masking is absent or strongly diminished. The level of a masking tone must be about 20 dB louder than in a normal ear for the CAP response to a probe tone to decrease. CAP masking tuning curves can still be plotted; however, because the line-busy neural mechanism of masking, alone, persists: this is what allowed Q10s to be found similar in mutants and controls. Therefore, in the presence of a mixture of sounds, the mutant cochlea is no longer able to significantly act on the contrasts among components.

Stereocilin enters in the composition of hair-bundle fibrous links, the top connectors, bonding the apexes of stereocilia inside the bundle. In mutant mice, top connectors are absent and the tips of stereocilia in OHCs are more remote that in non-mutant mice.

So, suppressive masking and waveform distortion come with each other and can vanish even though OHC mechanotransduction channels provide normal amplification and filtering. This unusual experimental situation leads to conclude that the top connectors, and possibly the stereocilin-mediated contact of the stereocilia bundle to the tectorial membrane contribute to a major cause of distortion, larger than that in relation to the Boltzmann statistics of mechanotransduction channels. Stereocilin-dependent connectors could distort either as a result of an intrinsic property or indirectly by a constraint they might exert on the displacement of the stereocilia bundle or on the response to sound of some of its components.

We thus propose that in mutant mice as well as in normal ones, the operating curve of OHC mechanotransduction channels relating displacement to current exhibits a normal sigmoid shape because its becoming straighter would affect cochlear gain by negatively affecting channel sensitivity, which was not the case. Likely, this nonlinearity, on its own, is not large enough to generate measurable distortion. In normal mice, it is the presence of top connectors that enables waveform distortions, DPOAEs and suppressive masking to show up in standard measurements. In mutant mice, the same measurements detect none of these properties even though the cochlear amplifier works, thanks to a normally nonlinear mechanotransduction in OHCs.

Stereocilin mutants show that dissociation between normal auditory thresholds and missing DPOAEs is possible, if not commonplace. Previous work on acute cochlear ischemia has shown, conversely, that DPOAEs can persist and keep many of their normal properties although cochlear gain has vanished (Avan et al., 2003). Put together, these observations should warn clinicians against too systematic attempts at interpreting DPOAEs in terms of cochlear amplification and hearing sensitivity.

Membrane-based amplification in hearing

*by William E. Brownell**

Acoustic vibrations enter and neuronal action potentials leave the inner ear. An interplay of mechanical and electrical energy results in hair-cell receptor potentials that ultimately trigger neurotransmitter release at the afferent synapse. The diffusion of neurotransmitter across the synaptic cleft depolarizes 8th nerve terminals and initiates action potentials that travel to the central nervous system. The action potentials encode information about the spectral and temporal content of environmental sounds. The ability to localize predator or prey is improved by analyzing sounds over a wide range of frequencies resulting in an evolutionary selection pressure for detecting ever higher frequencies. Nature has incorporated diverse strategies to overcome physical constraints for high-frequency hearing. The constraints include: (1) viscous damping by inner ear fluids; (2) electrical filtering by cell membranes; and (3) temporal limitations imposed by chemical cascades at the synapse. The mechanisms that overcome viscous damping have been called the "cochlear amplifier" in mammalian ears and an "active process" in vestibular and other hair cell systems. These must work in concert with mechanisms for increasing membrane bandwidth and assuring the temporal precision of afferent fiber action potentials if high-frequency hearing is to be achieved.

It is likely that the cochlear amplifier originated in the stereocilia bundle of early vertebrates. Several mechanisms for bundle motility have been proposed but it is the one responsible for fast voltage-dependent bundle movement or flicks (Cheung and Corey, 2006) that suggests an evolutionary origin for the voltage-dependent somatic motility of the outer hair cell. In order for high-frequency voltage-dependent electromechanical transduction to take place in either the bundle or the soma there must be a mechanism that increases the electrical bandwidth of the membrane. Membrane flexoelectricity and converse flexoelectricity are suited for high-frequency bundle and somatic motility as well as increasing membrane bandwidth. A flexoelectric based "synaptic amplifier" may also help to assure the temporal precision of afferent fiber action potentials.

When outer hair cell electromotility was first observed (Brownell et al., 1985) it was a strong candidate for the mammalian cochlear amplifier. The OHC is unique to the mammalian cochlea and is perhaps the most exotically specialized hair cell (see Fig. 2). Morphological and molecular features of its lateral wall endow it with the ability to generate mechanical force at high frequencies (Frank et al., 1999). The force generating mechanism is located in the lateral wall plasma membrane where the transmembrane electric field is converted directly into mechanical force. Biological membranes are soft, thin ensembles of lipids, proteins, and other molecules. The proportions of the components vary but lipids dominate reaching 10^2 lipid molecules for every protein in some membranes. Membrane constituents diffuse freely within the plane of the membrane unless they are anchored to the cytoskeleton. Membranes are very thin (typically ~5 nm) yet cover large surface areas (>10^3 μm^2 in the case of the plasma membrane). Living cells expend metabolic energy to sustain electrochemical gradients (~100 mV) across their membranes and the associated transmembrane electric field is large (>10 MV/m – compare to the ~3 MV/m fields associated with atmospheric lighting). Living cells also expend energy to maintain a characteristic asymmetry in the number of lipid associated fixed charges on the inner and outer surfaces of their membranes. Integral membrane proteins can contribute to the electrical charge difference at the two surfaces. The net charge asymmetry of the membrane gives rise to an intrinsic electrical polarization that sets the stage for a piezoelectric-like force generation (Brownell, 2006). The electrical field is converted directly into mechanical stress and charge displacement is converted into mechanical strain. Experimental evidence demonstrates that electromechanical coupling occurs naturally in lipid bilayers where it is called the flexoelectric effect (Petrov, 2006; Sachs et al., 2009). This phenomenon is an analogue of the electromechanical

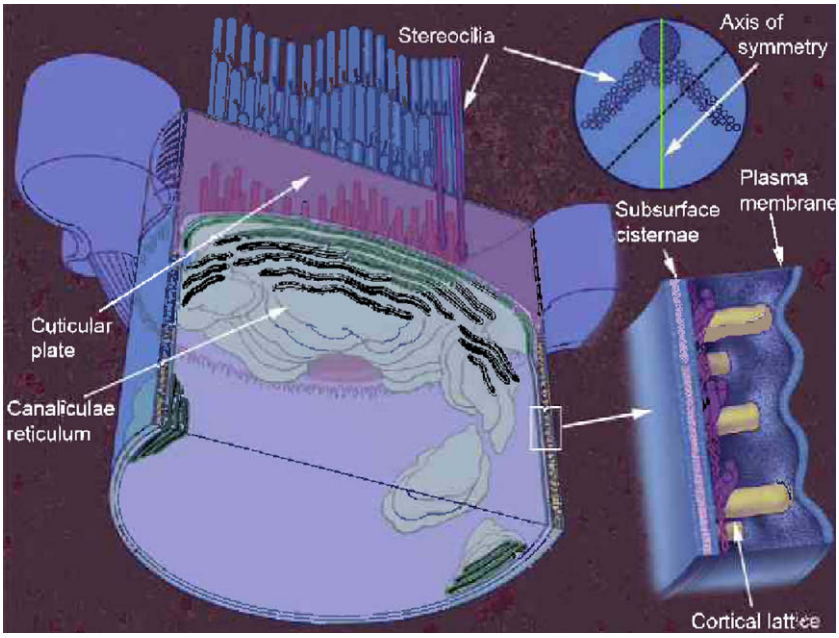

Fig. 2. Membrane organization of the outer hair cell stereocilia bundle and lateral wall. Both the apical pole and the lateral wall are composed of three layers. The plasma membrane is the outermost layer in both locations. The innermost layer is composed of a membrane bound organelle called the canalicular reticulum in the apex and the subsurface cisterna in the lateral wall. In between outer and inner membrane layers is a cytoskeletal structure called the cuticular plate at the apex and the cortical lattice in the lateral wall. Insert on the right portrays a high power rendering of the outer hair cell lateral wall. Insert at upper right is a view of the apical end showing the plane at which the outer hair cell has been opened. Adapted from Fig. 1 in Brownell, 2002.

behavior of piezoelectric crystals. Two kinds of flexoelectricity are typically discussed: (1) the direct flexoelectric effect describes changes in the electrical polarization of the membrane resulting from changes in curvature; and (2) the converse flexoelectric effect is the reciprocal phenomena in which the membrane curvature changes in response to applied electric fields. Both somatic (Raphael et al., 2000) and stereocilia bundle (Breneman et al., 2009) motility have been modeled to arise from converse flexoelectricity.

While membranes can produce high-frequency mechanical force (Anvari et al., 2007; Frank et al., 1999; Ludwig et al., 2001; Zhang et al., 2007) in response to experimentally applied electric fields the functional significance of this ability has been questioned because commonly studied cell membranes are considered to be low-pass electrical filters and therefore unable to sustain transmembrane receptor potentials at high frequencies. A solution for the low-pass constraint is provided by coupling electrical and mechanical energy. The ready conversion of one form of energy to the other endows the membrane with a biological piezoelectricity that pushes the cell membrane cutoff frequency to higher frequencies (Rabbitt et al., 2009; Spector et al., 2003; Weitzel et al., 2003).

Prestin is an integral membrane protein belonging to the Slc26A family of anion transporters that enhances the piezoelectric properties of transfected test cells (Ludwig et al., 2001; Zhang et al., 2007; Zheng et al., 2000). Prestin-associated charge movement is at least three orders of magnitude larger and qualitatively different than the nonlinear charge movement of untransfected cells (Farrell et al., 2006). Electromotile force production, in contrast, is increased by well under an order of magnitude (Anvari et al., 2007; Ludwig et al., 2001). The large prestin-associated non-ohmic, reactive displacement currents are thought to arise from the movement of cytoplasmic anions such as chloride and bicarbonate into and out the membrane. A model of the electrodiffusion of anions into a model protein is able to quantitatively reproduce several features of this charge movement (Sun et al., 2009). Prestin may help overcome the low-pass problem by facilitating a phaseshifted charge movement that compensates for membrane capacitance in a manner similar to the negative-capacitance circuits found in voltage-clamp amplifier headstages.

Both outer hair cell electromotility and neurotransmission at the inner hair cell synapse are rapid, membrane-based, mechanical events that are controlled by the hair cell receptor potential. Since neurotransmitter release can be synchronized to high frequencies (approaching 10 kHz) in some species, broad-band electrical properties are also required to allow synaptic stimulation. The magnitude of inner hair cell receptor potentials varies with stimulus intensity yet the timing of neural discharge is intensity invariant for both clicks and best frequency tones (if neurotransmitter release were only a function of current it would occur at different times as the intensity changed). Temporal invariance in the presence of receptor potentials of increasing magnitude argues for a feedback mechanism resembling that of the cochlear amplifier on basilar-membrane vibrations. OHC mechanical feedback preserves the temporal fine structure of basilar-membrane vibrations throughout a wide range of intensities (Shera, 2001). Temporal shifts of basilar-membrane vibration zero-crossings and local peaks and troughs would occur in the absence of mechanical feedback and these shifts are not observed experimentally (Recio and Rhode, 2000). Membrane flexoelectric mechanisms could provide an electromechanical feedback to exocystosis at the afferent synapse and help to insure intensity independent temporal precision (Brownell et al., 2003). The cochlear amplifier, broad-band electrical properties and the synaptic amplifier could all benefit from membrane electromechanics.

There are several experiments whose results could validate or disprove the flexoelectric concepts presented in this section. High-frequency axial displacements of the stereocilia bundle similar to those observed in membrane tethers (Zhang et al., 2007) is required to determine if converse flexoelectricity is contributing to the bundle motor. Experimental confirmation of the inverse relation between the radius of curvature of the membrane and electromechanical force production by the membrane is also

required. Such an experiment would require ultramicroscopic measures of the curvature. High resolution structural information for prestin is required to unravel its precise role in the outer hair cell somatic motor. The existence of acoustically evoked, non-ohmic, displacement currents in cochlear fluids is predicted by the prestin-associated charge movement measured in isolated cells. Experimental confirmation of cochlear displacement currents could explain the discrepancy between maximal hair cell receptor currents in isolated hair cells and those predicted from earlier cochlear current density measures (Zidanic and Brownell, 1990).

Feedback in the cochlea

by Peter Dallos

Science thrives on controversy and scientists love a good clean fight. Students of how mammalian "cochlear amplification" comes about have been in the ring for more than 30 years; more than 60 if we consider Gold's (1948) initial suggestions. The development of two schools of thought, championing outer hair cell (OHC) somatic motility and OHC ciliary motility as the means of amplification, is amply documented and need no review here (Dallos, 2008; Hudspeth, 2008). The common thread, that OHCs are the amplifier elements, arose early on the basis of experiments with chemical ablation of OHCs using ototoxic agents and the examination of resulting behavioral threshold shifts and alterations of neural tuning curves (Ryan and Dallos, 1975; Dallos and Harris, 1978; Liberman and Dodds, 1987). Inner hair cell (IHC) stereocilia have no firm contact with the tectorial membrane (Lim, 1980), consequently these cells are unlikely to participate in mechanical amplification.

Here I briefly list a few items that have been adduced as supportive or contrary to either amplifier schemes, which I consider to be less than deal breakers.

Probably the most often cited problem with somatic motility being the amplifier is its voltage dependence (Santos-Sacchi and Dilger, 1988). Inasmuch as the passive OHCs' lateral membranes are electrical low-pass filters with low cutoff frequencies (<1 kHz; Housley and Ashmore, 1992; Preyer et al., 1996) the receptor potential, which presumably drives electromotility, is attenuated at high frequencies. This seemingly fatal problem for electromotility-based amplification has been attacked by a whole host of schemes. These are in four major categories. One approach is to see if gross cochlear potentials might be sufficient to provide the voltage gradients for OHCs at high frequencies (Dallos and Evans, 1995; Fridberger et al., 2004; Iwasa and Sul, 2008), or if the cochlear electroanatomy is sufficiently influential (Mistrik et al., 2009). The second is based on the realization that the OHC is a reciprocal electromechanical system (Weiss, 1982). As a consequence, its effective time constant is not what is simply measured by electrical means in an isolated cell, but one modified by the reflection of the mechanical elements upon the electrical side of the network during contractile activity (Mountain and Hubbard, 1994; Spector et al., 2003; Ramamoorthy et al., 2007). The third possibility is that the collective action of a group of OHCs in a negative feedback circuit provides amplification at high frequencies even if individual OHCs are limited in their frequency response range (Lu et al., 2006 b). Finally, local activation of motor molecules by basolateral ionic current has been proposed as a means of avoiding the low-pass filter conundrum (Rybalchenko and Santos-Sacchi, 2003; Spector et al., 2005). While full experimental verification of any of these schemes is yet forthcoming, they, individually or collectively in some combinations, are sufficiently compelling as to render the principal objection to the somatic motility mechanism much less troublesome. The speed of stereociliary motility has been addressed as well. While the forward mechanotransducer channel activation is extremely fast (Corey and Hudspeth, 1983), fast adaptation of the channel, which is associated with the fast feedback process, is slower (Ricci et al., 2005). The development of force associated with OHC transducer channel activity has been measured (Kennedy et al., 2005). Negative stiffness (departure from linear stiffness) develops over time, but, while not proven, it is possible that *in vivo* the time course is adequately fast.

The second widely cited objection to the dominant role of somatic motility is that this process itself is not tuned. The context of this issue is the often-stated question: what tells an OHC to provide amplification for a given stimulus? The usual formulation is to postulate a need for a second system of graded filters, different from the traveling wave, which would provide the input to appropriately located amplifying OHCs. Tectorial membrane resonance is one of the favored means of such filtering (Allen, 1980; Zwislocki and Kletsky, 1979; Gummer et al., 1996). Another possibility is to enlist the inherent band-pass nature of the ciliary amplifier as a pre-filter to somatic motility (Ricci, 2003; Hudspeth, 2008). Tuning of ciliary motile processes (Martin and Hudspeth, 1999) may be a significant advantage, by itself, to this means of amplification.

One question raised about ciliary amplification pertains to the adequacy of the force that this source can deliver into the cochlear mechanical load. It is now reasonably certain that the collective action of circumscribed groups of OHCs can produce enough force to displace the cochlear partition, including the basilar membrane (Hudspeth, 2008; Dierkes et al., 2008). While the force produced by somatic motility is significantly greater, this should not preclude ciliary motility as a mechanism for amplification.

Are there definitive experiments that rule out the contribution of either candidate mechanism in the mammal? The short answer is no. There *are* experiments that suggest some combined operation of the two systems (Kennedy et al., 2006). The work of Chan and Hudspeth, 2005 intimates that ciliary motility is sufficient to provide cycle-by-cycle amplification, with slow somatic motility serving as an adjustor of the former system's operating point. A difficult problem in all experiments that attempt to parcel the process into its two possible components is eliminating one while sustaining the other. *In vitro*, the complete suppression of either process is difficult and may not have been achieved. *In vivo*, inasmuch as the cochlea operates as a feedback system any alteration of the feedback loop will affect the response of all components. Thus the difficulty of interpreting the results derived from mouse models in which the OHC motor molecule (prestin) was absent has been appreciated. In the absence of prestin from OHCs in the prestin knockout mouse, the cells become shorter and more compliant (Liberman et al., 2002; Cheatham et al., 2004; Dallos et al., 2008). Consequently, raised thresholds and lack of tuning in these mice could result from non-existing somatic motility, altered ciliary motility due to changed mechanical load, or a combination. While the model does not yield unequivocal results, the electrophysiological phenotype is essentially the same as one obtains in the absence of OHCs. In order to overcome incidental changes attendant to the lack of prestin, a mouse model was developed that incorporated the V499G/Y501H mutation in its prestin molecules (Dallos et al., 2008). OHCs in 499/501 mice have normal lengths and stiffnesses, but the prestin-produced somatic motility is more than 90% reduced. These animals have hearing loss and lack of tuning, not unlike the knockout mice. It was concluded that the presence of functional prestin is essential for the full expression of cochlear feedback. The result could be explained two ways. Somatic motility is the entire feedback amplifier and its elimination negates all gain. Alternatively, ciliary motility produces the feedback, but it is under tight control by somatic motility. At this time, further experimental refinement of the choice is lacking. The ubiquity of ciliary feedback-based amplification among vertebrates and indeed in some insects speaks for the primacy of this mechanism (Manley, 2001:

Hudspeth, 2008). Its suggested control by somatic motility in the mammal is more problematic. The commonly postulated low-frequency adjustment of the ciliary amplifier by somatic motility (e.g., Chan and Hudspeth, 2005) is not likely to occur due to the fact that, at low-levels, where amplification is most pronounced, the high-pass filter nature of mechanotransducer-channel fast-adaptation should virtually eliminate DC mechanical inputs to OHCs. Simply stated, the putative controlling DC signal in cochlear mechanics is significantly reduced. Of course, one should also ask what evolutionary pressure could have produced the voltage-activated prestin motor that has the demonstrated and unique capability of operating at ultrasonic frequencies (Frank et al., 1999), if its function would be effective only at DC?

Bottom lines: Prestin-based somatic motility and ciliary motility may both contribute to the total cochlear feedback. Examination of the cochlear output of genetically modified mice suggests that without functional prestin essentially all amplification is eliminated. The many suggested schemes to counteract OHC membrane filtering of receptor potentials suggest the possibility that the filter is not a necessary impediment to somatic motility providing the feedback at any frequency. It is unlikely that OHCs can provide DC control of a ciliary amplifier.

Coupled hair-bundles could endow the cochlear amplifier with sharp frequency tuning and nonlinear compression

*by Kai Dierkes, Benjamin Lindner, Frank Jülicher**

The key signatures of the auditory amplifier are (i) a frequency tuned and sensitive response to weak stimuli, (ii) a compressive nonlinear response over a large amplitude range, and (iii) spontaneous otoacoustic emissions (Dallos, 1992; Hudspeth, 2008). These signatures are reflected in observed basilar-membrane vibrations (Robles and Ruggero, 2001) and can be understood as the consequence of the presence of nonlinear dynamic oscillators operating in a critical regime (Camalet et al., 2000; Eguiluz et al., 2000; Duke and Jülicher, 2003). This suggests that the working of the cochlear amplifier is based on nonlinear oscillators. It is commonly thought that active amplification is mediated by mechano-sensory hair cells (Dallos, 1992; Hudspeth, 1997; Manley et al., 2001; Fettiplace and Hackney, 2006). Two important features of hair cells have been suggested to contribute: (i) outer hair cell electromotility can provide mechanical feedback to the basilar-membrane vibrations (Brownell et al., 1985; Santos-Sacchi, 2003; Ashmore, 2008; Dallos et al., 2008) and (ii) mechanosensitive hair-bundles have been shown to be active elements which can generate spontaneous movements and noisy oscillations (Crawford and Fettiplace, 1985; Martin and Hudspeth, 1999; Martin et al., 2001; Kennedy et al., 2005). Individual hair bundles can act as nonlinear oscillators capable to amplify stimuli (Martin and Hudspeth, 1999,Martin and Hudspeth, 2001) albeit with restricted performance which is limited by intrinsic noise at the cellular scale (Nadrowski et al., 2004). This limitation as well as the small forces associated with movements of individual hair-bundles have put doubts on the role of active hair-bundle motility for the cochlear amplifier.

In many vertebrate inner ear organs hair-bundles are linked to overlying elastic membraneous structures, such as otolithic and tectorial membranes (see Fig. 3a) and (Freeman et al., 2003). This introduces the possibility that the cooperation of hair-bundles plays a role to enhance the properties of hair-bundle-mediated amplification (Manley and Köppl, 2008). Recently, we have shown that small groups of hair bundles which are coupled by elastic elements can respond much more sensitively to periodic stimuli than isolated hair-bundles. Furthermore, such groups of hair-bundles display spontaneous movements with sharply peaked power spectra and behave as sharply tuned amplifiers that exhibit compressive nonlinearities over a wide range of signal amplitudes (Dierkes et al., 2008).

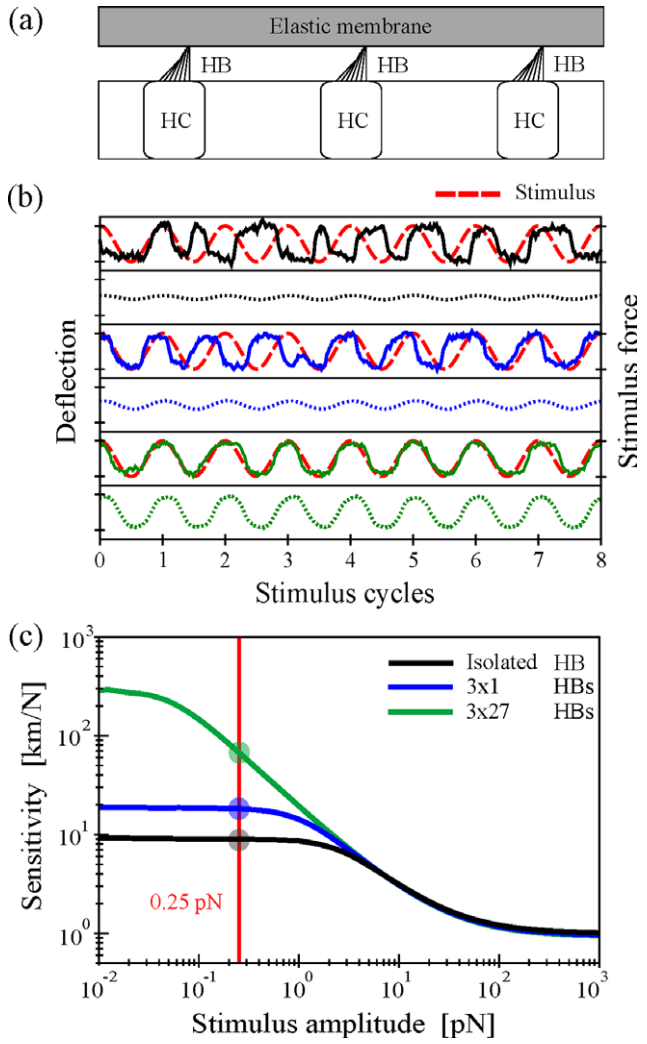

Fig. 3. (a) Schematic of three hair cells (HC) with their hair-bundles (HB) coupled elastically via an overlying membrane. (b) Illustration of phase-locking for an isolated (black) and the central hair-bundle of groups of coupled hair-bundles (3×1 HBs, blue; 3×27 HBs, green). Sample trajectories of simulation results (solid lines) are shown together with the periodic stimulus force $F(t) = A\cos(2\pi f_0 t)$ with $A = 0.25$ pN (broken red line) for coupling stiffness matched to stereociliar pivotal stiffness, $K = K_{SP} = 0.6$ pN/nm. Each system is driven at its characteristic frequency f_0 ($f_0 = 8.91$ Hz (1×1), 9.90 Hz (3×1), 10.54 Hz (3×27)). The respective time-dependent average responses over many repetitions of the stimulus are shown as dotted lines below. Distance between ticks is 40 nm for deflection and 0.5 pN for stimulus force. (c) Nonlinear response of coupled hair-bundles. For the three systems studied in (b) the sensitivity (average response amplitude divided by stimulus amplitude) is displayed as a function of stimulus amplitude. The red vertical line indicates the stimulus force used in (b). Note that the sensitivity to weak stimuli and the amplitude range of nonlinear compression increase with increasing system size.

In our study we employed a model of the single hair-bundle that can account quantitatively for its active mechanical properties and the stochastic features of hair-bundle motility (Nadrowski et al., 2004; Tinevez et al., 2007). The model incorporates stereociliar pivotal stiffness, channel gating elasticity, the properties of adaptation motors, as well as calcium feedback on these motors. Fluctuations reflecting thermal interactions of the hair-bundle with the surrounding fluid, stochastic transitions of transducer channels and adaptation motors are also taken into account. Limitations of the single hair bundle's ability to respond faithfully to an external stimulus (see Fig. 3b, broken red lines) are consequences

of these fluctuations (see Fig. 3b, black solid line). Fluctuations thereby limit the detector's sensitivity to weak stimuli and also the sharpness of frequency tuning, as well as the amplitude range over which nonlinear amplification occurs.

Our results were obtained by considering groups of $N \times M$ hair-bundles that are arranged on a square lattice with their excitatory directions aligned along the same lattice axis. Coupling is described by linear springs of stiffness K that connect nearest neighbors including diagonal connections. Homogeneous systems of identical hair-bundles as well as heterogeneous systems of hair-bundles with varying characteristic frequency were considered. In the homogeneous case the quality of spontaneous oscillations exhibits a threshold-like dependence on coupling strength K. A sudden increase of quality occurs for $K \approx K_{SP}$, with K_{SP} denoting the stereociliar pivotal stiffness. When a group of hair-bundles is driven by a weak periodic stimulus at the characteristic frequency (see Fig. 3b, broken red lines), the system shows an enhanced phase-locking to the external signal (see Fig. 3b, cf. blue and green solid lines to black solid line). This higher degree of phase-locking leads to an increase of the time-dependent average of the response amplitude (see Fig. 3b, dotted lines). Thus coupling of hair-bundles increases the sensitivity (defined as the ratio of the mean response amplitude to the stimulus amplitude) in response to a weak stimulus (see Fig. 3c). For increasing stimulus amplitude, the sensitivity decreases, indicative of the compressive nonlinear response of the system. The range of stimulus amplitudes over which this nonlinear response is observed increases for increasing system size (see Fig. 3c). The response to strong stimuli is determined by the passive stiffness of the single hair-bundles and does not depend on system size. As a consequence the amplification gain, which is the ratio of sensitivities to weak and strong stimuli, increases almost linearly with system size. For a system of 81 hair bundles a gain of up to 400 is obtained for optimal coupling strength.

In the mammalian cochlea, nonlinear compression of the basilar-membrane vibration amplitude in response to stimuli at the local characteristic frequency have been reported, that range up to four orders of magnitude of sound pressure amplitude (Robles and Ruggero, 2001). The corresponding amplification gains are of the order of 1000 (Robles and Ruggero, 2001). These properties can be understood as resulting from the combination of a global excitation of the basilar membrane (the traveling wave) and the effects of nonlinear active elements which govern the basilar-membrane vibration in the vicinity of the characteristic place (Nobili and Mammano, 1996; Duke and Jülicher, 2003). While the properties of the active elements in the cochlea exceed by far the abilities of an isolated hair-bundle, our work suggests that groups of coupled hair-bundles can approach their performance.

In the mammalian cochlea the basilar membrane exhibits a graded profile of characteristic frequencies and the sensory hair cells display a morphological gradient (Dallos et al., 1996). This raises the question whether enhanced signal detection due to coupling can also work in heterogeneous systems. We thus performed simulations of systems of 3 times 27 hair-bundles (representing three rows of outer hair cells) with varying intrinsic frequencies, resulting from a gradient of pivotal stiffness. For intermediate coupling strength $K \approx \overline{K}_{SP}$, where $\overline{K}_{SP}$ is the average pivotal stiffness of the hair-bundles, the amplification gain is still enhanced by coupling, while a frequency gradient is also maintained (Dierkes et al., 2008). This implies that in order to make use of mechanical coupling in the cochlea the elasticity of the overlying membrane has to be locally adjusted to the hair-bundle pivotal stiffness. It has been shown that hair-bundle stiffness as well as tectorial membrane stiffness vary gradually along the cochlea in such a way that coupling strength and the stereociliar stiffness could indeed be matched (Strelioff and Flock, 1984; Gueta et al., 2006; Richter et al., 2007).

What does the above imply about the cochlear amplifier? There is strong evidence that outer hair cell electromotility plays an important role in cochlear amplification (Dallos et al., 2008). Electromotility introduces an electromechanical feedback that couples hair-bundle movements back to basilar-membrane vibrations (Ashmore, 2008; Nowotny and Gummer, 2006). However, electromotility does not exhibit significant nonlinearities for physiological voltage variations and it does not show frequency tuning (Ashmore, 2008). In contrast, small groups of hair bundles do show all the necessary features: sharp frequency tuning, high sensitivity and compressive nonlinearity (Dierkes et al., 2008). However, there are two limitations. Firstly, the high amplification gain observed in the cochlea is not easily reached in our model if at the same time a frequency gradient is maintained. Secondly, hair-bundle movements may be inefficient to significantly drive basilar-membrane vibrations. These issues could be resolved by regarding the cochlear amplifier as a combination of outer hair cell electromotility and active motility of locally coupled hair-bundles. In this scenario, the frequency selectivity and the compressive nonlinear properties of the cochlear amplifier are provided by coupled hair-bundles. Outer hair cell electromotility is a largely linear element that may allow hair-bundle movements to efficiently drive basilar-membrane vibrations. By varying properties of the electromotile feedback the sensitivity and amplification gain of the amplifier could be adjusted. Careful regulation of nonlinear amplification is important to guarantee the stable operation of nonlinear oscillators in the inner ear (Camalet et al., 2000) and thereby to enhance the detection of complex sounds in varying environments. The electromotile feedback is well suited to mediate such a regulation. This may explain why outer hair cells receive signals from the brain via efferent fibers which could influence electromotility.

The origin of the cochlear amplifier

by Robert Fettiplace, Carole M. Hackney*

The mammalian cochlea is a unique cellular array the properties of which vary systematically along the organ. These range from the stiffness and size of gross features such as the basilar and tectorial membrane and the dimensions of the outer hair cells (OHCs) (Lim, 1986) to the amplitude of the mechanotransducer channels (Beurg et al., 2006). All features must ultimately conspire to establish the tonotopic map. Passive mechanical tuning is augmented by the cochlear amplifier which endows sharp frequency selectivity and accounts for the 20–60 dB of extra tip to the tuning curves measured for vibrations of the mammalian basilar membrane (Robles and Ruggero, 2001). The amplifier incorporates a compressive nonlinearity such that the gain and sharpness of tuning are diminished at higher sound levels. The underlying process is thought to involve electromechanical feedback by the OHCs probably through a filter whose frequency characteristics change along the tonotopic axis (Fig. 4). Work over the past 20 years has demonstrated voltage-dependent contractility of the OHCs underpinned by aggregation of the motile protein, prestin, in the lateral membrane (Zheng et al., 2000). However, somatic deformation of the OHC is only one step in a feedback pathway that also includes motion of the tectorial membrane and hair-bundles, mechanoelectrical transduction and generation of a receptor potential to drive the prestin motor. It is assumed that OHC contractions supply force to boost the vibrations of the basilar membrane. A primary argument for the somatic motor is that molecular modifications or knock out of prestin largely abolish amplification (Liberman et al., 2002; Dallos et al., 2008). A criticism of this approach is that interfering with prestin merely alters a feedback loop, any part of which could be the site of amplification. For example, knock out of the mechanotransducer channel protein (although not currently feasible) would presum-

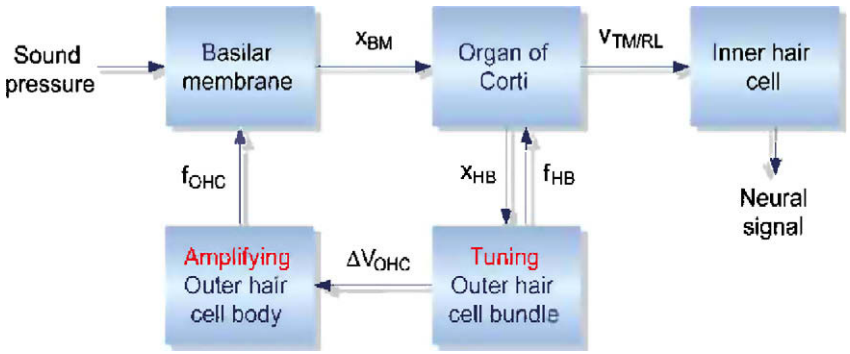

Fig. 4. Process involved in the cochlear amplifier. Sound causes displacements of the basilar membrane, X_{BM}, and organ of Corti leading to deflection of the OHC hair-bundle, X_{HB}. Activation and adaptation of the mechanotransducer channels generate a tuned transducer current culminating in a change in OHC membrane potential, ΔV_{OHC} , that drives the somatic motor. The force, f_{OHC}, produced by OHC electromotility augments basilar membrane motion and may also deform the organ of Corti (Mammano and Ashmore, 1993). Gating of the mechanotransducer channels may generate sufficient force, f_{HB}, to move the hair-bundles (the hair-bundle motor) and the organ of Corti. The inner hair cell bundles are stimulated by the relative velocity, $v_{TM/RL}$, between tectorial membrane and reticular lamina.

ably also eliminate amplification. An alternative view is that amplification is linked to active hair-bundle motion, powered by calcium influx promoting fast adaptation of the mechanotransducer channels (Ricci et al., 2000). To appreciate the contributions of the different processes, it is necessary to understand the micromechanics of the organ of Corti and how forces generated by the OHC somatic and hair bundle motors vibrate the basilar membrane and are transmitted to inner hair cells that also exhibit similar sharp tuning.

The prevailing view, that the somatic motor is at the heart of cochlear amplification, is strongly endorsed by recent work mutating prestin or proteins of the tectorial membrane (Dallos et al., 2008; Mellado Lagarde et al., 2008). However, there are several details not fully explained. How is the somatic motor controlled on a cycle-by-cycle basis at high frequencies where the periodic component of the receptor potential will be filtered by the OHC time constant? Several solutions have been proposed (summarized in Ashmore, 2008) but none has been fully confirmed experimentally. How does the somatic motor supply frequency selective feedback? In many attempts to simulate the sharp basilar membrane tuning, an additional filter or phase shift is introduced to match simulations with experimental results but somatic motility itself is not inherently frequency selective. The extra filter invoked in modeling is often assigned to a resonant tectorial membrane (Nobili and Mammano, 1996). Although the properties of the tectorial membrane, both stiffness and mass, change substantially along the cochlea (Richter et al., 2007), the evidence for membrane resonance is controversial. Finally, how do OHC properties change to generate the necessary forces at high frequencies to counter the increase in viscous load and basilar membrane stiffness? Again in simulations, the force achieved by OHC contraction is assumed to increase (sometimes >100-fold; Lu et al., 2006a) in progressing from the low- to high-frequency end of the cochlea. However, there is no evidence for such an increase in force generation (Iwasa and Adachi, 1997) and if the prestin concentration in the OHC lateral membrane shows little variation with cochlear location (Mahendrasingam et al., 2008), force production remains constant despite different cellular dimensions. Most of the direct evidence for performance of the somatic motor has accrued from measurements on isolated OHCs which invariably lack forward transduction. The operation of the motor may be clarified by studying OHC mechanics in an intact organ of Corti preparation.

The case for a role of the hair-bundle motor is based on its properties in non-mammals. In those animals it can amplify the extrinsically induced hair-bundle vibrations in a frequency-selective manner (Martin et al., 2000; Ricci et al., 2000). The frequency selectivity stems at least partly from tonotopic variation in the fast adaptation time constant for mechanotransduction. Why should it be less important in mammals? Perhaps the bandwidth of the process is insufficient to cope with the extension of the frequency range in mammals. A similar problem exists with electrical tuning of the receptor potential based on gating of potassium channels which is the major source of auditory frequency selectivity in non-mammals (Fettiplace and Fuchs, 1999). Although there is no direct evidence, it seems likely that hair-bundle amplification is employed in the high-frequency region of the avian cochlea, up to 9 kHz in owls (Köppl and Yates, 1999), in which short hair cells (analogous to OHCs) lack prestin or somatic contractility (He et al., 2003). Because the hair-bundle motor is driven by gating of the mechanotransducer channels, it does not suffer the frequency dependent attenuation imposed by the membrane time constant. The mechanotransducer channels must open and close on a microsecond time scale to explain transduction in animals such as bats and cetaceans that hear up to 120 kHz. However, the hair-bundle motor is thought to be coupled to fast channel adaptation (Ricci et al., 2000) which may itself be frequency limited due to the kinetics of calcium binding and unbinding (Nam and Fettiplace, 2008). Speed restrictions to the process remain an open question because attempts to measure active hair-bundle motion in mammalian preparations are currently limited by the bandwidth of force delivery using flexible fiber stimulation (Beurg et al., 2008). Nevertheless, amplification mediated by calcium influx via mechanotransducer channels has been observed in an isolated mammalian cochlea (Chan and Hudspeth, 2005). To fully characterize the hair-bundle motor in OHCs, the speed of the measurement techniques must be improved to ascertain whether the primary mechanical event is a recoil (negative feedback; Ricci et al., 2000) or a release (positive feedback; Martin et al., 2003; Kennedy et al., 2005) synchronous with fast adaptation. A drawback of the hair-bundle motor is the small force it can generate, a few hundred pN at most, more than 10-fold less than the prestin motor. Nevertheless, the feedback can be frequency tuned unlike that for the somatic motor. Furthermore, the force developed will increase with location due to a decrease in height and increase in number of stereocilia per bundle (Lim, 1986). Such frequency selectivity may be enhanced by tight coupling of the hair-bundles to the tectorial membrane (Nam and Fettiplace, 2008).

The most reasonable conclusion is that both somatic and hair-bundle motors collaborate to produce cochlear amplification and that the hair bundle motor has not been discarded but rather supplemented in extending the frequency range. Mechanoelectrical transduction in the hair-bundle may largely confer frequency

selectivity and the compressive nonlinearity, whereas the somatic motor may be the major force generator (Fig. 4). However, the relative importance of the two mechanisms may change between base and apex which differ in the shapes of their basilar membrane tuning curves and degree of low-level amplification and nonlinearity (Robles and Ruggero, 2001). To apportion the contributions of the two motors, the most promising experimental approach is to assay hair cell responses and cochlear mechanics in an *in vivo* preparation (Nuttall et al., 2009). However, these techniques may still have insufficient resolution to define the motion at specific points within the organ of Corti. In the long run, an understanding of the micromechanics will be needed to determine the efficacies of the two motors in vibrating the basilar membrane at both low- and high-frequency locations.

A critical need in hearing

by Pascal Martin, A.J. Hudspeth*

One may investigate the basis of the active process in either of two ways. Most studies have focused on the subcellular and molecular details of the candidate mechanisms, membrane-based electromotility and active hair-bundle motility. Despite the present uncertainties in the field, such detailed mechanistic investigations must ultimately reveal the origins of the four cardinal aspects of the active process: amplification, frequency tuning, compressive nonlinearity, and spontaneous otoacoustic emission (Manley, 2000).

A second approach is to inquire, not about mechanistic details, but instead about the principles underlying the active process. What feature of the active process accounts for the unusual phenomena associated with hearing? What is the connection between the four manifestations of the active process observed in amphibians, reptiles including birds, and mammals? We contend that the answers to these questions are the same: critical oscillation at a Hopf bifurcation.

A physical system displays a Hopf bifurcation when its behavior changes abruptly from quiescence to spontaneous oscillation as the value of a control parameter varies (Strogatz, 1997). If the control parameter is poised at or near the critical value at which spontaneous oscillation emerges, the system is termed a critical oscillator. Any critical oscillator is endowed with generic properties that do not depend on the specific mechanism that produces the oscillatory instability (Choe et al., 1998; Camalet et al., 2000; Eguiluz et al., 2000; Jülicher et al., 2001; Duke and Jülicher, 2008).

Precisely what phenomena can be explained by a critical oscillator?

(i) A critical oscillator can mobilize internal resources of energy to compensate for frictional losses and provide power gain, the defining feature of the cochlear amplifier.
(ii) The amplification of a critical oscillator is tuned to a narrow band of frequencies centered at the characteristic frequency of spontaneous oscillation. In addition, the bandwidth of this active resonance is inversely related to the intensity of the stimulus; weak stimuli are amplified with sharper frequency selectivity.
(iii) As observed in basilar-membrane recordings (Ruggero et al., 1997), the response of a critical oscillator to sinusoidal stimuli near resonance displays a compressive nonlinearity such that the amplification preferentially boosts weak signals. In contrast, the response is linear for stimulus frequencies that differ significantly from the characteristic frequency of critical oscillation.
(iv) As it traverses the Hopf bifurcation, a critical oscillator becomes unstable and enters into limit-cycle oscillation, a likely cause of spontaneous otoacoustic emission.
(v) Like the human ear (Goldstein, 1967), a critical oscillator displays "essential" nonlinearity in the sense that distortion products persist even for weak acoustic stimuli, decreasing more-or-less linearly with the amplitude of stimulation until they reach the threshold of detectability.
(vi) The responsiveness of a critical oscillator to a sinusoidal stimulus is diminished by the presence of a second stimulus at a nearby frequency, a phenomenon akin to psychoacoustical masking, or two-tone suppression, in the human ear.

A ubiquitous feature of vertebrate hair cells, active hair-bundle motility has been observed *in vitro* in the eel (Rüsch and Thurm, 1990), frog (Benser et al., 1996; Martin et al., 2003; Tinevez et al., 2007), turtle (Crawford and Fettiplace, 1985; Ricci et al., 2002), chicken (Hudspeth et al., 2000), and rat (Kennedy et al., 2005). In the frog's sacculus, active hair-bundle motility exhibits each of the six characteristics listed above (Martin and Hudspeth, 2001; Martin et al., 2001; Barral and Martin, unpublished observations). If intrinsic hair-bundle fluctuations are taken into account, a simple critical-oscillator model quantitatively emulates the observed behaviors (Nadrowski et al., 2004). Although intrinsic noise seriously limits amplification at the single-cell level, most hair-bundles are mechanically coupled by overlying membranous structures. By effectively reducing noise, cooperation among a few tens of neighboring hair-bundles apparently allows active hair-bundle motility to achieve a dynamic range of responsiveness compatible with that of hearing (Dierkes et al., 2008). The functional unit of the active process may thus comprise a small cluster of coupled hair cells with similar characteristics, which together achieve critical oscillation at a particular frequency.

Precisely because critical oscillation is generic, any dynamical system operating near a Hopf bifurcation must display the same properties. The mammalian lineage, which diverged from those of the other amniotes some 320 million years ago, has had ample opportunity to find novel ways of achieving critical oscillation. The phenomenon of membrane-based somatic electromotility, which is unique to mammalian outer hair cells, has been implicated in the production of active basilar-membrane movements (Dallos et al., 2008; Mellado Lagarde et al., 2008). Electromotility cannot operate alone, however, for this process is nearly linear over a physiological range of membrane potentials and lacks frequency selectivity (Ashmore, 2008). The nonlinearity and frequency selectivity of the cochlear amplifier are usually thought to emerge from respectively the saturating nonlinearity of mechanoelectrical transduction by the hair-bundle and passive mechanical resonance within the cochlear partition (Nobili and Mammano, 1996). Modeling studies suggest that electromotility can provide negative friction to turn each segment of the cochlear partition, described as a spring-mass system, into a highly tuned resonator (Nobili et al., 1998). If negative damping overcomes passive sources of friction, the system is expected to become unstable and oscillate spontaneously. We suspect that successful cochlear models have been adjusted to operate in a stable regime near an unrecognized Hopf bifurcation. If the simulated behaviors are generic, the success of a given model does not necessarily validate the underlying assumptions; this difficulty may explain why no particular model of cochlear amplification has yet been accepted as definitive.

Models that rely only on passive resonance to set the characteristic frequency of each segment of the cochlear partition confront an important problem. The measured range of stiffness along the cochlear partition does not suffice to account for the thousandfold frequency range of mammalian hearing (Naidu and Mountain, 1998). It is more likely that the frequency is set, at least in part, by the local active process (Duke and Jülicher, 2003). Active hair-bundle motility, which occurs in the mammalian cochlea (Chan and Hudspeth, 2005; Kennedy et al., 2005), may provide both the

necessary nonlinearity and the frequency selectivity of the active process.

The critical-oscillator hypothesis also bears on the propagation of signals within the cochlea. The cochlear partition may be viewed as a set of oscillator modules with characteristic frequencies tonotopically distributed along the longitudinal axis of the cochlea. Although the traveling wave that results from hydrodynamic coupling of these modules is doubtlessly important in distributing sound energy to appropriately tuned hair cells, critical oscillators can account for the sharp peaking of the wave at the characteristic place. When critical oscillation is invoked, relatively simple models of cochlear hydrodynamics suffice to capture the known qualitative features of the traveling wave (Duke and Jülicher, 2003; Kern and Stoop, 2003; Magnasco, 2003).

The actual behavior of the mammalian cochlea differs in four ways from the abstract representation of a single critical oscillator (Fig. 5). First, the presence of intrinsic noise limits the amplification of faint stimuli; the gain saturates at a constant value below some threshold level, whereas the gain of a critical oscillator formally diverges at resonance for vanishingly small stimuli. Next, the restricted dynamic range of some process, perhaps active hair-bundle motility, implies that amplification wanes at very high stimulus levels. Third, by curtailing responsiveness to stimuli above the characteristic frequency, the traveling-wave mechanism introduces a sharp asymmetry in real tuning curves. Finally, longitudinal shifts of the tuning curve at increasing stimulus levels, as well as nonlinear modifications of the pressure stimulus traveling from the cochlear base to the characteristic place, can distort the power-law behaviors that are typical of the compressive nonlinearity generated by a single critical oscillator. We expect generic behaviors to emerge most clearly by following the peak of basilar-membrane response and relating the magnitude of this response to the local pressure.

The wealth of experimental observations on mammalian hearing implies that few experimentally accessible tests of the critical-oscillation hypothesis remain to be performed. Put another way, the strength of the hypothesis lies less in its predictive ability than in its capacity to accommodate a broad range of existing observations in a unified model. There are nevertheless striking predictions from the hypothesis that could lead to its falsification. Because the various manifestations of the active process are posited to emerge together from critical oscillation, they should be coupled obligatorily. If a control parameter can be adjusted systematically, for example by pharmacological manipulations (Martin et al., 2003) or genetic engineering (Holt et al., 2002), the strengths of the several effects should rise or fall together. Moreover, if conditions can be found in which some features of the active process are definitely suppressed while others clearly persist, the critical-oscillator hypothesis must be modified or abandoned.

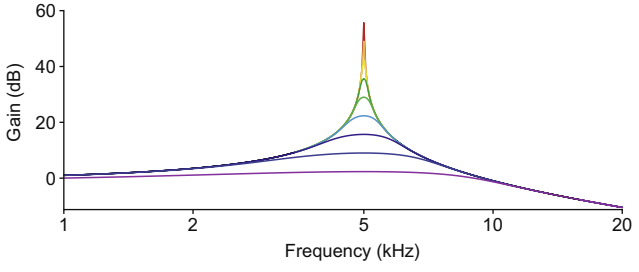

Fig. 5. The characteristic features of a critical oscillator emerge in a doubly logarithmic plot of the relation between stimulus frequency and gain for a series of sinusoidal stimuli. Gain is defined as the ratio of the oscillator's sensitivity to a given stimulus to that evoked by intense stimulation at the same frequency. A weak stimulus evokes a sharply tuned response with high gain. As the stimulus level rises in 10-dB increments, the gain at the characteristic frequency of 5 kHz declines as the two-thirds power of the stimulus amplitude and the bandwidth of amplification increases. Although the system displays compressive nonlinearity near resonance, its behavior remains linear for stimulus frequencies that differ significantly from the characteristic frequency of the critical oscillator.

Predicting the role of OHC somatic motility and HB motility in cochlear amplification using a mathematical model

by Julien Meaud, Karl Grosh*

Introduction

Outer hair cells (OHC) have been shown experimentally to exhibit somatic electromotility at frequencies covering the entire mammalian frequency range (Frank et al., 1999). To predict the high sensitivity of the mammalian cochlea to low-level acoustic stimulus, previous mathematical models have included OHC somatic motility as in Mammano and Nobili, 1993 and Ramamoorthy et al., 2007. These models can predict the high gain as well as the sharp tuning of the frequency response of the basilar membrane (BM) to low-level acoustic input. When these models were developed there was no experimental evidence of active hair-bundle (HB) motion in the mammalian cochlea. However, activity (as evidenced by distortion products and spontaneous otoacoustic emissions) and amplification without any OHC somatic motility in the hearing organ of non-mammalian vertebrates have been demonstrated. Experimental and theoretical studies have shown that the non-mammalian HB can produce a force due to the action of a calcium dependent process. This active force production is linked to the fast adaptation of the transduction current (Ricci et al., 2000) and can amplify an external stimulus (Martin and Hudspeth, 1999). Moreover, recent experiments show that the mammalian HB also exhibits fast adaptation of the transduction current (Kennedy et al., 2003) and can produce a force in a submillisecond time scale (Kennedy et al., 2005). This new evidence provides an alternative to the prevailing theory that somatic motility is the basis of the cochlear amplifier. In our mathematical model, we selectively include OHC somatic motility, HB motility and a combination of both, with the goal of understanding the role of these two active sources in the mammalian cochlea.

Model

Our mathematical model is based on a box model of the guinea pig cochlea with a 3 D representation of the fluid, as described in Ramamoorthy et al., 2007. Viscous dissipation in the subtectorial space is included. The BM interacts with the fluid via linearized Euler relation and with the organ of Corti which is coupled to the tectorial membrane (TM). Each cross-section of the TM is modeled as a rigid body with two degrees of freedom corresponding to the motions in a transverse and radial direction (see Fig. 3 in Ramamoorthy et al., 2007). Electrical conduction in the scalae of the cochlea is represented by longitudinal cables which allow current to pass down the length of the cochlea as well as into the transduction channels of the OHC (see Fig. 2 in Ramamoorthy et al., 2007). The system is linearized about the stationary point to predict the response of the system to low-level acoustic stimulation. We consider time harmonic vibrations ($e^{-i\omega t}$ time dependence). Somatic electromotility is modeled by linearized piezoelectric relations between the OHC deformation, u_{OHC}^{comp}, the fluctuating part of the transmembrane voltage, $\Delta\phi_{\mathrm{OHC}}$, the OHC force (per unit length of the BM), F_{OHC}, and the current (per unit length of the BM), I_{OHC}, which

$$F_{\mathrm{OHC}} = K_{\mathrm{OHC}} U_{\mathrm{OHC}}^{comp} + \epsilon_3 \Delta\phi_{\mathrm{OHC}} \tag{1}$$

$$I_{\mathrm{OHC}} = \frac{\Delta\phi_{\mathrm{OHC}}}{Z_m} - i\omega\epsilon_3 U_{\mathrm{OHC}}^{comp} \tag{2}$$

where K_{OHC} is the stiffness (per unit length of the BM) of the OHC, ϵ_3 is the electromechanical coupling coefficient of the OHC and Z_m is the impedance of the basolateral portion of the OHC.

In a nonlinear physiological model of HB transduction and motility, the dynamics of the HB are fairly complicated. In the linearization of such a model the properties are expected to be frequency dependent (as discussed in Ricci et al., 2000). Here, however, we use frequency independent properties and assume the transduction channel conductivity to be directly proportional to the stereocilia deflection, u_{HB}. Further the HB force is taken to be proportional to the HB deflection u_{HB} and velocity $-i\omega u_{HB}$. In this simple model, if the HB is to add energy to the system in a cycle-by-cycle manner, the real part of the HB impedance must be negative (i.e., some form of negative damping). Hence the HB force in the shear or radial direction is:

$$F_{HB} = k_{HB}u_{HB} - i\omega c_{HB}^{act}u_{HB} \quad (3)$$

where k_{HB} is the HB stiffness and c_{HB}^{act} is the (negative) active damping coefficient. The constant is c_{HB}^{act} chosen to provide forces and energies that are in the physiologically relevant ranges, limited by experimental evidence given in Kennedy et al., 2005 and Choe et al., 1998, respectively. The energy is assumed to arise from a calcium binding event that is not included in other models (Mammano and Nobili, 1993; Ramamoorthy et al., 2007).

Results

The response of the BM to acoustic stimulation is plotted as a function of frequency in Fig. 6. The green dashed line represents the response of the BM in the passive system, i.e., neither somatic nor HB motility are included. It compares very well to the experimental measurements of de Boer and Nuttall, 2000 at 100 dB SPL (shown in blue dashed line).

When we add somatic motility to the model (thick blue dashed line), we see an increase in the gain and in the sharpness of the tuning and a shift of about half an octave in the peak frequency as seen in the experimental data at 10 dB SPL. In the results presented here, the electromechanical coupling coefficient was chosen such that the model that includes both somatic and HB motility is stable. With this value of ϵ_3, the predicted magnitude of the BM gain of the model only including somatic motility is lower than the experimental value. However, if we use a slightly higher value for ϵ_3 (about 16% higher), the prediction for the magnitude of the BM gain when only somatic motility is included can match the experimental value as shown in Ramamoorthy et al., 2007. Despite the basolateral RC filtering of the transmembrane voltage, somatic electromotility can amplify the BM motion thanks to an electromechanical resonance in the organ of Corti and the high sensitivity of the transduction channels.

If we add HB motility to somatic motility (thick black solid line), there is another 10 dB increase in the gain. For a 0.5 nm displacement (which corresponds approximately to a 20 dB acoustic input), the magnitude of the OHC somatic force is about 60 pN (on the order of magnitude predicted by Iwasa and Adachi, 1997) and the power added to the system by HB motility is about 60zJ, which is lower than the maximum value postulated by Choe et al. (about 2000zJ, Choe et al., 1998). For this case (somatic motility and HB motility) as well as the previous one (somatic motility only), the tuning of the response is not due to an intrinsic tuning of somatic or HB motility, but to an electromechanical resonance in the organ of Corti.

However, when only HB motility is included (red solid line) using the same active damping coefficient, c_{act}^{HB}, as in the previous calculations, the response is similar to the passive model response, with a low gain and broad tuning. When the active damping coefficient is increased, the model becomes unstable before the maximum gain of the BM reaches the experimental value for low-level sounds.

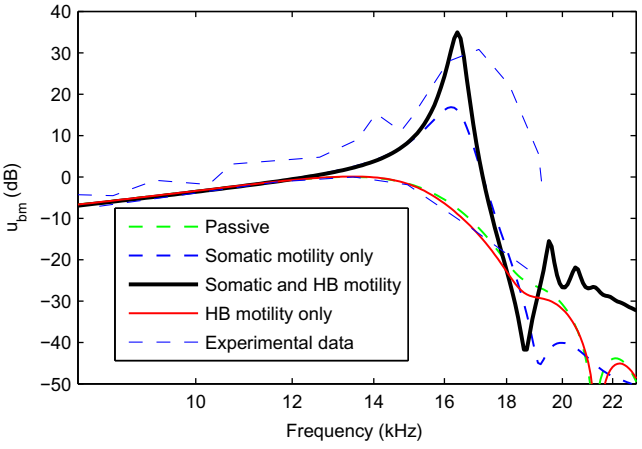

Fig. 6. Response of the BM to acoustic stimulation at the 17 kHz best place. The responses are normalized to the maximum passive BM response. The model predictions are compared to measurements from de Boer and Nuttall, 2000 at 20 and 100 dB SPL (thin blue dashed lines). The parameters used in the simulations are, for OHC somatic motility, $\epsilon_3 = -8.4 \times 10^6$ N/m/mv and for HB motility $c_{act}^{HB} = -3.15\times^{-8}$ N/m/s. The passive model prediction (thick green dashed line) follows closely the measurements at 100 dB SPL. When somatic motility is included (thick blue dashed line), the peak gain is about 20 dB higher than in the passive case and the tuning of the response is sharper as in the experimental data at 20 dB SPL. When somatic motility and HB motility are included (thick solid black line), the gain is about 10 dB higher than in the previous case. For the case when only HB motility is included (red solid line), the response is almost the same as the passive model response.

Conclusions

In these preliminary results with a simple HB model, OHC somatic motility is necessary for cochlear amplification whereas HB motility is not. This is consistent with measurements on prestin-knockin mice (Dallos et al., 2008) which also show that prestin-based somatic motility is necessary for normal cochlear function. With the parameters used here and the current experimental data, HB motility does not appear to be necessary to predict the BM gain to acoustic stimulus. However, as our results suggest, HB motility could still play a significant role and work in synergy with somatic motility to provide a higher BM gain and sharper tuning than with OHC motility alone. A more realistic HB model needs to be developed in order to make more conclusive remarks about the relative roles of OHC somatic and HB motility.

New experiments needed to change or elaborate our claims

- *In vitro* measurements of the mechanical response of mammalian HB to a stimulus more rapid than the time course of adaptation: The mechanical response of mammalian HB have only been measured with a stimulus having a time constant similar to or greater than the adaptation time constant. Measurements of HB with a faster time scale and/or with a small harmonic stimulation in the 5–20 kHz frequency range would help to find realistic parameters for a linearized HB model in the mammalian auditory frequency range, which are needed for a more precise prediction of the role of HB motility.
- *In vivo* measurements of the BM response to acoustic input in a cochlea perfused with salicylate: Current evidence that prestin somatic motility is necessary for normal cochlear function is based on prestin-knockin mice. The transduction channel of

these mutant mice appear to be normal. However the genetic mutation could affect other properties of the mouse cochlea during the development of the animal. Moreover data is only available for mice since genetic mutation have only performed on mice. Measurements of the BM response in a cochlea perfused with salicylate could be another way to block somatic motility while not affecting HB motility. It could potentially validate or invalidate the claim that prestin is necessary for cochlear amplification.

- Measurements of the BM frequency response to acoustic stimulation with endolymph with reduced calcium concentration: Using a perfusing scheme similar to Zheng et al., 2007, a controlled alteration of the endolymphatic calcium concentration can be reversibly applied. Reduction of the calcium concentration should slow down adaptation, increase the transduction current and reduce the magnitude of the active HB force. Because of the increase in the transduction current, it should also increase the magnitude of the OHC somatic force. Since OHC somatic motility is the main source of cochlear amplification according to our present model, we expect a net increase of the gain of the BM (provided the phase of the increased current is not deleteriously altered).
- *In vivo* measurements simultaneous measurements of the motion of the BM, the TM and the different structures of the organ of Corti in response to acoustic stimulation: Our mathematical model of the cochlea predicts the relative amplitude of the motion of the TM and the BM that are difficult to verify due to the lack of experimental data. In our results the gain of the BM and TM are similar. If HB motility had a greater effect than what we predict, we should expect the TM to have a much higher gain than the BM since the HBs are attached directly to the TM and can apply a force in the TM shear direction.

The mammalian cochlear amplifier done

by J. Santos-Sacchi

Introduction

I think we all agree that mammalian cochlear amplification must arise from the activity of OHCs, and that such activity must be physically coupled to the cochlear partition. The upshot of this is that (1) the evolution of OHCs to perform this special job arguably might have included a design to improve on extant mechanisms, namely, to recruit a new cellular component or modify an existing one, and (2) whether a somatic or stereociliar mechanism, it must link to the partition. Indeed, stereocilia embed in the tectorial membrane and the OHC soma join apically to the reticular lamina and basally to the basilar membrane via Deiters' cells. These required connections potentially allow OHC mechanical activity to provide a boost of stimulus to the inner hair cell stereocilia. Methods to uncouple these links or immobilize the underlying mechanics will tell which rules in the mammal.

Stereocilia drive the mammalian cochlear amplifier … not

There are clear examples showing that prestin generated mechanical responses underlie mammalian amplification. Included are (1) our results (Santos-Sacchi et al., 2006) that BM sensitivity and tuning is modulated by anion control of prestin, and (2) definitive knockout results from the Dallos lab (Dallos et al., 2008). Another key observation is that when the coupling between stereocilia and the tectorial membrane is abolished, the BM behavior characteristic of normal amplification evoked by electrical stimulation is unaffected (Mellado Lagarde et al., 2008). Furthermore, it is not clear to me that evidence for bundle contributions (Chan and Hudspeth, 2005; Kennedy et al., 2005; Kennedy et al., 2006) cannot be explained by underlying prestin-based mechanisms (Jia and He, 2005). I note that the use of salicylate as a tool to remove prestin effects is not absolute, as we have previously shown that residual mechanical responses remain in OHCs after such treatments (Kakehata and Santos-Sacchi, 1996). Given that the preponderance of evidence indicates that a prestin-based mechanism is responsible for mammalian amplification, we hope to put this issue to rest and focus on how this amazing protein prestin works at the cellular and molecular level.

Anions work as prestin's voltage sensor … not

The initial suggestion that anions influence the electrical signature of prestin, nonlinear capacitance (NLC), because they subserve voltage sensation by a dysfunctional prestin transporter (Oliver et al., 2001) is not supported by many pieces of data. These include (1) conformational state of the motor is altered by anions at fixed voltage, (2) effects on the motor depend not simply on the presence of anions, but also on anion species and structure, (3) there is not the expected relationship between anion valence and motor unitary charge, (4) prestin is an anion transporter, (5) intrinsic amino acid residue charge contributes to voltage sensing, and (6) mutations of prestin can divorce NLC and anion transport capabilities (Bai et al., 2009; Rybalchenko and Santos-Sacchi, 2003; Rybalchenko and Santos-Sacchi, 2008; Song et al., 2005). We view the effects of anions working in an allosteric fashion, just as allosteric actions of voltage and Ca^{2+} ions control the behavior of the Ca–K channel, for example (Horrigan et al., 1999). For the OHC, this allosteric mechanism may rival the well known allosteric effects of Ca^{2+} on the stereociliar MET conductance (Fettiplace and Ricci, 2003).

How I envision the ear's works working

Enhanced tuning exists within cochleae that possess prestin-endowed OHCs; however, how such sharpening occurs requires more than amplification of a passive travelling wave. We suggested that interactions among coupled OHCs could provide such sharpening and give rise to nonlinearities characteristic of the amplifier (Zhao and Santos-Sacchi, 1999). Interestingly, a recent model of coupled hair-bundle activity suggests that improvements in tuning and amplification can result from interacting adjacent hair cell bundles (Dierkes et al., 2008). I think that just as the generic Hopf bifurcation model for bundle function can be usurped to understand the action of the electromotility nonlinearity, so too can this new coupling model. In fact, I think there are many analogies between proposed bundle mechanisms of amplification and prestin-driven amplification. Ironically, work on the bundle may help us understand how electromotility might work! One notable hypothesis that we suggested was the possible action of an ion underlying the mechanical event that drives amplification. Thus, in analogy with the process whereby Ca^{2+} influx through the molecularly-unidentified transduction channel conductance (G_{met}) drives bundle movements, we suggested that Cl^- could be fluxed via the molecularly-unidentified, mechanically-active lateral membrane conductance (G_{metL}) to effect prestin conformation change (Rybalchenko and Santos-Sacchi, 2003). We have shown that manipulation of Cl^- flux across the lateral membrane can reversibly alter cochlear amplification on the BM *in vivo* (Santos-Sacchi et al., 2006). Clearly, if such flux could be effected at acoustic rates, such a mechanism would bypass the membrane filter problem identified as a consequence of the voltage-dependence and nonlinear nature of electromotility (Santos-Sacchi, 1989). In this regard, we did show that G_{metL} is gated at acoustic rates (Rybalchenko and Santos-Sacchi, 2003), and it was shown that

deformations of the OHC soma occur during acoustic stimulation (Fridberger and De Monvel, 2003) – a possible stimulus for G_{metL}.

I am now captivated by a possibility that stereocilia and the prestin-based mechanism may team up to overcome the membrane time constant problem intrinsic to the conventional concept of prestin activation (Fig. 7). Interestingly, a correspondence between apical and lateral membrane activities has been observed *in vivo*, where bundle biasing and other manipulations appeared to affect OHC mechanical activity (Kirk, 2001; Kirk and Yates, 1998). Possible mechanisms included an alteration of intracellular chloride levels. Could the bundle influence cochlear amplification in a manner unrelated to active bundle mechanics and not due to the direct action of voltage on the motors? Could this involve chloride? I see two possibilities in this regard. One challenging dogma and another recently identified.

Here is the first scenario. Ion channels are characterized by their selectivity, their ability to pass particular ions over others based on size and/or charge. For all channels, the selectivity is not perfect, and ranges from extremely poor selectivity (e.g., (Oliver et al., 2001; Singh et al., 2007; Ubl et al., 1988)), to high selectivity (e.g., K channels).

Still, the most select ones can pass small amounts of ions that are generally considered impermeable (e.g., <1%). It is true that the bundle channel is nonselective for cations (Corey and Hudspeth, 1979; Crawford et al., 1991; Ohmori, 1985), but even though the replacement of chloride with sulfate has no apparent effects on cation currents (Valli et al., 1979), detailed anion selectivity has never been studied. It may sound like heresy today, but concessions that anion selectivity of the transduction channel require further investigation have been made (Hudspeth, 1983; Ohmori, 1985), and I am unaware of any further investigations on this topic since that time, especially in OHCs. Interestingly, one of the most abundant proteins in sterocilia, CLIC5 (Gagnon et al., 2006), only nominally an intracellular chloride channel, has recently been shown to form channels in bilayers (Singh et al., 2007). Selectivity for charge is mildly cationic, with poor specificity, and measured multi-conductance levels of ~105 and ~17 pS. OHC MET channel conductance is about 110 pS (Geleoc et al., 1997). Imagine if CLIC5 were the MET channel! It is modulated by F-actin (Singh et al., 2007), making it potentially sensitive to Ca^{2+} and tension. It would be ironic should hair cells use only a few of an abundant supply of molecules – what redundancy for a critical mission! Let's check these ideas!

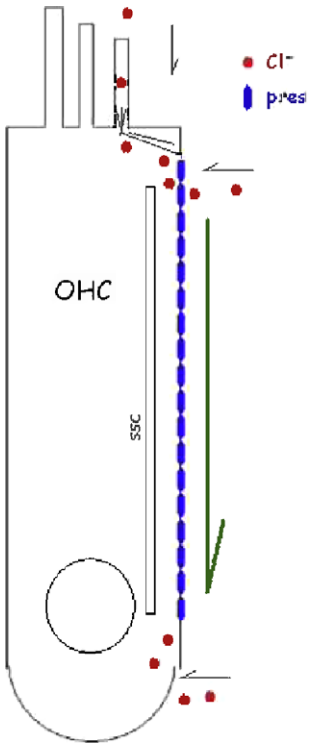

Fig. 7. The OHC is anion driven. The schematic illustrates the possible routes that chloride may take to alter prestin activity intracellularly. We have already shown that the expanse of the lateral membrane fluxes chloride via the mechanically active G_{metL} (Rybalchenko and Santos-Sacchi, 2003). Other proposed routes are through the stereociliary MET channel directly, or via rootlet perturbations of G_{metL}. Chloride may also flux during efferent activation of GABA receptors.

The second scenario involves an observation (Furness et al., 2008) made this past year showing that stereociliar rootlets insert into the junctional region of the apical lateral membrane. The potential for mechanical perturbation of the lateral membrane by the bundle therefore exists. Thus, bundle displacement, via the rootlets, is hypothesized to mechanically activate G_{metL}, promoting a flux of Cl^- at the apical region of the OHC. One further exciting prediction arises – a travelling wave of chloride flux along the apical to basal extent of the lateral membrane. This will occur because activation of G_{metL} causes a local flux of Cl which in turn causes the motor to change conformation, thereby mechanically triggering adjacent G_{metL} conductances to gate. A regenerative wave of activity should spread basally down the lateral membrane! An anion permeant MET channel could do the same. Let's measure it!

Finally, we should remember that efferent control of the cochlear amplification may also benefit from the modulation of chloride levels via GABA receptors at the cell's base (Maison et al., 2003; Plinkert et al., 1993). Here I can imagine a reverse travelling wave of contracture moving apically! Let's look for it!

Summary

Here, in this short space, I have revealed some of my inner most thoughts on mammalian cochlear amplification and associated problems. I have touched on why I think prestin rules, but offer up as consolation that the bundle may work with prestin. We need bold ideas like this and others (electro-osmosis, flexoelectricity) in our field to drive us to uncover the truth – to truly understand how hearing happens.

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