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Mechano- and electrophysiological studies on cochlear hair cells and lateral line cupulae

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Chapter 5

Signal processing by transducer channels in mammalian outer hair cells

Dinklo, T., van Netten, S.M., Marcotti, W. and Kros, C.J. (2003)
In *Biophysics of the Cochlea: From Molecule to Model* 73-79.

ABSTRACT

Transducer channels of mammalian outer hair cells may not be fully silenced during stimulation of the hair bundle into the inhibitory direction (< -50 nm). A recently formulated three-state model, assuming a state-dependent mechanical engagement of the transducer channel, accounts for this incomplete deactivation (van Netten and Kros, 2000). Moreover, the model suggests a specific function for calcium in controlling the transducer current, by modulating the energy gaps between the conformational states of the channel.

Combining this differentially activating model with experimental results on the gating of transducer currents, we attempted to estimate the consequences of this mode of engagement for the processing of mechanical signals by sensory hair cells.

We found that the channels transduce small mechanical signals most efficiently into transducer currents when the hair bundle is deflected some tens of nanometers away from its equilibrium position. The results are in line with a specific role of calcium in optimising the transducer efficiency and are possibly related to the calcium-dependent phenomenon of adaptation in mechano-electrical transduction.

INTRODUCTION

Hair cells encode the mechanical signals received by their hair bundles into electrical signals for subsequent transmission to the brain. The first stage in the cascade of events that underlies this mechano-electrical transduction process consists of a mechanically induced change in open probability of the hair cell's transducer channels. These channels are located in the hair bundle's tip region and are most likely engaged by elastic elements that are tensioned in response to a positive deflection of the hair bundle.

Recently, in mammalian hair cells, in addition to the gating compliance (Howard and Hudspeth, 1988; van Netten and Khanna, 1994; Ricci *et al.*, 2002), a discontinuous step in the bundle's mechanics was found when deflected more negatively than approximately -50 nm (van Netten and Kros, 2000; Géléoc *et al.*, 1997). This discontinuity was interpreted as a mechanical disengagement of the transducer channels and could be directly linked to the measured displacement-independent (finite) open probability of the transducer channels in this deflection range (van Netten and Kros, 2000). A new differentially engaging three-state (C_1, C_2, O) model was proposed that accurately describes this mechanical aspect of elastic activation of the transducer channel.

The model is based on six parameters that directly relate to the underlying physics: three engaging positions (X_{C1}, X_{C2}, X_O), defining the bundle's deflection at which each of the individual states is being engaged, the spring constant (K_{gs}) of the engaging elastic element, which is assumed to be the same for all states, and finally the two values of the energy gaps ($\varepsilon_{2,1}, \varepsilon_{0,2}$) that energetically separate the three conformational states in the deactivated range (see Fig.1). Variation of these energy gap values has been shown (van Netten and Kros, 2000) to describe the experimentally observed effects that variation of extracellular calcium concentration has on the open probability, $p_o(X)$ (Crawford *et al.*, 1991). Increasing the (summed) energy gap, $\Delta\varepsilon$, corresponds to increased calcium concentrations, which cause transducer current-displacement curves to shift to the right along the displacement axis, a process that physiologically has been described as adaptation. Lowering the energy gap value has the opposite effect.

In the present paper the performance of the first step in hair cell transduction is investigated by estimating the signal-to-noise ratio of outer hair cell transducer channels. Related new experimental results are shown to be adequately described by the differentially activating model. It is concluded that the process of adaptation may be involved in adjusting and possibly optimising the signal-to-noise performance of this early stage in mechano-electrical transduction in hair cells.

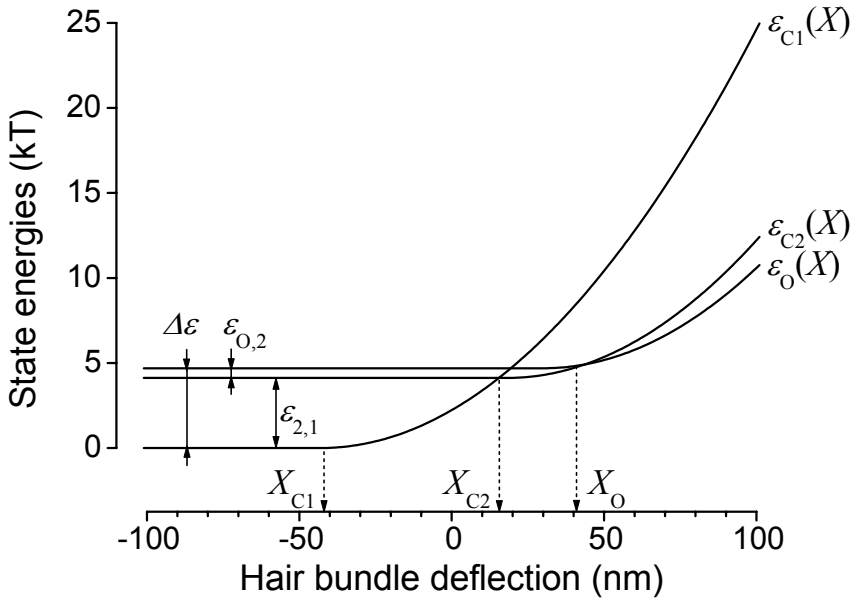


Figure 1: State energies of the three conformational states defined by the differentially activating model of the transducer channel (van Netten and Kros, 2000). Parameters are: $X_{C1} = -42$ nm; $X_{C2} = 16$ nm; $X_0 = 41$ nm; $K_{gs} = 7.3$ $\mu\text{N/m}$; $\varepsilon_{2,1} = 3.6$ kT; $\varepsilon_{0,2} = 0.9$ kT. Using these parameters, the open probability, $p_o(X)$, is completely dictated by the Boltzmann distribution. Increasing $\Delta\varepsilon = \varepsilon_{2,1} + \varepsilon_{0,2}$ simulates increased extracellular calcium and shifts the resulting $p_o(X)$ to the right, decreasing $\Delta\varepsilon$ has the opposite effect.

METHODS

The signal-to-noise ratio of hair cell transducer channels

The transducer current signal, $S_1(X)$, of a hair cell containing n identical operational transducer channels in response to a small change, ΔX , around a static displacement, X , of its hair bundle is defined here as:

$$S_1(X) = in \frac{dp_o(X)}{dX} \Delta X, \quad (1)$$

with, i , the current flowing through one transducer channel with a unitary conductance of 112 pS (Géléoc *et al.*, 1997), which amounts to about 9.4 pA at the holding potential used in this study (-84 mV). Equation 1 expresses that the change in the hair bundle's position is assumed to be the relevant signal to be processed and not its absolute position. In the experiments ΔX was about 10 nm. The results

of S_1 are displayed as the current change caused by a change in hair bundle position of 1 nm.

The hair cell transducer channel is assumed to possess 3 conformational states with just 2 conductance levels, open or closed. The probability of populating the open state is $p_o(X)$, that of the 2 closed states is $(1 - p_o(X))$. The total (r.m.s.) current-noise, $N_1(X)$, of n independent channels can then be calculated:

$$N_1(X) = i\sqrt{n p_o(X)(1 - p_o(X))}, \quad (2)$$

which combines with Eq. 1 in the (power) signal-to-noise ratio of the summed transducer channels of a hair cell:

$$\left[\frac{S_1(X)}{N_1(X)} \right]^2 = n \frac{\left(\frac{dp_o(X)}{dX} \right)^2}{p_o(X)(1 - p_o(X))} (\Delta X)^2. \quad (3)$$

Experiments

Acute preparations of 6 to 7 day old CD1 mice (P6-7) were used to record transducer current in response to fluid jet stimulation of the hair bundle. Transducer current of outer hair cells was measured using the tight seal whole cell configuration, with a holding potential of -84 mV. Extracellular solution contained (mM): 135 NaCl, 5.8 KCl, 1.3 CaCl₂, 0.9 MgCl₂, 0.7 NaH₂PO₄, 2 NaPyruvate, 5.6 D-glucose and 10 HEPES, pH 7.5, vitamins and amino acids were added from concentrates. Intracellular solution contained (mM): 147 CsCl, 2.5 MgCl₂, 2.5 Na₂ATP, 5 HEPES, 1 EGTA-NaOH, pH 7.3. Pipettes were pulled from soda glass and coated with wax. Hair bundle displacement was measured using a differential photodiode system and was recorded simultaneously with the transducer current (Géléoc *et al.*, 1997). The RC-time imposed on the measurements by the series resistance of the patch restricted current measurements to a bandwidth of approximately 10 kHz.

Fluid jet stimuli were generated and measured signals recorded using a Power 1401 data acquisition board in combination with the Signal software package (CED, Cambridge, UK). Stimuli were filtered at 2 kHz. Current and displacement recordings were filtered at 5 kHz and sampled at 50 kHz.

A step protocol displaced the bundle with incrementing steps of about 20 nm during 150 ms, alternating positive and negative hair bundle deflection. Current-displacement curves were constructed by displaying the step current response as a function of the simultaneously measured step displacement of the hair bundle between 50 and 100 ms after stimulus onset (Fig. 2A, triangles). Another protocol (double-sine), consisting of a sum of two sine waves ($f_1 \sim 49$ Hz and $f_2 \sim 1563$ Hz),

displaced the bundle quasi-statically through a large part of its dynamic range at frequency f_1 , while the transducer channel's signal sensitivity, $S_I(X)$, was probed at X , with frequency f_2 and an amplitude of about 10 nm.

Current-displacement relationships using the double-sine protocol were constructed by extracting the current response at f_1 from the total current and displaying it as a function of the associated hair bundle displacement at f_1 (Fig. 2A, squares). The envelope of the remaining f_2 component was calculated and plotted as a function of the associated f_1 displacement to obtain signal-displacement curves, $S_I(X)$, (Fig. 2B, squares). All experiments were performed at room temperature.

RESULTS AND DISCUSSION

Figure 2 shows the results as obtained from a mouse apical outer hair cell (P6). In panel A the current-displacement functions are shown as obtained using the two different stimulus protocols described in the methods. The double-sine protocol (squares) produces steeper slopes, while similar currents are obtained at smaller hair bundle deflections in comparison to the step protocol (triangles). A plausible explanation may lie in the different degree of adaptation evoked by the two protocols. The double-sine protocol, specifically designed to directly measure the signal, S_I , of transducer channels, obviously comprises a less adaptive way to induce transducer currents than the static step stimuli. The parameters used to fit the two curves (solid lines) also reflect a difference in adaptation. The (summed) energy gap, $\Delta\varepsilon$, is higher for the step-evoked currents (4.9 kT) than the double-sine-evoked currents (4.5 kT). It has been shown previously that an increase of $\Delta\varepsilon$ can simulate an increased extracellular calcium concentration or a more adapted state (van Netten and Kros, 2000). In addition, the engaging position of the open state, X_O , is shifted 10 nm to the left for the step-evoked currents as compared to the double-sine-evoked situation.

Figure 2B shows the signal, S_I , as measured with the double-sine protocol. It shows that under this condition the transducer channels are most sensitive at a hair bundle displacement of about 40 nm, reaching a maximum of about 15 pA/nm.

The measured signal (Fig. 2B squares) was modelled (Fig. 2B solid curve) by applying Eq. 1 to the solid curve in Fig. 2A, describing the current in response to the (slow) f_1 component (~ 49 Hz) of the double-sine protocol. Values so obtained had to be multiplied by a factor of 1.5 (Fig. 2B, solid curve) to fit the measured signal (Fig. 2B, squares), reflecting the smaller degree of adaptation at the higher f_2 component (~ 1563 Hz).

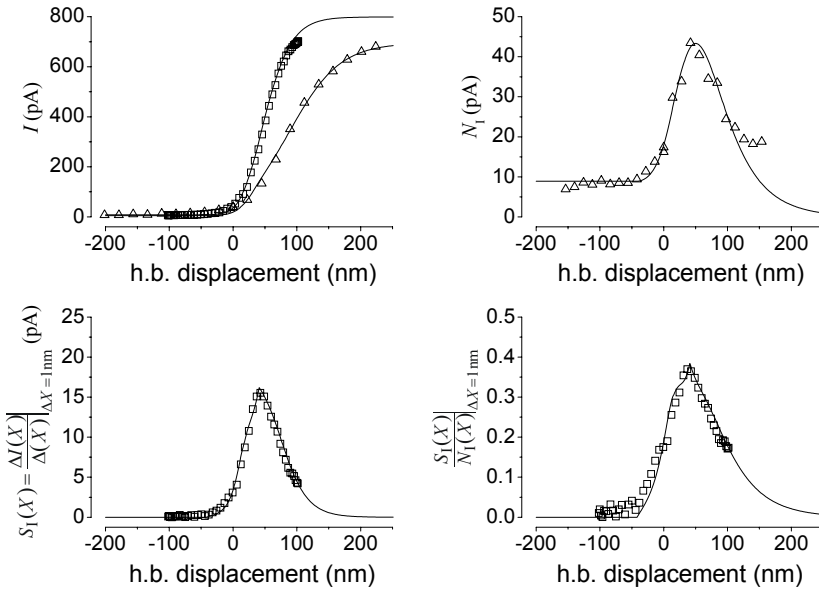


Figure 2: Signal, noise and their ratio of current through transducer channels in a mouse apical OHC.

(A) Transducer current of an OHC in response to a fluid jet producing forces with increasing steps (triangles) and, measured continuously, in response to a fluid jet producing low frequency (49 Hz) forces using the double-sine protocol (a small selection of data points is shown as squares). (B) Current signal in response to small variations ($\Delta X = 1\text{nm}$) of the hair bundle around a quasi-static position X , measured using the double-sine protocol in the same OHC as shown in A. (C) Noise (r.m.s.) of the same OHC measured during the step responses displayed in A (triangles). To facilitate comparison with the responses measured with the double-sine protocol, the noise measured during the step stimuli is displayed with a contracted X -axis (factor 1.6) commensurate with the apparent difference in the two transfer-curves in A. (D) Signal-to-noise ratio of transducer current obtained by dividing the signal displayed in B by the noise shown in C. Apart from the solid line through the triangles in A (step protocol), all solid lines in A, B, C and D are based on the parameter set of the model given in the caption of Figure 1 and Eqs. 1,2 and 3. $n = 85$. The solid line through the triangles (step protocol) is based on: $X_{C1} = -42\text{ nm}$; $X_{C2} = 16\text{ nm}$; $X_0 = 31\text{ nm}$; $K_{gs} = 7\text{ }\mu\text{N/m}$; $\mathcal{E}_{2,1} = 3.2\text{ kT}$; $\mathcal{E}_{0,2} = 1.7\text{ kT}$; $n = 74$.

Figure 2C depicts the r.m.s. noise of the same outer hair cell, as measured during the step responses over a 50 ms time period. The values are displayed as a function of hair bundle displacement, which was reduced with a factor of 1.6 with respect to the actual measurement. This rescaling of the X -axis effectively describes the differences between the two measurement protocols and facilitates a proper comparison of the measured noise data with the signal, S_I , obtained using the double-sine protocol. Also, the measured r.m.s. noise was actually a factor 2.13

less than plotted in Fig. 2C. This multiplication factor was used to obtain a best match between the measured and calculated noise (Fig. 2C solid curve). A probable explanation lies in the limited bandwidth (0-5 kHz) that was used to measure the current noise during the step responses. The noise spectrum of a three-state channel can be shown to consist of a double Lorentzian with cut-off frequencies related to the rate constants of the transitions between the states (Colquhoun and Hawkes, 1995). Transducer channel current noise spectra have been measured in frog saccular- and turtle auditory hair cells with cut-off frequencies of 250 and 1185 Hz respectively (Holton and Hudspeth, 1986; Ricci, 2002). The present OHC current noise was found to be flat within the filter bandwidth used (5 kHz). Since cochlear outer hair cells may have rate constants that exceed this bandwidth, we may have measured only a fraction of the current noise present. An estimate based on the ratio (0.22) between measured and calculated (power) noise predicts a channel noise spectrum bandwidth of at least 20 kHz, suggesting relatively fast rate constants in mammalian hair cells. In this estimate we neglected the effect of bundle noise and other sources that could contribute to the current noise.

Figure 2D finally shows the signal-to-noise ratio as obtained by taking the ratio of the measured data shown in panel B and the solid curve in panel C. The solid line depicts the signal-to-noise ratio resulting from the model. Both results show that the signal-to-noise ratio peaks at a hair bundle deflection of about 40 nm where it reaches a value of 0.37. It should be noted that this is the signal-to-noise in response to a change in hair bundle position of 1 nm. Another way to characterise this result is to define the noise-equivalent stimulus amplitude (NESA) as the change in hair bundle position that produces a response signal equal to the noise. The NESA ranges from a minimum of about 2.7 nm around a static deflection of 40 nm to very high values at negative deflections. At the equilibrium position of the hair bundle, the NESA amounts to 5.3 nm.

Adaptation has been shown to effectively shift a hair cell's operational point and may also affect the slope of current-displacement curves. The related signal-to-noise ratio will therefore also be controlled by adaptation. The present results were obtained at extracellular calcium concentrations of 1.3 mM. However, the calcium concentration of the cochlear endolymph is much lower (~30 μ M; Bosher and Warren, 1978). At such low extracellular calcium concentrations the current-displacement curve may shift several tens of nanometers to the left (Crawford *et al.*, 1991). Under *in vivo* conditions hair cell transducer channels may therefore be expected to reach the optimal signal-to-noise ratio closer to the resting position of the hair bundle than found under the present experimental conditions.