Thermal-Noise-Limited Transduction Observed in Mechanosensory Receptors of the Inner Ear

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Simultaneous recordings of the spontaneous mechanical input fluctuations and the electrical output fluctuations in hair cells that serve as biological microphones show that the Brownian motion of the mechanoceptive organelle, the hair bundle, is often strongly correlated with the fluctuations of the transmembrane voltage. This correlation stems from transduction of the spontaneous input noise and shows that these cells can almost reach the sensitivity limit imposed by the thermal noise at their input.

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Biological receptors for light[1] and for some chemicals[2,3] are known to perform close to the ultimate sensitivity given by the physical constraints inherent in optical and molecular stimuli. This means that photoreceptor cells can "see" single photons and olfactory receptor cells can "smell" single molecules. But the nature of the limits to the ultimate sensitivity of mechanosensory receptors, particularly the auditory organs, has remained undetermined and controversial.[4] In this Letter we present results of simultaneous measurements of the spontaneous thermal input noise and of the electrical output noise in those cells that perform the crucial task of converting a mechanical stimulus into an electrical signal. Our results show that most of the output noise consists of transduced input noise and that the cells consequently have the capability to "hear" even the thermal motion at their input.

Thermal motion is to be expected to physically limit mechanoreception because the thermal energy (k_BT) is between 10^8 and 10^{12} times larger than the single-quantum energy ($\hbar\omega$). In contrast the reverse is true for the stimuli of transduction for photonic and olfactory receptors where the single-quantum energies by far exceed the thermal energy so that shot-noise-limited performance is possible. The situation in hearing is also complicated by the fact that auditory sensory systems are not merely passive detectors; some are known to emit sound energy spontaneously[5] and some mechanosensory cells are capable of generating force and displacement in response to electrical stimulation.[6–8] The existence of a source of mechanical energy which compensates for the inevitable losses due to viscous damping and thus allows high-Q values of a mechanical resonance had been suggested forty years ago on theoretical grounds to account for the observed acuity of frequency discrimination.[9]

The first step in the process of sensory detection of a mechanical stimulus is the mechanical conversion of the primary input such as sound, or linear or angular acceleration into a deflection of the hair bundle, a specialized, microscopic organelle on the surface of hair cells. The next step is the transduction of this still mechanical signal into a modulation of the intracellular voltage, which occurs because the transmembrane resistance is sensitive to deflection of the hair bundle.[10] At this step the mechanical input power ($x^2/2\kappa$) is also amplified by 20 to 40 dB so that the Johnson noise becomes insignificant compared to the electrical output power ($v^2/2R$); here x is the bundle deflection and v is the ensuing intracellular voltage change and κ and R are the mechanical impedance of the hair bundle and the electrical impedance of the cell membrane, respectively. The change in intracellular voltage in turn modulates the activity of a synapse which connects the hair cell with higher levels of the nervous system.[11]

The hair bundle itself consists of an organ-pipe-like array of about sixty stereocilia (each 1–50 μm long, 0.2–1 μm in diam) protruding from one end face of the cylindrically shaped hair cell.[12] The tips of these protrusions (hairs) are connected by filamentous links[13] which are strained upon deflections of the whole bundle along its "sensitive" axis thus presumably leading to the opening of ion channels across the cell membrane thereby reducing the membrane resistance.[14]

To characterize the mechanoelectrical response and the sources of noise we recorded simultaneously the spontaneous thermal fluctuations of the hair-bundle deflection [input noise $x(t)$] and the fluctuation of the intracellular voltage [output noise $v(t)$] and correlated both signals. In the case of an ideal transducer-amplifier output noise arises only from transduction and amplification of input noise and both signals must therefore be completely correlated. For any real transducer and amplifier this correlation will be reduced by the noise added in the process of transduction and amplification.

To measure the hair-bundle motion we developed and constructed a laser differential microinterferometer[15–18] which combines confocal optics with the principle of differential interference contrast (DIC).[19,20] The movements of a microscopic object in the plane of focus are detected by determining the differential phase shift between two mutually coherent laser beams of orthogonal polarization focused into two closely spaced overlapping

foci ($\delta x \approx 0.2$ μm) in which the object is positioned. Shot-noise-limited sensitivities of close to 1 pm/(Hz)$^{1/2}$ were achieved with test objects such as latex beads 0.3 μm in diameter as well as with hair bundles.[16] This sensitivity is more than sufficient to detect and record the Brownian motion of those hair bundles.

The experiments described in this Letter were performed on saccular hair cells from the frog inner ear, which sense linear accelerations by detecting the displacement of an elastically suspended dense body (the otonica).[21] Those cells are very similar to the cells sensing sound or those sensing rotational acceleration and have thus served as a model to study the cellular biology of the detection of mechanical stimuli in vertebrate inner ears.[14] Sheets of hair cells were obtained by a microsurgical dissection procedure from freshly killed frogs of the species *Rana catesbeiana* or *Rana pipiens* and were maintained in artificial saline solution.[22] The displacement fluctuations of hair bundles were measured along the direction of maximum mechanoelectrical transduction sensitivity. To record simultaneously the intracellular voltage we impaled the same cell with a saline filled glass microelectrode using standard techniques.[23] The results reported here are based on correlation measurements on 41 cells and are part of a more extended study of mechanical and transduction properties using fluctuation measurements on hair cells.[8,16,24,25] The data presented in Fig. 1 were chosen to show the high degree of noise correlation and to show the sensitivity of the intracellular noise and the impulse response to the intracellular dc voltage.

We found[26] that the spontaneous motion of the hair

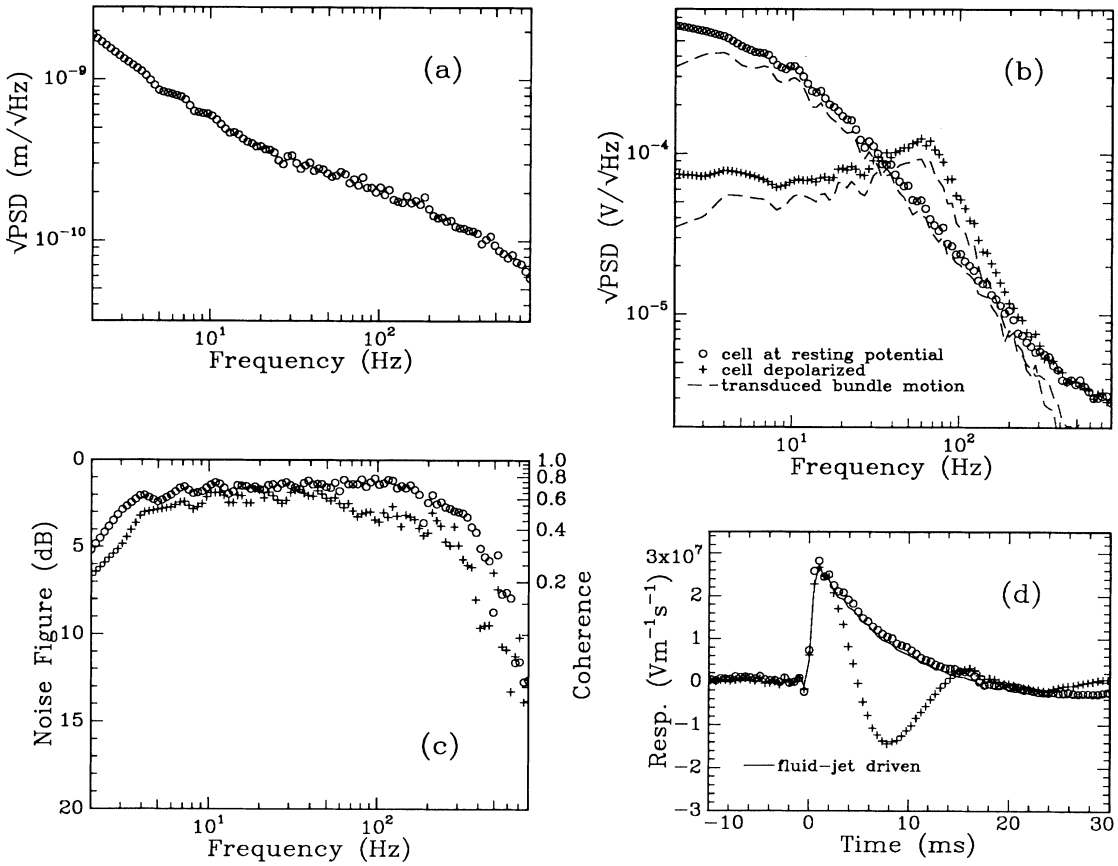

FIG. 1. Results of a spontaneous noise correlation experiment on a saccular hair cell from a frog of the species *Rana catesbeiana*: (a) square root of the power spectral density (PSD), $(2\langle x_f^* x_f\rangle)^{1/2}$, of the bundle's position fluctuations, (b), $(2\langle v_f^* v_f\rangle)^{1/2}$, of the intracellular voltage fluctuations, (c) coherence C_f between electrical and mechanical fluctuations and the corresponding noise figure N_f, and (d) the impulse response function $I(t)$ describing the linear electrical response to a δ-function mechanical stimulus. The dashed lines in (b) indicate that part of the electrical noise that is due to transduced Brownian motion. Note that the electrical noise (b) and the impulse response (d) are very sensitive to changes in the intracellular dc voltage. The current injected in this case was 70 pA, leading to an estimated depolarization by only a few mV. The impulse response function obtained by driving the cell mechanically with an oscillatory fluid jet is also shown for comparison (d). Data points at the calibration frequency (50 Hz) (Ref. 17) and at harmonics of the line frequency (60 Hz) were removed for clarity.

bundles vaguely resembled that of an overdamped harmonic oscillator with rms amplitudes around 4 nm and knee frequencies between 300 and 1000 Hz. This spontaneous motion appears to consist primarily of thermal excitation, i.e., Brownian motion; its rms amplitude is consistent with the hair bundles's stiffness measured using flexible microprobes.[27] Under certain circumstances nonthermal spontaneous bundle motion has been observed.[8,28] The spectral shape of the intracellular electrical noise[8,29] [Fig. 1(b)] varied amongst cells from a low pass ($20\ \text{Hz} \leq f \leq 80\ \text{Hz}$) to a sharply tuned bandpass ($Q \geq 10$, $50\ \text{Hz} \leq f \leq 150\ \text{Hz}$). In some cells the spectral shape of the intracellular electrical noise, but not of the bundle motion, could be changed by varying the intracellular dc voltage [Fig. 1(b)]. In many cells the spontaneous bundle motion and the fluctuations of the intracellular voltage were correlated [Fig. 1(c)] as expected because the Brownian motion of the hair bundle is transduced as any other stimulus that leads to a deflection of the hair bundle.

The properties of any linear transducer can be considered separately for each frequency. The quality of transduction can then be represented by a dimensionless measure, the coherence function C_f, which is defined in terms of cross spectral densities[30] ($2\langle x_f^* v_f\rangle$ and its complex conjugate $2\langle v_f^* x_f\rangle$) and power spectral densities ($2\langle x_f^* x_f\rangle$ and $2\langle v_f^* v_f\rangle$):

$$C_f = \langle x_f^* v_f\rangle\langle v_f^* x_f\rangle / \langle x_f^* x_f\rangle\langle v_f^* v_f\rangle .$$

Here x_f and v_f are Fourier transforms of concurrent time records and $\langle\ \rangle$ denotes expectation values or averages. The more familiar noise figure (N_f) is related by[31]

$$N_f = -10 \log_{10}(C_f) .$$

For an ideal transducer and amplifier (noiseless and linear) $C_f = 1$ and for any real device $0 \leq C_f < 1$ [Fig. 1(c)]. We found 41 cells that showed a peak C_f of at least 0.05. The maximum of C_f exceeded 0.50 for 10 cells and 0.25 for 22 cells. The largest value observed for C_f was almost 0.75 (Fig. 1), corresponding to a noise figure of 1.25 dB.

With the hypothesis that the correlation between deflection and voltage is solely caused by transduced Brownian motion, the mechanoelectrical transfer function (T_f) can be calculated from the data according to the relation

$$T_f = \langle x_f^* v_f\rangle / \langle x_f^* x_f\rangle .$$

Fourier transformation of T_f generates the impulse response function $I(t)$. Consistent with the transduction hypothesis, T_f obeys the Kramers-Kronig dispersion relations for most cells, as can be seen in the vanishing of $I(t)$ for $t < 0$ [Fig. 1(d)]. We found, however, a few cells for which causality seemed to be violated [$I(t) \neq 0$ for $t < 0$]. By injection of current into some of these cells and observation of the ensuing bundle motion we showed that these cells do possess an inverse (i.e., electromechanical) transduction mechanism.[8]

The hypothesis that much of the electrical noise is due to the transduction of mechanical motion is further supported by our observation that the correlation between the electrical and the mechanical noise is abolished by the pharmacological agent streptomycin, a known blocker of the mechanoelectrical transduction property of those cells.[32] We also confirmed for several cells that an identical mechanoelectrical transfer function was obtained by stimulating the hair bundles mechanically with an oscillating fluid microjet [Fig. 1(d)]. The values for the coherence that we obtained are almost certainly lower limits for the values in the living animal because the damage done to the transduction mechanism and to the cell membrane by dissection and impalement is expected, as is frequently the case in physiological preparations, to degrade transduction efficiencies as well as to increase intracellular voltage noise. Although our measurements were performed on cells that had been enzymatically detached[33] from the otholitic membrane through which the stimulus is conveyed in the intact organ we are confident that the bundles's Brownian motion was not changed dramatically as our own measurement on attached bundles indicate.[34]

We conclude that at least some of these sensory receptor cells that perform the crucial step of converting a mechanical stimulus into an electrical signal are fully capable of detecting the spontaneous thermal motion at their inputs. Although we measured transduction noise in only one cell type, these cells have served as a model for all hair cells due to their archetypal properties[12,14] and our results may thus be valid for hair cells in general. We have thus shown that there are hair cells that can "hear" the thermal motion at their inputs and consequently any stimulus above the thermal background. They are therefore as sensitive as the physical nature of the stimulus will permit.

The amplitude of the basilar membrane motion in the mammalian cochlea[35] at the auditory threshold is comparable to the rms amplitude of the hair-bundle Brownian motion as we have measured it in the frog sacculus. To understand the psychophysical detection threshold of hearing, the properties of the mechanical conversion preceding and of the neural processing succeeding transduction must also be taken into account.

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