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Cochlear amplification, outer hair cells and prestin

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Mechanical amplification of acoustic signals is apparently a common feature of vertebrate auditory organs. In non-mammalian vertebrates amplification is produced by stereociliary processes, related to the mechanotransducer channel complex and probably to the phenomenon of fast adaptation. The extended frequency range of the mammalian cochlea has probably co-evolved with a novel hair cell type, the outer hair cell and its constituent membrane protein, prestin. Cylindrical outer hair cells are motile and their somatic length changes are voltage driven and powered by prestin. One of the central outstanding problems in mammalian cochlear neurobiology is the relation between the two amplification processes.

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Introduction

The structural and functional complexity of the mammalian hearing organ has bedeviled investigators throughout the long history of hearing science. While the basic principles of operation, indeed most of its details, are well understood for many other sensory systems, at least one fundamental issue about the functioning of the cochlea remains only partially mastered. This pertains to amplification, a common phenomenon in various types of sense organs. For example, it is a general property of systems that are based on G-protein-mediated second-messenger cascades. Whether it is ubiquitous in systems based on ionotropic receptor processes is not yet determined. In such systems, amplification was first proposed to occur in the mammalian cochlea; recent work suggests that it may be present in some invertebrate mechanoreceptors [1] and is probably operative in all hair-cell-based organs [2,3]. In mechanoreceptors amplification is integrated into the mechanical cascade that leads to the transduction of the effective stimulus in sensory receptor cells or sensory neurons. By definition, amplification is a process whereby the incoming biologically relevant signal modulates a local energy source so that the energy content of the transduced signal is higher than that arriving from the environment. The obvious analog is a battery-powered radio. The energy content of the electromagnetic wave, which is picked up by the antenna, is low. In order that the speaker is able to produce an audible sound, a higher energy level is needed. This is obtained by having the incoming alternating current (AC) signal modulate the direct current (DC) battery power. The key concept is increased energy, not increased amplitude. Amplitude increase can be had by using a transformer, such as a simple mechanical lever or, for hearing, the middle ear apparatus. These are passive systems in which transformer action occurs as a tradeoff. While one variable, say displacement, is increased, another, say force, is decreased in accordance with the principle of conservation of energy. In the context of the mammalian ear, we ask if the mechanical energy associated with the displacement of stereocilia (the mechanotransducer organelles of hair cells) is greater than the acoustic energy contained in the sound stimulus. Inasmuch as this question is largely refractory to experiments, substitute measures are used. In the radio analogy, these are reduction or elimination of the battery power or disruption of the circuit.

In the context of mammalian hearing, the origin of the concept of active amplification is usually attributed to Thomas Gold [4]. This is correct, but some caveats are necessary. Gold's 1948 theoretical publication was forgotten and had no influence on the field during the 1970s when the experimental work showing the need for amplification was conducted and when the concept re-emerged *de novo*. This re-emergence grew out of a failed but stimulating idea: the 'second filter' [5]. The first filter is von Békésy's traveling wave [6], a hydromechanical disturbance on the basilar membrane (BM), which produces a rudimentary spectral analysis in the cochlea. The second filter was invoked to reconcile the apparent poor frequency selectivity (tuning) of the traveling wave with the exquisitely sharp tuning of auditory nerve fiber responses. A whole host of imaginative schemes were invented to give corporeal existence to the second filter, but these involved passive filtering and did not recognize the need for amplification. A detailed review of this period is given in [7], and a more contemporaneous reflection in [8]. Suffice it to say that contemporary measurements of BM and neural tuning not only show difference in frequency selectivity [9], but also indicate that the mechanical tuning is intimately related to the

metabolic state of the cochlea [10]. The present consensus is that a mechanical feedback process exists in the cochlea, whereby signals generated in mechanoelectric (forward) transduction feed back signals (reverse transduction) so that the closed-loop response is more energetic and larger in amplitude than the open-loop response [11]. The energy source is the stria vascularis epithelium (see [12] for an overview), which is responsible for the unusual ionic content and positive resting polarization of the extracellular fluid, endolymph, which bathes the hair cell stereocilia and their mechanoelectric transducer (MET) channels. The generally cited consequences of cochlear feedback are heightened sensitivity, enhanced frequency selectivity, nonlinear distortion, and spontaneous otoacoustic emissions [3,8]. The existence of spontaneous emissions, sounds of inner-ear origin that are radiated by the eardrum, is often taken as a signature of the feedback process and used to infer the presence of amplification in a variety of vertebrates [13]. Using nonlinearity of the response as an indicator of amplification needs to be done with care. Even if the feedback loop is opened, nonlinearities normally evident in high-level electrical responses (hair-cell generated or neural) should be maintained. Mechanical nonlinearities that depend on feedback, such as compression, should disappear. While amplification in the form of feedback is generally accepted to function in the mammalian cochlea, the identity of the feedback path is still controversial.

A distinguishing feature of the mammalian cochlea is the frank dichotomy between two types of sensory receptor cells, inner hair cells (IHC), and outer hair cells (OHC). The two groups are spatially segregated and morphologically distinct. Their innervation also differs. While OHCs have a sparse and divergent afferent innervation by thin non-myelinated fibers, IHCs are connected to some 90–95% of the thick myelinated auditory afferents in a punctate manner [14]. Efferent innervation is also different. Descending neurons from the medial aspect of the superior olivary complex establish large terminals on the OHC soma. By contrast, lateral olivary neurons form axodendritic synapses with IHC afferents [15]. Mammals hear with their IHCs—the true sensory receptors of the cochlea. The role of OHCs in hearing emerged gradually in the 1960s and 70s. In 1958, Hallowell Davis, the leading light of mid-century auditory neurobiology, likened them to retinal rods, and IHCs to cones, and assumed that they process low-level and high-level sounds, respectively [16]. Experiments in the 1970s using chemical ablation of OHCs indicated, however, that in the absence of OHCs hearing threshold was elevated by some 50 dB [17] and frequency selectivity was compromised or eliminated [18–20]. Knowing the innervation patterns, such results led to the suggestion that OHCs amplify signals that are subsequently processed by IHCs [17,18]. Discovery of spontaneous otoactoustic emissions [21] and demonstration of mechanical consequences of OHC stimulation via efferents [22] clearly indicated that the amplification was mechanical.

Two discoveries in 1985 still reverberate in today's arguments. Crawford and Fettiplace [23] showed that ciliary bundles of turtle hair cells are capable of producing active mechanical reactions to incoming mechanical stimuli as well as spontaneous movements. Numerous follow-up experiments amply demonstrated that ciliary bundles of various animals, including mammals, can produce active movements [24–29]. A radically different mechanical response was discovered by Brownell *et al.* [30] and elaborated by others [31–34]. This, so called somatic electromotility, entails the elongation/contraction of the OHC's cylindrical cell body in response to membrane hyperpolarization/depolarization. The novel membrane-spanning motor protein that powers electromotility, prestin, was identified in 2000 [35]. The two mechanisms, ciliary and somatic motility, provide alternative and/or complementary substrates for cochlear amplification. Inasmuch as ciliary motility is ubiquitous among vertebrate hair cells, it has the potential to produce amplification in *any* hair cell. By contrast, somatic motility, being intimately tied to OHCs and thus to mammals, can amplify only in this restricted class. Hence, there have been arguments that ciliary motility is the general amplifier and that in mammals somatic processes have evolved to control/adjust the amplifier [36–38]. The two mechanisms are schematically depicted in Figure 1.

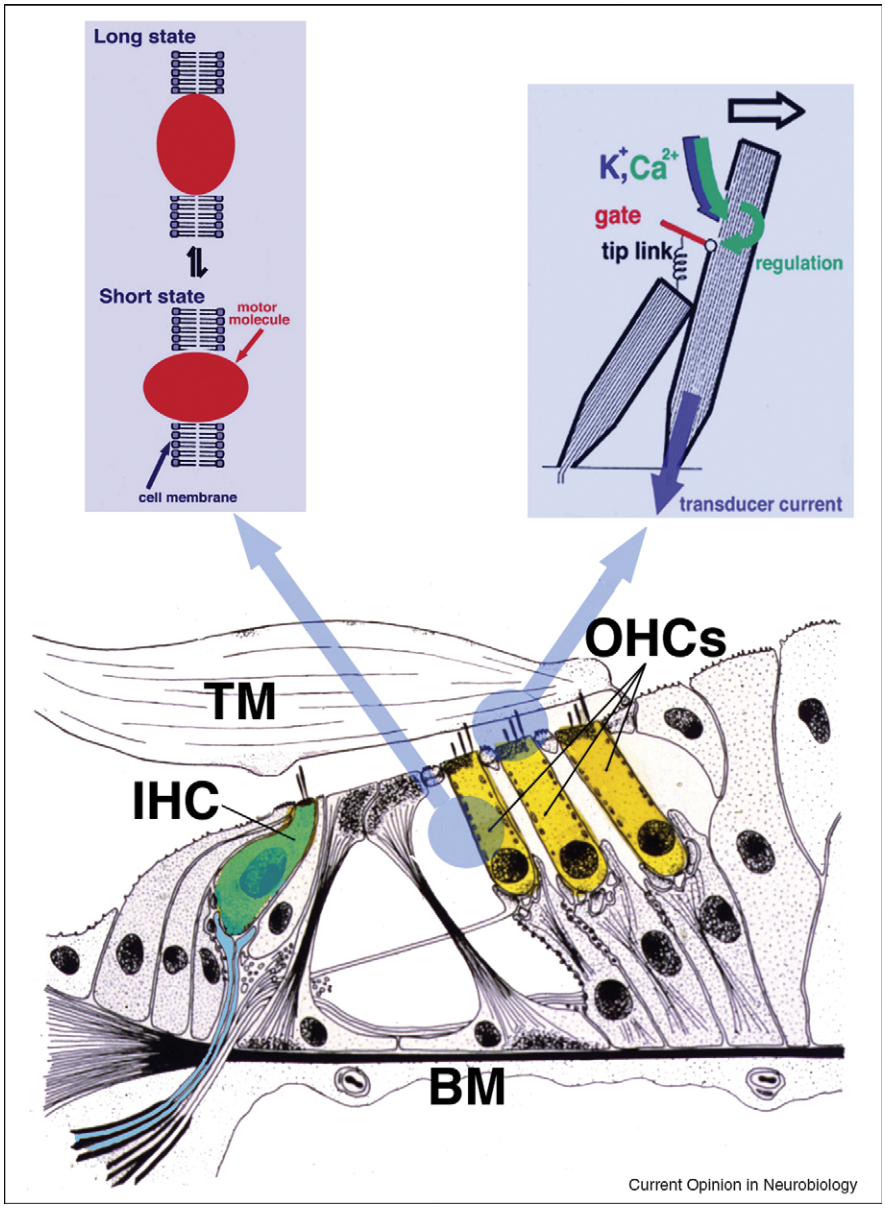
Outer hair cell motility and prestin

A masterly review of this subject was recently published [39•] and thus it is unnecessary to provide a detailed account here. Other recent books and reviews are also recommended [40–42,43•,44]. Some current issues about prestin's operation are briefly discussed here.

Prestin (SLC26A5) is a member of the SLC26A family of membrane antiporters that transfer anionic molecules across the cell membrane [45]. While it has been reported that prestin is best modeled as an anion transporter [46], recent work shows that mammalian prestin does not undergo a full transport cycle, in contrast to non-mammalian prestin [47•]. Instead, it is an incomplete transporter, failing to unload a bound anion at the extracellular face of the protein [44,48].

Aside from voltage-dependent shape changes – the originally observed electromotility – highly correlated somatic stiffness changes were also reported [49–51]. Hallworth [52] recently challenged these findings; in short basal OHCs from the gerbil he failed to observe voltage-dependent stiffness. Considering the extensive effort expended in the original work to rule out any artifact [50], it is plausible that the differences are due to sampling error. Not all cells show the phenomenon, and 'healthier' cells tend to show it more consistently.

Figure 1



A schematic cross-section of the organ of Corti is shown. BM: basilar membrane, TM: tectorial membrane, IHC: inner hair cell, OHC: outer hair cell. The two putative mechanisms of the amplification process are shown in the inserts. On the left, the OHC membrane is shown with the incorporated motor (prestin) molecular complex, possible a tetramer, in two conformational states. On the right, stereocilia and the mechanotranducer channel are shown in cartoon form. Deflection toward the taller stereocilium opens the channel via the tip link, cations (K^+ and Ca^{2+}) enter, driven principally by an electrical gradient of some 160 mV, due to the summing of the positive endocochlear potential and the cell's negative resting potential. Potassium current dominates the transducer current and it produces the cell's receptor potential. The voltage change facilitates neurotransmitter release from IHCs and drives prestin motors in OHCs. Calcium current regulates slow and fast adaptation, probably through different mechanisms. Fast adaptation is thought to relate to amplification by controlling channel open probability and thereby producing ciliary deflection.

Because basal cells are notoriously difficult to isolate, they may not show a change in stiffness because of deterioration in their condition.

When OHC membranes are examined in freeze fracture, densely packed ~11 nm diameter particles are revealed [53]. It has been a consistent assumption that the particles consist of some multimer of the motor protein inasmuch as the 744 amino acid prestin molecule is too small to produce an 11 nm monomer. Using FRET, Navaratnam *et al.* [54] found that homodimerization of prestin depended on an intact N-terminus (but see also [55]). In addition, Zheng *et al.* [56] used chemical cross-linking and PFO-PAGE to demonstrate a tetrameric structure of

prestin. They concluded that monomers are covalently linked by disulfide bonds and that dimers associate to form tetramers. By contrast, the experiments of Detro-Dassen *et al.* [57], while acknowledging dimers as the functional form, deny that these are formed by covalent bonds. They show that dimers are the dominant stoichiometric form for a variety of eukaryotic and prokaryotic SLC26 proteins. They further confirm prestin's quaternary structure as consisting of 12 helices, in line with early suggestions [48,58] but in contrast with [54].

The computational technique of evolutionary trace analysis has been used to show positive selection for prestin in mammals [59•] and for identifying candidate residues for mutational studies [60]. Using similar techniques, and approaches of comparative and evolutionary biology, Okoruwa *et al.* [61] sought to predict the polypeptide motif responsible for prestin's unique motile capability. A preliminary indication of prestin's shape was provided by Mio *et al.* [62] who expressed prestin particles in Sf9 cells, purified them and used electron microscopy to image them at 2 nm resolution. Their result is consistent with prestin being a tetramer, having a large cytoplasmic domain and assuming a 'bullet shape.' Further details of prestin's shape and structure remain obscure.

A recent development in the study of prestin is the discovery of its orthologs. Indeed, when assessed by sequence similarity, the closest homolog of mammalian SLC26A5 is that found in the zebrafish hearing organ [63,64••]. Remarkably, zebrafish prestin (zprestin) shows voltage-mediated charge movement, expressed as gating currents and investigated as nonlinear capacitance (NLC). This property was hitherto assumed to belong only to mammalian prestin. Contrasting the latter whose maximal charge transfer occurs close to −70 mV (the *in vivo* resting potential of OHCs), zprestin is most sensitive at +96 mV and its voltage dependence is much shallower (∼53 mV vs. ∼34 mV for an e-fold change). Other differences are slower kinetics, existence of electrogenic anion transport [47•] and, most importantly, lack of motility. Thus zprestin appears to be an intermediate form, performing voltage-dependent, chloride-sensitive charge dislocations, including full anion transport, but not having acquired motility.

Amplification in the cochlea

Mechanical amplification is a generally accepted feature of mammalian cochlea and may operate in some form in all hearing organs [1,2,13]. However, in the mammalian context, it is still not fully resolved how best to apportion the task of amplification between the two candidate mechanisms (Figure 1). It was thought that the production of a prestin knockout (ko) mouse should settle the issue, inasmuch as the ko fully eliminates one of the candidates. Indeed, the physiological profile of the ko matched that of the no-OHC phenotype, suggesting that prestin-mediated somatic OHC motility is the amplifier [65,66]. Unfortunately, there are three problems with this conclusion. First, strong support for the ciliary mechanism has recently emerged and this is considered below. Second, the ko exhibits certain features that make it of possibly limited use. To wit, ko OHCs are only ∼60% of the normal length [65] and it has been shown that their somatic stiffness is reduced to approximately 20–25% of normal [67••]. These findings allow for altered passive micromechanics in the organ of Corti. The ko is also susceptible to accelerated age-related hearing loss [66,68]. Third, inasmuch as amplification involves some cochlear feedback loop, modifications at any stage will affect the loop's output. Thus if ciliary processes were the amplifier and somatic motility were to crucially adjust the amplifier's properties, eliminating prestin could simulate lack of amplification [69]. In spite of these caveats, recent work shows that normal prestin function is essential for normal sensitivity and frequency selectivity. By mutating two amino acids in prestin's sequence and producing a knockin (ki) mouse expressing the altered protein, we demonstrated several features [67••]. These mice had normal OHC length and stiffness and their forward transduction apparatus, as assessed from transducer currents and measures of adaptation, was also wild-type like. Yet, these mice had ko-like sensitivity and no frequency selectivity. A limited examination of prestin's role as an operating-point adjustor of the ciliary amplifier was also done with the aid of another ki mouse [70]. In this preparation, the voltage sensitivity of the protein shifted but its AC motility did not change. By contrast, its DC motile component was reversed in polarity. In spite of this reversal, the mice had entirely normal cochlear function. While the exercise shows that prestin's own DC response is immaterial, it says nothing about he protein's ability to transduce the receptor potential's DC component and thereby potentially adjust the ciliary amplifier's operating point. The *in vivo* experiment of Santos-Sacchi *et al.* [71] in which perilymphatic chloride content was manipulated also underscores the necessity of normal prestin function.

A variety of theoretical work was designed to examine the often-cited speed limitation of electromotility, attributed to the low-pass filtering of potentials by the OHC's membrane. Using different approaches, investigators show that in the unique environment of OHCs and with prestin's reciprocal behavior, the speed limitation may be overcome and should not constitute a 'fatal flaw' in prestin's putative role as the amplifier [72,73]. A brief review of the variety of schemes proposed to overcome membrane filtering is included in [74].

What may be the most provocative recent experimental result is a measurement of BM motion in prestin ko mice [75••]. The data suggest that lack of prestin does not affect sensitivity, but sensitizes responses for frequencies

below best frequency, thereby broadening the tuning curve. These mechanical data suggest a hitherto unsuspected role of prestin and, if confirmed, may require a paradigm shift in the amplification debate. While the authors suggest an explanation for their results, its theoretical basis is not fully evident. Other significant influences on prestin function or amplification have been seen by manipulating cholesterol content of OHC membrane [76] and by using amphipathic ions in order to change membrane curvature [77] and/or OHC stiffness [78].

The alternative to amplification based on somatic motility, the ubiquitous transducer channel, or cilia-based mechanism, continues to receive a great deal of attention. The tour de force experiment of Chan and Hudsepth [38], in which an *in vitro* cochlear segment was contrived to have *in vivo* like ionic and electrical environment, indicated that as long as forward transduction was maintained while prestin function was eliminated or at least reduced, some degree of amplification remained. While it cannot be ruled out that the small amplification reflected residual somatic motility [79,80•], it is reasonable that some feedback from ciliary mechanisms was detected. Kennedy *et al.* [29] demonstrated that the ciliary amplifier is capable of producing sufficient force and that the gradual buildup of the force could be fast enough *in vivo* to account for amplification. Subsequent work by the same group [80•] argues for the combined operation of both candidate mechanisms. A somewhat different reconciliation is espoused in [67••,81], assigning all amplification to prestin based somatic electromotility and arguing that, inasmuch as ciliary feedback is inherently frequency selective [28], it could serve as a pre-filter. Such filtering would select the appropriate group of OHCs to perform their amplificatory role.

Conclusions

In spite of significant progress, many questions remain incompletely answered. Principal among these are the apportioning of amplification between somatic and ciliary motility, identification of the molecular constituents of the MET channel complex, and the solving of prestin's structure.

Acknowledgements

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