Neurobiology of Cochlear Hair Cells

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ABSTRACT

Transduction processes in mammalian inner and outer hair cells are examined, along with the influence of the basolateral cell membrane upon the receptor potentials. The characteristics of the receptor potentials, as revealed by *in vivo* recordings are considered. Finally, outer hair cell motility is discussed.

KEYWORDS

Cochlea, hair cells, transduction, ion channels, receptor potentials, hair cell motility.

INTRODUCTION

Several strategies have developed to subserve vertebrate hearing. This review is concerned with the mammalian specialization based on the apparent dichotomy of rôles of inner (IHC) and outer hair cells (OHC). This specialization is probably necessitated by the high-frequency requirements of mammalian hearing and the likely inability of other extant mechanisms to operate beyond a few kilohertz. A great deal of our available knowledge about hair cells is accrued from non-mammalian preparations. Thus, while the present emphasis is on the neurobiology of IHCs and OHCs, information from these studies on lower vertebrates is incorporated when appropriate.

Differences in innervation and morphology between IHCs and OHCs are too well known to require detailed review. **Figures 1a** and **b** provide the necessary background information in schematic form. It is apparent from the anatomical dissimilarities alone that the likely rôle of the two cell populations is different. The present "party line" identifies IHCs as the sensory receptor cells of the mammalian cochlea and relegates the OHC to the status of a mechanical effector in a feedback loop that controls the input to IHCs (**Fig. 2**). Sensory function for OHCs is not commonly assumed in this scheme.

The review is divided into three segments. The first deals with the delivery of mechanical stimulus to the hair cell cilia and the transducer process. The second considers the properties and determinants of the receptor potential. Finally, the motile properties of OHCs are considered along with the putative role of motility *in vivo*. The following reviews are recommended as background and complementary reading: Dallos (1985a), Kim (1986), Dallos (1988a,b), Roberts et al. (1988), Hudspeth (1989), Patuzzi and Robertson (1989), Fettiplace (1990); Holley (1991) and Dallos and Corey (1991).

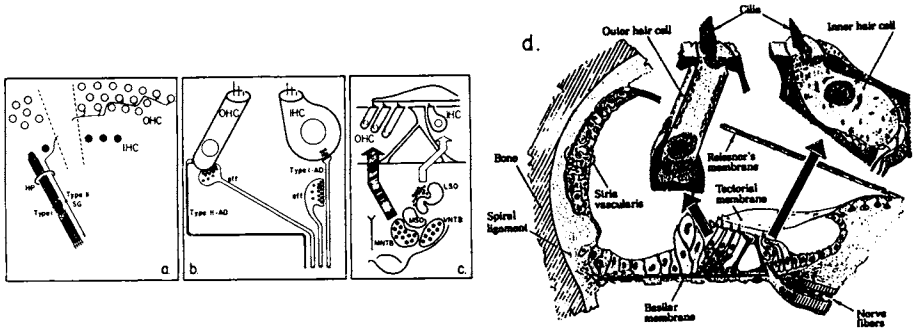

Fig. 1a. Schematic of afferent innervation of OHCs and IHCs. **Figs. 1b,c.** Schematic of efferent innervation of OHCs and IHCs (from Dallos, 1988a; after Spoendlin and Warr) **Fig. 1d.** Cross-section of the cochlear duct with IHC and OHC enlarged (from Dallos, 1985b).

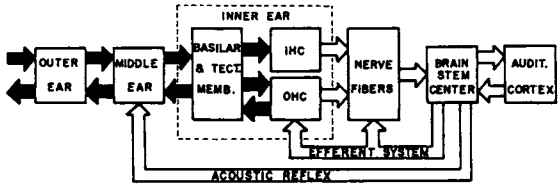

Fig. 2. Block diagram of the auditory system (from Dallos, 1988b).

INPUT MACHINERY AND TRANSDUCTION

Transmission of forces to cilia.

The excitatory proximal stimulus to any hair cell is the bending of the ciliary tuft toward the kinocilium (Hudspeth and Corey, 1977) or, in case of mammalian hair cells that lack kinocilia in the adult, toward the tallest row of stereocilia (Russell and Richardson, 1987). Forces exerted on the ciliary bundle may derive directly from a relative displacement between the reticular lamina, the roof of the organ of Corti anchoring the hair cell apices, and the tectorial membrane to which some of the cilia are attached. Alternatively, flow of endolymph in the narrow gap between the two moving surfaces may derive from the aforementioned relative motion and deliver viscous forces to the cilia (Billone and Raynor, 1973; Freeman and Weiss, 1990). The latter forces are more efficient in stimulating cilia that are not attached to the tectorial membrane. In OHCs only the tallest row of cilia are connected to the tectorium and it appears that IHC cilia are either entirely free-standing or only tenuously attached.

As a result of the viscoelastic coupling of forces to IHC cilia, their displacement is likely to be proportional to basilar membrane velocity at low frequencies (Billone and Raynor, 1973; Freeman and Weiss, 1990). There is experimental evidence to support this claim, both indirect through cochlear microphonic data (Dallos, 1973) and direct from intracellular recordings from IHCs (Nuttall et al., 1981). At higher frequencies, above 300-500 Hz, the ciliary displacement becomes proportional to basilar membrane displacement. The low-frequency velocity-dependence, aside from imposing a dynamic characteristic upon the IHC response, has a more profound consequence. Because of the coupling properties of IHC cilia, very low-frequency (DC) signals are probably ineffective in stimulating IHCs. In other words, DC displacements of basilar and/or tectorial membranes (LePage, 1987) are

not likely to produce an IHC response (Dallos and Cheatham, 1990). Inasmuch as high-frequency receptor potentials are shunted by the IHC's basolateral RC-filter (Russell and Sellick, 1978), they cannot be effective in stimulating synaptic transmitter release. It follows that changes in stimulus-related discharge-rate in primary afferents and in their precursor transmitter exocytosis, must be governed by DC receptor potentials produced in the nonlinear response of the IHC itself. This DC is likely to arise in the rectifier properties of the IHC transduction process (Russell and Sellick, 1983; Dallos, 1986; Dallos and Cheatham, 1989), but may be enhanced by nonlinear properties of the cell's basolateral membrane (Dallos and Cheatham, 1990).

Transduction

The stereocilia are exvaginations of the sensory cell, bounded by the plasma membrane and consisting of some 3000 parallel actin filaments that are extensively cross-linked by fimbrin (Flock and Cheung, 1977; Tilney et al., 1980;). Adjacent cilia of a bundle are connected to one-another by multiple linkages (Howard and Hudspeth, 1987). The cilia are very stiff and rotate about their insertion into the cell, where the number of actin filaments is reduced by two orders of magnitude. The entire bundle tends to rotate as a unit when micromanipulated (Flock et al., 1977), while adjacent cilia slide with respect to one-another. Within a given bundle the cilia appear in a staircase arrangement with the tallest rows facing toward the outside of the cochlear spiral. Ciliary height is graded from the high-frequency cochlear base to the low-frequency apex from 1 to about 6 μm, depending on cell type and row (Lim, 1980). Mechanical resonance of cilia is implicated in frequency tuning in some reptilian cochleae possessing free-standing bundles (Frishkopf and DeRosier, 1983; Holton and Hudspeth, 1983). While these bundles are much longer (10-30 μm) than their mammalian equivalents, it is not inconceivable that some mechanical tuning may be imposed upon cochlear IHCs due to their graded ciliary height.

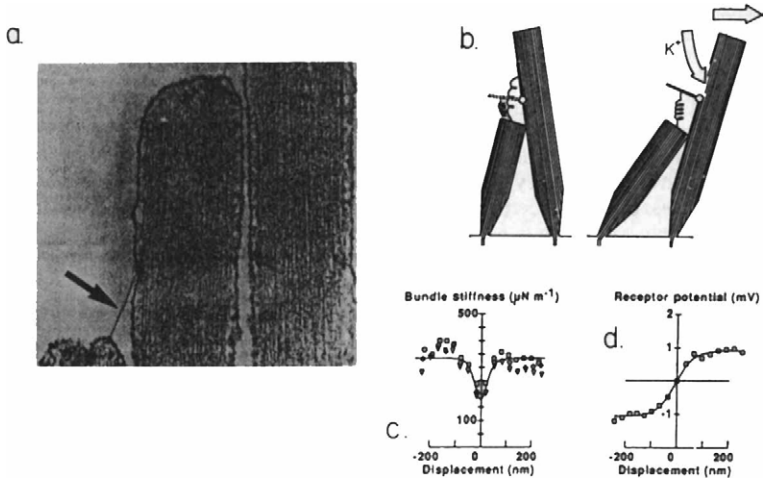

Fig. 3a. Electron micrograph of cilia and connecting tip link (arrow). **Fig. 3b.** Cartoon of cilia, gating spring and mechanically gated channel in two bundle positions. **Fig. 3c.** Bundle stiffness and (**Fig. 3d.**) receptor potential at different bundle deflections (After Hudspeth, 1989).

An attractive and coherent hypothesis of hair cell transduction implicates mechanically gated channels located at the tips of stereocilia that are controlled by changing tension in attached elastic elements, the gating springs (Hudspeth, 1989). Slender filamentous tip links (**Fig. 3a,** arrow) reach up from the top of short cilia to the side of adjacent tall cilia in a bundle, parallel to the mirror-symmetry axis (Pickles et al., 1984). Extracellular current measurements indicate that the sink for transducer current is at the top of the ciliary bundle (Hudspeth,1982), even though fura-2 indication of Ca^{2+} entry into the cell can

not be associated with this location (Ohmori, 1988). The arrangement of tip links is consistent with the cell's directional sensitivity, whereby stretching the links by displacing the bundle toward the tallest cilia is excitatory, movement in the opposite direction is inhibitory, while orthogonal displacement is ineffective (Shotwell et al., 1981). Approximately 50% of the hair bundle's total stiffness (~300 μN/m) may be attributed to its basal pivot, the rest resides in the gating springs. The latter depends on bundle position (**Fig. 3c**). Stiffness is minimal at displacements (about the resting position) where transduction gain is highest (**Fig. 3d**), suggesting an association between gating stiffness and open-closed states of transducer channels (Howard and Hudspeth, 1988). The cartoon included in **Fig. 3b** represents a version of the mechanical gating process of channels via the tip link (gating spring) connection. The number of channels per cilium is estimated to be few (Howard and Hudspeth, 1988) and the "swing" of the gate about 4 nm. Due to its direct activation by the mechanical stimulus, the opening and closing times of the gate are very short, certainly less than 50 μs and likely much shorter in animals in need of very high frequency hearing, such as chiropterans (Hudspeth, 1989).

The transducer channels themselves are non-selective aquaeous pores of at least 0.7 nm diameter, that permit the passage of both monovalent and divalent cations (Corey and Hudspeth, 1979) and probably possessing single-channel conductance of ~50 pS (Ohmori, 1985). In all inner ear systems the cilia, and hence the transducer channels, are in contact with endolymph, a fluid high in K^+ and very low in both Na^+ and Ca^{2+}. The likely carrier of transducer current is thus potassium. The driving force for K^+ is the electrical gradient across the ciliary membrane, produced by the sum of the cell's resting potential and the positive endocochlear potential, the latter being some +80~+100 mV. In the absence of stimulation about 5-15% of the transducer channels are open (Hudspeth and Corey, 1977; Crawford and Fettiplace, 1981; Russell et al., 1986). As a consequence, there is a standing current producing a "biased epithelium" similar to that seen in the retina and in electroreceptors, in that the cell's is normally depolarized to bring it near the range of activation of voltage-gated channels of the basolateral cell membrane. The resting potential of cochlear IHCs is between -40 and -50 mV *in vivo* (Russell and Sellick, 1978; Dallos, 1985b).

Transducer current in response to ciliary deflection has been measured for different hair cells. The relationship between current and ciliary rotation is sigmoidal and indicate great asymmetry for deflections in opposing directions. The gating process underlying transduction is probably describable as a three-state Boltzmann function (Corey and Hudspeth, 1983). Strong saturation is seen for bundle displacements in excess of 0.5 μm, corresponding to a rotation of about 5°. Large, 200-400 pA maximum currents were seen in the saturation region, in contrast to observed maximum transducer currents of about 50 pA in isolated mammalian OHCs (Ashmore, 1990). If measured in an endolymph-like medium, the transducer current in turtle hair cells may increase some three-fold (Crawford et al., 1991). In the best hair cells the transducer conductance is 2.5-5 nS and the sensitivity of the process is about 600 pA/μm (Crawford et al., 1989). Patch clamp recordings reveal single channel currents of ~9 pA and single transducer channel conductance of ~106 pS (Crawford et al., 1991). Assuming a hair cell input resistance *in vivo* of ~50 MΩ (Russell and Sellick, 1978), the receptor potential produced may be of the order of 0.1 mV at 1 nm ciliary displacement; of the same order that is seen experimentally around zero dB SPL (Dallos, 1985b).

It is noted that virtually all information about transducer channels and transducer gating comes from work on submammalian vertebrates. It is likely that the process of transduction is highly conserved for all hair cells and that the above information is applicable to the mammalian hearing organ, presumably to both IHC and OHC. In contrast, as we note below, the properties of the hair cell's basolateral membrane are likely to show considerable divergence, not permitting easy generalizations.

THE BASOLATERAL MEMBRANE AND RECEPTOR POTENTIALS

Membrane properties.

Voltage drops produced by the receptor current may be shaped and altered by voltage-dependent conductances found in the cells' basolateral membrane. The measured receptor potential is the result of interaction among all active conductances. In some hair cells, turtle cochlear hair cells serving as

prime example, the basolateral conductances dominate the character of the cell's voltage response (Crawford and Fettiplace, 1981). In contrast, the dominant character of the receptor potential of mammalian IHC is likely determined by the transducer conductance itself (Kros and Crawford, 1990). One form of ubiquitous influence by the basolateral membrane is due to the inevitable low-pass filtering, owing to the parallel capacitance-resistance of this membrane segment. The filter shunts those frequencies that are above its corner frequency: $f_0=1/2\pi RC$, and consequently limits the size of the dc receptor potential at high frequencies. It is widely assumed that limitations on neural phase-locking are largely a consequence of this pre-synaptic filtering action (Palmer and Russell , 1986; Weiss and Rose, 1988). Inasmuch as R (incorporating time- and voltage-dependent conductances) is dependent on the basolateral membrane voltage (Dallos and Cheatham, 1990; Russell and Kössl, 1991), the corner frequency changes with receptor potential (stimulus) level (Kros and Crawford, 1990). The larger the input and hence the receptor potential, the higher frequencies may be transmitted by the hair cell. Even if these conductances are fully activated, it is unlikely that the corner frequency would much exceed 1 kHz, setting a maximum lower limit for the deterioration of phase-locked transmitter release.

Voltage-gated Ca^{2+} channels and Ca^{2+}-gated K^+ channels have been identified in amphibian (Lewis and Hudspeth, 1983; Pitchford and Ashmore, 1987), reptilian (Crawford and Fettiplace, 1981) and avian hair cells (Fuchs et al., 1988), as well as in mammalian OHCs (Ashmore and Meech, 1986; Santos-Sacchi and Dilger, 1988). In lower vertebrate hair cells the rapidly activating Ca^{2+} inward current and delayed $K^+(Ca^{2+})$ outward current interact together and with the membrane capacitance to produce damped oscillations with high Q. These cells tend to show a range of best frequencies, established by the membrane resonance, that is determined by the kinetics of the $K^+(Ca^{2+})$ channels (Art and Fettiplace, 1987; Hudspeth and Lewis, 1988). The positive feedback provided by the depolarization due to the rapid Ca^{2+} inward current generates high-Q resonance. There is no evidence for resonant behavior in mammalian cochlear hair cells, except for a very rapidly damped resonance in IHCs at driving current levels that are unlikely to be physiological (Kros and Crawford, 1990). In anuran hair cells the Ca^{2+} channels and the $K^+(Ca^{2+})$ channels cluster together with preference to the synaptic region, probably corresponding to presynaptic active zones (Roberts et al., 1990). In these same cells only transducer conductances are found in the cell's ciliated apex. The uneven division of ion channels between the apical and basolateral surfaces is a common characteristic of secretory and sensory epithelia. A preliminary report on OHCs indicate the possibility of the presence of conductances other than those of the transducer in the apical membrane (Gitter et al., 1986).

Basolateral membrane conductances in IHCs (Kros and Crawford, 1990) are dominated by two different voltage-gated K^+channels. One is rapidly activating (0.15-0.35 ms) and TEA-sensitive, the other slowly activating (2-10 ms) and 4AP-sensitive. Both channels are active in the -60 to -20 mV membrane potential range that encompasses all values seen *in vivo*. The two K^+ outward currents shape the receptor potential in that a very rapid initial decline is a consequence of the activation of the TEA-sensitive conductance and a gradual, adaptation-like decline is produced by the activation of the 4AP-sensitive conductance. Neither potassium current appear to be dependent on calcium influx into the cell. Isolated IHCs have zero-current membrane potentials of approximately -67 mV and and a conductance of about 2 nS. The conductance increases to 300-500 nS when fully activated; it decreases to as low as 0.5 nS when the cell is hyperpolarized. A cartoon of IHC, shown in **Fig. 4**, depicts the various currents and channels that dominate IHC electrical phenomena. OHC basolateral membranes are endowed with two types of Ca^{2+}-activated K^+ channels, one with conductance of ~230 pS, the other with ~45 pS (Ashmore and Meech, 1986). It is likely that the basolateral membrane also possesses Ca^{2+} channels, inasmuch as blocking the K^+ current by internal Cs, reveals a voltage-dependent inward current (Santos-Sacchi and Dilger, 1988). The zero-current membrane potential of isolated OHCs ranges from -10 to -68 mV (Santos-Sacchi and Dilger, 1988),"up to" -70 mV (Gitter et al., 1986) and from -15 to -40 mV (Ashmore and Meech, 1986). Apparently a rapid loss of internal K^+ occurs upon isolation and is responsible for the variable and low membrane potentials. If K^+ channels are blocked by BAPTA and the cytoplasm is loaded with K^+, the resting potentials stabilize around -60 mV (Ashmore and Meech, 1986). The mean conductance around this resting potential is 9.2 nS (Santos-Sacchi and Dilger, 1988).

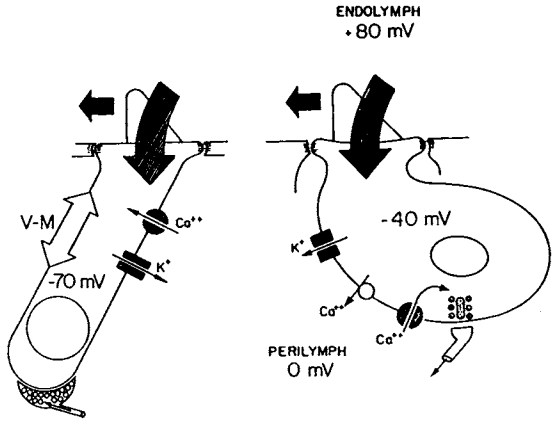

Fig. 4. Cartoon of OHC (left) and IHC (right), showing K^+ receptor current, basolateral ion channels and ion pumps. OHC motility is symbolized by the axial arrow marked V-M, for voltage-to-movement converter.

Receptor potentials.

The first successful intracellular recordings from *in vivo* mammalian IHCs were obtained by Russell and Sellick (1977) and from OHCs by Dallos et al. (1982). The properties of these receptor potentials have been extensively characterized (Russell and Sellick, 1978; 1983; Cody and Russell, 1987; Russell, 1983; Nuttall, 1985; Dallos, 1985; 1986; Dallos and Cheatham, 1989; 1990; Zwislocki, 1989). Receptor potential measurements are also available from organotypic cultures of the mouse cochlea (Russell et al., 1986; Russell and Richardson, 1987). The schematic of **Fig. 5** depicts the two techniques employed. In the original method of Russell and Sellick (1978), the organ of Corti is approached from the scala tympani via the enlarged round window. The method permits excellent control over the orientation and direction of electrodes, but is applicable to only the ~16 to 20 kHz CF region With the technique developed by Dallos et al. (1982), the electrode reaches the organ of Corti through an opening on the lateral bony wall. In theory, this approach is usable in all cochlear turns and it has been applied to the 4th (CF≈200 Hz), 3rd (CF≈1000 Hz) and 2nd (CF≈4000 Hz).

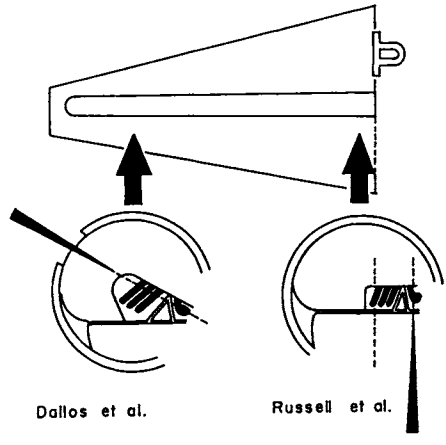

Fig. 5. Schematic depicting the two available techniques for *in vivo* recordings from hair cells in the mammalian cochlea.

Both recording techniques reveal similar membrane potentials, approximately -40 mV for IHCs and -70 mV for OHCs. Similarly, both techniques yield IHC receptor potentials that imply invariance in the behavior of these cells along the length of the cochlea. In response to tones all IHCs produce stereotyped receptor potentials. These contain a fundamental response, a dc component and a harmonic series. At low sound level the fundamental response increases in proportion to the signal level, while the dc response reflects square-law behavior. Harmonics also rise faster than the fundamental. All responses saturate at high levels. As the time patterns of **Fig. 6** show, the harmonic content is clearly manifested in the waveforms, generally as a flattening of the hyperpolarizing peaks and the sharpening of the depolarizing ones. The harmonic content plus the dc make the response waveforms exceedingly asymmetrical about the resting potential. The positive and negative peaks of the response plotted against sound pressure in the ±1 Pa range shows the asymmetry of the receptor potential. Particularly severe saturation is always evident in the hyperpolarizing direction. The properties of the receptor potential versus sound pressure functions seem to be common among hair cell responses in various systems and may be quantified as rectangular hyperbolas (Hudspeth and Corey, 1977; Crawford and Fettiplace, 1981; Russell and Sellick, 1983; Dallos, 1986).

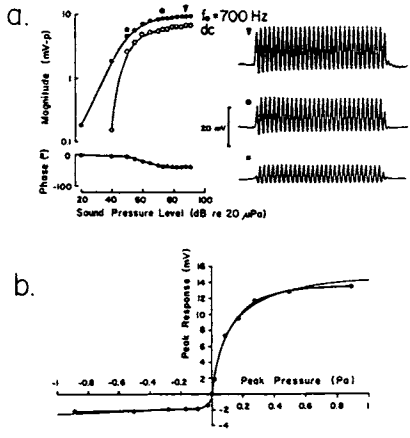

Fig. 6a. Response magnitude (fundamental and dc components) and phase versus sound level for a third-turn IHC at a frequency somewhat below CF. Waveforms at three levels (indicated by the symbols) are shown in the right panel. **Fig. 6b.** Positive and negative peaks of the response graphed in a linear plot against positive and negative peak sound pressure (from Dallos and Cheatham, 1989).

The pattern is similar for a given IHC at all stimulus frequencies, but the quantitative properties of the response differ. In terms of sensitivity and many nonlinear response properties, the IHC reflects its mechanical input. Thus the tuning of its place along the cochlea is the prime determinant of the quantitative features of the IHC's response. As **Fig. 7a** depicts, both fundamental and dc response components are tuned and the sharpness of tuning decreases with stimulus level. Response saturation is most evident at the CF and more linear responses are found far from CF. All such features reflect the properties of basilar membrane vibrations (e.g., Ruggero and Rich, 1991).

The low-level, low-frequency sharpening of the ac plots reflects the velocity-dependence of the IHC's low-frequency response. Concomitant with the slope change, there is an associated phase-lead, reaching 90° at the lowest frequencies (not shown). Aside from shape changes with level, there are also phase changes that depend on the relation between stimulus frequency and CF; this behavior also reflects the preceding mechanical nonlinearity (e.g., Sellick et al., 1982). We note that the magnitude function of the dc component is sharper, reflecting a square-law relation with the fundamental. It is also noted that no matter what the stimulus conditions, the dc response of IHCs is always depolarizing. When tuning curves (iso-response functions) are compared for ac and dc components, they have

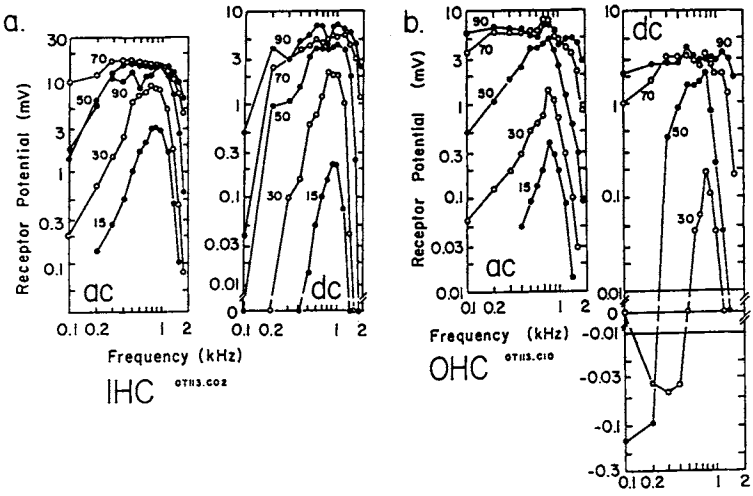

Fig. 7a. Fundamental and dc responses as a function of frequency at the indicated sound pressure levels from a third-turn IHC. **Fig. 7b.** As in **a.** but for an OHC from the same cochlea (from Dallos, 1985b).

the same shape both at low frequencies (Dallos, 1985b) and high frequencies, after correction for the filtering of the ac (Russell and Sellick, 1978). Inner hair cell receptor potentials reflect all well-studied nonlinear features of cochlear function, such as intermodulation distortion (Nuttall and Dolan, 1990), harmonic production (Dallos et al., 1986) and two-tone suppression (Sellick and Russell, 1979; Cheatham and Dallos, 1990).

Certain features of OHC receptor potentials are cochlear location and/or technique dependent. In brief, recordings from the low-frequency region of the cochlea with the lateral approach reveal that OHC and IHC receptor potentials are more similar than different. Thus, aside from two primary differences to be considered below, the previous description of IHC behavior could be applied to OHCs as well. However, recordings from OHCs in the high-frequency end of the cochlea via the scala tympani approach, reveal a fundamental difference in that at CF and low sound levels the receptor potential versus sound pressure functions are symmetrical. In other words, little or no dc is produced. The comparison between the two types of recording is highlighted with the aid of **Fig. 8**. First note that due to the basolateral membrane filters the ac component is negligibly small in the basal turn recording for either IHC or OHC. The dc response does not appear in OHCs until the sound level reaches ~90 dB, whereas from an IHC located at the same place the dc component is already very prominent at 10 dB SPL. In contrast, at the 1 kHz location both IHC and OHC dc responses are present at 30 dB. When measured in organotypic cultures of mouse cochleas (Russell et al., 1986), both IHC and OHC exhibit the type of receptor potential versus input level functions as exemplified in **Fig. 6b**, that is, they both cells show asymmetry. The common explanation of the different OHC behavior *in vitro* and *in vivo* invokes the lack of a tectorial membrane in the culture. This would produce "open loop" operation for the OHC *in vivo*, inasmuch as the presumed motile OHC (see below) could not feed energy back in the absence of the tectorial membrane and in the presence of direct ciliary stimulation. The suggestion is that the fundamental asymmetry of OHCs and IHCs is similar and is expressed in the culture. However, *in vivo* the OHC, via its cilia and the tectorial membrane, alter the response so as to minimize the dc component (Russell et al., 1986). In this light, lower frequency OHCs either do not participate in such a feedback process or their responses are compromised. Alternatively, the symmetry of

high-frequency OHCs *in vivo* may reflect their compromised state. Inasmuch as two methods have been used to gather high- and low-frequency information, it is not possible to state at this time whether it is the CF or the method that produces the discrepancies. Taking available information at face value, one consequence is that while it is generally agreed that extracellular ac responses (cochlear microphonics) are dominated by OHC receptor currents, it is now assumed by many that extracellular dc responses (summating potentials) are controlled by IHC receptor currents. Comparison of recordings of ac and dc responses from low-frequency IHCs and OHCs reveal two qualitative differences. First, there is a ~6 dB/octave low-frequency slope difference coupled with a ~90° phase difference between frequency responses of OHCs and IHCs. Both features reflect the basilar membrane displacement-dependence of OHCs and velocity-dependence of IHCs. Second, while IHCs produce only depolarizing dc responses, OHCs generally show a frequency and level-dependent transition between hyperpolarizing and depolarizing asymmetries. The frequency response plots of **Fig. 7b** depict ac and dc responses at several levels and the below-CF, low-level hyperpolarizing responses are amply evident. Such hyperpolarizing responses are more prominent in basal-turn OHCs below the CF (Cody and Russell, 1987).

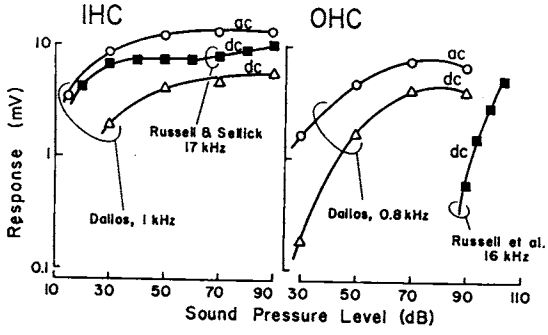

Fig. 8. Comparison of intracellular recordings from IHCs and OHCs. Third turn data are compared with first-turn data (from Dallos, 1988b).

OUTER HAIR CELL MOTILITY

Various mechanical responses of hair cells have been observed. Several types of electrically- and chemically-induced motile events are recordable and mechanical stimulation of certain hair cells results in a reaction force exerted by the cilia upon the driver. These phenomena are discussed separately.

Somatic electromotility.

Fast motility. When placed in an electric field or have its membrane potential changed, an isolated living outer hair cell is capable of longitudinal contraction or elongation (Brownell, 1983; Brownell et al., 1985). This electrically induced somatic motility is capable of following voltage changes into the kilohertz range (Kachar et al., 1986; Ashmore, 1987). On this basis alone, it is apparent that the motile mechanism is not based on conventional molecular motors, such as an acto-myosin reaction. The motile response is not dependent on ATP or Ca^{2+} (Kachar et al., 1986; Holley and Ashmore, 1988a) and persists in the presence of agents directed against actin and tubulin polymerization (Holley and Ashmore, 1988a). The force-generating process has been associated with the plasma membrane and its associated cortical structure (Holley and Ashmore, 1988b; Dallos et al., 1991), with the subsurface cisterns playing a significant, but as yet undetermined, function (Evans, 1990; Brownell, 1990). Fast motility is driven by transmembrane voltage changes, in contrast to either transmembrane current flow or longitudinal potential gradients along the cytoplasm (Ashmore, 1987; Holley and Ashmore, 1988a); Santos-Sacchi and Dilger, 1988; Dallos et al., 1991). Isolated OHCs possess positive intracellular pressure that largely determines their resting stiffness (Holley and Ashmore, 1988b) and without which fast motility is degraded (Brownell et al., 1985; Ashmore, 1987).

Several theories of fast electromotility have been advanced. Probably the most parsimonious of these associates the "motors" with charged proteins associated with the plasma membrane (Ashmore, 1989; Dallos et al., this volume), whose conformational change may underlie the detection of "gating" currents seen during motility (Ashmore, 1989; Santos-Sacchi, 1990). One may inquire about the adequacy of force generated by this process. The longitudinal stiffness of the average isolated OHC has been estimated as $5x10^{-4}$ N/m (Holley and Ashmore, 1988b). Energy required to compress this "spring" by 2 nm is ~10^{-21} Joule. Such a 2 nm motile response is produced by approximately 1 mV transmembrane voltage (Santos-Sacchi, 1990; Evans et al., 1989). Assume that each intramembrane particle of the OHC lateral membrane that can be seen in freeze-fracture (Forge, 1989) carries one net charge. Their estimated density from Forge's pictures is ~6400/μm^2. Consequently, for a 70 μm long, 10 μm diameter cell there are ~$2x10^7$ particles. If they all move across 1 mV then the total energy available is ~$3.2x10^{-15}$ Joule, vastly in excess to that required for the compression of the cell itself and conceivably enough to work against external loads. Of course, charge movement in the membrane along the field needs to be translated into longitudinal displacement. Presumably the elaborate submembrane cortex could perform this transformation (Holley and Ashmore, 1990) at some cost of energy The identification of the molecular motor and its association with the OHC cytoskeleton is one of the most interesting unsolved problems in auditory neurobiology.

It is generally assumed that OHC motility *in vivo*, if it occurs, is driven by the cell's receptor potential to produce mechanical feedback upon the basilar membrane-tectorial membrane complex (**Fig. 9a.**). It is estimated that the latter is approximately 0.1 mV at 0 dB SPL for low-frequency hair cells. Can such receptor potential produce enough energy to drive motility? If the capacitance of the cell is ~40 pF (Santos-Sacchi, 1989), then 0.1 mV produces $2x10^{-19}$ Joule energy. In order that OHC motility should have a feedback effect, it ought to be commensurate with basilar membrane motion at 0 dB SPL, or approximately 0.1 nm. Using the same spring constant as before, 0.1 nm compression requires $2.5x10^{-24}$ Joule energy. Once again, it seems that there is ample movement available if driven by the receptor potential. Of course, the OHC basolateral low-pass filter attenuates the available receptor potential. The corner frequency is estimated to be between 50 Hz *in vitro* (Santos-Sacchi, 1989) and 1200 Hz *in vivo* (Dallos, 1984). Taking the more pessimistic estimate, 50 Hz, the attenuation at

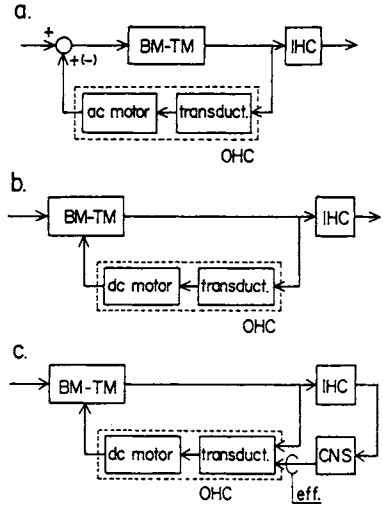

Fig. 9a. Schema of cycle-by-cycle (ac) feedback by OHCs upon cochlear micromechanics. **Fig. 9b.** Schema of parametric (dc) feedback, affecting the micromechanical operating point. **Fig. 9c.** Same as in **b.** but efferent drive of OHC is included (from Dallos, 1988a).

20 kHz is 52 dB, yielding an available membrane potential of only 0.00025 mV. This produces 1.25×10^{-24} Joule energy, similar to that required to compress the cell without any external load. A greater problem is that the filtered receptor potential would produce only 0.0005 nm displacement which is probably insufficient to interact effectively with tectorial membrane movement. Even if the higher corner frequency is correct, the cell motility amplitude at 0.1 mV receptor potential is only 0.0125 nm. There are several conceivable escapes from this quandary. It is possible that *in vivo* the "real" motile event is a ciliary motion or ciliary stiffness change. It has been pointed out that a more effective influence over cochlear micromechanics may be had by ciliary displacement than by somatic motility (Hudspeth, 1989). Such events have not yet been observed for isolated OHCs, even though some ciliary motion is seen in electrically stimulated organ of Corti fragments (Reuter and Zenner, 1990). Another possibility is that the prominent dc component of OHC motility (Evans et al., 1989; Santos-Sacchi, 1989), which is of course impervious to filtering, may act in a process of parametric feedback as sketched in **Fig. 9b** (Dallos, 1988a). Isolated outer hair cells show somatic motility upon mechanical stimulation of their basolateral membranes (Brundin et al., 1989). Conceivably elicited by electrical changes due to opening and closing of stretch-sensitive channels, the hallmark of these motile responses was their apparent sharp tuning, with best frequencies appropriate to cell length. If confirmed, these results are of great significance, inasmuch as theoretical considerations of cochlear amplification become more feasible in the presence of tuned effector elements.

Slow motility. Various agents can promote reversible OHC volume changes that, due to the cylindrical shape of the cell and its anisotropic cortical support (Holley and Ashmore, 1988c), result primarily in elongation or contraction (Zenner , 1986; Ulfendahl and Slepecky, 1988; Schacht and Zenner, 1987; Slepecky et al., 1988;Flock et al., 1986). Acto-myosin reactions may be involved in some of these slow shape changes. A particularly intriguing slow motile response is elicited by focal application of acetylcholine to the synaptic region of the OHC (Brownell, 1983; but see also Bobbin et al., 1990), presumably mimicking the action of efferent transmitters. Slow motile responses, if they occur *in vivo*, may have the ability to modulate the operating point of cochlear micromechanics (**Fig. 9c.**).

Stereocilia stiffness change and movement.

The stiffness of the ciliary bundle is altered in the presence of Ca^{2+} and ATP (Orman and Flock, 1983). This observation led to numerous suggestions that cochlear micromechanics may be actively modulated via such stiffness effects, presumably under control by the receptor potential. In lower vertebrates membrane potential-controlled movements of the stereocilia, or a reaction upon the driver producing deflection of the cilia have been observed (Crawford and Fettiplace, 1985; Howard and Hudspeth, 1988; Assad et al., 1989). Such stereociliary reactions or active movements may be best suitable to subserve cochlear amplification.

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Commentaries

PICKLES

You say that intracellular a.c. voltage responses are negligibly small in basal turn hair cells at high frequencies, and yet that fast motility (which we think particularly important for sharp tuning in this region) is driven by the trans-membrane voltage changes. How do you reconcile these views ?

DALLOS

The small size of the a.c. receptor potential in basal turn outer hair cells at high frequencies presents a serious challenge to our understanding of the cochlear amplifier. The issue was considered during the Discussion Session of the Keele meeting (Wilson and Kemp, 1989). One explanation is that in vitro measurements under-estimate the gain of OHC motility.

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PUJOL

The recent results you mentioned from the Brighton group have been obtained in an immature preparation and there is a crucial difference. Actually, at this stage of development OHCs are not completely differentiated in motile elements as they are in adults. Moreover their innervation pattern is similar to that of IHCs. Then, it is not surprizing to find at this stage similar electrical properties (of "sensory cell" type) in both IHCs and OHCs.