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An application of atomic force microscopy to study the mechanical properties of the lateral membrane of cochlear outer hair cells

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Atomic force microscopy when used in its imaging mode is a technique which allows surface structures of biological membranes to be resolved, in principle, with molecular resolution (Fotiadis et al. 2002). By using the reflection from the scanning cantilever, sub nanometre changes in the surface height can be detected during scanning. This signal forms the feedback signal intrinsic to the scanning microscope. In addition to XY imaging modes, the technique has been used to study hair cell lateral membrane elasticity (Zelenskaya et al. 2005). The time dependence of surface fluctuations can also be measured and it has been reported that yeast cell membranes undergo oscillations of 0.8 -1.6 kHz (Pelling et al. 2004).

In order to investigate whether hair cells also show membrane properties found in yeast,and important to the understanding of some models of cochlear mechanics, we have investigated the surface membrane of guinea pig cochlear outer hair cells microdissected from the organ of Corti. Isolated outer hair cells from the apical (low frequency) turns are cylindrical and 50-80 μm in length. To hold the cells during scanning and limit rolling, tapered 100 μm long slots 10 μm deep etched onto quartz slides were used to prevent cell movement during the cantilever contact. Force-distance curves could thus be obtained from the lateral membrane. Using the quadrant detector of the microscope (Nomad, Quesant Instrument Corp., CA, USA), both the z-motion (flexion) and the x-y (tilt) motion of the cantilever were measured. Standard V shaped cantilevers (stiffness 0.01 N/m) were used attached to a purpose-built holder which allowed scanning under solution. Spectral peaks distributed between 1-7 kHz were recorded in the noise of the reflected light in both flexion and tilting motion of the cantilever. Tilting (x-y) motion noise peaks were prominent in measurements made when the cantilever was either immersed in solution or touching the hair cell membrane. The power corresponding to tilting motions was reduced by over 20 times when the cantilever was withdrawn into air and z-deflections mainly occurred. In measurements made on cantilevers placed in solutions with viscosity between 1-800 cP (0.001-0.8 Pa.s), a redistributon of noise power was also found between tilting and flexion modes. The results indicate that understanding the complex dynamics of such cantilevers (Sader, 2003) may be critical in biological applications to prevent attribution of spurious signals.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

DC2

Viscous damping of acoustic resonance with a restricted zone of wall compliance

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The basis of cochlear frequency analysis lies in the compliance of the basilar membrane, separating two fluid chambers that are subjected to acoustic pressure differences. Modelling of the viscous damping associated with resonances in this structure (Gold, 1948) has led to the widely accepted conclusion that passive resonance could not be sharp enough to be consistent with psychophysical and physiological measurements. Active mechanisms (capable of generating force), associated with outer hair cells, may therefore be critical in the establishment of sharp resonance. Though this account may be correct, it is not the only way in which active mechanisms could be relevant to acoustic function of the cochlea. For example, they could modulate rather than cancel the viscous damping, they might limit the resonant after-effects of brief transients, or they might somehow act to restrict and concentrate the energy absorption of the Organ of Corti in zones where it will have greatest effect.

Gold's model was based on calculations treating a zone of the basilar membrane as analogous to a piano string immersed in water. An alternative is to consider a portion of the basilar membrane (responsive to a particular frequency), as a small compliant zone of the wall separating two chambers with otherwise relatively rigid walls. This begs the question of in what sense, or perhaps by what active mechanism, the rest of the basilar membrane could be considered rigid for the purposes of analysing the dynamics of a single zone, but it provides an alternative model for analysing the ultimate constraint that viscosity places on the sharpness of resonance in a cochlear structure.

The model adopted here considers a circular compliant zone of membrane of radius R and zero mass, with a relatively large chamber on each side subjected to distant pressure variation. Resonance involves alternating transfer between potential energy associated with extension of the compliant membrane and kinetic energy (KE) of fluid movement towards and away from the membrane. The KE and viscous dissipation are both mainly in fluid within a radius of the membrane, where velocities are highest. Measurements with ×10 scale models and calculations with simplified flow patterns suggest that the time constant (T) for energy loss with a compliant zone of radius R = 0.1mm can exceed 2ms (yielding for example a 3 dB resonance bandwidth equal to 4% of a center frequency f = 2 kHz, $Q_{3dB} = 2\pi fT = 25$). The value of T due to viscosity scales with R^2/K (where K = kinematic viscosity, ca. 0.7×10^{-6} m^2/s at 37°C). With plausible dimensions this would appear to be able to account passively for frequency selectivity substantially greater than is inferred at any frequency from physiological and psychophysical data (Moore, 2003). A key issue would be how energy could be directed to optimal vibration modes for maximum sensitivity and selectivity.

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