Membrane-based amplification in hearing

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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. These 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 et al., 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 Figure). 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 ($>10^3 \mu\text{m}^2$ 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 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 enhance 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 non-linear 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 phase-shifted 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 et al., 2000). Membrane flexoelectric mechanisms could provide an electromechanical feedback to exocytosis 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 et al., 1990).

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Figure.

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 canaliculae 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 Figure 1 in Brownell 2002(Brownell, 2002).