Time course and extent of mechanotransducer adaptation in mouse utricular hair cells: Comparison with frog saccular hair cells

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ABSTRACT

Whole-cell transduction currents were recorded from hair cells in early postnatal mouse utricles in response to step deflections of the hair bundle. For displacement steps delivered by a stiff probe (1-ms rise time), half-maximal responses decayed monoexponentially with a mean time constant of 30 ms. Adaptation and other transduction properties did not vary systematically with hair cell type (I vs. II) or region (striola vs. extrastriola). Thus, regional variation in the phasic properties of utricular afferents arises through other mechanisms. When bundles were deflected by a fluid jet, which delivers force steps, transduction currents decayed about three-fold slower than during displacement steps. A simple model of myosin-mediated adaptation predicts such slowing through forward creep of the bundle during a force step.

For a faster stiff probe (rise time 200 µs), step responses of both mouse utricular and frog saccular hair cells decayed with two exponential components, which may correspond to distinct feedback processes. For half-maximal responses, the two components had mean time constants of 5 and 45 ms (mouse) and 2 and 18 ms (frog). The fast and slow components dominated the decay of responses to small and large stimuli, respectively.

Abartation shifts the instantaneous operating range in the direction of the adapting step. In frog saccular hair cells, the operating range shift is a constant percentage of the displacement. In mouse utricular hair cells, the percentage shift increases for large displacements, extending the range of background stimuli over which adaptation can restore instantaneous sensitivity.
INTRODUCTION

The time course of vestibular afferent responses to head movements varies according to the location of the afferent terminals within the sensory epithelium. In the rodent utricle, afferents that innervate a narrow strip of epithelium called the striola have more phasic response properties than those that innervate the surrounding extrastriola (Goldberg et al. 1990b). This difference can be seen in the frequency dependence of responses to sinusoidal head movements. Below 2 Hz, the highest frequency examined, the response gain tends to rise with frequency for striolar afferents and remain flat with frequency for extrastriolar afferents.

Such differences in afferent responses can arise at any preceding stage, from the macromechanical response of the utricular accessory structures (otoconia and gel layer) to hair cell synaptic transmission. (Goldberg et al. (1984) showed that differences in afferent response dynamics are unlikely to reflect differences in spike generation in the afferent nerve.) Holt et al. (1997, 1998, 1999) found that transduction currents in mouse utricular hair cells adapt to step bundle deflections with a time course that, in the frequency domain, causes the currents to increase with stimulus frequency over the range from DC to 5 Hz (high-pass filtering). Whether this behavior varies with hair cell location in the sensory epithelium was not determined. In the present study, we asked whether the transduction stage contributes to regional variation in the response kinetics of utricular afferents, by comparing the responses of extrastriolar and striolar hair cells to step bundle deflections. We have also compared the responses of the two morphologically distinct hair cell types, type I and type II, that are found in the vestibular organs of all amniotes.

In previous studies in which bundles were deflected with a fluid jet (Holt et al., 1997, 1998, 1999), adaptation of mouse utricular hair cells was slower than that evoked in frog saccular (Shepherd and Corey 1994) and turtle cochlear hair cells (Ricci et al. 1998) by deflecting bundles with stiff probes. It was not clear whether this difference arose from physiological or technical differences in the experiments. The
former seemed plausible because the rodent utricle operates down to lower frequencies than do the turtle cochlea and frog saccule. Turtle auditory afferents have best frequencies between about 30 and 700 Hz (Crawford and Fettiplace 1980). The frog saccule differs from mammalian otolith organs in that it is designed to detect relatively high-frequency substrate vibrations (Narins and Lewis 1984); frog saccular afferents have best frequencies between 20 and 300 Hz. While the upper end of the rodent utricle’s frequency range is not established, chinchilla utricular afferents produce robust responses to stimulus frequencies from 2 Hz down to steady state (Goldberg et al. 1990a).

Technical differences in the stimulus method may also have affected the time course of adaptation. Stiff probes apply displacement steps, clamping bundle position, while fluid jets apply force steps. Adaptation is known to be accompanied by stiffness changes in the bundle (Howard and Hudspeth 1987). By Hooke’s law, a stiffness change during a force step will change bundle displacement; thus, fluid-jet stimuli are more complex in terms of bundle displacement. To permit comparisons with published frog and turtle data obtained with stiff probes, we have stimulated mouse utricular hair bundles with stiff probes. To eliminate the possibility of technical differences affecting the results obtained from different hair cell organs, we have used identical methods to record from frog saccular hair cells. To investigate the differences between fluid-jet and stiff-probe stimulation, we have recorded with both methods from individual mouse utricular hair cells.

Experiments on in vitro preparations of hair cells of the frog saccule and turtle cochlea have provided evidence for two Ca$^{2+}$-dependent processes that affect the decay of transduction current in response to step bundle deflections (Eatock 2000; Holt and Corey 2000; Howard and Hudspeth 1987; Wu et al. 1999). Whether these processes are referred to as adapting or amplifying mechanisms depends on the experimental conditions. In millimolar external Ca$^{2+}$, the fast and slow components cause decay (adaptation) of the transduction current with time constants of milliseconds or less and of tens of milliseconds, respectively. When Ca$^{2+}$ bathing the bundle is lowered to endolymph levels (50 - 100 µM),
both components may be seen to act as tuning mechanisms (Choe et al. 1998; Howard and Hudspeth 1988; Martin et al. 2000; Ricci et al. 1998; reviewed in Fettiplace et al. 2001 and Hudspeth et al. 2000). Experimentally this is seen as the transformation of the step response from one that simply decays following a peak to one that undergoes a damped oscillation. It is hypothesized that the fast component reflects channel closure in response to Ca\(^{2+}\) binding to a site on or near the transduction channel (Choe et al. 1998; Crawford et al. 1991; Howard and Hudspeth 1987). The slow component has been modeled as a myosin-driven movement of the transduction channel and associated gating spring along the stereocilium, which tends to restore tension on the gating spring toward resting levels (Assad et al. 1991; Howard and Hudspeth 1987; Shepherd and Corey 1994). The gating springs contribute a substantial fraction of the total bundle stiffness (Howard and Hudspeth 1988; Ricci et al. 2002; van Netten 1997) and their relaxation during the slow process decreases the total bundle stiffness.

The responses of mouse utricular hair cells to fluid-jet steps (Holt et al. 1997) and the data we present here for a stiff probe with a 1-ms rise time are both consistent with the slow component. We show that a faster stiff probe reveals a fast component in both mouse utricular hair cells and frog saccular hair cells.

In hair cells of the turtle cochlea and frog saccule, the rate of decay of transduction current during a step decreases strongly with increasing step size. In the turtle cochlea, this change has been explained as an increase in the amplitude of the slow kinetic component with increasing positive displacement (Wu et al. 1999). In the frog saccule, this change has been interpreted in terms of the effects of adaptation on the instantaneous and steady-state current-displacement (I(X)) relations. The decay of the transduction current reflects a shift in the hair cell’s instantaneous I(X) relation in the direction of the imposed deflection (Corey and Hudspeth 1983; Eatock et al. 1987). Thus, adaptation acts to re-align the instantaneous operating range with the steady bundle position. In the frog saccule, the steady-state shift of the instantaneous I(X) relation is a constant percentage (~80%) of the adapting step. As described
by Shepherd and Corey (1994), this behavior produces a steady-state operating range that is five-fold broader than the instantaneous operating range. Here we report that in many mouse utricular hair cells, in contrast, the percent shift increases with size of the adapting step, with dramatic effects on the steady-state relation.

METHODS

Preparation

Mouse utricle. Semi-intact preparations of the mouse utricle were made as described previously (Holt et al. 1997; Rüsch and Eatock 1996a). Procedures involving animals were approved by the animal care committee of Baylor College of Medicine. Briefly, utricles of early postnatal mice (postnatal days (P) 0 - 10, birth = P0; ICR outbred strain, Charles River Laboratories, Wilmington, MA) were exposed by opening the medial wall of the otic capsule, then bathed for 20 minutes in standard extracellular solution (below) containing 100 µg/ml protease type XXVII (Sigma, St. Louis, MO). The protease facilitates removal of the otoconia and otolithic membrane overlying the hair bundles. The utricle was then dissected out, mounted in an experimental chamber and viewed on an upright microscope (Axioskop FS; Zeiss, Oberkochen, Germany) with water-immersion objectives (40 or 63×) with differential interference contrast optics (Fig. 1). All preparations and recording were done at room temperature (22-25°C).

The extracellular solution for dissection and perfusion contained (in mM): 144 NaCl, 0.7 NaH₂PO₄, 5.8 KCl, 1.3 CaCl₂, 0.9 MgCl₂, 5.6 D-glucose, 10 HEPES-NaOH, vitamins and minerals as in Eagle's MEM; pH 7.4, ~320 mmol/kg. The recording pipette contained (in mM): 140 KCl, 0.1 CaCl₂, 10 EGTA-KOH, 3.5 MgCl₂, 2.5 Na₂ATP, 5 HEPES-KOH, 0.1 Li-GTP, 0.1 Na-cAMP; pH 7.4, ~290 mmol/kg. The free Ca²⁺ concentration is estimated as 710 pM by MaxChelator software (WEBMAXC Standard; http://www.stanford.edu/~cpatton/maxc.html; Bers et al. 1994).
Cell type: Hair cells were classified as type I if they were innervated by a partial or full calyx terminal (Wersäll 1956; see Fig. 1) or if the type I-specific conductance, \( g_{K,L} \), was present (Correia and Lang 1990; Ricci et al. 1996; Rüsch and Eatock 1996a). Before P8, a hair cell in the mouse utricle that does not have \( g_{K,L} \) can be either a type II cell or an immature type I cell (Rüsch et al. 1998). In a previous study (Holt et al. 1997) and the present study, such cells showed no systematic variation with age between P0 and P10 in the time course of adaptation or other transduction properties (linear regression of the adaptation time constant, \( \tau_A \), as a function of age yielded \( r^2 = 0.07; n = 18 \), data in Table I). For simplicity, then, we refer to all hair cells lacking \( g_{K,L} \) and calyces (partial or complete) as “type II”.

Cell region: Hair cells were also classified as either striolar or extrastriolar. The striola, an arc-like strip running approximately through the middle of the sensory epithelium, differs from the extrastriola in many morphological features (Lindeman 1973; Lysakowski and Goldberg 1997; Rüsch et al. 1998). Based on data from the chinchilla utricle (Fernández et al. 1990), we classified hair cells as striolar if they were within six cells of the line of hair bundle reversal. Recent data from Zeh et al. (1999), in which regions were classified according to patterns of calretinin staining, suggest that the striola in the mouse utricle is about seven cells wide, rather than twelve. Our ‘striolar’ sample therefore includes hair cells from what Fernández et al. (1990) called the ‘juxtastriola’, a strip about five hair cells wide around the striola. We classified hair cells as extrastriolar if they were within 12 hair cells of the edge of the sensory epithelium. This is in reasonable agreement with the calretinin staining study of Zeh et al. (1999).

Frog saccule. Some experiments were performed on hair cells in a semi-intact epithelium of the frog saccule. Leopard frogs, *Rana pipiens* (Kons Direct, Germantown, WI), were deeply anesthetized by chilling, double pithed and decapitated. The saccules were dissected out and treated with 50 µg/ml protease type XXIV (Sigma, St. Louis, MO) in extracellular solution (below) for 20 minutes. The otolithic membrane was removed and the preparation mounted as for the mouse utricle. The extracellular solution for dissection and perfusion contained (in mM): 120 NaCl, 2 KCl, 4 CaCl₂, 5 CsCl, 3 D-glucose,
5 HEPES; pH 7.25, ~250 mmol/kg. The recording pipette contained (in mM): 120 CsCl, 0.1 CaCl₂, 10 EGTA, 2 MgCl₂, 2 Na₂ATP, 5 HEPES; pH 7.25, ~290 mmol/kg. Again, all procedures were done at room temperature.

**Recording**

Pipettes were pulled from R6 glass (Garner Glass, Claremont, CA) and coated with sylgard (Dow Corning Corporation, Midland, MI). Their resistances in standard solutions were 3-5 MΩ. Positive pressure was applied to the recording pipette as it was lowered into the epithelium and advanced toward the hair cell of interest; the outflow of pipette solution cleaned the pipette tip and the cell’s basolateral membrane. The positive pressure was released just before making contact with the membrane, then suction was applied to form a seal and rupture the membrane, entering whole-cell voltage clamp mode. The currents were amplified with an Axopatch 200A or 200B amplifier (Axon Instruments, Union City, CA). Hair cells were voltage clamped at $-64 \text{ mV}$, near the mean resting potential for type II and neonatal cells in this preparation ($-66 \text{ mV}$, Rüsch et al. 1998). Voltage-clamp protocols and stimulus waveforms were controlled by pClamp 8.0 software (Axon Instruments). Currents were low-pass-filtered at a corner frequency, $f_c$, of 2-10 kHz (8-pole Bessel filter) and digitized at 10-100 kHz ($> 2 \times f_c$) with a 12-bit acquisition board (Digidata 1200, Axon Instruments) and stored on disk. Data analysis and fitting were done with Origin 6.0 (Microcal Software, Northampton, MA), which uses a Levenberg-Marquardt least-squares fitting algorithm. Results are presented as means ± SEM. Comparisons of transduction and adaptation properties across cells were tested for significance with the Student’s t-test.

**Stimulation**

Transduction currents were elicited by hair bundle displacements effected with either a stiff probe or a fluid jet.
Stiff Probe. Borosilicate pipettes (Sutter Instrument Company, San Rafael, CA) were pulled to a final diameter of 1-2 µm and mounted on a piezoelectric bimorph (Corey and Hudspeth 1980). The stiff probe was brought into contact with the short edge of the hair bundle and used to push or pull the bundle to deliver positive or negative step displacements, respectively (Figs. 1B, 3A). The probe was driven by voltage protocols generated with pClamp 8.0 and the Digidata 1200 and low-pass-filtered by an 8-pole Bessel filter (Model 902, Frequency Devices, Haverhill, MA), with \( f_c \) below the probe’s resonant frequency. Probe displacement as a function of applied voltage was calibrated from videotaped images of the displacements evoked by 1-s voltage steps. For each new probe, the waveform of probe movement in response to a voltage step was recorded with a photodiode. According to these recordings, stimulus ‘creep’ (further movement in the direction of the applied step, after the step onset) was less than 10% of the initial step, as found in other studies (Corey and Hudspeth 1980). For the probes used for comparisons of type I and type II cells and hair cells from different regions, input voltages were usually filtered at \( f_c = 500 \) Hz, giving a 10-90% rise time of 1 ms. For comparison with the fluid jet stimulus, we slowed the rise time to 2 ms by filtering at 200 Hz. To deliver faster stimuli (rise time \( \leq 200 \) µs), we increased the probe’s resonant frequency by making it lighter and filtered the voltage input at 1.5-2.5 kHz.

Fluid Jet. We used a fast pressure-clamp system (McBride and Hamill 1995) to deliver suction or pressure steps to the back of a wide-bore (10 µm) pipette filled with extracellular solution (Holt et al. 1997). The fluid-jet pipette was placed tens of microns from the hair bundle. Piezoelectric valves, driven by the output of the Digidata 1200 board, controlled a mixture of vacuum and air that was supplied to the back of the pipette. The feedback circuit included a strain gauge that measured the pressure at the back of the pipette. The waveform shown for the fluid jet stimulus (Fig. 3B) is the output of the strain gauge, calibrated by measuring steady-state displacement near the top of the bundle from videotaped images of bundle movement during 1-s fluid jet steps. This method does not reveal dynamic...
changes in bundle position that may occur as a result of adaptation (Howard and Hudspeth 1987; see Hair bundle model, below, and Fig. 3D).

Stimulus protocols and data analysis

Figure 2 illustrates how we measured properties of transduction and adaptation from the transduction currents evoked by families of step displacements of the hair bundle. Steps were 100, 350 or 400 ms.

TIME COURSE OF ADAPTATION. By adaptation, we mean the decay of the transduction current during step deflections of the bundle. Decays were fit with single-exponential or double-exponential functions (Equations 1 and 2) (Fig. 2A).

\[
I(t) = A e^{-t/\tau_1} + I_{ss} \quad (1)
\]

\[
I(t) = A_{fast} e^{-t/\tau_{fast}} + A_{slow} e^{-t/\tau_{slow}} + I_{ss} \quad (2)
\]

I is current, \( t \) is time, \( A \) or \( A_{fast} \) and \( A_{slow} \) are the amplitudes of each exponential term, \( \tau_1 \) or \( \tau_{fast} \) and \( \tau_{slow} \) are the time constants of each term, and \( I_{ss} \) is the current level at steady state. Percent decay of the transducer current was measured at steady state, \([100 \times (\text{peak current} - I_{ss}) / \text{(peak current)}]\).

CURRENT-DISPLACEMENT RELATIONS. We generated instantaneous and steady-state current-displacement relations, \( I(X) \) relations, from the peak currents and \( I_{ss} \) values, respectively, evoked by families of steps. Examples of instantaneous \( I(X) \) relations are shown in Figure 2C. \( I(X) \) relations were fit with a second-order Boltzmann function (Equation 3):

\[
I(X) = \frac{I_{max}}{1 + e^{A_1(P_2 - X)/(P_1 - X)}} \quad (3)
\]

\( I_{max} \) is the maximum current level, \( X \) is displacement, \( A_1 \) and \( A_2 \) are constants that determine the steepness of the function and \( P_1 \) and \( P_2 \) are constants that set the position of the function along the X-axis.

From the Boltzmann fits of \( I(X) \) relations, we took the resting open probability, \( P_0 \): the percentage of the maximum current at resting bundle position, \( X = 0 \); and the operating range: the range of
displacements corresponding to growth of the I(X) relation from 10% to 90% of $I_{\text{max}}$. We refer to the operating range of the instantaneous I(X) relation as $OR_{\text{inst}}$. For between-cell comparisons of the time constants of decay and percent decay of the transducer current, we fit the responses to $X_{1/2}$: the displacement corresponding to the half-maximal response $[(I_{\text{max}} - I_{\text{min}})/2]$ (Fig. 2A).

**Adaptive shift of the instantaneous I(X) relation.** In hair cells of the frog saccule (Assad and Corey 1992; Eatock et al. 1987) and mouse utricle (Holt et al. 1997), adaptation of the transduction current during a step reflects a shift of the instantaneous I(X) relationship along the displacement axis. To measure this shift, we applied families of test steps before and 250 ms after the onset of the adapting step (Fig. 2B). The *percent shift* is the shift of the I(X) relation expressed as a percentage of the adapting step. Note that this is the same as the “extent of adaptation” used in studies of frog saccular hair cells (Shepherd and Corey 1994) and is strongly correlated with but not identical to the percent decay of the transducer current.

In some cases, the steady-state I(X) relation was fit with a stretched version of the instantaneous I(X) relation:

$$
I(X) = \frac{I_{\text{max}}}{1 + e^{A_2(P_2 - (X/s))}/[1 + e^{A_1(P_1 - (X/s))}]} \quad (4)
$$

where $s$ is the stretch factor applied. $s$ was allowed to vary; all other parameters were fixed at values obtained by fitting Eq. 3 to the instantaneous I(X) relation of the same cell. In frog saccular hair cells (Shepherd and Corey 1994), the stretch required to fit the steady-state I(X) relation is related to the percent shift, according to the following equation:

$$
s = \frac{1}{(1 - \text{shift})} \quad (5)
$$

Hair bundle model
The motor model of hair cell adaptation (Assad and Corey 1992; Howard and Hudspeth 1987) predicts that the transduction current decay evoked by a force step will be slower than that evoked by a displacement step to the same steady-state displacement. To illustrate this, we used a simple version of the model, comprising two parallel, Hookean springs and a motor element (illustrated in Fig. 3D). One spring represents the stiffness of the stereociliary pivots and the other spring represents the stiffness of the gating springs, hypothetical elastic elements that apply force to the mechanosensitive transduction channels. The adaptation motor is in series with the gating springs. After the onset of a displacement or force step toward the tall edge of the bundle (a positive step), the adaptation motor moves in such a way as to reduce the stretch across the gating springs. If $X_S(t)$ is the stereociliary (bundle) displacement at time $t$ and $X_M(t)$ is the position of the motor, then the displacement applied to the gating springs at time $t$ is $[X_S(t) - X_M(t)]$. Then the forces sensed by the springs are:

$$F_S = K_S X_S \quad (6)$$

$$F_G = K_G (X_S - X_M) \quad (7)$$

and the total force,

$$F_T = F_S + F_G \quad (8)$$

This model does not include the gating compliance term that reduces bundle stiffness as a result of channel opening and closing (Howard and Hudspeth 1988).

The movement of the adaptation motor is modeled as a single exponential process that acts to restore a target force, $F_{G,\infty} = K_G (X_{S,\infty} - X_{M,\infty})$, across the gating spring:

$$X_M (t + \Delta t) = X_{M,\infty} - [(X_{M,\infty} - X_M(t)) \ast e^{(-\Delta t/\tau_{XM})}] \quad (9)$$

$X_{S,\infty}$ and $X_{M,\infty}$ are the target positions of the bundle and the motor and $\tau_{XM}$ is the intrinsic time constant of the motor. After each $\Delta t$, $X_S$ is updated as $X_S (t+\Delta t) = (F_T + K_G X_M) / (K_S + K_G)$.

According to the model, the time constant of the operating range shift evoked by a displacement step
is the intrinsic time constant of the motor (Assad and Corey 1992). For small steps that do not saturate
the instantaneous operating range, the time constant of the operating range shift and the time constant
of transduction current decay are similar. Therefore, for our simulation we assigned to $\tau_{XM}$ the average
time constant of transduction current decay for stiff-probe bundle displacements evoking half-maximal
responses (30 ms; Table I). This value is similar to the time constant of the operating range shift
measured for stiff-probe displacements of mouse utricular hair bundles (25 ms; Holt et al. 1997). We
used $\Delta t = 1$ ms or 0.1 ms.

RESULTS

Transduction and adaptation properties do not vary systematically with cell type and location

We performed a series of experiments to determine whether transduction and adaptation properties
map onto location or cell type in the utricular sensory epithelium. The hair bundles were deflected with
a stiff glass probe with a 10-90% step rise time of 1 ms.

We fit the time course of adaptation to steps at half-maximal displacements ($X_{1/2}$) with a single-
exponential function (Eq. 1; Fig. 2A). The fit provided the time constant of adaptation, $\tau_A$, and the
percent decay at steady state. There were no systematic differences across cell types and cell locations
(Table I). The mean $\tau_A$ was $30 \pm 2$ ms (range 9 - 75 ms; normal distribution) and the mean percent
decay at steady state was $76 \pm 3\%$. In the frequency domain, a monoexponential adaptation process acts
like a high-pass filter with $f_C (Hz) = 1/(2\pi\tau_A)$ ($\tau_A$ in seconds). For our sample, $f_C$ had a mean value of 5
Hz and ranged from 2 to 18 Hz. If percent decay is similar in vivo to its average value here, then $\sim 25\%$
of the peak response would be available to report steady head position and head movements below 0.1
Hz.

Instantaneous $I(X)$ relations were generated and fit as illustrated in Figure 2 and described in
METHODS. Again, no systematic differences were seen in the parameters obtained from fits of the
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Adaptation is faster with a stiff probe than with a fluid jet

For type II cells, the mean $\tau_A$ in the present study is half that obtained previously with a fluid-jet stimulus ($61 \pm 8$ ms; 28 cells; Holt et al. 1999). The fluid jet provides a force step rather than a displacement step, and has a slower rise time: 2-3 ms vs. 1 ms for the experiments summarized in Table I. Wu et al. (1999) showed that $\tau_A$ in turtle cochlear hair cells increases with the rise time of deflections effected by a stiff probe. To investigate the relative importance of stimulus rise time and stimulus type (displacement vs. force step) in setting $\tau_A$, we compared the responses of individual cells to a fluid jet and to a stiff probe with two rise times. The input to the probe was filtered so that its rise time would be either 1 ms or 2 ms, closer to the rise time of the fluid jet (mean rise time $2.82 \pm 0.03$ ms, $n = 9$...
As shown for one cell in Figure 3A, slowing the rise time of the displacement step to 2 ms had no effect on adaptation time course. Mean $\tau_A$'s were 32 ± 5 ms and 34 ± 6 ms for the 1-ms and 2-ms rise times, respectively (n = 9 cells; p = 0.75, paired t-test). When the same hair bundles were deflected with the fluid jet, however, the mean $\tau_A$ was significantly slower and had a larger variance, 90 ± 23 ms (p = 0.02 for a pair-wise comparison with the data from the 2-ms probe, Fig. 3C). The percent decay of the transducer current was slightly smaller with the fluid jet (70 ± 5%) than with the 2-ms probe stimulus (82 ± 4%; p = 0.02). During individual experiments, $\tau_A$ was similar for stiff-probe deflections given before and after the fluid jet stimulus (Fig. 3B), showing that the slowness of the decay in response to the fluid jet did not reflect bundle damage.

Thus, in this comparison, the type of stimulus, rather than its rise time, affected the measured properties of adaptation (shortening the rise time below 1 ms affects adaptation time course, below). During a fluid jet step, the bundle position depends on bundle stiffness, which may vary dynamically as a result of transduction and adaptation processes (Hudspeth et al. 2000). Note that any effects of such stiffness changes on bundle position are not shown in our stimulus traces, which represent the pressure output of the fluid jet calibrated by the steady-state bundle deflection recorded on videotape.

We made a simple mechanical model of the bundle similar to that of Assad and Corey (1992), with two parallel springs corresponding to the passive bundle springs and the gating springs and an adaptation motor in series with the gating spring (see METHODS). For a displacement step, the bundle displacement, $X_s$, is constant and the force across the gating springs, $F_G$, changes exponentially with the time constant of motor movement, $\tau_{XM}$ (Fig. 3D). Thus, for a step that is small relative to the instantaneous $I(X)$ relation, the open probability of the transduction channels and the transduction current decay with the same time constant, $\tau_{XM}$. For a force step, the total force, $F_T$, is constant but the motor movement reduces $F_G$, causing a proportional increase in the force across the
stereociliary pivots, $F_S$ (Eq. 8). As a consequence, $X_S = F_S/K_S$ increases with time, i.e., the bundle creeps forward (Fig. 3D). Change in $X_S$ changes the force across the gating spring (the input to the motor), so that the motor continues to move, slowing the rate of transduction current decay.

With this model, the ratio of the time courses of the motor movement in the force step and displacement step conditions equals the ratio of the summed stiffnesses over the stereociliary stiffness. Thus, for approximately equal stiffnesses, as found by Howard and Hudspeth (1988) and Ricci et al. (2002), the time course of the relaxation during a force step is twice that during a displacement step. The two-fold difference between our mean time constant for all cells and that obtained in the fluid jet study by Holt et al. (1997) is consistent, therefore, with the gating springs accounting for about half of the bundle stiffness. In the sample for which we have both fluid jet and stiff probe data, linear regression of stiff-probe $\tau_A$ against fluid-jet $\tau_A$ yields a slope of 0.3 (Fig. 3C). This slope and the ratio of the mean $\tau_A$ values (34/90) are consistent with the gating springs contributing almost two-thirds of the total bundle stiffness. In the simulation shown in Figure 3D, we set the time constant of the adaptation motor equal to the mean time constant of transduction current decay (30 ms; Table I). To triple the time constant of motor movement during a force step relative to a displacement step, we set $K_G = 2K_S$.

Another difference between the fluid-jet data and the stiff-probe data is the rounded onset of the fluid-jet response (Fig. 3A; see also examples in Holt et al. 1997, Fig. 2). In Géléoc et al. (1997), a similar rounded onset was seen in both the transduction currents and the hair bundle motion evoked by fluid-jet steps. Such rounding may reflect low-pass filtering of the force stimulus by passive bundle mechanics, such that bundle deflection takes longer than the fluid velocity step illustrated in the stimulus trace. In Géléoc et al. (1997), rounding of the onset response was much less evident for the stiffer bundles of mouse outer hair cells, consistent with a faster mechanical response time.

The nine cells in this study comprised five type I cells and four type II cells; seven were from the striolar region. In this small sample, as in the total data set (Table I), adaptation rates were similar for
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type I and type II cells. In contrast, in the fluid-jet study of Holt et al. (1998), only six of 14 type I cells showed any response decay during 100 - 300 ms steps, and for these six cells, the mean $\tau_A$ was very large (230 ± 39 ms). It is not clear why the two studies differ in this regard. The average postnatal ages of the type I cells were not significantly different (4.9 ± 0.6 days, $n = 18$, in the present study vs. 5.9 ± 0.6 days, $n = 14$ in Holt et al. (1998); $p = 0.3$); furthermore, $\tau_A$ did not change systematically from PO to P10 ($r^2 = 0.09$ for type I cells in the present study). While it is possible that the two studies sampled from different epithelial regions (region was not specified in the earlier study), there is no indication in our data of strong regional differences. Type I cells from the striola and extrastriola adapted with mean $\tau_A$ values of 26 ± 4 ms ($n = 10$) and 32 ± 8 ms ($n = 6$), respectively ($p > 0.4$). A third possibility is that distinct subsets of type I cells - e.g., with different bundle morphologies - were selected in the two studies.

A faster step revealed two components of transduction current decay

In hair cells, the fastest time constants of adaptation (<1 ms) have been measured in the turtle cochlea with a stiff probe with a rise time of about 100 $\mu$s (Ricci and Fettiplace 1997). To determine if our measured $\tau_A$ values were slowed by stimulus rise times in the 1-2 ms range, we built a stiff probe with a rise time of 200 $\mu$s. The decay of the transduction current was faster for the faster stiff probe. If we fit the response at $X_{1/2}$ with a single-exponential function, the mean $\tau_A$ was significantly shorter: 18 ± 3 ms ($n = 44$) vs. 30 ± 2 ms ($n = 36$) for the 1-ms probe (Table I) (data from different sets of experiments). This would not be expected for a single exponential process, and suggests that the faster probe revealed an additional fast component. Moreover, at $X_{1/2}$ the decay was better fit by a double-exponential function than by a single-exponential function in 90% (41/44) of cells, as judged by eye and by $\chi^2$ value (Fig. 4A). Although the increased number of free parameters would be expected to produce better fits, our data for small-to-intermediate stimuli showed a clear early fast component that is not
accommodated by single-exponential fits. At $X_{1/2}$, the average fast and slow time constants from the double-exponential fits, $\tau_{\text{fast}}$ and $\tau_{\text{slow}}$, were $5.2 \pm 0.7$ ms (range 0.8 - 22.8 ms) and $45.6 \pm 4.5$ ms (range 6.3 - 171.2 ms). Adaptation time course did not vary systematically with transduction current amplitude. Linear regressions of $\tau_A$ vs. $I_{\text{max}}$ had $r^2$ values of 0.028 (fast probe) and 0.023 (1-ms probe).

Because the faster probe completed the step faster than the time constant of the fast component, the average $I_{\text{max}}$ value obtained with the fast probe was significantly larger than that obtained with the 1-ms probe (Table I): $199 \pm 10$ pA vs. $155 \pm 16$ pA, $p = 0.02$. Assuming a reversal potential of $+3$ mV for the transduction channels (Kros et al. 1992), the mean $I_{\text{max}}$ for the 1-ms probe corresponds to an average maximum conductance ($g_{\text{max}}$) of $3.1 \pm 0.3$ nS, similar to that obtained by Géléoc et al. (1997) in their study of hair cells in cultured neonatal mouse saccules and utricles. For the single-channel conductance that Géléoc et al. (1997) calculated for mouse cochlear hair cells, 112 pS, the average $g_{\text{max}}$ corresponds to 28 transduction channels per bundle. The maximum transduction current we recorded was 450 pA, or 6.7 nS and 60 channels. For hair bundles of neonatal mouse saccules and utricles, Géléoc et al. (1997) found an average of 38 ± 2 stereocilia with 31 ± 2 potential tip links ($n = 15$ bundles). Thus, our average and largest peak currents correspond to 0.9 and 1.9 transduction channels per tip link, respectively.

We also examined the transduction currents evoked in frog saccular hair cells by the fast stiff probe for evidence of two decay components. Although a fast component has been described in responses to deflections imposed by flexible fibers (Benser et al. 1996; Howard and Hudspeth 1987), its time course has not been characterized for a displacement step. Again, at $X_{1/2}$, double-exponential fits of transduction current decays were better than single-exponential fits in four of the five cells tested (Fig. 4B). At $X_{1/2}$, the mean time constant from the single-exponential fit, $\tau_A$, was $3.0 \pm 0.8$ ms, and the mean $\tau_{\text{fast}}$ and $\tau_{\text{slow}}$ values were $2.3 \pm 1.0$ ms and $18.1 \pm 3.9$ ms. The slow component has a similar time course to the adaptation previously reported for the responses of frog saccular hair cells to bundle displacement (20 -
30 ms; Shepherd and Corey 1994). In our experiments, the use of a faster probe revealed an additional fast component.

As in the turtle cochlea (Wu et al. 1999), the fast component was most prominent for small displacements in both frog and mouse hair cells. This tendency can be seen in Figure 4A, where the largest step has a negligible fast component. Figure 5 shows the effects of bundle displacement on the time constants (A), the size of the slow component as a fraction of the total decay (B), and the percent decay (C). Note that the displacement axis is three-fold broader for the mouse data, reflecting the relative sizes of the operating ranges expressed in terms of bundle displacements part-way up the rake of the bundle (Fig. 1). In both cells, the fractional amplitude of the slow component grew from 0.1-0.2 for the smallest step to 1 for the largest steps (Fig. 5B).

Percent shift of the operating range increases with adapting step size in mouse utricular hair cells

With increasing displacement, $\tau_{\text{fast}}$ decreased, $\tau_{\text{slow}}$ increased and the percent decay at steady state decreased markedly in frog saccular hair cells (Fig. 5A,C). Similar changes in $\tau_{\text{slow}}$ and percent decay with step size occur in turtle cochlear hair cells (Wu et al. 1999). This can be understood in terms of the slow adaptation process. In frog hair cells, the adaptive shift of the instantaneous I(X) relation (Fig. 2C) is a constant 80% of the adapting step (Shepherd and Corey 1994). As shown schematically in Figure 6A, as adapting step size increases (arrows), the 80% shifted operating range lies increasingly negative to the imposed bundle position. As a result, the percent decay of the transduction current decreases with adapting step size (Figs. 5C, 7A). For very large steps (not shown), the 80% shift is not large enough to bring the $\text{OR}_{\text{inst}}$ into register with the new bundle position, and therefore there is no decay in the transduction current.

Figure 6A shows schematically how a constant percent shift produces a steady-state I(X) relation (grey curve) that is a stretched version of the instantaneous I(X) relation (Shepherd and Corey 1994). We
confirmed this behavior in five frog saccular hair cells (Fig. 7A). The mean $OR_{\text{inst}}$ was $280 \pm 32$ nm. To fit the steady-state $I(X)$ relations, the instantaneous relations were stretched by $s = 5.6 \pm 1.0$ (Eq. 4). This corresponds to a percent shift of $82 \pm 2\%$ (Eq. 5), in agreement with the $80\%$ reported by Shepherd and Corey (1994).

In mouse utricular hair cells, the average percent shift of the instantaneous $I(X)$ relation was $86\%$ (Table I). If the mouse cells behaved like frog cells, then the steady-state $I(X)$ relations would be fit by stretching the instantaneous $I(X)$ relation $\sim 7$-fold (Eq. 5). Instead, the mean steady-state operating range was not significantly larger than $OR_{\text{inst}}$: $1181 \pm 224$ nm vs. $762 \pm 49$ nm ($n = 37; p = 0.06$). This reflects the fact that fewer than half of the mouse steady-state $I(X)$ relations could be fit by stretching the instantaneous $I(X)$ relations. Two examples that could be fit are shown in Figure 7B. In many of these cases, the steady-state currents were so small that very large stretch factors ($>20$) were required (Fig. 7B, right panel). In contrast, in $26/37 (70\%)$ of the mouse cells, the steady-state $I(X)$ relations saturated at current levels far below the $I_{\text{max}}$ values of the instantaneous $I(X)$ relations (Fig. 7C). Correspondingly, the percent decay did not decrease with increasing displacement; in some cases, it increased with displacement (see the mouse hair cell data in Fig. 5C).

Because frog and mouse hair cells differ in how percent decay changes with adapting step size, we investigated whether percent decay in mouse cells follows directly from percent shift of the instantaneous operating range ($OR_{\text{inst}}$) as it does in frog cells. Figure 8B shows that this is the case. Figure 8B also shows, however, that the percent shift increased with step size ($4\%$ per $OR_{\text{inst}}, r^2 = 0.51$; Fig. 8B,C). This is in contrast to the frog data, where the percent shift was constant ($80\%$) over a large range of step sizes (Shepherd and Corey 1994). (For mouse data obtained with the slower probe, which covered a smaller range of step sizes ($0.4 - 1.4 \times OR_{\text{inst}}$; Table I), the percent shift also tended to increase with step size: $12\%$ per $OR_{\text{inst}}, r^2 = 0.19$.) Figure 6B shows schematically how a percent shift that increases with displacement affects the steady-state operating range. This explains why steady-state $I(X)$ relations of
mouse cells were not fit by stretching the instantaneous I(X) relations (Fig. 7C) and why the percent decay did not decrease with step size (Fig. 5C): the OR$_{\text{inst}}$ shifted to align with the new bundle position even for very large adapting steps.

For very large adapting steps, small reductions in $I_{\text{max}}$ may also contribute to compression of the steady-state I(X) relation. For five cells in which instantaneous I(X) relations were measured in the presence of multiple adapting steps, the mean instantaneous $I_{\text{max}}$ for the largest adapting steps (mean size = $(3.8 \pm 0.7) \times \text{OR}_{\text{inst}}$) was $90.58 \pm 0.01\%$ of the initial (pre-adapted) instantaneous $I_{\text{max}}$. In Figure 8B, there was a 12% decrease in $I_{\text{max}}$ for the largest (2.7-µm) step. In contrast, there was no compression for adapting steps the size of the instantaneous operating range (mean $(1.0 \pm 0.2) \times \text{OR}_{\text{inst}}$); for these, the mean instantaneous $I_{\text{max}}$ was $99 \pm 1\%$ of the pre-adapted instantaneous $I_{\text{max}}$.

In addition, for very large adapting steps, reductions in the slope of the instantaneous I(X) relation contribute to compression of the steady-state I(X) relation. For steps over twice the initial OR$_{\text{inst}}$, OR$_{\text{inst}}$ increased by $28 \pm 12\%$ (range: 5 to 77%). For example, in Figure 8B, the instantaneous I(X) relations obtained initially and after adaptation to the largest step had OR$_{\text{inst}}$ values of 0.99 µm and 1.75 µm, respectively. The broader OR$_{\text{inst}}$ reflects a more gradual positive saturation and more current at displacements negative to the adapting displacement. Similar shape changes were seen in the OR$_{\text{inst}}$ of an isolated frog saccular hair cell when its bundle was subjected to a large adapting step by a stiff probe (Assad and Corey 1992; their Fig. 11C). Large adapting steps delivered by fluid jet also caused broadening and compression of OR$_{\text{inst}}$ in a previous study of mouse utricular hair cells (Holt et al. 1997). Thus, large displacements may recruit distinct processes that affect transduction.

Figure 9 compares the instantaneous and steady-state operating ranges of a mouse utricular hair cell and a frog saccular hair cell. What stands out is the high instantaneous sensitivity and small OR$_{\text{inst}}$ of the frog saccular bundle. *In vivo*, the stimulus is likely to be applied near the tip of the bundle, so that the operating range as we measure it is relevant to thinking about the normal input to the hair cells. The high
instantaneous sensitivity of the frog saccular hair cells befits their role in detecting minuscule, high-
frequency substrate vibrations (Narins and Lewis 1984). The utricular afferents of mammals are reported
to be insensitive to vibrations (Fernández and Goldberg 1976). The difference in instantaneous
sensitivity between the frog and mouse hair cells may reflect differences in bundle geometry more than
intrinsic differences in the gating springs or channel sensitivity (Géléoc et al. 1997; Holt et al. 1997). For
a given displacement, $X$, at a height above the base of the bundle, $h$, the extension of tip links is
approximated by $X \gamma$, where $\gamma = (\text{inter-stereociliary distance})/h$. For neonatal mouse utricular hair bundles,$\gamma$ has been estimated as 0.047 (Holt et al. 1997), just one-third its average value in frog saccular hair cells
(0.14; Jacobs and Hudspeth 1990). This difference can therefore account for the 2.9-fold difference in
our mean OR$_{\text{inst}}$ values for mouse utricular and frog saccular hair cells: 749 ± 59 nm ($n = 31$; Table I) and
280 ± 32 nm ($n = 5$). To convert to angular deflections, we assume that the probe was half-way up the
bundle and use average bundle heights of 6.7 µm (frog saccule; Jacobs and Hudspeth 1990) and 13.2 µm
(mouse utricle; Holt et al. 1997), yielding operating ranges of 4.8° and 6.5° angular deflection,
respectively.

Our estimate for mouse utricular bundles is more than three times the estimate by Géléoc et al.
(1997) for mouse utricular and saccular bundles stimulated with fluid jets (2.0 ± 0.6°, $n = 5$). The mean
bundle height in their sample (9.3 µm) is shorter than the value we used (13.2 µm), possibly because they
included saccular bundles in their study. Our estimates will err on the large side if our probe was
positioned more than half-way up the bundle. The mean OR$_{\text{inst}}$ that we obtained with the fluid jet
(1996 ± 436 nm, $n = 8$) was about twice that obtained with the stiff probe (1010 ± 173 nm, $n = 9$).
Since we measured fluid-jet displacement by focusing on the bundle tip, this comparison suggests that
the stiff probe was about half-way up the bundle. But the displacements used to measure OR$_{\text{inst}}$ for the
fluid jet were actually steady-state displacements (see METHODS), which may have included significant
forward creep relative to the onset displacement as a result of adaptation (see Fig. 3D). Correcting for
such a creep would place the probe closer to the bundle tip and reduce the instantaneous operating range by as much as one-third.

**DISCUSSION**

*Comparison with afferent data*

Adaptation is sensitive to stereociliary Ca\(^{2+}\) levels and presumably to temperature. In our experiments, the bundles were bathed in 1.3 mM Ca\(^{2+}\) and the pipette solution contained 10 mM EGTA. Endolymphatic Ca\(^{2+}\) is on the order of 10-100 µM (Bosher and Warren 1978; Corey and Hudspeth 1983; Crawford et al. 1991) and stereociliary Ca\(^{2+}\) buffering may be equivalent to 1 mM BAPTA or less (Roberts 1993; Ricci et al. 1998). The low external Ca\(^{2+}\) in vivo might be expected to substantially slow the Ca\(^{2+}\)-dependent adaptation processes. For low-frequency turtle cochlear hair cells, adaptation rates in in vivo-like conditions (70 µM apical Ca\(^{2+}\) and endogenous Ca\(^{2+}\) buffering) were one-third those obtained in conditions similar to ours (1 mM apical Ca\(^{2+}\) and 10 mM EGTA in the pipette) (Ricci et al. 1998). In mouse utricular hair cells, however, these effects may be offset in vivo by the effects of increased temperature on adaptation rate.

The slow adaptation that we have measured is roughly consistent with the frequency dependence of responses from chinchilla utricular afferents with irregular and intermediate spontaneous discharge (Goldberg et al. 1990a), which tend to innervate the striolar and juxtastriolar zones of the macula (Goldberg et al. 1990b). For such afferents, the response gain rises from its steady-state value to a value 2-10-fold higher at 2 Hz, with no sign of leveling off. Both the steady-state response and the rise in gain with frequency up to 2 Hz are expected from our hair cell data. The steady-state transduction current for the mouse utricular cells was about 25% of the peak current (Table I). Such currents can strongly depolarize type II hair cells, even after activation of their outwardly rectifying K\(^{+}\) conductances (Rüsch and Eatock 1996b; Fig. 3 in Eatock 2000; M.A. Vollrath and R.A. Eatock, unpublished results). Therefore,
the fully adapted current is adequate to carry low-frequency signals.

The response gains of afferents that innervate the extrastriola are constant with head movement frequency up to 2 Hz (Goldberg et al. 1990b). We therefore hypothesized that a population of extrastriolar hair cells would not adapt to bundle deflections. Instead, we found that virtually all hair cells adapted, with no systematic differences in transduction and adaptation properties between hair cells of different type or location. There are at least two kinds of explanation.

First, the regional variation in afferent data may arise at sites other than the transduction channel, including bundle mechanics and voltage-gated conductances. Bundle morphology varies with hair cell type and location in mammalian vestibular epithelia (Bagger-Sjöbäck and Takumida 1988; Denman-Johnson and Forge 1999; Lim 1976; Rüschi et al. 1998;), and the otoconia and otolithic gel of the striola and extrastriola differ (Lindeman 1973; Xue and Peterson 2002). The mechanical consequences of these regional variations would not be revealed in experiments in which bundles are deflected by rigid probes. There is also regional variation in the outwardly rectifying $K^+$ currents that repolarize the type II hair cell’s membrane following a depolarizing current step: inactivation kinetics are faster in the extrastriola than in the striola (Holt et al. 1999; M.A. Vollrath and R.A. Eatock, unpublished observations). Inactivation of outward $K^+$ current may offset adaptation of the transduction current, such that the receptor potential adapts less than the transduction current.

The second kind of explanation is that regional variation in mechanotransduction does occur in vivo, but that the conditions of our experiments did not reveal it. Possible factors include:

1) The immaturity of our preparation: The first week or so after birth is a time of dramatic changes in bundle morphology (Denman-Johnson and Forge 1999), $Ca^{2+}$-binding proteins (Dechesne et al. 1993, 1994; Rüschi et al. 1998) and voltage-gated conductances of rodent vestibular hair cells (Rüschi et al. 1998). Although we did not record from hair cells with frankly immature bundles, it remains possible that the mechanosensitive apparatus had not fully differentiated in our preparation.
2) Whole-cell recording conditions: The ruptured-patch recording configuration may eliminate between-cell variations that arise through different concentrations or types of intracellular Ca\textsuperscript{2+} buffers. Ca\textsuperscript{2+} concentration inside the stereocilia sets the rate of adaptation and other properties such as the slope of the I(X) relation and the resting open probability of the transduction channel (Assad et al. 1991; Crawford et al. 1991; Eatock et al. 1987; Ricci et al. 1998). In vestibular epithelia, Ca\textsuperscript{2+} binding proteins vary with hair cell type, location and stage of development (Baird et al. 1997; Dechesne et al. 1994). At present the properties of Ca\textsuperscript{2+} buffers in mouse vestibular hair cells are not known. Previous experiments on mouse vestibular hair cells used lower EGTA concentrations in the internal solution, for estimated free Ca\textsuperscript{2+} concentrations of 170 nM (Holt et al. 1997, 2002) and 10 nM (Géléoc et al. 1997), vs. 710 pM in our experiments and 130 pM in the experiments on frog saccular hair cells (Shepherd and Corey 1994). Evidence from turtle cochlear hair cells (Ricci et al. 1998) suggests that the fast component is relatively impervious to the pipette’s Ca\textsuperscript{2+} buffer, possibly because it is mediated by Ca\textsuperscript{2+} binding on or close to the channel. The adaptation time constants of turtle cochlear hair cells, which appear to principally reflect the fast component, are similar for 1 and 10 mM EGTA, 0.1 mM BAPTA and the endogenous buffer as measured in perforated-patch whole cell recordings. The slow component, however, may be mediated by Ca\textsuperscript{2+} binding at a more distant site from the channel (e.g., on calmodulin associated with myosin) and therefore may be more sensitive to changes in internal Ca\textsuperscript{2+} buffers.

3) The stimulation method: The otolithic gel layer that normally delivers the stimulus to hair bundles is likely to be less stiff than the probes used in our experiments (Benser et al. 1993). Thus, the transduction current decay may be slowed by micromechanical rearrangements within the bundle (reflecting gating, Ca\textsuperscript{2+} re-closure and myosin-dependent movements of the gating springs; see Hudspeth et al. (2000) for a review). As discussed next, such an effect is seen upon switching from a stiff probe to a fluid jet.
Fluid jet vs. stiff probe

Use of the fluid jet increased $\tau_A$ about three-fold over its value with the stiff probe in the same cell (Fig. 3C). The critical difference is not rise time, and therefore is likely to be that the fluid jet delivers a force step rather than a displacement step. Howard and Hudspeth (1987) showed that slow adaptation to a positive bundle deflection is accompanied by a decrease in bundle stiffness, and argued that the loss of stiffness reflects the relaxation of force across the gating springs during adaptation. When the bundle is coupled to a relatively flexible glass fiber, which is then stepped to a new position, the bundle first jumps forward, reflecting the instantaneous bundle stiffness, then 'creeps' further forward with the same time course as slow adaptation reduces the tension on the gating springs. A similar result may be expected in the case of the fluid jet, and indeed, such forward creep is evident in records of mouse vestibular bundle displacement during fluid-jet steps (Géléoc et al. 1997; their Fig. 2). The corresponding transduction currents did not decay, but the lack of creep in the transduction current indicates that some adaptation occurred. Moreover, the 50-ms steps may have been too short to clearly reveal slow adaptation, the only type that we saw with fluid-jet stimuli. This is illustrated in the fluid-jet responses of Figure 3: While decay of the transduction current is clear when 320 ms of the step response is shown (Fig. 3A), it is not at all obvious over the first 50 ms (Fig. 3B).

In our cells, the use of a force step rather than a displacement step tripled the mean time course of transduction current decay. For a simple two-spring model of the bundle, this result is consistent with gating spring stiffness being two-thirds of the total bundle stiffness. The one cell in our sample for which the fluid-jet $\tau_A$ did not exceed the stiff-probe $\tau_A$ had a relatively fast $\tau_A$ (Fig. 3C). It is possible that in this one case the fast adaptation component dominated, so that there was little myosin-mediated creep.

Two components of transduction current decay
In most mouse utricular hair cells, the fast probe revealed two kinetic components of decay in response to step displacements, which may correspond to the fast and slow processes that have been identified in other hair cells. The slow component has the same time course as a component that was recently shown to be mediated by myosin-1c (Holt et al. 2002). The faster component in mouse utricular cells resembles the fast adaptation in the turtle cochlea and the ‘twitch’ of the frog saccular bundles in two ways: it occurs on a similar time scale and is most prominent for small displacements. In turtle, this process does not depend on myosin motors (Wu et al. 1999) and is postulated to reflect Ca$^{2+}$-mediated re-closure of transduction channels (Crawford et al. 1989; Fettiplace et al. 2001). Ricci et al. (2002) found evidence that the relative importance of the two components is influenced by the steady position of the turtle bundle. Depolarization evokes bundle movements by changing intracellular Ca$^{2+}$. At the resting bundle position, depolarization evoked forward movements, reflecting the fast Ca$^{2+}$ feedback process. In the presence of a steady bias of the bundle toward the kinocilium, this movement reversed and slowed, consistent with the slow Ca$^{2+}$ feedback process.

In frog, it has been proposed that the twitch is mediated either by Ca$^{2+}$-mediated channel re-closure (Choe et al. 1998; Howard and Hudspeth 1987) or by an interaction between channel gating and the same myosin motors that are believed to mediate slow adaptation (Martin et al. 2000). The latter possibility was ruled out by the results of Holt et al. (2002), who blocked myosin-1c in mouse utricular hair cells. This eliminated slow adaptation but revealed a fast component in a subset of cells, with the same time constant as the fast component that we have measured. Thus, mouse utricular hair cells have a fast component that is independent of the slow adaptation motor.

In turtle cochlea and frog saccule, one or both processes tune the transduction current to the acoustic and vibrational best frequencies, respectively, of the hair cells (Fettiplace et al. 2001; Hudspeth et al. 2000). With intracellular and apical Ca$^{2+}$ at in vivo levels, both fast and slow components can participate in resonances (Benser et al. 1996; Martin et al. 2000; Ricci et al. 1998,
2000). The $\tau_{\text{fast}}$ and $\tau_{\text{slow}}$ values that we measured in frog saccular hair cells correspond to low-pass corner frequencies of about 80 and 8 Hz, respectively, within the range of best frequencies of frog saccular afferents (Narins and Lewis 1984). With similar methods, we measured $\tau_{\text{fast}}$ and $\tau_{\text{slow}}$ values in mouse utricular hair cells corresponding to corner frequencies of about 30 and 3.5 Hz. While in vivo conditions may change these time courses, it is not clear in which direction (see discussion of temperature and Ca$^{2+}$ effects, above).

The slow component as measured is clearly in the frequency range of head movements (Wilson and Melvill Jones 1979), while the fast component is above the frequency range traditionally associated with the vestibular system. There is, however, energy at frequencies up to 20 Hz in human angular head movements during natural locomotion (Grossman et al. 1988). Recent work on otolith-driven and canal-driven reflexes in monkeys (Angelaki 1998; Huterer and Cullen 2002; Minor et al. 1999) and the responses of chinchilla canal afferents (Hullar and Minor 1999), however, has revealed robust vestibular afferent signals for stimuli up to 25 Hz. A resonance in the hair cell transduction process at some tens of Hertz may serve to amplify stimuli that are low-pass filtered by the inertia of the head.

**Extent of adaptation as a function of step size**

The resting tension on the mechanosensitive channel, and therefore open probability, has been modeled as a Ca$^{2+}$-regulated balance between slipping and climbing of a transduction complex (gating spring, channel and adaptation motor) relative to the actin core of the stereocilium (Assad and Corey 1992; Howard and Hudspeth 1987). This motor model explains the shift of the instantaneous operating range in the direction of the applied step. In order to explain why the shift in frog saccular hair cells is about 80% of the applied step rather than 100%, Shepherd and Corey (1994) modified the model by adding a linear extent spring in parallel with the adaptation motor. Because the shift is a
constant percentage of the adapting step in the frog cells, the stiffness of the extent spring was assumed constant for adapting steps of different size.

In most mouse utricular hair cells, in contrast, the percent shift of the instantaneous $I(X)$ relation increased with large steps (Fig. 8C). As a result, mouse utricular hair cells can report novel stimuli over a very broad range of background stimuli (Fig. 6B). This result is accommodated by modifying the Shepherd and Corey (1994) model such that the stiffness of the extent spring decreases as steps get larger. Such nonlinear behavior is seen in titin, the giant elastic protein that dominates myocardial passive stiffness (for review, see Granzier and Labeit 2002). Another possible mechanism is tilting of the cuticular plate, a structure just below the apical surface of the hair cell into which the stereocilia insert. Each stereocilium is anchored in the cuticular plate via a subset of its actin filaments (the stereociliary ‘rootlet’). The cuticular plate contains filamentous actin, at cross angles to the rootlets (DeRosier and Tilney 1989), and other proteins, including myosins (Dumont et al. 2002; Hasson et al. 1997). Transduction stimuli lead to increases in $\text{Ca}^{2+}$ at the base of the bundle (Ohmori 1988), which might interact with cuticular plate proteins to change the attitude or stiffness of the hair bundle.
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Table I. Parameters of adaptation and the instantaneous I(X) relation did not vary with region or cell type.

<table>
<thead>
<tr>
<th></th>
<th>ADAPTATION</th>
<th>Instantaneous I(X) RELATION</th>
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<tr>
<td></td>
<td>$\tau_A$ (ms)</td>
<td>% Decay</td>
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<td><strong>REGIONAL COMPARISON</strong></td>
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<tr>
<td>Extrastriolar</td>
<td>32 ± 5 (10)</td>
<td>72 ± 7 (10)</td>
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<tr>
<td>Striolar</td>
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<td>78 ± 3 (18)</td>
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<td><strong>CELL TYPE COMPARISON</strong></td>
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<td>Type I</td>
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<tr>
<td>Type II*</td>
<td>31 ± 4 (18)</td>
<td>79 ± 3 (18)</td>
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<tr>
<td>TOTAL</td>
<td>30 ± 2 (36)</td>
<td>76 ± 3 (36)</td>
</tr>
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Mean ± SEM (Number of cells); * includes immature hair cells (see METHODS). $P_0$: current at the resting position of the hair bundle as a fraction of the total transduction current. $\text{OR}_{\text{inst}}$: For the instantaneous I(X) relation, the range of bundle displacements corresponding to the current range from 10% to 90% of $I_{\text{max}}$. 
FIGURE LEGENDS

FIG. 1. Recording transduction current from a type I hair cell in the mouse utricle.  
A: About 10 hair bundles in the striolar region are visible; the surface of the utricular epithelium is not flat, so that the focus is at the base of bundles on the left and near the tip of bundles at the right.  
B: Stimulus probe in contact with the short edge of the hair bundle of a type I cell.  Deflections toward the tall edge of the bundle (toward the top of the page) increase the open probability of the transduction channels.  
C: Focusing deeper into the epithelium reveals the nucleus of the type I cell.  
D: A patch pipette on the same hair cell.  Recordings were made in the whole-cell ruptured-patch mode.  
E: The stalk of the calyx below the hair cell.  
F: Schematic of a profile view of a type I hair cell, with recording pipette and stimulus probe in place.

FIG. 2. The effects of adaptation on step responses and instantaneous I(X) relations in mouse utricular hair cells.  Transduction currents were elicited with a stiff probe with a 1-ms rise time.  
A: Transduction currents (top) evoked by a family of 350-ms step deflections of the hair bundle (bottom).  The current trace corresponding to half-maximal activation of the transducer current (at X_{1/2}) is fit with a single-exponential function (solid grey line; Eq. 1;  \tau_A = 25 \text{ ms}, A = -449 \text{ pA}, I_{ss} = -18.1 \text{ pA}; \% \text{ decay} = 84\%).  Scale bar: 100 \text{ pA}.  
Cell 000417A; striolar, type I, P7.  
B: Protocol to test the effects of adaptation on the instantaneous I(X) relation.  Families of test steps were delivered before, and 250 ms after, the beginning of an adapting step.  Scale bar: 200 \text{ pA}.  
Cell 000120D; extrastriolar, type II, P1.  
C: Instantaneous I(X) relations were made from the peak currents (in B) evoked by test steps before (\blacktriangle) and during (\bigcirc) the adapting step, and fit with a 2^{nd}-order Boltzmann function (Eq. 3; solid curves).  The instantaneous I(X) relation shifted by 89\% of the 400-nm adapting step (arrow).  P_0 = 0.13; I_{max} = 405 \text{ pA}; \text{ operating range, OR}_{inst} = 568 \text{ nm}.  


FIG. 3. Measured adaptation is faster when bundles are deflected by a stiff probe than by a fluid jet. **A, B:** Transduction currents evoked by a series of four different bundle deflections, shown at two time scales. The bundle deflections (**B, bottom panel**) were all $+1.2 \, \mu m$ and were applied in the following order: stiff probe, 1-ms rise time; stiff probe, 2-ms rise time; fluid jet, 2.7-ms rise time; stiff probe, 1-ms rise time. Fits of each response with Eq. 1 (not shown) yielded $\tau_A$ values of 31, 29, 65 and 36 ms, respectively. The step waveforms show, for the stiff probe, the voltage input to the piezoelectric bimorph on which the probe is mounted; and for the fluid jet, the voltage output of the strain gauge at the back of the stimulus pipette. Displacement values were determined by off-line calibration, from videotaped images, of the voltage dependence of steady-state displacements of the rigid stimulus probe or, in the case of the fluid jet, the hair bundle. **Cell 000524C, type I, P1. C:** Comparison of $\tau_A$ values obtained with the fluid jet to those obtained with a stiff probe, in 9 cells. $\tau_A$ values for the two rise times of the stiff probe were similar in all cells (1 cell had only 1-ms stiff probe data), but those for the fluid jet were clearly longer in 7 of 9 cells. **Dashed line:** Slope = 1. **Solid line:** Linear fit of $\tau_A$ (2-ms probe) as a function of $\tau_A$ (fluid jet), constrained to go through the origin: slope = 0.31, $r^2 = 0.65$. **D:** Modeling the different bundle motions during steps delivered by a stiff probe (displacement step) and a fluid jet (force step). **Top,** Schematic showing the hair bundle modeled as two parallel springs with stiffnesses $K_S$ and $K_G$, corresponding to the stereociliary pivots and the gating springs, respectively (see METHODS). $K_G$ is in series with an adaptation motor that operates with a time constant $\tau_{XM}$. $X_S$ and $X_M$ are the displacements of the hair bundle tip and the motor, respectively. The motor moves until a target force ($F_{G,\infty}$) is established across the gating springs. **Middle and bottom panels,** Positions of the hair bundle motor, $X_M$, and the bundle, $X_S$, as functions of time following a displacement step (black line) and a force step (thick grey line). Arbitrary units. For the displacement step, the motor approaches its steady state position with its intrinsic time constant ($\tau_{XM} = 30 \, ms$). During the force step, the movement of the adaptation motor changes $X_S$ with time, which feeds back onto the adaptation motor, slowing the approach of $X_M$ to steady state. Model parameters: $F_T = 1; \Delta t = 1 \, ms, \, F_{G,\infty} = 0.2, \, K_S = 1, \, K_G = 2,$ and
\[ \tau_{XM} = 30 \text{ ms.} \] In this simulation, the time constant of the motor movement is tripled for a force step (90 ms).

**FIG. 4.** Use of a fast probe (~200 \( \mu s \) rise time) revealed two kinetic components of adaptation in hair cells from the mouse utricle (A) and the frog saccule (B). Transduction currents were evoked by three different displacements. Double-exponential fits (Eq. 2, red lines) to the decay of transduction current are superior to single-exponential fits (Eq. 1, cyan lines) (see \( \chi^2 \) values given with fit parameters, below). In each case, \( \tau_{\text{fast}} \) and \( \tau_{\text{slow}} \) differ by an order of magnitude or more. *Scale bars:* 5 ms, 50 pA.

Fit values:  
**A:** Mouse utricular hair cell 010330D, type II, P3.  
*Small step:* Single-exponential fit: \( \tau_A = 9.3 \) ms, \( \chi^2 = 6.9 \). Double-exponential fit: \( \tau_{\text{fast}}, \tau_{\text{slow}}; 1.3, 29.1 \) ms; amplitude ratio \( [A_{\text{slow}}/(A_{\text{slow}} + A_{\text{fast}})] \) 0.38, \( \chi^2 = 3.2 \).  
*Intermediate step:* Single-exponential fit: \( \tau_A = 12.5 \) ms, \( \chi^2 = 16.5 \). Double-exponential fit: \( \tau_{\text{fast}}, \tau_{\text{slow}}; 1.3, 22.9 \) ms; amplitude ratio 0.44, \( \chi^2 = 3.5 \).  
*Large step:* Single-exponential fit: \( \tau_A = 28.9 \) ms, \( \chi^2 = 8.8 \). Double-exponential fit: \( \tau_{\text{fast}}, \tau_{\text{slow}}; 1.3, 30.4 \) ms; amplitude ratio 0.87, \( \chi^2 = 7.5 \).

**B:** Frog saccular hair cell 000803F.  
*Small step:* Single-exponential fit: \( \tau_A = 3.6 \) ms, \( \chi^2 = 10.3 \). Double-exponential fit: \( \tau_{\text{fast}}, \tau_{\text{slow}}; 0.9, 8.2 \) ms; amplitude ratio 0.12; \( \chi^2 = 5.9 \).  
*Intermediate step:* Single-exponential fit: \( \tau_A = 2.1 \) ms, \( \chi^2 = 28.7 \). Double-exponential fit: \( \tau_{\text{fast}}, \tau_{\text{slow}}; 1.2, 18.0 \) ms; amplitude ratio 0.56, \( \chi^2 = 6.9 \).  
*Large step:* Single-exponential fit: \( \tau_A = 8.4 \) ms, \( \chi^2 = 18.7 \). Double-exponential fit: \( \tau_{\text{fast}}, \tau_{\text{slow}}; 0.9, 9.8 \) ms; amplitude ratio 0.64, \( \chi^2 = 10.9 \).

**FIG. 5.** Changes in the properties of adaptation with step size, for the frog and mouse hair cells of Figure 4. Step responses to different bundle displacements were fit with a double-exponential function (Eq. 2). Note that the displacement scale for the frog cell is one-third that for the mouse cell.  
**A:** For both hair cells, \( \tau_{\text{fast}} \) decreased with increasing displacement; at \( X > X_{1/2} \), where the fast component was smaller than the slow component (see B), we constrained \( \tau_{\text{fast}} \) to be \( \geq 1 \) ms (Wu et al. 1999). For the frog cell, but not the mouse cell, \( \tau_{\text{slow}} \) increased with step size.  
**B:** The amplitude of the slow
component relative to the total amplitude of both components increased with step size, for both mouse and frog data. The relations were fit with a single-order Boltzmann function. C: Percent decay of the transduction current at steady state. With increasing step size, percent decay decreased for the frog cell, from ~90% to ~60%, and increased for the mouse cell, from ~70% to ~80%. Fit values at $X^{1/2}$: Mouse cell: $\tau_{\text{fast}} = 0.9$ ms, $\tau_{\text{slow}} = 39.2$ ms, % decay = 58.4. Frog cell: $\tau_{\text{fast}} = 1.0$ ms, $\tau_{\text{slow}} = 7.9$ ms, % decay = 84.6.

Fig. 6. Schematics showing the steady-state I(X) relation (thick grey curve) if an adapting step causes a pure shift in the instantaneous I(X) relation (black curves) that is either (A) a constant percentage of the adapting step, as in frog hair cells or (B) an increasing percentage of the adapting step, as in many mouse hair cells. Instantaneous relations are shown from the resting bundle position (thick black curve) and after shifting (thin black curves) in response to adapting steps of the amplitudes shown by the arrows below. As shown in B, even a modest increase in the percent shift, from 80% to 90%, significantly compresses the steady-state I(X) relation. Note that in both A and B, the smallest adapting step lies in the most sensitive part of the shifted OR$_\text{inst}$ (left dashed lines). The largest steps, however, lie near the middle of the shifted OR$_\text{inst}$ in B but near the positive saturation of the shifted OR$_\text{inst}$ in A (dashed lines). Thus, only the cell in B is sensitive to deflections superimposed on the largest adapting steps.

Fig. 7. Comparison of instantaneous and steady-state I(X) relations in mouse and frog hair cells. Frog steady-state relations (A) and some mouse steady-state relations (B) can be fit be a stretched version of the instantaneous I(X) relation, as described for frog saccular hair cells by Shepherd and Corey (1994). This does not work, however, for most steady-state relations in mouse cells (C). A-C: The middle panels of instantaneous (■) and steady-state (○) I(X) relations were generated from the transduction current data shown in the left panels (plus additional traces). The right panels shows instantaneous and I(X) relations from an additional hair cell of each type. Solid lines are fits. The instantaneous I(X) relations
were fit (black lines) with Eq. 3. The steady-state I(X) relations were fit (grey lines) with Eq. 4, using parameter values obtained by fitting the instantaneous I(X) relation from the same cell, and allowing just the stretch factor, s (Eq. 5), to vary. A: Steady-state I(X) relations from two frog hair cells have s values of 5 (left) and 7.4 (right). Cells 000816B and 000803F (same frog cell as in Figs. 4, 5). B: Steady-state relations of two mouse hair cells that were fit by stretching the I(X) relation have s values of 4.7 (left) and 23 (right). Large s values, found in mouse but not frog data, correspond to large percent shifts (Eq. 5); s = 23 corresponds to a shift that is 96% of the adapting step. Cells 011106D, type II, P3; 011115F, extrastriolar, P1. C: Data from two mouse cells for which steady-state I(X) relations could not be fit by stretching the instantaneous I(X) relations. Cells 010330D, extrastriolar, type I, P4; 010329B, type II, P3.

FIG. 8. The shift of the instantaneous I(X) relation, expressed as percentage of the adapting step, increased with size of the adapting step. The data in A, B are from a mouse crista hair cell (Cell 020206I, P4). Similar results with fewer step sizes per hair cell were obtained from several utricular hair cells, as shown in C. A: Transduction currents evoked by a family of steps. B: A steady-state I(X) relation (grey circles), generated from the data in (A), plus 5 instantaneous I(X) relations: one from the resting bundle position (black squares) and the others (open symbols) from test step responses generated near the end of 4 different 250-ms adapting steps (see step protocol in Fig. 2B). The adapting steps (arrows above the X-axis) were 0.3, 0.8, 1.7 and 2.7 μm. Solid curves show the second-order Boltzmann fit of the instantaneous relation at the resting position, plus the same curve shifted by 0.2 μm (67% of the adapting step), 0.6 μm (75%), 1.4 μm (82%) and 2.5 μm (93%) to line up with the instantaneous relations measured during each adapting step. Note that for a particular adapting step size, steady-state current equals the current at that displacement in the shifted instantaneous I(X) relation (see black circles at the intersection s of the instantaneous and steady-state curves). Thus, the shape of the steady-
state relation is largely predicted by the shift in the instantaneous relation. The instantaneous relation for the largest adapting step (□), however, differs in shape from the relations for the smaller adapting steps and has a 12% smaller $I_{\text{max}}$ and 77% broader $\text{OR}_{\text{inst}}$. $C$: The percent shift of the instantaneous $I(X)$ relation, normalized to $\text{OR}_{\text{inst}}$, for adapting steps of different amplitudes, in 5 mouse vestibular hair cells (4 utricular cells plus the crista cell from $A$, ■). On average, the percent shift increased with step size by 4% per $\text{OR}_{\text{inst}}$ (from the linear regression, thick grey line; $r^2 = 0.51$).

**Fig. 9.** Comparison of instantaneous and steady-state $I(X)$ relations for a frog saccular hair cell and a mouse utricular hair cell. Currents are normalized to the peak instantaneous current and fit with second-order Boltzmann functions (solid curves; Eq. 3). The instantaneous and steady-state operating ranges are 235 and 1166 nm for the frog cell, and 1015 and 950 nm for the mouse cell. Data collected with the fast probe. *Frog saccular cell 000816B; mouse utricular cell 010329B, type II, P3.*
Fig. 1, Vollrath & Eatock
Vollrath and Eatock, Figure 3

A

B

C

D

\[ A \]

\[ B \]

\[ C \]

\[ D \]
Fig. 5, Vollrath & Eatock

A. Displacement, frog cell (µm)

B. \( A_{\text{slow}} / (A_{\text{slow}} + A_{\text{fast}}) \)

C. % Decay

Displacement, mouse cell (µm)
Fig 6, Vollrath & Eatock

A

B
Fig. 7, Vollrath & Eatock

A  Frog

B  Mouse

C  Mouse

Time (ms)

Displacement (µm)

Current (pA)
Current (pA) vs Displacement (µm)

A)

B)

C)

Fig. 8, Vollrath & Eatock
Fig. 9, Vollrath & Eatock

- **Normalized current**
- **Displacement (µm)**

- **Mouse instantaneous**
- **Mouse steady state**
- **Frog instantaneous**
- **Frog steady state**