

The cochlear amplifier is a surface acoustic wave resonator

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Abstract:

A companion paper (Bell, 2001) formulated a model of the cochlea as a surface acoustic wave (SAW) resonator. This supporting paper seeks to give a working account of the sensing elements in the ear and how they operate together to create a SAW resonator. A key feature of any such device is that the interdigital transducers alternate in polarity, an arrangement ideal for launching and detecting surface waves. Translated to the ear, the three rows of outer hair cells (OHCs) are conjectured to be the interdigital transducers. In the simplest (degenerate) SAW resonator, only a single set of three electrodes is required to create resonance between the fingers, a situation presumed to apply in the cochlea, where OHC2 is assumed to respond in antiphase to OHC1 and 3. The antiphasic response is not to displacement, but to intracochlear fluid pressure. An examination of the literature interprets OHCs as responding directly to pressure via their cell bodies, and two populations, with opposite response polarities, are observed. Whether an OHC behaves in one way or the other depends on its membrane potential and turgor pressure, so it is conjectured that OHC1/3 operate at a membrane potential of about -70 mV, whereas in OHC2 it is about -50 mV. At low sound pressure levels, two mechanisms for creating an electrical response in OHCs are identified: one involves the piezoelectric response of the OHC wall to pressure, the other a transient sodium current which acts as a biological ‘transistor’ to amplify the transducer voltage.

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I. INTRODUCTION

In Bell (2001), it was speculated that the cochlear amplifier was based on a SAW resonator, a device familiar from solid-state electronics. Normally, a SAW resonator generates electromagnetic ripples between two sets of electrodes placed on the surface of a quartz substrate, but in the biological analogue the acoustic ripples are generated on the undersurface of the tectorial membrane between rows of outer hair cells. The active cochlear model was called an ‘underwater piano’ after the description of Gold (1987), who portrayed the cochlea’s predicament of possessing individual resonant ‘strings’ that needed to operate in a fluid environment having high damping.

The model can be described most succinctly by reference to **Fig. 1**, a three-dimensional view of the cochlear partition showing a single excited ‘string’ of the piano, represented as a resonant cavity in the tectorial membrane (TM). The string is a standing wave generated between the first and third row of outer hair cells, and can sometimes be detected in the ear canal as the continuous faint ringing called spontaneous otoacoustic emission. Unlike conventional traveling wave theories where stimulation of the OHC stereocilia is deemed the primary stimulus, the theory considers that, at low sound pressures, an additional sensitivity to acoustic pressure variations, sensed by the body of the hair cell, is more important. Cells respond to acoustic pressure variations with amplified movement, which generates capillary waves, or ripples, on the lower surface of the TM.

The dual sensitivity of OHCs to both pressure and displacement allows them to be simultaneous sensors and effectors, and causes ripples, once generated, to reverberate back and forth between the outer rows, as detailed below (Section IV). The result is a resonating cavity, like the plucked string of a piano, but in this case cellular energy is used to sustain the reverberation, enabling high Q ’s to exist in a watery environment.

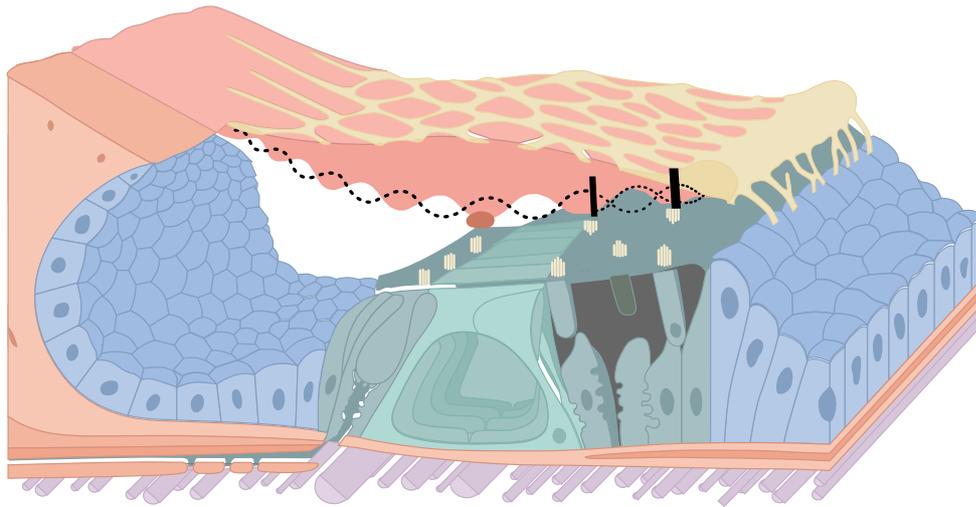


FIG. 1. A three-dimensional view of the cochlear partition showing a single string of the ‘underwater piano’, represented as a resonant cavity in the tectorial membrane. The ‘string’ is a standing wave (shown as solid black lines between vertical marks) generated between the first and third row of outer hair cells. The waves are conjectured to propagate as capillary waves, or ripples, on its lower surface. The ripples are produced by piezoelectric outer hair cells, which directly sense incoming sound pressure and, in response, change length cycle by cycle. Inner hair cells, *middle*, detect the ripples escaping the cavity.

Because the two outer rows of OHCs are spaced one wavelength apart, a resonant cavity is formed in which standing waves are sustained by the dual sensory/effector properties of the OHCs. This process, which acts like a surface acoustic wave (SAW) resonator, allows high Q 's to exist in a fluid environment (hence, the reference to Gold's description of the ear as ‘an underwater piano’).

A SAW resonator generates electromagnetic ripples between two electrodes placed on the surface of a solid-state substrate (see Fig. 2), and structural analogies with it are seen in the cochlea. Thus, the OHCs are the interdigital transducers, Hensen's stripe (dark red, above IHC) and the marginal band (yellow, at right) are absorbers, and the covering net (yellow, top) is a diffuser of unwanted propagation modes.

For the sake of clarity, the ripple amplitude in Fig. 1 is shown without attenuation, although in practice it will diminish with distance, particularly at Hensen's stripe. Nevertheless, if the ripples remain unextinguished, they will be reflected off the sharp edge of the inner spiral sulcus (top edge of blue area on left), so that when the returning wave reenters the cavity, an echo or evoked otoacoustic emission will result.

(Drawing by Tara Goodsell, RSBS Graphic Design, ANU, after Lim (1980, Fig. 3) *J. Acoust. Soc. Am.* 67, p. 1686. Used with permission of the author and the Acoustical Society of America.)

Like a laser cavity, oscillations can escape the resonant cavity, and in this case they travel across the lower surface of the TM to the inner hair cells (IHCs), where stereocilia are deflected, generating signals about changes in the strength of the emerging energy. In brief, incoming sound energy, transmitted as pressure variations through the cochlear fluids, is sensed and amplified by OHCs then detected by IHCs, all in a manner similar to how a radio senses, amplifies, and detects electromagnetic waves using a regenerative receiver arrangement, a topology again suggested for the ear by Gold.

The earlier paper described how the SAW resonator model answered a number of questions in current hearing theory, and this paper sets out a possible mechanism for its operation.

II. BASICS OF SAW RESONATORS

Surface acoustic wave devices have become established in solid-state electronics as useful, compact components for signal processing applications (Bell and Li, 1976), with the flexibility to be configured as resonators, delay lines, convolvers, filters, and frequency analysers (Maines and Page, 1976). The simplest SAW device is the delay line, in which two sets of parallel finger-like electrodes are placed on the surface of a piezoelectric substrate, normally quartz or lithium niobate, forming a cavity between them (**Fig. 2**). The thin metal electrodes are interleaved, with reverse polarity applied to every second finger. An electrical signal applied to one set of interdigital electrodes (a port) gives rise to slowly propagating waves (Rayleigh waves) on the surface of the solid. The second port senses the electric field of the propagating wave. For the SAW resonator, which is our principal interest here, simple feedback to the delay line creates continuous oscillation.

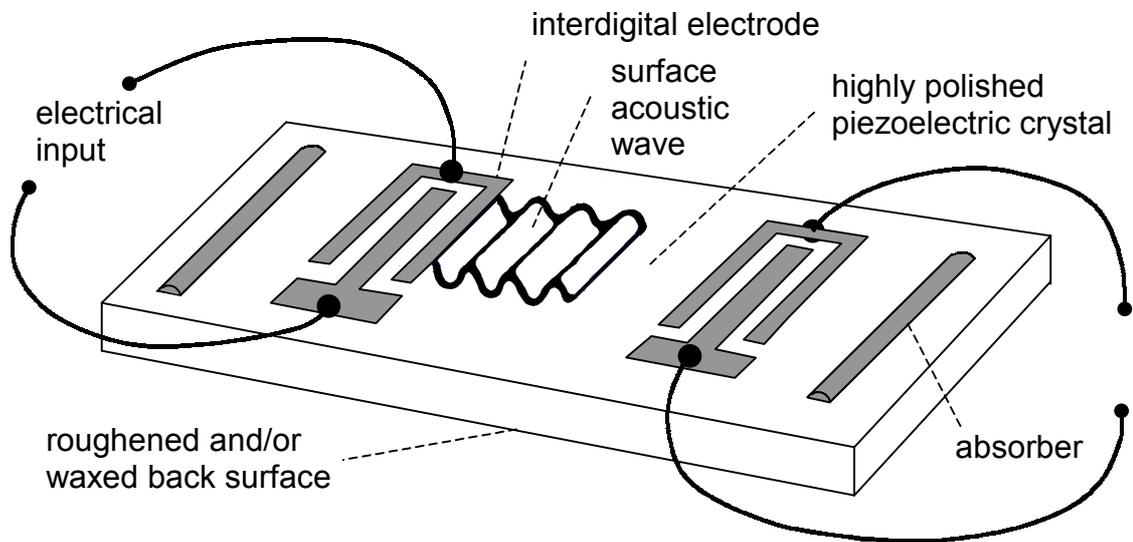


FIG. 2. The basic elements of a surface acoustic wave delay line (after Smith, 1981). Surface acoustic waves are launched when an electrical signal is applied to an interdigital electrode on the surface of a piezoelectric crystal; the system is designed so that the period of the interdigital transducer matches the wavelength of the surface acoustic wave. The waves are detected by a similar electrode set after an appreciable delay. A resonator is formed by feeding the output back to the input; alternatively, with driving and sensing functions combined, a resonator can be made using a single set of electrodes. The latter is the topology expressed by the three rows of outer hair cells.

A typical electromagnetic SAW wave has a frequency in the megahertz range, an amplitude of about 0.1 nm, and propagates five orders of magnitude slower than it would in a cable, a compression made use of in signal processing applications. An important property of the cochlear version is that it offers a similar speed reduction: some 30 mm/s compared to the speed of sound in water, 1500 m/s, again a reduction of five orders of magnitude (see Bell, 2001).

The interdigital spacing of the electrodes is usually arranged such that the interdigital period is equal to the acoustic wavelength. In this way, each of the waves will add in phase. The simplest form of resonator comes from using the interdigital transducer as a one-port resonator (Bell and Li, 1976) in which the resonance takes place between the fingers of the one transducer, which operate as combined generator and detector of the electrical signal. The minimum number of fingers is three, and it is this configuration which is presumed to apply to the cochlea with its three rows of OHCs. It is significant that the cochlea sometimes has four or more rows, especially near the apex, but never less. Some animals such as the echidna have five or more rows (Pickles, 1992), but it is hypothesised that the same interdigital transducer mechanism operates. Additional rows provide extra gain and sensitivity, and reduced bandwidth.

On either side of a SAW resonator, acoustic absorbers (Fig. 2) are usually placed to absorb stray wave energy that passes beyond the electrodes (Smith, 1981). These typically comprise a bulge of material sitting on top of the substrate. Similarly, the base of the substrate is usually roughened – waxed or sandblasted – to discourage reflection of bulk waves that would interfere with the main surface propagation mode (Fig. 21 of Smith, 1981). It is therefore significant that the edge of the TM has a characteristic bulge – the marginal band – on its outer edge and another strip of dense material – Hensen's stripe – in the middle

above the inner hair cell. Hensen's stripe is ideally placed to absorb acoustic energy emerging from the resonant cavity and deliver it to the stereocilia of the inner hair cells directly underneath. Moreover, the upper surface of the tectorial membrane is covered with a net of fibrous material, which again would act to absorb energy escaping from the surface.

In the context of SAW resonators, it is notable that the cochlear version has a sharp impedance discontinuity where the tectorial membrane overlies the vestibular lip. If some wave energy did pass Hensen's stripe, it would be expected to be reflected at this point and return towards the inner hair cell after an appreciable delay – judging from published cross-sections of the cochlea, after about twice 5, or 10, cycles (counting 1 wavelength as the distance OHC1 to OHC3). In this respect, reflection from the sharp vestibular lip, and its associated long delay which (as suggested in Bell, 2001) might give rise to evoked emissions, could have significance as a delay line in the ear's processing of speech.

These analogies are sufficiently strong to prompt a closer examination of how rows of OHCs could act as a SAW resonator.

III. DUAL SENSORS/EFFECTORS

OHCs exhibit one key property that allows rows of them to act like the interdigital electrodes of a one-port SAW resonator, and that is that, like their electronic counterpart, they are both sensors and effectors. As sensors they sense deflection of their stereocilia or of imposed pressure; as effectors their cell bodies can almost instantaneously change length in parallel with transmembrane voltage, a unique characteristic known as 'mechanomotility' (Evans and Dallos, 1993) which owes its origins to a cell constituent called prestin (Zheng et al., 2000). At hearing threshold, sinusoidal movement of the stereocilia by $\pm 0.05^\circ$ (a 1 nm displacement) gives synchronised length changes of 10–30 nm, an amplification process that

can be understood in terms of stereocilia deflection changing the cell's membrane potential, and, in turn, a voltage sensor in the cell wall driving a fast molecular motor.

In a feedback loop in which one cell senses the movement of a neighbour, a system of two adjacent OHCs can, given appropriate phase delay and gain, resonate. Consider a wave propagating from one row to the next so that there is a 360° phase delay and no attenuation: sensing of the wave would give positive feedback and, if the movement were detected and propagated back to its source, continuous oscillation would occur. The rows would form a cavity that would resonate like a pipe open at both ends and sounding in its whole-wavelength mode. Capillary waves (surface tension waves, or ripples) on the TM are an ideal propagation mode for this because their very slow speed offers large phase delays over small distances. As calculations in the companion paper indicate, the speed required in the cochlea is less than that of ripples on the surface of water – of the order of centimetres per second. Such a speed implies that the surface tension of the tectorial membrane – endolymph interface is less than that between water and air, a realistic requirement. The companion paper cites observations of the TM's surface tension, and, more importantly, its extreme flexibility (low stiffness) which allows this characteristically gelatinous structure to act more like a liquid than a solid.

The amplification process described would be facilitated if, like a SAW resonator, there were in fact three or more rows and successive rows alternated in polarity – that is, with a 180° phase difference. Populations of mammalian OHCs do not show phase and antiphase responses to stereocilia deflection,¹ but, as is shown below, there is good reason to believe they behave this way in response to a co-existing stimulus, acoustic pressure.

IV. OHCs HAVE SENSITIVITY TO DISPLACEMENT AND PRESSURE

It is natural to assume – and has been assumed since the first theory of auditory excitation by Hensen – that because OHCs bear stereocilia they are displacement detectors. However, this perception tends to disguise their additional pressure sensitivity, an idea that has periodically been put forward. Pohlman (1933) suggested that “the auditory cells are directly affected through the vibrations in the liquid” (p. 183); Davis et al. (1934), in formulating a piezoelectric theory of cochlear microphonics, supposed that the potential was developed between the upper and lower ends of the cells by mechanical deformation; more recently, the studies of Brundin et al. (1989) on isolated OHCs “demonstrate that the sensory cells of the inner ear *per se* are sharply frequency-specific in the absence of the basilar membrane [and its travelling wave]” (p. 815), although it was left open as to which part of the cell was responding to the fluid-jet stimulus. In the cochlear model of Kolston et al. (1989), excitation of the OHC was taken to come from movement of its body rather than bending of its stereocilia.

It is an important aspect of the SAW resonator theory that OHCs, as well as sensing movement, can also sense acoustic pressure through their cell bodies, and the idea will be examined in detail below. The design of mammalian OHCs is that of a pressure vessel, a structural arrangement which also happens to be suited for good performance as a pressure sensor. The special sensitivity of OHCs to intracochlear pressure allows for a mixture of in-phase and anti-phase responses to occur between an OHC and its neighbour, a feature that many animals make use of in their acoustic processing (see footnote 1).

Because of an OHC’s dual sensitivity to both displacement and pressure, amplification of a ripple can happen in two ways, the first involving the OHC’s stereocilia and the second its cell body. At this point it needs to be recognised that a point on a surface

upon which a transverse wave is propagating traces out an elliptical locus. This elliptical motion can be broken down into a vertical and a horizontal component, with the phase difference governing whether the wave is propagating to the right or left.

If a ripple passes over an OHC, the stereocilia will be deflected by the horizontal component. Similarly, the vertical displacements will create an alternating pressure on the body of the cell. Both movements contribute to the detection of the wave by the cell. (The special case of OHC2 is discussed below.)

Supporting the notion of OHCs being dual sensors, Zenner et al. (1988) observed that OHCs display two distinct effector properties: an oscillating electrical field was seen to cause cells to change length (a well-observed phenomenon) but also to produce active tilting of the cuticular plate of 14° or more. According to the ideas being developed here, the length changes relate to the vertical component of the wave and sensing of pressure, whereas the tilting of the cuticular plate relates to sensing of the horizontal component. That is, forces acting on stereocilia can lead to tilting of the cuticular plate as well as bending of stereocilia, and, with OHC being reversible transducers, the active tilting mechanism assists in the launching of ripples on the TM.

Evidence for the separate function of the two motile mechanisms in OHCs is given by Zenner et al. (1988). By rotating the applied electric field by 90° with respect to the long axis of the cells, each motile response – length change or cuticular plate tilting – could be favoured in turn.

A. Amplification based on stereocilia sensitivity

If an OHC senses a wave with its stereocilia, its polarisation changes and this causes a length change, a process called ‘mechanomotility’ (Evans and Dallos, 1993) which is regarded as the basis of the cochlear amplifier (Davis, 1983). It is assumed there is a voltage

sensor of some kind in the cell wall which drives movement of the cell's cytoskeletal spring (called a 'voltage-to-movement converter' in Evans et al., 1991). In terms of the hypothesis under consideration here, it is assumed that the spring is part of a tight feedback loop and actively returns to its equilibrium position with greater force than it was extended, a process that will pump energy into the system. A 'kick back' effect has been identified in OHC stereocilia by Flock (1988). The stronger return stroke reflects and amplifies the wave disturbance, creating continuous oscillation between the rows. As with an etalon, multiple reflections naturally lead to high Q (the more reflections, the higher the Q).

This arrangement is sufficient to create oscillation between two rows of OHCs, and is a mechanism that can be ascribed to even-numbered cavities (L_0, L_2, L_4 , etc.; see Bell, 2001), which contain but two hair cells. It is a mechanism that can explain the genesis of spontaneous otoacoustic emissions. However, it is not a process that can be ascribed to the odd-numbered cavities: here there is an alignment of three OHCs and the middle cell, OHC2, cannot deflect in both directions simultaneously, which it would be required to do if a 360° phase change between the outermost rows were to be maintained (that is, while keeping two resonant cavities – OHC1/OHC2 and OHC2/OHC3 – back to back). The alternative would be to have a 720° phase change in this case, but this is not possible because, as inspection of Fig. 3 of Bell (2001) will make clear, the outermost hair cells in such a triplet form two-hair-cell cavities with yet other hair cells.

B. Amplification based on pressure sensitivity

By returning to the SAW resonator analogy, a second, more powerful resonant mechanism can be constructed which relies on the pressure sensitivity of OHCs. Just as the three-finger one-port resonator has its central electrode in antiphase to its flanking electrodes, a similar behaviour can be expected in its OHC counterpart, with OHC2

mechanomotility acting in antiphase to OHC1 and 3. At this point, the antiphasic behaviour is assumed; evidence for this supposition is put forward below.

Pressure sensitivity allows the common-mode acoustic pressure in the cochlea to stimulate an OHC, again causing a membrane potential change and producing a vertical movement in the OHC; this launches a ripple which propagates to neighbouring OHCs via the tectorial membrane. Again, these subsequent cells amplify the ripple by responding with increased vigour to the initial stretching and compressing of their cytoskeletal spring. But because of the antiphasic response of OHC2, this amplification process involves the cooperation of three cells, rather than two. It is therefore a process that only occurs in the odd-numbered cavities (L_1, L_3, L_5 , etc.).

C. Integration of vertical and horizontal

Of course, although a ripple may be initiated by a vertical movement after a response to pressure, it will also naturally acquire a horizontal component. At the same time, the mechanomotility of the OHC is tightly linked to both its stereocilia/cuticular plate and its cytoskeletal spring, so that a change in membrane potential results in the launching of a wave in the TM: the cuticular plate tilts (to give a horizontal component) while simultaneously the whole cell changes length (to generate the vertical component).

The result of the coupling between the stereocilia/cuticular plate and the cytoskeletal spring is that there will be a continuous interplay between the horizontal- (displacement-) sensing stereocilia and the vertical- (pressure-) sensing cell body – together these components create the circular locus of a point on a wave, and naturally the reverse process as well. In summary, OHC are wave detectors and wave generators, and they do this by intimate coupling of the horizontal detector/effector and the vertical detector/effector. The result is that a wave can be generated at one OHC row and transmitted to a neighbouring

row, where it is detected some time later (with a phase change), amplified, and returned to its source, creating a simple self-sustaining (and self-limiting) oscillation. Gain in the system is controlled by the intrinsic sensitivities of the transducer stages, but also, as described in Section VB, by differential activity between rows, a factor that is conjectured to depend on resting membrane potential. This potential is assumed to be set by efferent activity.

In situations where additional gain might well be required, such as at low frequencies (at the apex), four or more rows are sometimes found (Bredberg, 1968); it is observed that constant inter-row spacing is retained, meaning that additional amplifying cavities have been added in series. Animals with multiple rows – such as in birds, frogs, and lizards – are proposed to have a similar resonating mechanism involving rows of alternating polarity (although in these animals the dual polarities may be achieved by simply facing the cells and their stereocilia in opposite directions rather than having direct pressure sensitivity – see footnote 1).

A characteristic of the wave-generating mechanism is that tilting of the cuticular plate tends to launch a wave in the direction of tilting. This means that when an acoustic pressure pulse enters the cochlea, ripples are projected towards the modiolus from both OHC1 and OHC3. This directional asymmetry allows the cavity to gather wave energy; if there were no asymmetry, there would be no favoured direction and waves would propagate in both directions equally, effectively canceling and blocking any build-up of energy.

In discussing their observations of the tilting of cuticular plate under electrical stimulation, Zenner et al. (1998) note that “the simultaneous occurrence of both longitudinal and tilting motile responses suggests that the [cuticular plate] may actively transform longitudinal OHC motions into shearing motions of the hair bundle” (p. 238). Clearly, this process would also serve to transform pressure-stimulated length changes into modiolus-directed ripples. They continue: “These shear motions could explain a mechanical radial

coupling (fluid and/or TM coupling) of OHCs and IHCs thus allowing a mechanical transfer from OHCs to IHCs” (p. 238).

Earlier, mention was made that OHC2 cannot usefully respond to stereocilia deflection: if it (bidirectionally) responded to horizontal movement then the tendency would be for the three-cell cavity to split in two. To make a SAW resonator, OHC2 should only respond to vertical movements, that is, pressure. Indeed, as shown in Section VII, there is evidence that there are OHCs that respond only in a limited way to a stimulus (the adapting or unipolar cells of Russell et al., 1992). It is therefore proposed that OHC2 are designed to respond to pressure only, whereas the other two rows respond to both pressure and displacement. Such a distinction in function between rows would explain the observation that some OHC fail to be stimulated by stereocilia displacement (Canlon and Brundin, 1991; Ashmore, 1988). As will be outlined in Section VII, evidence indicates that OHCs with dual sensitivity may be able to change – by undergoing a change in membrane potential (and turgor pressure) – to a type with pressure sensitivity only.

A particularly revealing finding is that about two-thirds (50–70%) of the 504 OHCs examined by Zenner et al. (1988) displayed an “angular” cuticular plate – the plate appeared to cap the cell with an overhang – a characteristic associated with the ability of the plate to tilt. It is presumed that these angular cells derive from the two of the three rows (the outer rows) which are designed to sense deflection, whereas the remaining inner row, without a hinged cuticular plate, detects pressure only.

The sensitivity of OHCs to intracochlear pressure is crucial for the theory because it creates a positive-feedback pathway to the primary stimulus. Thus, when a triplet of OHCs respond biphasically to intracochlear pressure and initiate a ripple on the TM, the subsequent passage of this wave to and fro between the three rows of OHCs, will, as Fig. 9 of the companion paper illustrates, push down and pull up on the OHCs *in phase* with the

surrounding pressure. The vertical component of the ripple thereby provides positive feedback to the pressure, and, like any resonance process, the amplitude builds up cycle by cycle. The amplitude of the ripple will grow until the process reaches a level of amplification appropriate to the magnitude of the surrounding acoustic pressure. This process defines a regenerative receiver of acoustic energy in the cochlea, just as Gold (1948) specified. The cavity will resonate in its whole wavelength mode just like a three-finger one-port resonator or a guitar string plucked in its middle.

Pressure sensitivity allows the cochlea to respond to extremely small stimuli: those that are so weak that they cannot even begin to move the partition bodily. In contrast, if the threshold stimulus were movement of the partition (generated by pressure difference across it), the energies involved would be larger and there would be no clear positive feedback path. Having the stereocilia respond to such a displacement would create no SAW cavity, for there is no purpose in OHC2 stereocilia acting in antiphase to the others (and there is no evidence of it). Such a biphasic response would create an initial transitory ripple, but there would be no positive feedback path to the initial stimulus (partition movement or differential pressure), and so the amplitude of the ripple could not grow.

While specifying the positive feedback path in this way, it is also worth noting that runaway is tamed by the high attenuation of capillary waves, particularly at acoustic frequencies (which is why ripples on water are commonly observed to carry only low frequencies). In the cochlea, the occurrence of acoustic-frequency ripples is made possible only because of the very short distances involved.

V. SENSITIVITY OF OHCs TO PRESSURE

A. Piezoelectric property of OHCs as the basis of the cochlear amplifier

Outer hair cells are known to be piezoelectric – that is, they produce electrical responses when stresses are applied to their cell walls (Gale and Ashmore, 1994; Mountain and Hubbard, 1994; Tolomeo and Steele, 1995). Indeed, their piezoelectric constants are much greater than those of common piezoelectric materials (Tolomeo et al., 1996). However, this behaviour has not been considered of primary importance; instead, the stress in the wall is assumed to be a secondary response to deformations created by initial movement of the partition, which both compresses the cells and deflects the stereocilia. There is thus a choice of two feedback paths: via deformation of the cell body (Kolston, 1989), followed by a piezoelectric response; or, more commonly, via deflection of the stereocilia (de Boer, 1996), which changes membrane potential and hence cell length (via the voltage-to-length converter). In contrast, the present hypothesis sees the piezoelectric response as the primary step in transduction (at least at low SPLs), and not a secondary feedback response. The supposition is that the OHCs respond directly to the acoustic pressure oscillations generated in the cochlear fluids by the displacement of the stapes.

Previously, this process has been overlooked as a primary transduction mechanism because the sensitivity of isolated OHCs to pressure seemed too low. However, once it is seen that pressure is a signal in a positive feedback loop, the sensitivity problem is immediately overcome. Acoustic pressure initiates small ripples, but the amplitude of those ripples builds as the wave reverberates back and forth between the rows. That regenerative process is no less than the cochlear amplifier at work, so that the growth in ripple amplitude – sensed by the OHCs as the gain in the vertical component of the ripple pressing up and down on them – is the gain of the cochlear amplifier. Its gain near threshold is about 1000

(Nilsen and Russell, 1999), so the initial acoustic pressure (ripple amplitude) has been multiplied 1000 times. Whereas an isolated OHC may give only a small response to imposed pressure, that should be regarded as an open loop figure, and when the OHCs join together into a SAW resonator the response is greatly magnified, enough to make the identified pressure sensitivity sufficient to be the adequate stimulus in the cochlea. The threshold pressure is some 20 μPa in the ear canal, which is magnified 20–30 times by the middle ear to give a pressure of about 0.5 mPa in the cochlea. If the cochlear amplifier gives a pressure gain of 1000 over the isolated cell, then at threshold, OHCs will be experiencing a pressure of about 0.5 Pa. There is clear evidence (Section V) that isolated OHCs do respond appreciably to such a pressure (that is, with receptor potentials and length changes appropriate to hearing threshold).

B. Origin of the piezoelectric response: membrane capacitance

Holley and Ashmore (1988) studied isolated OHCs with a patch-clamping protocol, and observed length changes elicited by a change in membrane potential. To explain their results, they suggested a model in which the surface tension of the cells' plasma membrane due to charge accumulation is controlled by membrane potential, a mechanism that had previously been put forward to account for pressure changes in the squid axon during action potentials. These authors point out that for a cylindrical cell of radius r , surface tension (γ) acting on the rounded surface produces a force γ/r which tends to lengthen the cell and another (larger) force $2\gamma/r$ acting on the ends which tends to shorten the cell. Naturally, to preserve the cell's shape, these pressures will be resisted by its internal turgor pressure and by elastic elements within the cell's structure.

Their simple model suggests that a pressure change δP will be produced by a voltage change of $-(3/2r) C_m (\delta V)^2$, where C_m is the membrane capacitance and δV is the change in membrane potential. If the intrinsic capacitance of the cell is typical for cell membranes ($1 \mu\text{F}/\text{cm}^2$), then for a long tube $10 \mu\text{m}$ in diameter, the pressure will change by 0.1 Pa for every 1 mV of membrane potential change. Actual cells with a finite length will have a lesser sensitivity because of the counteracting effect of the ends. Here it is suggested that by reversing the mechanism, the cell will behave as a piezoelectric transducer, with pressure changes causing shifts in membrane potential. Most common piezoelectric materials are bidirectional, the degree of coupling between mechanical and electrical properties governing the efficiency of the forward and reverse transduction. An ideal transducer will be perfectly reversible, and it is assumed in the following that the OHC is such a transducer.

A number of authors have already examined the piezoelectric properties of OHCs and assumed that they are ideal (Mountain and Hubbard, 1994; Adachi and Iwasa, 1999). Given this ideal coupling, a pressure difference of 100 mPa between inner and outer will create a membrane potential change of something less than 1 mV . Therefore, if a threshold pressure sensitivity of 500 mPa were applied to the cell then a membrane potential of some millivolts would result, well above the receptor potentials of $15\text{--}100 \mu\text{V}$ measured at threshold (Santos-Sacchi, 1989). From this theoretical perspective, it can be concluded that, based on a threshold receptor potential of $100 \mu\text{V}$, OHCs should be able to detect pressures as small as 2 mPa , close to the 0.5 mPa figure calculated above; for $15 \mu\text{V}$, the corresponding figure is below threshold pressure.

One property of this model is that surface tension on the cell ends is countered by surface tension on the circumferential wall, turgor pressure, and internal cell stresses. Note that for a cell of length l , the surface tensions balance for $l = 2r$ making turgor pressure and internal stresses zero. All OHCs are longer than $2r$, so that passive cells with no internal

pressure will tend to lengthen in response to a positive external pressure step, and the longer the cell the closer the pressure change will be to the limiting (infinitely long) case specified by the equality above.

1. Non-linear cell capacitance and the origin of antiphase behaviour

A key property of OHCs is that in addition to the normal, linear membrane capacitance, typically 20 pF for an average cell, they possess a voltage-dependent non-linear component which has a bell-shaped characteristic that is shifted by resting membrane potential (and resting turgor pressure). The non-linear capacitance normally substantially exceeds the linear component. The required antiphase behaviour of the SAW resonator can be derived from this non-linear capacitance.

Takehata and Santos-Sacchi (1995) applied channel blockers to isolated OHCs to eliminate ion currents and studied the remaining non-linear component of the membrane capacitance. This capacitance shows itself as a displacement current which can be compared to the traditional much smaller gating current that occurs when ion channels open. The displacement current can be viewed as the movement of charge associated with the piezoelectric transducer. The size of this current is voltage-dependent, and the authors of this study measured how the capacitance changed with membrane potential and with pressure applied by a patch pipette. Positive internal (negative external) pressure was found to reduce length and increase radius and hyperpolarised the cell. They concluded that the OHC motor senses tension directly, instantaneously affecting cell length, and suggest that the motor and sensor are either tightly coupled or in fact one entity.

The non-linear membrane capacitance was found to vary with membrane potential, producing a bell-shaped curve, with the peak occurring at voltages ranging from 0 to -80 mV (Santos-Sacchi et al., 1994; Takehata and Santos-Sacchi, 1995). The result of one

such experiment is shown here as **Fig. 3**. Altering the internal turgor pressure also shifted the peak of the non-linear capacitance to different voltages, an important consideration because OHCs have a positive internal turgor pressure which varies over the range 0.17–1.9 kPa (Ratnanather et al., 1993). Kakehata and Santos-Sacchi (1995) conclude that the variability of the voltage at which peak capacitance is observed results from variability in OHC turgor pressure. Whereas this may be true in general, their Fig. 8 shows that the same peak voltage may be accompanied by different turgor pressures, indicating that the two variables are not completely codependent (and that the peak potential need not correspond with the resting membrane potential). Other factors, such as various basal conductances, must also play a role in setting the location of the peak.

Of relevance to the SAW resonator mechanism, it is seen in Fig. 1 of Kakehata and Santos-Sacchi that the displacement currents *change polarity* on either side of the peak (Fig. 3). That is, if the turgor pressure of OHC2 is such that the cell's resting membrane potential is on the hyperpolarized section of the curve, say at -80 mV, and the turgor pressures of OHC1/3 are such that the resting membrane potential is on the depolarised part of the curve, say at -50 mV, then the non-linearity of the capacitance will mean that there will be an antiphasic response of the sets of cells. Displacement currents (as indicated in Fig. 3) represent the sum of positive and negative responses, so translating this to positive and negative pressure pulses means that each cell responds non-linearly in opposite directions. This means that, with a positive pressure pulse, OHC2 may give an expanded response (both electrically and, via electromotility, mechanically), while the two other rows give a compressed response; similarly, with a negative pulse, OHC2 will compress while the others expand. The result is that the set of three cells will give an oscillating differential response sitting on top of a constant offset (the linear response). Put another way, a positive pressure pulse will always lengthen all three cells and a negative pulse will always shorten

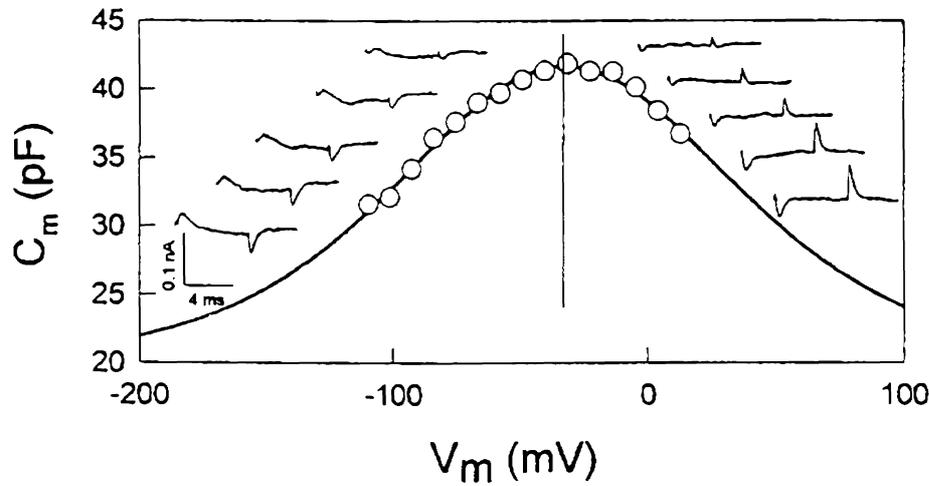


FIG. 3. Capacitance of an isolated outer hair cell varies with membrane potential (from Kakehata and Santos-Sacchi, 1995). The inset waveforms are gating currents derived by applying fixed voltage steps to the cell at various holding potentials; note the reversal of the gating current on either side of the capacitance peak. Translated to the cochlea, where the cell operates as a piezoelectric transducer, this response characteristic means that a pressure pulse applied to a cell will produce voltage responses of different polarities depending on whether the polarization and turgor pressure of the cell places its operating point on one side of the peak or the other, and this is conjectured to be a key difference between OHC row 1/3 and row 2.

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them, but non-linearities will generate an anti-phasic component sitting on top of a linear response. Despite the d.c. offset, the oscillating component will ensure that the SAW resonator will function as required. The relative magnitude of the d.c. and a.c. components will depend on where the cell is on the operating point of the curve relating non-linear capacitance to membrane potential. Moreover, a possible additional mechanism outlined in Section VIII (which invokes amplifying currents only in OHC1 and 3) has the potential to greatly increase the a.c. component.

2. Another perspective on turgor pressure and non-linearity

From study of the equations governing the interplay between membrane capacitance and pressure (Holley and Ashmore, 1988), it can be seen that both membrane potential (V) and turgor pressure (T) affect surface tension (γ). Surface tension and membrane potential are related by:

$$\gamma(V) = \gamma(0) - \frac{1}{2}C_m V^2 \quad (1)$$

where C_m is the membrane capacitance and $\gamma(0)$ is the surface tension at zero voltage. Turgor pressure will alter the charge density on the membrane, and so $\gamma(0)$ will also depend on turgor pressure. By treating the cell wall as piezoelectric, it can be seen that the inward force F_w acting on the walls is:

$$F_w = [\gamma(V)/r - T] \times l \times 2\pi r \quad (2)$$

and this force will generate the stress in the cell wall that leads to charge displacement. Note that when $T = \gamma(V)/r$, the stress in the wall will be zero. Tolomeo and Steele (1995) have examined the piezoelectric properties of the cell wall of OHCs and concluded that they are orthotropic, that is, the stiffness in the circumferential direction is several times that in the radial direction. They note that turgor pressure acts to prestress the cell wall. Depending on whether the turgor pressure is greater than, or less than, $\gamma(V)/r$, it can be seen from

Equation 2 that the stress, and the corresponding non-linear capacitance of this piezoelectric transducer, can be either positive or negative.

C. Experimental evidence for pressure responses of OHCs

Some direct observations of the effect of static pressure on OHCs have been made. Ashmore (1987) observed that small positive pressures of 2–5 Pa applied to the inside of an OHC via a patch-clamp pipette caused the cell to shorten by 10–20%. Zenner et al. (1992) and Gitter et al. (1993) applied negative pressures to the outside of OHCs held in capillary tubes and observed elongation of the cells. However, in both of these studies, the time scale of reaction is not reported, meaning that protracted osmotic effects (over the scale of seconds or minutes) cannot be ruled out as underlying the change.

Adachi and Iwasa (1999) patch-clamped guinea pig OHCs and varied the internal pressure via a pipette. They found that the peak membrane capacitance of the cells varied with membrane potential and that peak was controlled by pipette pressure. In this way they found a relationship between voltage shift and pressure of about 150 mV/kPa, which translates to 0.15 μ V for a threshold intracochlear pressure of 1 mPa. This pressure sensitivity is at least 100 times less than the 15–100 μ V quoted earlier, but then this is a static, open-loop figure for a single sensing element; when the cells are working together in a positive feedback loop at audio frequencies, then the static figure would need to be multiplied by the feedback gain of the system, that is, the gain of the cochlear amplifier. According to recent figures (e.g., Nilsen and Russell, 1999), the gain of the cochlear amplifier approaches 52–66 dB near threshold, giving sufficient sensitivity for the pressure transducer to supply the observed threshold voltage.

The most apt studies are those where isolated OHCs were subjected to a.c. pressure variations – acoustic stimulation from a minihydrophone – and a direct response observed. In

these experiments, isolated cells immersed in liquid were subjected to acoustic pressure variations by placing an oscillating water jet near them. Significantly the cells changed in length in response to this stimulation, even when the stereocilia were absent or were protected from stimulation by a surrounding pipette (Canlon et al., 1988; Brundin et al., 1989; see also Canlon and Brundin, 1991; Brundin and Russell, 1993; Brundin and Russell, 1994). In fact, Canlon and Brundin (1991) noted that length changes could not be elicited when the stereocilia were stimulated directly.

Results showed that the length changes had both tonic and phasic components, and were tuned to specific frequencies. At low stimulus levels, the phasic component was larger than the tonic, and, depending on the cell studied, led to either tonic lengthening or shortening. High frequency (short) cells tended to shorten, whereas low frequency (long) cells tended to lengthen. Cells of intermediate length could either lengthen or shorten, a result interpreted here as reflecting the level of the turgor pressure (and resting membrane potential). The important point is that the phasic behaviour of some cells is presumably in antiphase to that of others, a position supported by the statement of Brundin and Russell (1993) that “[t]he mechanically induced tonic length change is a non-linear rectification and amplification of the phasic mechanical response” (p. 187). Another supporting observation is Fig. 5 of Brundin and Russell (1994) where the phase of the isolated cell’s oscillatory response (measured as cuticular plate displacement) changed abruptly by 180° as the stimulus intensity reached a “high” level. Interestingly, the phase reversal was only apparent after one cycle of the 100-Hz stimulus, a result that could be interpreted as the time required for the polarization of the cell to drop in response to excess stimulation.

Brundin and Russell (1993) make the suggestion that in the cochlea the OHC may respond directly to intracochlear pressure, but their idea has not been taken further, possibly because of a perceived lack of sensitivity. The authors calculate that a threshold 1 nm

movement could be elicited by a pressure of 8–46 mPa (or 49–64 dB SPL), which, allowing for the pressure gain by the middle ear of about 30 dB, brings the response to within 19–34 dB of threshold.

VI. A 21-dB DISPARITY?

Santos-Sacchi (1989) makes a comparison between receptor potentials and mechanical activity of OHCs at threshold, and calculates that the mechanical activity appears to be about 21 dB less sensitive than the electrical (see also Santos-Sacchi, 1991; Kakehata and Santos-Sacchi, 1995). This calculation is based on a receptor potential at threshold of 15–100 μV (depending on frequency) measured in the OHCs of guinea pig cochleas; if this figure is multiplied by the slope of the voltage–displacement characteristic (2 nm/mV at a polarisation of -70 mV according to Santos-Sacchi's data), a corresponding displacement of 0.03 nm is derived. This last figure is 21 dB below observed displacements of 0.35 nm at threshold.

However, this voltage–displacement characteristic was derived from measurements on isolated, patch-clamped OHCs. The figures are therefore unlikely to correspond with physiological conditions. In particular, the cells could well have suffered in sensitivity from the isolation process, and would not experience the same ionic concentrations as the actual endolymph and perilymph which surrounds the cells in vivo (in this context, the results of Konishi et al, 1966, show that replacing endolymph with perilymph in the live cochlea abolishes extracellular potentials). Nevertheless, the figures are appropriate for closed loop conditions in that the receptor potentials were observed in the cochlea where the cochlear amplifier was functioning², and the voltage–displacement characteristic will apply under most conditions.

However, there are additional limitations attaching to measurements on patch-clamped cells. Kakehata and Santos-Sacchi (1995) mention the unknown turgor pressure of cells *in vivo*, and that if it were less than previously thought the apparent disparity might disappear. They note that high turgor pressures may well be a result of the isolation process, in that OHCs often swell when they are placed *in vitro*. Under culture conditions, cells are also missing their controlling efferent connections and the 60 mV endolymphatic potential they would experience in a cochlea. Most importantly, cells would suffer various degrees of impairment from being pierced by an electrode. If OHCs are indeed pressure sensors, then piercing them is likely to be a particularly damaging treatment, and would explain why successful intracellular recordings from OHCs are so difficult (Dallos, 1985). Santos-Sacchi (1991) noted that impalement would release the internal turgor pressure and adversely affect sensitivity. Kakehata and Santos-Sacchi (1995) noted that great care was needed when establishing a patch clamp not to break the membrane with negative pressure on the pipette (which could lead to an immediate loss of turgor pressure); instead, these authors broke through the membrane with a high-voltage pulse applied to the electrode tip, a procedure that tended to prevent leakage of OHC turgor pressure from around the pipette–cell junction.

Kros (1996) says it is ‘naïve’ to expect to find the mechanisms of sharp tuning to be intact in isolated cells. “The microelectrodes used for *in vivo* recording damage the cells, obliterating evidence of the basolateral membrane currents that are so important in shaping the receptor potentials. On the other hand, isolating the cells to allow the more gentle whole-cell patch recordings is also likely to damage the cells and irreversibly change their properties.” (p. 375). Other related difficulties are also documented (pp. 360, 366, 368).

Given these difficulties, it is reasonable to conclude that the 21 dB disparity may be an experimental artefact and that OHC electromotility matches receptor potentials right down to threshold. Impairment of normal cell function by invasive recording techniques

would explain why antiphasic responses to sound, which rely on hyperpolarisation and high turgor pressure, have not been seen at low SPLs (Dallos, 1985, but see later discussion; Cody and Russell, 1987). Only studies on relatively intact cells (for example, those of Canlon et al., 1988) have provided evidence of two response polarities to acoustic stimulation, and even these may be suspect because of the isolation procedures employed. Nevertheless, as Section VII shows, there are a number of studies that indicate that OHCs show either a wide range of cell properties, dual response polarities to electrical stimuli, or both, circumstances that support the notion that one row of OHCs responds differently to sound pressure compared to the others.

VII. TWO CLASSES OF OHC

To complete the analogy to a SAW resonator, this work has sought evidence for the antiphasic behaviour of OHC2 compared to OHC1/3. The clearest evidence is taken be that of Canlon et al. (1988) where some OHCs lengthened, and some contracted, in response to oscillating acoustic pressure. In similar experiments where the tuning of the response was reported, Fig. 3 of Brundin et al. (1989) illustrates that the response in 1 of 7 cells was a lengthening, whereas the others shortened.

When isolated OHCs are placed in an electric field, some 80% elongate under positive potential gradients, while the remainder contract (Kachar et al., 1986). For a 5-Hz alternating field this resulted in a fraction of the cells changing length in antiphase to others (their Fig. 3), a result which strongly supports the hypothesis here. Under similar conditions but with an oscillating electric field of variable frequency, Zenner et al. (1988) noticed that the cuticular plate of 62% of motile OHCs tilted when the plate was closest to the ground electrode and 38% tilted when the plate was closest to the active electrode. As pointed out

earlier, two-thirds of OHCs display an angular cuticular plate associated with an ability of the plate to tilt by up to 14°. Tilting of the cuticular plate follows, cycle by cycle, the imposed frequency of an imposed oscillating field (to at least 500 Hz).

There are a number of other lines of evidence in the literature which point to either two distinct classes of outer hair cell, or which show such wide variation in properties and responsiveness that an explanation in terms of two overlapping ranges is possible.

A wide range in the resting membrane potentials of OHCs have been noted, ranging from 0 to –80 mV (Santos-Sacchi et al., 1994). Russell and Kössl (1992) noted that the majority of OHCs they observed had a resting membrane potential of –65 to –70 mV; however, a minority of their cells, and cells from others' data sets, fell in the range –52 to –60 mV. As discussed in Section VB1, Kakehata and Santos-Sacchi (1995) matched this variability in membrane potential with variation in turgor pressure and explained the range of resting membrane potentials of isolated OHCs (Santos-Sacchi et al., 1994) as resulting from a corresponding range of internal turgor pressures.

No direct measurement of differences between the rows of cells has been made in the living cochlea, although measurements made by Russell and Richardson (1987) on an organ culture of the mouse showed there was no obvious differences between the resting membrane potentials of the three rows. Significantly, perhaps, the organ culture OHCs did not display the level-dependent reversal in their d.c. responses to direct low-frequency stimulation of their stereocilia which is seen *in vivo*. Key differences here are likely to be the lack of a supplied pressure stimulus and no efferent connections.

Another organ culture experiment, that of Russell et al. (1989), is highly indicative of a dual responsiveness of OHCs. This work, which involved displacing sensory bundles with fine glass probes, identified two distinct classes of OHCs. One class they called 'non-adapting' and the receptor potential of these showed a positive response when the probe

moved the stereocilia in the excitatory direction, and a negative response when the probe moved in the opposite (inhibitory) direction (their Fig. 2). The receptor potentials did not adapt, even for the smallest stimuli. By way of contrast, in the other class, called ‘adapting’, they observed cells that only gave an excitatory response (seen in their Fig. 1A, although the percentage of such cells is not reported). A better name for each of the two classes may be ‘bipolar’ to recognise the dual response polarities, and ‘unipolar’ to label the unresponsiveness in one direction.

Clearly, if OHC2 were in one class, and OHC1/3 in the other, there would be a major difference in electrical polarisation, and the SAW resonator would be strongly energised.

This result is also of interest because there is a known association between the adaptive response and deflection of stereocilia, which are well known to adapt to stimulation and to respond predominantly to deflection in the excitatory direction only (e.g., Hudspeth and Corey, 1977; Hudspeth, 1989). However, the lack of adaptation in one class of hair cells invites the conjecture that the response is actually due to pressure exerted on the cell by tilting of the cuticular plate. This supposition is strengthened by the observation that the adaptive response decayed, but did not disappear, settling at a fixed (non-adaptive) plateau; this behaviour could be explained as the stimulating probe opening both stereocilia-responsive channels and putative pressure-sensing channels. Put simply, it is proposed that the adapting response was due to bending of stereocilia (in the excitatory direction only), whereas the non-adapting (‘bipolar’) response was due to pressure. In an actual cell, the responses are combined, because the bending of stereocilia is always accompanied by internal pressure variations (due to tilting of the cuticular plate). The existence of a bipolar (pressure-sensitive) response – in isolation from a stereociliar response – is therefore highly significant, for it ties in with the supposition, raised in Sections IVB and IVC above, that

OHC2 responds only to pressure (because a stereocilia response cannot assist in energising a SAW cavity, it can only serve to ‘split’ the cavity).

The notion that deflection of the stereocilia stimulates both bending transducers in the stereocilia and – via the pressure transferred to the cell by tilting of the cuticular plate – activates pressure transducers located in the cuticular plate or wall of the cell, solves a number of puzzles in cell micromechanics. Thus, Russell et al. (1992) noted that the stiffness of hair bundles on mouse OHCs dropped by 14% with an excitation that opened 50% of the ion channels, and puzzlingly, increased by a similar amount when the stimulus was an inhibitory one closing but 10% of the channels, a result in conflict with the simple gating-spring model of hair cell transduction. However, the anomaly could be reconciled if deflection activates both displacement and pressure transducers. A discussion of the location of the pressure transducers is set aside for a later paper, but the suggestion is that ATP-activated non-selective cation channels in the cuticular plate are sensitive to pressure.

Perhaps the most startling property of the cell culture is the discovery that “after prolonged intracellular recording, adapting OHCs may depolarise and transform into non-adapting OHCs” (Russell et al., 1989, p. 66). That is, the stereociliar response may be switched off by depolarisation, justifying the hypothesis that OHC2 cells are depolarised with respect to cells in the other rows.

VIII. ROLE OF VOLTAGE-SENSITIVE IONIC CURRENTS

The electromotility of OHCs depends on a voltage sensor in the cell wall. It is known that cell length changes are controlled by voltage, not cellular currents, because the motility does not exhibit a reversal potential (Evans et al., 1991). Nevertheless, because the operation of the SAW resonator calls on non-linear receptor potentials created between rows of cells

by differences in membrane potential, it is possible that voltage-sensitive ionic currents may also play a role. Stretch-sensitive channels in the basolateral membrane could also contribute to setting the operating point of the cell.

In this connection, the above discussion has pointed to turgor pressure as a key variable in adjusting the system to operate around the non-linear slopes of the pressure–membrane potential curve. Stretch-sensitive receptors have been observed in the basolateral membrane (Ding et al., 1991; Iwasa et al., 1991), and Kakehata and Santos-Sacchi (1995) in fact suggested that their purpose is to set the cell’s turgor pressure. Ding et al. (1991) applied pressure to isolated OHCs via a pipette and found pressure-activated channels (two types, of high- and low-conductance, about 150 pS and 40 pS) in the membrane wall, which they supposed was caused by membrane tension (stretch). It seems these receptors were too few in number and had too low a conductance to operate as acoustic pressure sensors.

Iwasa et al (1991) used patch-clamp techniques on isolated OHC from the guinea pig and on applying a pressure of -1000 Pa to their pipettes found that the lateral wall was “a mechano-receptor”. Suction caused an activation of depolarising potassium channels with a conductance of 130 pS. There seemed to be some 95 channels per OHC, comparable to the number of channels associated with stereocilia. When the cell was placed under voltage clamp, measured currents followed the imposed osmotic stress, again suggesting the action of a pressure-transducer. Cell length was measured, and it too followed the induced current. It is noteworthy that the current (and length extensions) varied with holding potential, and reversed at about -80 mV.

Other observations of pressure-induced whole-cell currents have been reported. The most direct and unambiguous result is that of Gale and Ashmore (1992). When a pressure pulse of 600 Pa was applied to an isolated guinea pig OHC via a patch-clamp pipette, the cell responded with an *outward* whole-cell current of some 40 pA (a near-threshold current

according to Russell et al., 1986) at a clamping potential of -20 mV. This sensitivity is much below that which would be adequate, although the cell's environment was not physiological (see below). However, of particular interest, the recorded response had two different components, an inward and an outward current, which varied with the holding potential of the cell. Thus, when the cell was hyperpolarised to a holding potential of -72 mV, the current reduced to zero and, with increased hyperpolarisation, reversed direction, showing an *inward* current of 20 pA.

Gale and Ashmore (1992) conclude that the two distinct whole-cell currents they detected may be activated by membrane stretch, a possibility already suggested by other workers.

Later work by these authors (Gale and Ashmore, 1994) looked at stretch sensitivity in more detail by holding a single cell with a patch pipette at its base and directly stretching the cell by applying a vibrating probe attached near its apex. The cell behaved piezoelectrically in that vibration of the probe at 5 kHz with an amplitude of about 10 nm generated a 10 pA current in the patch electrode at the same frequency. In this case, the pressure sensitivity was much improved, with their calculations indicating that a 1 nm stretch would give a potential shift of 1.6 μ V, not far below observed intracellular threshold voltages of 15–100 μ V. The authors conclude that, in accordance with their previous simple capacitance model, the currents they observed derived from intramembrane charge movement (a gating charge) rather than ion movement, although they sometimes did observe an additional current, presumed ionic, with a reversal potential close to 0 mV which was not examined in detail.

It therefore seems possible that, as well as setting turgor pressure, voltage-sensitive ionic currents could contribute to the pressure sensitivity of OHCs, serving to magnify the initial piezoelectric response.

A. Na currents: the cochlea's transistor?

Of most relevance is the behaviour of the enigmatic voltage-sensitive sodium current, I_{Na} . This rapid, transient inward current is inactivated at voltages more positive than about -60 mV and, while present in the outer hair cell, no obvious role for it in cell function has so far been found (Kros, 1996; Witt et al., 1994). I_{Na} is common in neonatal OHCs, and declines in strength and prevalence with development, but it still persists in an appreciable fraction of mature OHCs, so that half of the cells in 18-day-old rats possessed the current at a level of about 50 pA (Oliver et al., 1997), and in about 10% of normal laboratory guinea pigs (Witt et al., 1994). Kros et al. (1993), in a culture of cochlear cells from neonatal mice, found the current in about half the cells they examined. Its unusual properties, particularly its very high voltage sensitivity near the resting potential of the cell, make it a prime candidate as an active amplifier of transducer current – indeed, its transconductance places it in the same category of amplifying devices as the transistor. Hudspeth and Corey (1977) note that an endogenous action potential mechanism (based on a regenerative sodium current) could serve as an amplifier of receptor potentials. A mechanism like this (except involving potassium ions and leading to internal cell oscillation) occurs in the modified acousto-lateralis cells of the skate electroreceptor, which operates in a region with a negative transconductance of -5 nA/mV (Clusin and Bennett, 1979). Hudspeth and Corey (1977) also note that cochlear hair cells, lacking kinocilia, may operate in a different manner from most other hair cells.

If this inward current were only present in OHC rows 1/3, while OHC2 possessed just the normal outward current, which is generated by stereocilia deflection or opening of pressure-sensitive channels, then a polarity reversal between rows would occur when the cochlea received a stimulus. The transconductance characteristic would greatly enhance the sensitivity of the SAW resonator – which, until this point, was deemed to rely for activation

on non-linearities in gating current. I_{Na} could amplify both stimulus-induced ionic currents and the piezoelectrically generated displacement current. The relative sizes of these currents are not known, but, on design grounds, pressure is more likely to give larger piezoelectric responses than other currents.

Witt et al. (1994) studied the transient sodium current in the OHCs of mature guinea pigs, where it was found in 11 of 106 cells. They suggested that its infrequent incidence may be due to its expression in only a subpopulation of OHCs, and put forward the possibility that this may mean only in certain rows. An alternative explanation they gave was that the current is expressed in a large fraction of OHCs but is normally non-conducting; this idea gained support from their observation that, in one experiment, 5 out of 5 OHCs from the same cochlea expressed a sodium current. Both conjectures can in fact be reduced to one if it is specified that the membrane potential should vary between the rows.

That is, since I_{Na} has a very negative activation voltage and is only activated at potentials between about -100 and -70 mV, it is required that row OHC1/3 have a membrane potential more negative than -70 mV (it has already been stipulated that OHC2 have a low membrane potential of about -50 mV to make it into a pressure-only transducer). In OHCs from a mouse cell culture, Jagger et al. (1999) found that the current was completely inactivated at holding potentials more positive than -70 mV. It seems no coincidence that, of all mammalian cell types, only OHCs have sufficiently negative resting membrane potentials to allow I_{Na} to activate. Measurements of normal resting membrane potentials suggest that typical values are -50 to -70 mV, with the highest values in the range -70 to -85 mV (Dallos, 1985; Cody and Russell, 1987). The actual *in vivo* values are difficult to judge because, as pointed out earlier, the usual patch-clamping procedures and electrode penetrations are likely to impair the turgor pressure and polarization of these cells (Kros, 1996, p. 369). Evans et al. (1991) point out that it is probable that “isolated cells are

often depolarised compared to their *in vivo* counterparts” (p. 298) and that “patch clamping depressurises OHCs, which are naturally turgid ... and that reducing the internal pressure can diminish or even abolish the motile response” (p. 298).

As noted, a most remarkable property of the sodium current is that, in the region surrounding its half-activation point, it has an extremely high transconductance – that is, it has a steep, and negative, voltage–current characteristic, not unlike a transistor. Data from Witt et al., (1994) show that a change in membrane potential of just 10 mV changes the current by –2 nA: that is, a transconductance gain (g_m) of –200 pA/mV. Of course, the sodium current is transient, and operates concurrently with the stimulus-induced current – the normal outward potassium current (e.g., Oliver et al., 1997). Thus, a small pressure- or displacement-induced current would lead to a small depolarisation, and this change in membrane potential would mean that the elicited sodium current would be correspondingly larger. That is, a small voltage has led, transistor-like, to a large change in current.

Like the active regenerative properties of the related sodium current responsible for action potentials in nerve cells, there is a risk of voltage runaway. This potential for instability led Evans and Fuchs (1987) to conclude, after studying sodium currents in the hair cells of alligator cochleas, that “even the small Na conductance remaining at the resting potential could contribute to excitability in these cells.” Similarly, Witt et al. (1994) were puzzled by the purpose of the sodium current and were concerned that “[t]he presence of a regenerative voltage-dependent sodium current in the OHC could initiate an inappropriate electromotile event that would disrupt cochlear mechanics.” Perhaps that is why they deemed it ‘physiologically silent’, even though the resting membrane potentials of OHCs are sufficient to activate it. However, seeing the current as a means of amplifying the receptor current, and feeding it into the SAW resonator, provides an elegant mechanism for furnishing the ear with its exquisite sensitivity.

Figure 4A shows the voltage–current characteristic of the sodium current obtained by Witt et al. (1994) from voltage-clamp experiments on the OHCs of mature guinea pigs at room temperature. The major feature is the steep slope of the curve between -60 and -70 mV, the region of resting membrane potential. Over this range, the size of the current changes by some 2 nA, giving a gradient of -200 pA/mV. **Figure 4B** (from Oliver et al., 1997) shows the fast response of the current, a feature necessary if it is to work satisfactorily at audio frequencies. The current can be fully activated within 1 ms. Note that at the same time as the inward sodium current is being activated, an outward potassium current is also operating. The studies of Evans and Fuchs (1987) indicate that it is a Ca-activated K current, such as would be associated with normal transducer action. Their work also clearly shows (their Fig. 1) that, as expected for two independent currents, the (negative) amplitude of the sodium current increases when the (positive) potassium current is blocked and the two no longer counteract each other. This independence will ensure that the rows with I_{Na} operating (OHC1/3) will generate a differential voltage to the row with I_{Na} inactivated (OHC2).

Current-clamp experiments of Oliver et al. (1997) show the amplification process in a complementary way. **Figure 4C** shows that when the cell was held at a point just off the steep portion of the characteristic (-90 mV), a small (10 pA) step in current lead to a very large change in membrane potential – depolarising the cell down to nearly 0 mV. Note that at threshold, receptor currents have been estimated to be in the region of 60 pA (Russell et al., 1986).

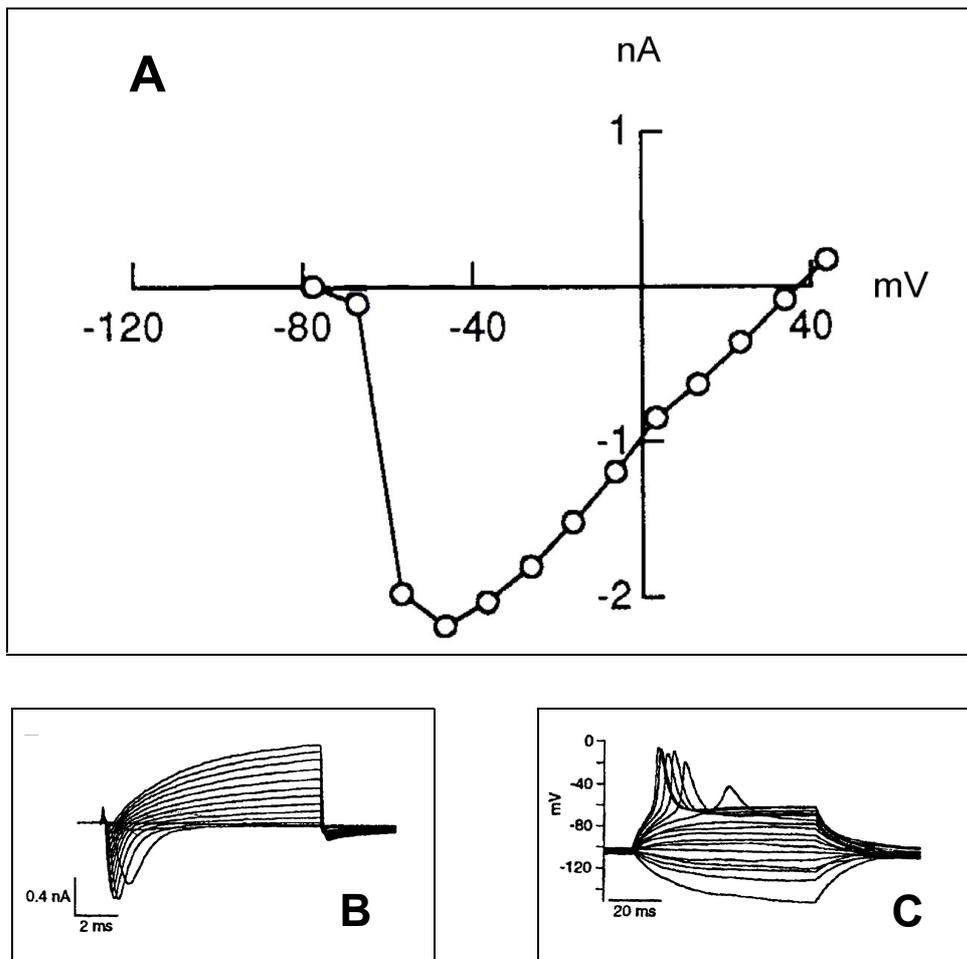


FIG. 4. **A.** Electrical characteristic of the cochlea's transistor – a voltage-activated inward sodium current (observed by Witt et al., 1994) in a voltage-clamped outer hair cell of a mature guinea pig. Note the steep negative slope between -60 and -70 mV, corresponding to a transconductance of -200 pA/mV. Under a resting membrane potential of -65 mV, presumed to occur in OHC1 and 3, this sodium current will provide regenerative amplification of any voltage change in the cell, such as produced by transducer action. The current will be switched off by depolarisation of the cell to -50 mV, and will provide differential action between OHC1/3 and OHC2, since the latter is presumed to be relatively depolarised. **B.** Time traces of transient sodium currents in a neonatal rat OHC during voltage clamp (from Oliver et al., 1997). The initial spike is the inward sodium current; upward is potassium current. If the sodium current is blocked, only the outward potassium current remains. **C.** Voltage responses of transient sodium current under current clamp (from the same source). Upward spikes show the sodium current's regenerative action when the holding potential includes its activation range.

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IX. SELF-LIMITING BEHAVIOUR OF THE COCHLEAR AMPLIFIER

A description of the cochlear amplifier has been given in which the amplification is provided by positive feedback within a surface acoustic wave resonator. The gain of this device depends on the antiphase response of OHC2 compared to that of OHC1/3.

Takehata and Santos-Sacchi (1995) have shown that the displacement current (and electromechanical gain) of an OHC varies with membrane potential, which as suggested by these authors gives a way of controlling the gain of the cochlear amplifier. All that is needed to drop the gain of the amplifier is for the non-linearities to disappear, or for them to change from being of opposite sign to being of the same sign. Adjusting the turgor pressure, or shifting the resting membrane potential, would accomplish this.

The significance of the rich efferent connections of OHCs should now be apparent in that it allows the membrane potential (or turgor pressure) of each row to be precisely set. Efferent stimulation is known to cause cell hyperpolarization (via a calcium-sensitive potassium current) and to cut the gain of the cochlear amplifier (Guinan, 1996), and this would be particularly effective if all the OHC rows were caused to hyperpolarize equally.

One of the distinctive properties of the cochlear amplifier is that it ceases to function within minutes when the cochlear nerve is cut (Rhode, 1973). This is unusual in that OHC motility in response to imposed stimuli is known to continue for hours after isolation from the cochlea. What, therefore, has caused the amplifier to shut down in this case? One possibility is that the blood supply to the cochlea has been severed along with the nerve; however the rapid loss of amplification should be contrasted with the maintained presence of non-linearity for an hour, indicating a continuation of some OHC function. The answer suggested by the SAW model is that severing the nerve causes control of the membrane

potential to be lost, so that the needed antiphase behaviour of the three rows is no longer possible. The cells are still alive, but no amplification results because their resting potentials and turgor pressures have become equal.

In the intact cochlea, if sound pressure levels are increased, the activity of outer hair cells increases, and more ion channels are opened, leading to more cell depolarisation. This will happen to all three rows of cells, and at some point the polarisation of all cells will fall to virtually the same level, a point at which the cochlear amplifier will cease to function. At this level, somewhere between 60 and 80 dB SPL, there is no need for a cochlear amplifier because at this level the pressure difference across the partition has become sufficient to cause the partition to move bodily up and down, and the traveling wave mechanism begins to function.

X. CONCLUSION

Evidence from a diverse literature has been advanced in support of the proposal that the cochlea functions as a surface acoustic wave resonator. Briefly, it would require a sensitivity to pressure and a reversed response in OHC2 compared to that in OHC1/3. The reversal need not be an absolute polarity reversal, but could originate from a non-linearity that caused the response in one row to have a d.c. offset to that in the others. The non-linear capacitance of the cell would fulfil that requirement. However, an absolute polarity reversal would be the most efficient way of exciting the SAW cavity, and the fast inward sodium current, with its amplifying properties and an activation range suited to the resting membrane potentials of OHC1 and 3, is an ideal candidate for this role.

Dallos (1985) reported the responses, *in vivo*, of guinea pig OHCs to low-frequency tone bursts, and found that “low sound levels evoke hyperpolarizing d.c. responses, whereas

high levels generate the more prevalent depolarising potentials”. Dallos comes to the conclusion that “it appears that two rectifying mechanisms operate in the cochlear mechanics-OHC system in opposite directions, with one dominating at low levels and low frequencies, and the other dominating under all other conditions” (p. 1597). His conclusion is based on how the receptor potentials of OHCs grow with sound pressure level, in particular how it is that the d.c. responses sometimes go negative below 60 dB SPL.

By themselves, of course, negative d.c. potentials do not provide simultaneous antiphasic behaviour of OHC2 compared to OHC1/3. If all cells behave the same, it would not matter how complicated the response is. For the one cell recording in Dallos (1985) that shows the two mechanisms operating (his Fig. 9), the important a.c. response is not an inverted version of that at higher levels, just a clipped version (so there is no positive response). Perhaps the sodium current that might have given an antiphasic response at lower levels was disabled by electrode penetration, or maybe this cell had a membrane potential that placed it the OHC2 category (and so lacked I_{Na}).

In summary, evidence from the literature indicates that there is likely to be sufficient pressure gain from OHCs when configured as a SAW resonator to account for observed partition movements at 0 dB SPL. The evidence appears sufficient to overcome doubts that there might be a 21 dB disparity between threshold receptor potential and the corresponding threshold electromotility. The hypothesis that the adequate stimulus for movement of the partition at low sound pressure levels is intracochlear pressure is consistent with known OHC properties. OHCs are specialised piezo-electro-mechanical transducers that convert pressure into voltage (one polarity for OHC2, the reverse for OHC1/3) which drives a fast mechanical response. The special arrangement of three OHCs as a SAW resonator defines the cochlear amplifier, a device that has become necessary to explain cochlear behaviour, but the construction and workings of which have not so far been placed within a detailed

physical model. Computer implementations of this model are now required to show the combined response of the organ of Corti system to the OHCs' sensitivity to intracochlear fluid pressure, simulations that will include more detail than has been possible to show in Fig. 7 of the companion paper. However, due to the extreme sensitivity of the ear, invasive studies are unlikely to show the unperturbed operation of this micromechanical masterpiece.

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FOOTNOTES AND REFERENCES

1. In birds and lizards, arrangements of hair cells with opposite morphological polarity are seen. That is, the polarity is defined as the direction in which the stereocilia receives an excitatory stimulus (towards the kinocilium), and on the sensory epithelium this polarity is found mixed (cells face in opposite directions). Of particular relevance, in the gecko three cells can be seen joined with a triangular TM formation (Morales and Garcia-Martinez, 1990, Fig. 6).
2. Despite some impairment from loss of intracochlear pressure due to an observing hole in the cochlear wall. See companion paper.

Adachi, M. and Iwasa, K. H. (1999) “Electrically Driven Motor in the Outer Hair Cell: Effect of a Mechanical Constraint,” *Proc. Natl. Acad. Sci. USA* **96**, 7244–7249.

Ashmore, J. F. (1987). “A Fast Motile Response in Guinea-Pig Outer Hair Cells: the Cellular Basis of the Cochlear Amplifier,” *J. Physiol.* **388**, 323–347.

Ashmore, J. F. (1988). “What Is the Stimulus for Outer Hair Cell Motility?” in *Basic Issues in Hearing*, edited by H. Duifhuis, J. W. Horst, and H. P. Wit (Academic Press, London), pp. 42–48.

Bell, A. (2001). “Helmholtz’s Piano Strings: Reverberation of Ripples on the Tectorial Membrane,” (*companion paper*).

Bell, D. T. and Li, C. M. (1976). “Surface-Acoustic-Wave Resonators,” *Proc. IEEE* **64**, 711–721.

Brundin, L., Flock, Å., and Canlon, B. (1989). “Sound-Induced Motility of Isolated Cochlear Outer Hair Cells is Frequency-Specific,” *Nature* **342**, 814–816.

Brundin, L. and Russell, I. (1993). “Sound-Induced Movements and Frequency Tuning in Outer Hair Cells Isolated from the Guinea Pig Cochlea,” in *Biophysics of Hair Cell Sensory Systems* edited by H. Duifhuis, J. W. Horst, P. van Dijk, and S. M. van Netten (World Scientific, Singapore), pp. 182–191.

Brundin, L. and Russell, I. (1994). “Tuned Phasic and Tonic Motile Responses of Isolated Outer Hair Cells to Direct Mechanical Stimulation of the Cell Body,” *Hear. Res.* **73**, 35–45.

- Canlon, B., Brundin, L., and Flock, Å. (1988). "Acoustic Stimulation Causes Tonotopic Alterations in the Length of Isolated Outer Hair Cells from Guinea Pig Hearing Organ," *Proc. Natl. Acad. Sci. USA* **85**, 7033–7035.
- Canlon, B. and Brundin, L. (1991). "Mechanically Induced Length Changes of Isolated Outer Hair Cells Are Metabolically Dependent," *Hear. Res.* **53**, 7–16.
- Clusin, W. T. and Bennett, M. V. L. (1979). "The oscillatory responses of skate electroreceptors to small voltage stimuli," *J. Gen. Physiol.* **73**, 685–702.
- Dallos, P. (1985). "Response Characteristics of Mammalian Cochlear Hair Cells," *J. Neurosci.* **5**, 1591–1608.
- Davis, H. (1983). "An Active Process in Cochlear Mechanics," *Hear. Res.* **9**, 79–90.
- Davis, H., Derbyshire, A. J., Lurie, M. H., and Saul, L. J. "The Electric Response of the Cochlea," *Am. J. Physiol.* **107**, 311–332.
- De Boer, E. (1996). "Mechanics of the Cochlea: Modeling Efforts," in *The Cochlea*, edited by P. Dallos, A. N. Popper, and R. R. Fay (Springer, New York), pp. 292–295.
- Ding, J. P., Salvi, R. J., and Sachs, F. (1991). "Stretch-Activated Ion Channels in Guinea Pig Outer Hair Cells," *Hear. Res.* **56**, 19–28.
- Evans, B. N., and Dallos, P. (1993). "Stereocilia Displacement Induced Somatic Motility of Cochlear Outer Hair Cells," *Proc. Natl. Acad. Sci. USA*, **90**, 8347–8351.
- Flock, Å. (1988). "Do Sensory Cells in the Ear Have a Motile Function?" *Prog. Brain Res.* **74**, 297–304.
- Evans, B. N., Hallworth, R., and Dallos, P. (1991). "Outer Hair Cell Electromotility: the Sensitivity and Vulnerability of the DC Component," *Hear. Res.* **52**, 288–304.
- Evans, M. G. and Fuchs, P. A. (1987). "Tetrodotoxin-Sensitive, Voltage-Dependent Sodium Currents in Hair Cells from the Alligator Cochlea," *Biophys. J.* **52**, 649–652.
- Gale, J. E. and Ashmore, J. F. (1992). "Pressure-Activated Whole-Cell Currents in Isolated Outer Hair Cells from the Guinea Pig," *J. Physiol.* **452**, 51P.
- Gale, J. E. and Ashmore, J. F. (1994). "Charge Displacement Induced by Rapid Stretch in the Basolateral Membrane of the Guinea-Pig Outer Hair Cell," *Proc. R. Soc. Lond. B*, **255**, 243–249.
- Gitter, A. H., Rudert, M., and Zenner, H.-P. (1993). "Forces Involved in Length Changes of Cochlear Outer Hair Cells," *Pflügers Arch.* **424**, 9–14.
- Gold, T. (1987). "The Theory of Hearing," in *Highlights in Science*, edited by H. Messel (Pergamon, Sydney).

- Guinan, J. J. (1996). "Physiology of Olivocochlear Efferents," in *The Cochlea*, edited by P. Dallos, A. N. Popper, and R. R. Fay (Springer, New York), pp. 435–502.
- Holley, M. C. and Ashmore, J. F. (1988). "On the Mechanism of a High-Frequency Force Generator in Outer Hair Cells Isolated from the Guinea Pig Cochlea," *Proc. R. Soc. Lond. B* **232**, 413–429.
- Horowitz, P. and Hill, W. (1980). *The Art of Electronics* (Cambridge University Press, Cambridge), pp. 64–67.
- Huang, G. and Santos-Sacchi, J. (1993). "Mapping the Distribution of the Outer Hair Cell Motility Voltage Sensor by Electrical Amputation," *Biophys. J.* **65**, 2228–2236.
- Hudspeth, A. J. (1989). "How the Ear's Works Work," *Nature* **341**, 397–404.
- Hudspeth, A. J. and Corey, D. P. (1977). "Sensitivity, Polarity, and Conductance Change in the Response of Vertebrate Hair Cells to Controlled Mechanical Stimuli," *Proc. Natl. Acad. Sci. USA* **74**, 2407–2411.
- Iwasa, K. H., Li, M., Jia, M., and Kachar, B. (1991). "Stretch Sensitivity of the Lateral Wall of the Auditory Outer Hair Cell from the Guinea Pig," *Neurosci. Lett.* **133**, 171–174.
- Jagger, D. J., Holley, M. C., and Ashmore, J. F. (1999). "Ionic Currents Expressed in a Cell Line Derived from the Organ of Corti of the Immortomouse," *Pflügers Arch.* **438**, 8–14.
- Kachar, B., Brownell, W. E., Altschuler, R., and Fex, J. (1986). "Electrokinetic Shape Changes of Cochlear Outer Hair Cells," *Nature* **322**, 365–371.
- Takehata, S. and Santos-Sacchi, J. (1995). "Membrane Tension Directly Shifts Voltage Dependence of Outer Hair Cell Motility and Associated Gating Charge," *Biophys. J.* **68**, 2190–2197.
- Keynes, R. D. and Rojas, E. "Kinetics and Steady-State Properties of the Charged System Controlling Sodium Conductance in the Squid Giant Axon," *J. Physiol.* **239**, 393–434.
- Kolston, P. J. (1989). "Realistic Mechanical Tuning in a Micromechanical Cochlear Model," *J. Acoust. Soc. Am.* **86**, 133–140.
- Kros, C. J. (1996). "Physiology of Mammalian Cochlear Hair Cells," in *The Cochlea*, edited by P. Dallos, A. N. Popper, and R. R. Fay (Springer, New York), pp. 318–385.
- Kros, C. J., Rüsch, A., Richardson, G. P., and Russell, I. J. (1993). "Sodium and Calcium Currents in Cultured Cochlear Hair Cells of Neonatal Mice," *J. Physiol.* **473**, 231P.
- Lin, T. and Guinan, J. J. (2000). "Auditory-Nerve-Fiber responses to High-Level Clicks: Interference Patterns Indicate That Excitation Is Due to the Combination of Multiple Drives," *J. Acoust. Soc. Am.* **107**, 2615–2630.

- Maines, J. D. and Paige, E. G. S. (1976). "Surface-Acoustic-Wave Devices for Signal Processing Applications," Proc. IEEE **64**, 639–652.
- Morales, J. and Garcia-Martinez, V. (1990). "Differentiation of Hair Cells in the Reptile Basilar Papilla," Adv. Otorhinolaryngol. **45**, 111–118.
- Mountain, D. C. and Hubbard, A. E. (1994). "A Piezoelectric Model of Outer Hair Cell Function," J. Acoust. Soc. Am. **95**, 350–354.
- Mountain, D. C. and Cody, A. R. (1999). "Multiple Modes of Inner Hair Cell Stimulation," Hear. Res. **132**, 1–14.
- Nilsen, K. E. and Russell, I. J. (1999). "Timing of Cochlear Feedback: Spatial and Temporal Representation of a Tone Across the Basilar Membrane," Nature Neurosci. **2**, 642–648.
- Nuttall, A. L., Guo, M., and Ren, T. (1999). "The Radial Pattern of Basilar Membrane Motion Evoked by Electric Stimulation of the Cochlea," Hear. Res. **131**, 39–46.
- Oliver, D., Plinkert, P., Zenner, H. P., and Ruppertsberg, J. P. (1997). "Sodium current expression during postnatal development of rat outer hair cells," Pflügers Arch. **434**, 772–778.
- Pickles, J. O. (1992). "Scanning Electron Microscopy of the Echidna: Morphology of a Primitive Mammalian Cochlea," Adv. Biosci. **83**, 101–107.
- Pohlman, A. G. (1933). "A Reconsideration of the Mechanics of the Auditory Apparatus," J. Laryngol. Otol. **48**, 156–195.
- Ratnanather, J. T., Brownell, W. E., and Popel, A. S. (1993). "Mechanical Properties of the Outer Hair Cell," in *Biophysics of Hair Cell Sensory Systems*, edited by H. Duifhuis, J. W. Horst, P. van Dijk, and S. M. van Netten (World Scientific, Singapore), pp. 199–206.
- Rhode, W. S. (1973). "An investigation of post-mortem cochlear mechanics using the Mossbauer effect," in *Basic Mechanisms in Hearing*, edited by A. R. Møller (Academic Press, New York), pp. 49–67.
- Rhode, W. S. and Recio, A. (2000). "Study of Mechanical Motions in the Basal Region of the Chinchilla Cochlea," J. Acoust. Soc. Am. **107**, 3317–3332.
- Russell, I. J. and Kössl, M. (1992). "Voltage Responses to Tones of Outer Hair Cells in the Basal Turn of the Guinea-Pig Cochlea: Significance for Electromotility and Desensitization," Proc. R. Soc. Lond. B **247**, 97–105.
- Russell, I. J., Kössl, M., and Richardson, G. P. (1992). "Nonlinear Mechanical Responses of Mouse Cochlear Hair Bundles," Proc. R. Soc. Lond. B **250**, 217–227.
- Russell, I. J., Richardson, G. P., and Kössl, M. (1989). "The Response of Cochlear Hair Cells to Tonic Displacements of the Sensory Hair Bundle," Hear. Res. **43**, 55–70.

- Santos-Sacchi, J. (1989). "Asymmetry in Voltage-Dependent Movements of Isolated Outer Hair Cells from the Organ of Corti," *J. Neurosci.* **9**, 2954–2962.
- Santos-Sacchi, J. (1991). "Reversible Inhibition of Voltage-Dependent Outer Hair Cell Motility and Capacitance," *J. Neurosci.* **11**, 3096–3110.
- Santos-Sacchi, J., Huang, G.-J., and Kakehata, S. (1994). "Variability of Voltage Dependency of the Outer Hair Cell (OHC) Motility Voltage Sensor," (Abstract) *J. Acoust. Soc. Am.* **95**, 2841.
- Smith, W. R. (1981). "Circuit-Model Analysis and Design of Interdigital Transducers for Surface Acoustic Wave Devices," in *Physical Acoustics*, edited by W. P. Mason and R. N. Thurston (Academic Press, New York), pp. 99–189.
- Stopp, P. E. (1982). "The Effect of Moderate-Intensity Noise on Cochlear Potentials and Structure," in *New Perspectives on Noise-Induced Hearing Loss*, edited by R. P. Hamernik, D. Henderson, and R. Salvi (Raven Press, New York), pp. 331–343.
- Tolomeo, J. A. and Steele, C. R. (1995). "Orthotropic Piezoelectric Properties of the Cochlear Outer Hair Cell Wall," *J. Acoust. Soc. Am.* **97**, 3006–3011.
- Tolomeo, J. A., Steele, C. R., and Holley, M. C. (1996). "Mechanical Properties of the Lateral Cortex of Mammalian Auditory Hair Cells," *Biophys. J.* **71**, 421–429.
- Witt, C. M., Hu, H.-Y., Brownell, W. E., and Bertrand, D. (1994). "Physiologically Silent Sodium Channels in Mammalian Outer Hair Cells," *J. Neurophysiol.* **72**, 1037–1040.
- Zenner, H.-P., Zimmermann, R., and Gitter, A. H. (1988). "Active Movements of the Cuticular Plate Induce Sensory Hair Motion in Mammalian Outer Hair Cells," *Hear. Res.* **34**, 233–240.
- Zenner, H. P., Gitter, A. H., Rudert, M., and Ernst, A. (1992). "Stiffness, Compliance, Elasticity and Force Generation of Outer Hair Cells," *Acta Otolaryngol.* **112**, 248–253.
- Zheng, J., Shen, W., He, D. Z. Z., Long, K. B., Madison, L. D., and Dallos, P. (2000). "Prestin is the Motor Protein of Cochlear Outer Hair Cells," *Nature* **405**, 149–155.