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Sensory Transduction and Adaptation in Inner and Outer Hair Cells of the Mouse Auditory System

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Abstract

Auditory function in the mammalian inner ear is optimized by collaboration of two classes of sensory cells known as inner and outer hair cells. Outer hair cells amplify and tune sound stimuli that are transduced and transmitted by inner hair cells. Although they subserve distinct functions, they share a number of common properties. Here we compare the properties of mechanotransduction and adaptation recorded from inner and outer hair cells of the postnatal mouse cochlea. Rapid outer hair bundle deflections of about 0.5 micron evoked average maximal transduction currents of about 325 pA, whereas inner hair bundle deflections of about 0.9 micron were required to evoke average maximal currents of about 310 pA. The similar amplitude was surprising given the difference in the number of stereocilia, 81 for outer hair cells and 48 for inner hair cells, but may be reconciled by the difference in single-channel conductance. Step deflections of inner and outer hair bundles evoked adaptation that had two components: a fast component that consisted of about 60% of the response occurred over the first few milliseconds and a slow component that consisted of about 40% of the response followed over the subsequent 20–50 ms. The rate of the slow component in both inner and outer hair cells was similar to the rate of slow adaptation in vestibular hair cells. The rate of the fast component was similar to that of auditory hair cells in other organisms and several properties were consistent with a model that proposes calcium-dependent release of tension allows transduction channel closure.

INTRODUCTION

Both inner and outer hair cells of the mammalian auditory system transduce deflections of their sensory hair bundles into electrical signals. Prior characterization of sensory transduction in the inner ear has focused on hair cells from physiologically accessible but genetically intractable model organisms including bullfrogs (Hudspeth 1997), turtles (Ricci et al. 2003), and rats (Beurg et al. 2006; Kennedy et al. 2003). A few studies have examined transduction in outer hair cells (Géléoc et al. 1997; Kros et al. 1992, 2002; Rüsch et al. 1994), but none has presented a systematic comparison of transduction and adaptation between inner and outer hair cells of the genetically tractable but physiologically challenging mouse auditory organ. As such, we sought to characterize transduction and adaptation in wild-type mouse cochlear hair cells for the following reasons. 1) There is a growing number of mouse models of genetic deafness and thus a need to characterize the normal response of wild-type mice to facilitate an interpretation of gene function in the genetic models. 2) Only a few reports have characterized the biophysics of mechanotransduction in mammalian inner hair cells as a function of hair

bundle deflection (Beurg et al. 2006; Kros et al. 1992; Michalski et al. 2007) and none has analyzed adaptation. 3) Although fast adaptation has been reported in a number of cochlear preparations, remarkably there are very few reports of slow adaptation in mammalian outer hair cells and none in inner hair cells. 4) Although the mechanism of slow adaptation has been established in vestibular hair cells, we were interested to examine the properties of adaptation to determine whether a similar mechanism may contribute to slow adaptation in cochlear hair cells. 5) Last, two distinct models for fast adaptation have been proposed: *a*) the calcium reclosure model, which suggests that calcium entry causes an active conformational change that forces the channel shut (Fettiplace and Ricci 2003; Howard and Hudspeth 1988), and *b*) the release model (Bozovic and Hudspeth 2003; Martin et al. 2003), which suggests that calcium evokes release of tension and passively allows the channel to close. Thus we were interested to examine the properties of fast adaptation in cochlear hair cells in search of evidence that might support either of these models.

To minimize the number of variables in our experiments, we chose to address these questions by examining a uniform population of cells and selected postnatal day (P) 6 to P8 hair cells located at the apical end of the mouse cochlea acutely excised without the use of enzymes that have documented detrimental effects on hair bundles. To effect rapid hair bundle deflections we designed a stimulator that used a piezoelectric element with a resonant frequency of >300 kHz and engineered stimulus pipettes shaped to fit snugly into the concave aspects of the distinctive morphologies of inner and outer hair bundles. We found differences in some of the properties of mechanotransduction between inner and outer hair cells and suggest that molecular heterogeneity may contribute to these differences. On the other hand, the properties of adaptation were remarkably similar between inner and outer hair cells and similar to those of vestibular cells, which may suggest that similar mechanisms and perhaps even similar molecules may be involved in both fast and slow adaptation in auditory and vestibular hair cells.

METHODS

Tissue preparation

Cochleae were harvested from P6–P8 mouse inner ears according to a protocol approved by the Animal Care and Use Committee at the University of Virginia (Protocol #3123). Briefly, the neonatal mice were rapidly decapitated and the heads were sectioned longitudinally. The bulla from each ear was excised and placed in room-temperature MEM (Invitrogen, Carlsbad, CA) supplemented with 10 mM HEPES, pH 7.4 (Sigma, St. Louis, MO). The organ of Corti was dissected away from the accessory structures and the tectorial membrane was gently removed without the use of enzymes. The tissue was then mounted on glass coverslips and secured beneath two glass fibers that were glued to the coverslip at one end. The tissue was subsequently processed for either imaging or electrophysiology experiments as subsequently described.

Confocal and scanning electron micrograph (SEM) imaging

For the confocal experiments, apical turns of P7 cochleae were prepared as described earlier. They were bathed in an external solution that contained 5 μ M of the styryl dye FM1-43 (Molecular Probes/Invitrogen, Carlsbad, CA). The cells were imaged immediately using an LSM 510 confocal microscope (Zeiss, Oberkochen, Germany) equipped with a $\times 63$, 1.4 NA oil-immersion objective with the pinhole set to one airy unit (AU). The number of stereocilia, number of putative tip-link sites (counted as pairs of stereocilia oriented along the axis of sensitivity), and interstereocilia distances were quantified using a Zeiss LSM Image Browser, Metamorph 6.2 (Molecular Devices, Sunnyvale, CA) and Photoshop 6.0 (Adobe, San Jose,

CA). Stereocilia heights were measured from projections of about 25 image stacks with focal planes separated by 0.25- μ m intervals.

For SEM experiments, the tissue was fixed for 1.5 h in 2.5% glutaraldehyde in 100 mM cacodylate buffer and 3 mM CaCl_2 , followed by three 10-min washes in PBS, a 1 h postfix in 1% osmium tetroxide, three 10-min washes in cacodylate buffer, and three 10-min washes in PBS. The tissue was subsequently dehydrated in an ethanol series, critical point dried in liquid CO_2 , sputter coated with gold/palladium, and imaged on a JEOL 6400 scanning electron microscope at 20 to 30 kV. Glass pipettes were sputter coated and imaged; the imaged inner hair cell pipette was the actual pipette used for bundle stimulation in 8 of 16 inner hair cells, whereas the outer hair cell pipette was fire-polished to a shape representative of the probes used for outer hair cell bundle stimulation.

Electrophysiology

Cochleae from P6–P8 CD-1 or C57/BL6 mice were excised and apical turns (~5–10% from the apex) were mounted on glass cover-slips. Cells were viewed on an Axioskop FS upright microscope (Zeiss) equipped with a $\times 63$ water-immersion objective and differential interference contrast (DIC) optics. Electrophysiological recordings were performed at room temperature in solutions containing the following (in mM): 137 NaCl, 5.8 KCl, 10 HEPES, 0.7 NaH_2PO_4 , 1.3 CaCl_2 , 0.9 MgCl_2 , 5.6 D-glucose, vitamins (1:100) and amino acids (1:50) as in MEM (Invitrogen), pH 7.4 (311 mOsm/kg). Recording electrodes (2–4 M Ω) were pulled from R-6 glass (Garner Glass, Claremont, CA) and were filled with the following (in mM): 135 KCl, 5 EGTA-KOH, 5 HEPES, 2.5 Na_2ATP , 2.5 MgCl_2 , and 0.1 CaCl_2 , pH 7.4 (284 mOsm/kg). The whole cell, tight-seal technique was used to record mechanotransduction currents using a Multiclamp 700A amplifier (Molecular Devices, Palo Alto, CA). Cells were held at –64 mV. Currents were filtered at 10 kHz with a low-pass Bessel filter, digitized at ≥ 20 kHz with a 12-bit acquisition board (Digidata 1322A), and recorded using pCLAMP 8.2 software (Molecular Devices).

Hair bundle stimulation

Cochlear outer hair cells were stimulated by a stiff glass probe with an angled and forged tip that was shaped to fit into the “V” of the hair bundle. Inner hair cells were stimulated by a stiff probe with a larger tip, rounded to fit against the slightly curved rows of bundles from inner hair cells located near the apex of the cochlea. After being used, the end of the stimulus probe was removed from the external solution and rinsed with distilled water before being placed back onto another cell. For both inner and outer hair cells positive hair bundle deflections were evoked by pushing the stereocilia from the concave side so that the bundle was obligated to follow the probe displacement. Deflections in the negative direction depended on adherence between the bundle and the glass probe. Movement of the stimulus probe was driven by a PICMA chip piezo actuator with an unloaded resonant frequency of >300 kHz (Physik Instruments, Waldbronn, Germany); a Teflon block with a bore sized to fit the stimulus pipette acted as a guide and ensured linear probe motion. The actuator was driven by a 400-mA ENV400 amplifier (Piezosystem, Jena, Germany); stimulus voltage steps were low-pass filtered with an eight-pole Bessel filter at 10 kHz to eliminate residual pipette resonance. The 10–90% rise time of the probe, determined with a four-quadrant photodiode, was 62 μ s. There was no off-axis motion of the probe. Hair bundle deflections were monitored by video microscopy using a CCD camera (Hamamatsu, Shizuoka, Japan), which allowed for spatial resolution of about 4 nm and temporal resolution at video rates or about 30 ms.

Data analysis

Analysis was performed using Clampfit 8.2 software (Molecular Devices) and OriginPro 7.0 (OriginLab, Northampton, MA). The following second-order Boltzmann equation was used to fit current–displacement [$I(X)$] data

$$I(X) = I_{\min} - (I_{\max} / \{1 + \exp[Z_2 \times (X - X_2)]\}) \times \{1 + \exp[Z_1 \times (X - X_1)]\} \quad (1)$$

where $I_{\min}$ and $I_{\max}$ are the minimum and maximum currents; Z_2 and Z_1 govern the slope of the curve; and X_2 and X_1 give the position of the curve along the x -axis. Extent–time [$X_e(t)$] curves were fit with the following equation with $time = 0$ defined for the peak transduction current

$$X_e(t) = X_{e(\text{fast})} \times [1 - \exp(-t/\tau_{\text{fast}})] + X_{e(\text{slow})} \times [1 - \exp(-t/\tau_{\text{slow}})] \quad (2)$$

where $X_{e(\text{fast})}$ and $X_{e(\text{slow})}$ are the extents of fast and slow adaptation and τ_{fast} and τ_{slow} are the respective time constants. We used the Levenberg–Marquardt least-squares fitting algorithm supplied with OriginPro 7.0 (OriginLab) to achieve the best fits to the data. Data are presented as the mean $\pm$ SD unless noted otherwise. We used all available data for analysis. Complete data sets were not available for every cell; thus the number of cells analyzed (n) is indicated in the text, tables, and figure legends.

RESULTS

Cytoarchitecture of cochlear hair bundles

A major goal of this work was to characterize the biophysical properties of transduction and adaptation in inner and outer hair cells by deflecting hair bundles with glass stimulus pipettes. Although vestibular hair bundles exhibit coherent motion of all stereocilia when the bundle is deflected (Kozlov et al. 2007), we suspect that may not be the case with cochlear hair bundles. The unique morphology of inner and outer hair bundles likely requires that each of the stereocilia in the tallest row be deflected to evoke coherent motion of the entire hair bundle. A lack of coherent motion in cochlear hair bundles can be demonstrated experimentally with small tipped stimulus pipettes (~1 micron), which when used to deflect stereocilia in one wing of the V-shaped bundle do not evoke coherent motion of stereocilia in the opposing wing (unpublished observation). As such, we sought to mimic in vivo tectorial membrane-evoked hair bundle deflection as closely as possible by engineering stimulus pipettes to match the distinct morphology of inner and outer hair bundles to engage tallest row stereocilia equally. Thus we began with a detailed analysis of hair bundle morphology. We imaged hair bundles excised from the apical turn of P7 mouse cochleae. For these experiments, 5 μ M FM1-43 was bath applied to illuminate the cell membranes that ensheath the stereociliary bundles. We reasoned that we could obtain more accurate measurements of hair bundle dimensions by imaging live cells stained with FM1-43 rather than by imaging fixed tissue, which is subject to shrinkage and distortion artifacts. Immediately after application of the FM dye, we imaged the tissue at high magnification using a Zeiss LSM 510 confocal microscope (Fig. 1, *A* and *B*). We quantified the number of stereocilia, height of the tallest row of stereocilia, interstereociliary distances (measured at stereocilia bases), and number of putative tip-link sites for inner and outer hair cells. Tip-link sites were estimated from the number of pairs of stereocilia oriented along the hair bundle's sensitive axis. For comparison, we also quantified the number of stereocilia and putative number of tip-link sites from SEM images of inner hair

cells. Notably, we found that the hair bundles of inner hair cells contained fewer stereocilia (~50 vs. ~80) and thus fewer tip-link sites (~30 vs. ~50), but that the bundles were taller by about 1 μm and the stereocilia were more widely spaced. The data are summarized in Table 1.

To facilitate the synthesis of glass stimulus pipettes that best matched the shape of the hair bundles we measured the mean angle formed by the “V” shape of the outer hair cell bundle (Fig. 1C). Lines drawn through the approximate middle of the left and right wings of outer hair cell bundles were superimposed on the digital confocal images of FM1-43-illuminated hair bundles. The mean angle formed by the two lines was $73 \pm 10^\circ$ ($n = 33$). To examine hair bundles of inner hair cells we used SEM to image the samples from above (Fig. 1D). The bundles were crescent shaped and not well described by measurement of a simple angle, as was the case for outer hair bundles. Instead, we found that they were better described by an arc of a semicircle. On average inner hair cell bundles had profiles that consisted of $79 \pm 18^\circ$ of a semicircle that had a radius of $3.4 \pm 0.8 \mu\text{m}$ ($n = 11$). Given the differences in inner and outer hair bundle geometry we used a microforge to generate glass stimulus pipettes that were shaped to match the two categories of bundles. To confirm a good fit of the stimulus pipettes with the hair bundles we superimposed semitransparent SEM images of stimulus pipettes on SEM images of inner and outer hair cell bundles (Supplemental Fig. 1).¹

Mechanotransduction in inner and outer hair cells

We used the whole cell, tight-seal technique to record mechanotransduction currents evoked by step deflections of hair bundles from inner and outer hair cells. The apical turns of P6–P8 mouse cochleae were excised and the organs of Corti were pinned beneath two parallel glass fibers glued at one end to coverslips mounted in the recording chamber. Bundles were viewed from above with DIC optics. Stimulus pipettes were shaped to fit snugly within the concave side of inner and outer hair cell bundles. Positive deflections were defined as toward the tallest row of stereocilia.

Square step deflections evoked rapidly activating transduction currents that decayed to steady state within the subsequent 50 ms. Qualitatively, we noted that the current decay consisted of both fast and slow components, which presumably reflected adaptation. In Fig. 2, A–D we show four representative families of transduction currents that bracket the range of responses we observed. The data of Fig. 2, A and B were recorded from inner hair cells and Fig. 2, C and D were from outer hair cells. Although all of the cells were of the same age (P6–P8) and were located in the same general tonotopic location, about 5–10% from the apical end of the cochlea, variation in both the rate and the extent of adaptation was observed. A family of transduction currents from an inner hair cell that exhibited some of the fastest adaptation and the greatest extent of adaptation is shown in Fig. 2A, whereas an inner hair cell with some of the slowest adaptation and lowest extent of adaptation is shown in Fig. 2B. Likewise, currents from an outer hair cell with some of the fastest adaptation and near-complete extent of adaptation is shown in Fig. 2C and data from an outer hair cell with some of the lowest extent of adaptation and slower adaptation rates are shown in Fig. 2D. When the hair bundles were deflected in the negative direction we noted a small decrease in the current amplitude; when it was returned to the rest position we often observed rebound currents. In most, but not all, cases rebound currents were observed in data from outer hair cells. In previous work with vestibular hair cells (Holt et al. 1997, 2002; Stauffer et al. 2005; Vollrath and Eatock 2003), the stimulus probe was well coupled to the hair bundle during negative displacements, as continuous suction was applied to the back of the stimulus pipette. This was not possible with auditory hair cells, due to the morphology of the hair bundle. However, because we usually observed rebound currents at the termination of a negative step in outer hair cells, it suggested that the glass stimulus probe was

¹The online version of this article contains supplemental data.

well coupled to the bundle (Fig. 2, *C*, *D*, and *F*). Rebound currents were observed less frequently in inner hair cells (Fig. 2, *A*, *B*, and *E*), possibly reflecting either a reduction in the adherence of the hair bundle to the stimulus probe or a lower channel open probability at rest. We did not observe the slow offset component that is apparent in transduction currents recorded from rat inner hair cells (Beurg et al. 2006). The source of variability among inner hair cells and also among outer hair cells of similar developmental stages and cochlear locations was not clear.

To generate a more general view of inner and outer hair cell responses we averaged transduction currents evoked by families of bundle deflections from nine inner hair cells (Fig. 2*E*) and separately from eight outer hair cells (Fig. 2*F*). The average maximum transduction current amplitude among all inner hair cells was 324 ± 118 pA and the average maximum transduction current amplitude of all outer hair cells was 311 ± 69 pA. The largest currents we recorded were 580 and 418 pA in inner and outer hair cells, respectively. Current–displacement [$I(X)$] relationships were plotted (Fig. 2, *G* and *H*) using the average transduction current data shown in Fig. 2, *E* and *F*, respectively, and were fit with three-state Boltzmann functions (Eq. 1). On average, outer hair cells had a steep current–displacement relationship, with a 10–90% operating range of 0.44 ± 0.12 μm , similar to that of rat outer hair cells (Kennedy et al. 2003). Inner hair cells, in contrast, had a significantly broader current–displacement relationship, with a 10–90% operating range of 0.86 ± 0.17 μm . We plotted $I(X)$ curves from two other examples that bracket the range of responses for both inner (Fig. 2*G*) and outer hair cells (Fig. 2*H*). The narrowest operating range we observed was 0.53 μm for inner hair cells and 0.3 μm for outer hair cells.

We wondered whether differences in hair bundle geometry might account for the differences in the mean operating ranges we observed for inner and outer hair cells. We used the geometrical gain factor, gamma, first characterized by Jacobs and Hudspeth (1990), to estimate the operating range of the transduction apparatus at the tips of the stereocilia. Gamma can be approximated from the interstereocilia distance divided by the height of the tallest stereocilia. We estimated a gamma of 0.14 and 0.12 for inner and outer hair bundles, respectively. The product of gamma and operating range yielded an estimate of the operating range over which gating springs are stretched: 120 nm for inner hair cells and 53 nm for outer hair cells. Next we converted the operating range, measured in deflection at the tip of the bundle, into rotation of the bundle around a pivot point, as was used to reconcile the difference between outer hair cells and vestibular hair cells (Géléoc et al. 1997). We calculated an operating range of 12.2 rotational degrees for inner hair cells and 7.1° for outer hair cells. Thus neither method was able to reconcile the difference in operating range. SEM images of inner hair cell stereocilia indicate that the tallest row of stereocilia is about twice the height of the second row (unpublished observation). Thus when we consider bundle movement at the site of transduction, i.e., at the tip of the second row of stereocilia, the measured operating range was 0.48 μm , very similar to that of outer hair cells. There is a relatively small difference in height between the tallest and second row of stereocilia in outer hair cells. Although this view of hair bundle morphology may account for the difference in operating range, the possibility remains that the difference may reflect heterogeneity in the mechanosensitivity of transduction molecules between inner and outer hair cells.

Slow and fast adaptation

To consider the effects of fast and slow adaptation on the shape and position of the $I(X)$ relationship we designed several stimulus protocols that consisted of conditioning steps with different durations and amplitudes. The conditioning steps were preceded by a family of test steps designed to map the position of the $I(X)$ at rest and were followed by an identical family of test steps that allowed us to sample the position and shape of the curve after the conditioning step. Figure 3, *A* and *B* shows families of currents recorded from the same inner hair cell evoked

by a small, brief conditioning step and a larger, longer conditioning step, respectively. Similar protocols, but with longer conditioning steps, were used to examine adaptation from an outer hair cell (Fig. 3, *D* and *E*). The resting $I(X)$ curves for both inner and outer hair cells are shown in Fig. 3, *C* and *F* (diamonds). The data were fitted with second-order Boltzmann functions (Eq. 1). We also plotted data evoked by the test steps that followed both the shorter (squares) and longer (circles) conditioning steps. However, rather than performing a new fit to the conditioned data we used the parameters generated from the best-fit Boltzmann curve to the resting $I(X)$ data and fixed the parameters that govern the shape of the curve (Z_1 and Z_2) but allowed the curve to shift along the x -axis until we achieved the best fit. Both the inner and outer hair cell $I(X)$ data were well fit by this method, which suggested that both small, brief bundle deflections that highlight fast adaptation and longer, larger deflections that highlight slow adaptation, functioned to shift the $I(X)$ relation in the direction of the applied stimulus without significant alteration to the shape of the curve, consistent with adaptation in bullfrog hair cells (Eatock et al. 1987). Because this method is time consuming and requires that we record from a cell for extended periods, we opted to expedite analysis of the temporal and extent characteristics of fast and slow adaptation in inner and outer hair cells by using the inferred-shift method (Hirono et al. 2004; Holt et al. 1997; Shepherd and Corey 1994; Stauffer et al. 2005).

The validity of the inferred-shift method rests on the assumption that adaptation evokes a shift of the $I(X)$ relation without a change in shape or a reduction in amplitude of the maximum currents as demonstrated in Fig. 3. For the inferred-shift method, each data point in a transduction current trace (Fig. 4*A*) is mapped onto the resting $I(X)$ curve (Fig. 4*B*). The extent of adaptation (X_e) is defined as the magnitude of the shift of the resting $I(X)$ curve required to align the curve with each data point (Fig. 4*B*). A plot of X_e as a function of time reveals the temporal pattern of the adaptive shift of the $I(X)$ curve (Fig. 4*C*). As a test of the method, the time constants of double-exponential least-square fits to the raw current traces with peaks equal to 50% of the maximum current amplitude were compared with double-exponential least-square fits to the inferred-shift data derived from the same current traces (Fig. 4, *D* and *E*, *top traces*). In theory, at the midpoint of the $I(X)$ curve the time constants measured from the two methods should be identical. In the examples from the inner hair cell shown in Fig. 4*D* (*top traces*) the time constants were 3.6 and 14.3 ms for raw current data and 3.8 and 14.8 ms for the inferred-shift data. For the outer hair cell data shown in Fig. 4*E* (*top traces*) the time constants were 2.4 and 12.6 ms for raw current data and 2 and 14.1 ms for the inferred-shift data. As such, we felt the method offers a good approximation of the adaptive shift of the $I(X)$ relationship for both inner and outer hair cells. The utility of the inferred-shift method is that it facilitates separation of fast and slow components of adaptation and often reveals the presence of significant adaptation not readily apparent in the raw current traces, particularly those evoked by supersaturating bundle deflections. This is illustrated for both inner and outer hair cells in the bottom pairs of traces in Fig. 4, *D* and *E*. Here the raw currents were evoked by supersaturating bundle deflections and the decay in current (black lines) is much slower than the adaptive shift of the $I(X)$ relation, as revealed by the inferred-shift analysis (green circles). For the inner hair cell, the raw current data were best fit with a double exponential that had time constants of 13.7 and 24.2 ms, whereas the inferred-shift analysis revealed time constants of 4.3 and 16.6 ms. For the outer hair cell, the raw current data were best fit with time constants of 3.2 and 21.2 ms and the inferred-shift data were best fit with time constants of 1.8 and 16.6 ms. The discovery that fast adaptation can be evoked even by supersaturating bundle deflections is novel and is inconsistent with the channel reclosure model of fast adaptation.

To examine the time course of the adaptive shift of the $I(X)$ relation over the entire operating range of the cell we used the inferred-shift method to analyze families of transduction currents evoked by a wide range of bundle deflections. Representative families of inferred-shift data are shown in Fig. 5, *A* and *B* from inner and outer hair cells. We found in all cases that the data

were well described by double-exponential equations (Eq. 2) with fast and slow time constants, τ_{fast} and τ_{slow} , and with amplitudes or extents of adaptation that corresponded to $X_{\text{e(fast)}}$ and $X_{\text{e(slow)}}$, respectively. The $X_{\text{e(t)}}$ data for both inner and outer hair cells were better fit by double-exponential functions, rather than by single-exponential functions, suggesting that both fast and slow adaptation, as defined by adaptive shifts of the current–displacement relationship, occur in both inner and outer hair cells.

We analyzed adaptation in 15 inner hair cells and 12 outer hair cells from P6 to P8 acutely excised mouse cochleae. The parameters from fits of Eq. 2 to the inferred-shift data were pooled and are presented in Fig. 5, C–F. In both inner and outer hair cells, we found that the extent of total adaptation was a linear function of displacement amplitude (Fig. 5, C and D, gray lines), consistent with our previous analysis of adaptation in frog and mouse vestibular hair cells (Stauffer et al. 2005). However, in contrast to the previous report where we observed saturation of fast adaptation for larger stimulus amplitudes, in cochlear hair cells we found a linear relationship between the extent of both fast and slow adaptation and deflection. The data were fit with a linear regression with a slope that indicated the extent of fast adaptation was 41% in inner hair cells and 43% in outer hair cells. Although slow adaptation has not been previously characterized in mammalian cochlear hair cells we found that the extent of slow adaptation was a significant fraction of total adaptation in both inner and outer hair cells, 29 and 31%, respectively. Thus the average total extent of adaptation in both cell types was about 70–74%, regardless of stimulus amplitude.

The time constants of fast and slow adaptation were also similar among the two cell types. For inner hair cells, the time constants of fast adaptation as a function of stimulus size were well described by a flat line that had a y-intercept of 1.5 ms, whereas slow adaptation had a y-intercept of 14.2 ms with a positive slope of 12.2 ms/ μm . For outer hair cells both fast and slow time constants were well fit with flat lines that had y-intercepts of 1.8 and 20 ms, respectively. The smallest fast adaptation time constants we measured were 0.66 and 0.46 ms for inner and outer hair cells, respectively. As such, the fast adaptation time constants we report here were much faster than those of frog and mouse vestibular organs (Stauffer et al. 2005) but of a similar range to those of rat cochlear hair cells (Beurg et al. 2006). The slow adaptation time constants, on the other hand, were within the same range as those reported for frog and mouse vestibular hair cells (Holt et al. 1997; Shepherd and Corey 1994; Stauffer et al. 2005; Vollrath and Eatock 2003).

As an estimate of adaptation rate we divided the extent of adaptation by the adaptation time constants for the fast and slow components in both inner and outer hair cells (Fig. 6, A and B). Whereas the largest adaptation rates in inner hair cells (circles, Fig. 6A) were about twice those of outer hair cells (circles, Fig. 6B), for a given step size, they were quite similar. For example, deflections of about 0.5 μm resulted in fast adaptation rates of 162 $\mu\text{m/s}$ in inner hair cells and 156 $\mu\text{m/s}$ in outer hair cells, again significantly faster than the rates of fast adaptation in frog and mouse vestibular hair cells. The slow adaptation rate constants were similar among inner and outer hair cells and ranged up to about 13 $\mu\text{m/s}$. Interestingly, the slow adaptation rates were remarkably similar to those of frog and mouse vestibular hair cells (Stauffer et al. 2005), which raises the possibility that cochlear hair cells may use a slow adaptation mechanism similar to that of vestibular hair cells.

The rates and extents of adaptation varied among individual inner and outer hair cells, despite the cells being from similar regions and mice of similar ages. We found neither a significant ($P > 0.1$) difference between the adaptation rate constants of inner and outer hair cells nor a correlation between the rates of fast and slow adaptation in individual cells (Fig. 6C, $R = 0.08$, $P > 0.5$). However, we did find an inverse correlation between the slopes of linear fits to the fast and slow extents of adaptation versus displacement plots. Figure 6D plots the slope of the

extent of fast adaptation as a function of the slope of the extent of slow adaptation for 16 inner and 10 outer hair cells. The data from inner and outer hair cells were not significantly different ($P > 0.5$) and thus were pooled and fit with a linear regression ($R = 0.48$, $P < 0.05$) that had a slope of -0.41 . The inverse correlation we note here was similar to that observed in a previous study of vestibular hair cells (Stauffer et al. 2005). Because adaptation is a dynamic process with fast adaptation proceeding more rapidly, its amplitude or extent directly affects the amplitude or extent of slow adaptation. As such, the data support the observation that cells with a greater extent of fast adaptation tended to have less slow adaptation (Fig. 5D). These data are similar to those from mouse utricle and frog saccule hair cells and are consistent with the release model of fast adaptation (Stauffer et al. 2005), whereby fast adaptation reduces the drive to slow adaptation. The channel reclosure model of adaptation, on the other hand, predicts the opposite result, a positive correlation: the greater the extent of fast adaptation, the greater the drive for slow adaptation.

DISCUSSION

Mechanotransduction in inner and outer hair cells

We examined inner and outer hair cells of a single developmental stage and cochlear region and noted some systematic differences between the biophysics of transduction and adaptation. One striking difference was that the operating range of inner hair cells was twice as broad as that of outer hair cells, 0.86 versus $0.44 \mu\text{m}$. Beurg et al. (2006) also noted that the operating range was broader in rat inner hair cells than that in outer hair cells. Differences in morphology between hair bundles from inner and outer hair cells may account for the difference in operating range, but we suggest that factors other than geometry may also contribute to this difference.

To our surprise, the mean maximal transduction currents in inner and outer hair cells (Table 2) were similar, despite the greater number of stereocilia in outer hair cells. By assuming a reversal potential of 0 mV and the single-channel conductances of rat cochlear hair cells (170 and 95 pS for inners and outers, respectively, at the apical end; Beurg et al. 2006) we estimated the number of functional transduction channels in our cells. Remarkably, the estimate revealed that inner hair cells had 30 functional channels and outer hair cells had 51, which corresponded exactly to the number of putative tip-link sites we estimated from our hair bundle images (Table 1). Thus we conclude that, on average, our cells contained one functional channel per tip link. Yet, we also note in the extreme case, a cell with the largest current (580 pA) and fewest number of tip-link sites (28), there may have been two functional channels per tip link. Based on the different operating ranges and the convergence of the maximal transduction current and the estimates of the number of tip links with the single-channel conductances from Beurg et al. (2006), we support the notion that there may be substantial differences in the molecular composition of the transduction apparatus between inner and outer hair cells, in particular among the molecules that contribute to transduction channel conductance and sensitivity.

Slow adaptation

We report, for the first time, the presence of slow adaptation in inner hair cells of the mammalian cochlea. Mouse outer hair cells also displayed evidence of slow adaptation as noted previously (Kros et al. 2002). The contribution of slow adaptation in both cell types was about 40% of the total. Interestingly, we found remarkably similar adaptation rates between inner and outer hair cells that were surprisingly similar to those of mouse vestibular hair cells (Stauffer et al. 2005). The similar rates suggest that the mechanism of slow adaptation in cochlear hair cells may be similar to that of vestibular hair cells. The motor model of slow adaptation posits that a cluster of molecules, perhaps myosins, move along the actin cores of the stereocilia to modulate gating-spring tension and thus channel open probability. In vestibular cells there is substantial evidence that myosin 1c contributes to slow adaptation

(Holt et al. 2002; Stauffer et al. 2005). However, this has not been addressed in cochlear hair cells where other myosins may be involved, including myosin 7a (Kros et al. 2002), myosin 15a (Stepanyan et al. 2006), or myosin 3a (Schneider et al. 2006). Whether myosin 1c contributes to slow adaptation in cochlear hair cells has yet to be determined.

That slow adaptation has escaped the attention of previous investigations is not surprising given its slower time course and the longer step stimuli required to measure its properties. Furthermore, because adaptation acts as a high-pass filter, the low rate probably prevents slow adaptation from contributing to active processing of sensory information in the cochlea. Unless it is substantially faster in vivo, the high-pass corner frequency imposed by slow adaptation (~ 10 Hz) is below the low-frequency hearing threshold in rodents (>4 kHz). However, because an important function of slow adaptation may be to allow hair cells to compensate for tonic offsets in bundle position, it may be advantageous for cochlear hair cells to perform this function at a rate below the auditory frequency range so that it does not interfere with normal stimulus processing.

Another potentially important role of slow adaptation may be active force generation that may function together with other hair bundle motors to tension the gating springs (LeMasurier and Gillespie 2005). Collectively, these motors may exert forces that bias the resting hair bundle in the negative direction and at the same time tension the transduction apparatus to the point where about 10% of the transduction channels are open. Based on estimated active force generation in rat outer hair cell bundles (~ 500 pN; Kennedy et al. 2005), our estimates for the numbers of transduction channels/cell and our estimate of the geometrical factor, γ , we suggest that hair bundle motors may tension individual gating springs with forces of 42–83 pN, which brackets the theoretical estimate of about 60 pN from LeMasurier and Gillespie (2005).

Fast adaptation

We also found evidence for significant fast adaptation in both inner and outer hair cells. On average the extent of fast adaptation was about 60% of total adaptation. The fastest time constants we measured were 0.65 ms from an inner and 0.46 ms from an outer hair cell. The average values were similar: 1.5 and 1.8 ms for inner and outer hair cells, respectively. That these values are larger than those reported for rat inner (Beurg et al. 2006) and outer hair cells (Kennedy et al. 2003) is not unexpected given the higher calcium concentration (1.5 vs. 1.3 mM) and the hyperpolarized holding potential (-84 mV) used for their experiments. Both factors contribute to greater calcium entry and thus faster adaptation (Ricci et al. 2003). Furthermore, we recorded from a single location at the extreme low-frequency end of the cochlea, i.e., with the slowest adaptation. If fast adaptation contributes to tuning of the responses of mouse cochlear hair cells to stimulus frequency as reported for rat outer hair cells (Beurg et al. 2006), we predict that adaptation may vary along the length of the cochlea with faster time constants toward the basal end.

Although the time constants of fast adaptation in mouse auditory hair cells were an order of magnitude faster than those of vestibular hair cells (Stauffer et al. 2005), we were surprised to find that the rate of fast adaptation was similar among auditory and vestibular hair cells (compare Fig. 6, A and B with Fig. 2L of Stauffer et al. 2005). Because adaptation rate was derived from the extent of adaptation divided by the time constant and because the absolute extents of adaptation (measured in microns) were also smaller in auditory hair cells, the adaptation rates that resulted were remarkably similar to those of vestibular cells. The similarity in fast adaptation rates raises the possibility that similar molecular mechanisms may contribute to fast adaptation in the various cell types.

Two distinct models for fast adaptation have been proposed: the calcium reclosure model (Denk et al. 1995; Howard and Hudspeth 1988) and the release model (Bozovic and Hudspeth 2003; Martin et al. 2003; reviewed by LeMasurier and Gillespie 2005). In mouse vestibular hair cells, Stauffer et al. (2005) found evidence that supported the release model for fast adaptation. In the present study our data from cochlear hair cells also support the release model for fast adaptation. Most important, perhaps, we found an inverse relationship between the extent of fast and slow adaptation for both inner and outer hair cells. The reclosure model suggests that calcium induces a conformational change that forces the channel shut, which is predicted to increase gating-spring tension. It follows that increased tension would provide a greater drive for slow adaptation and thus a positive correlation between the extent of fast and slow adaptation. However, activation of a release mechanism would decrease gating-spring tension and, in turn, decrease slow adaptation that would result in a negative correlation between the two. Indeed, we found a negative relationship in both inner and outer hair cells, consistent with the data of frog and mouse vestibular hair cells (Stauffer et al. 2005). Therefore we suggest that they may share a similar mechanism of fast adaptation, both consistent with the release model. In mouse vestibular cells, it was suggested that the release may result from a calcium-dependent, ATP-independent conformational change in myosin 1c (Stauffer et al. 2005). In theory, any calcium-dependent molecule in series with the transduction apparatus, including but not limited to myosin 1c, would be in a position to contribute to the release.

In contrast to vestibular hair cells, the extent of fast adaptation in mouse inner and outer hair cells did not saturate over the range of bundle deflections we examined (compare Fig. 5, *C* and *D* with Fig. 2, *D* and *J* of Stauffer et al. 2005). In cochlear cells larger deflections evoked greater extents of fast adaptation. If we consider hair bundle geometry, or gamma, we can translate the extent of fast adaptation into movement of a release element. Interestingly, despite the differences in extent, operating range, and hair bundle geometry, data from frog and mouse vestibular cells predicted releases of about 40 nm (Stauffer et al. 2005). Our data from mouse outer hair cells predicted a release of 36 nm for the largest bundle deflections and 38 nm for inner hair cells with similar bundle deflections, or about an order of magnitude larger than the movement of the channel gate (Denk et al. 1995; Howard and Hudspeth 1988). Although it is difficult to envision how a release of this magnitude could be accommodated by a parallel arrangement of myosin molecules, it could result from a serial arrangement (LeMasurier and Gillespie 2005; Stauffer et al. 2005). Alternatively, we suggest that both the dimensions of the release and the lack of saturation could be explained by the sequential unraveling of a coiled, calcium-dependent element that continues to unfold and release tension with larger displacements. Last, based on the lack of saturation of fast adaptation in our cells, the release model would predict a lack of saturation of fast adaptation–associated positive bundle movements, a result documented for fast adaptation–associated bundle movements in rat outer hair cells (Kennedy et al. 2005). Although these observations are consistent with the release model and inconsistent with the calcium reclosure model, the precise mechanism, molecular correlate, and physiological contributions of fast adaptation in auditory hair cells remain elusive.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIG. 1.

Confocal and scanning electron micrographs (SEMs) of inner and outer hair bundles. *A*: representative confocal image of an apical P7 inner hair cell, focused at the base of the hair bundle. Stereocilia were stained with FM1-43 in *A–C*. Scale bar = 1 μm and applies to *A–D*. *B*: representative confocal image of an apical P7 outer hair cell, focused at the base of the hair bundle. *C*: method of measuring the angle formed by the “V”-shaped hair bundle of a representative P7 outer hair cell. Angle for this particular cell was 74° . *D*: method of measuring the arc of a semicircle formed by the crescent-shaped hair bundle of a representative P7 inner hair cell SEM image. This particular cell had a profile consisting of 90° of a semicircle with a radius of 3 μm .

FIG. 2.

Families of transduction currents recorded from inner and outer hair cells. *A*: transduction currents from a representative inner hair cell exhibiting relatively fast adaptation and a large extent of adaptation. Stimulus protocol below *E* applies to *A*, *B*, and *E*. *B*: transduction currents from a representative inner hair cell exhibiting relatively slow adaptation and a small extent of adaptation; scale bar from *A* applies. Stimulus protocol below *F* applies to *C*, *D*, and *F*. *C*: transduction currents from a representative outer hair cell exhibiting relatively fast adaptation and a large extent of adaptation. Scale bar from *E* applies to *C*–*F*. *D*: transduction currents from a representative outer hair cell exhibiting relatively slow adaptation and a small extent of adaptation. *E* and *F*: transduction currents in response to the family of displacement steps

shown, averaged from 12 inner hair cells (*E*) and 9 outer hair cells (*F*). *G*: peak current–displacement relationship from average data shown in *E* (black squares); current data were normalized to open probability and fit with Eq. 1, a second-order Boltzmann function. Data from inner hair cells with the steepest (blue circles) and broadest (green triangles) current–displacement relationships are also shown. Inner hair cell parameter values: Average curve: $X_1 = 0.15 \mu\text{m}$, $X_2 = 0.51 \mu\text{m}$, $Z_1 = 15.69 \mu\text{m}^{-1}$, $Z_2 = 3.69 \mu\text{m}^{-1}$; Steepest curve: $X_1 = 0.10 \mu\text{m}$, $X_2 = 0.20 \mu\text{m}$, $Z_1 = 19.58 \mu\text{m}^{-1}$, $Z_2 = 5.67 \mu\text{m}^{-1}$; Broadest curve: $X_1 = 0.32 \mu\text{m}$, $X_2 = 0.66 \mu\text{m}$, $Z_1 = 10.67 \mu\text{m}^{-1}$, $Z_2 = 3.50 \mu\text{m}^{-1}$. *H*: peak current–displacement relationship from average data in *F* (black squares); current data were normalized to open probability and fit with Eq. 1. Data from outer hair cells with the steepest (blue circles) and broadest (green triangles) current–displacement relationships are also shown. Outer hair cell parameter values: Average curve: $X_1 = 0.10 \mu\text{m}$, $X_2 = 0.19 \mu\text{m}$, $Z_1 = 19.66 \mu\text{m}^{-1}$, $Z_2 = 7.67 \mu\text{m}^{-1}$; Steepest curve: $X_1 = 0.15 \mu\text{m}$, $X_2 = 0.07 \mu\text{m}$, $Z_1 = 19.32 \mu\text{m}^{-1}$, $Z_2 = 8.06 \mu\text{m}^{-1}$; Broadest curve: $X_1 = 0.09 \mu\text{m}$, $X_2 = 0.26 \mu\text{m}^{-1}$, $Z_1 = 18.31 \mu\text{m}^{-1}$, $Z_2 = 5.36 \mu\text{m}^{-1}$.

FIG. 3.

Adaptation in inner and outer hair cells. *A* and *B*: representative families of transduction currents recorded from an inner hair cell. Stimulus protocols (below) were designed to directly examine the adaptive shift of the current–displacement [$I(X)$] curve. *C*: peak transduction current values were plotted for a family of test steps (black diamonds) to create a resting $I(X)$ curve from the data shown in *A* and *B*. Resting $I(X)$ was fit with Eq. 1: $X_1 = 0.65 \mu\text{m}$, $X_2 = 1.01 \mu\text{m}$, $Z_1 = 18.30 \mu\text{m}^{-1}$, $Z_2 = 5.29 \mu\text{m}^{-1}$. Test $I(X)$ data were taken from the peaks following the conditioning steps shown in *A* (blue squares) and *B* (green circles) and were fit with the same Boltzmann curve shifted along the x -axis. *D* and *E*: representative families of transduction currents recorded from an outer hair cell. Stimulus protocols (below) were used to examine

the adaptive shift of the current–displacement [$I(X)$] curve. F : peak transduction current values were plotted for a family of test steps (black diamonds) to create a resting $I(X)$ curve from the data shown in D and E . Resting $I(X)$ was fit with Eq. 1: $X_1 = 0.19 \mu\text{m}$, $X_2 = 0.25 \mu\text{m}$, $Z_1 = 21.45 \mu\text{m}^{-1}$, $Z_2 = 7.99 \mu\text{m}^{-1}$. Test $I(X)$ data were taken from the peaks following the conditioning steps shown in D (blue squares) and E (green circles) and fit with the same Boltzmann curve shifted along the x -axis.

FIG. 4.

Inferred-shift analysis of adaptation in cochlear hair cells. **A**: representative transduction current trace evoked by an 80-ms, 0.27- μm bundle deflection. To illustrate the method, sample data points (circles) are superimposed on the current trace (black line). Horizontal and vertical arrows indicate the progression of time and the adaptive current decline, respectively. **B**: inferred shift of the $I(X)$ relationship was estimated by plotting the peak $I(X)$ relation (hollow circles), which was fit with a second-order Boltzmann curve (black line). Sample data points shown in **A** are plotted in **B** (circles) for the same bundle position (dashed line). Magnitude of the shift (horizontal arrows) of the Boltzmann curves required to align the curve with sample data points was calculated. **C**: shift or extent of adaptation (vertical arrows), derived from **B**, was plotted as a function of time (horizontal arrows), derived from **A** for the sample data points (circles) and entire trace (black line). **D** and **E**: comparison between raw transduction current traces (black lines) and data extracted from inferred-shift analysis of the raw current traces (colored symbols) for representative inner (**D**) and outer hair cells (**E**). Top pair of data sets were evoked by bundle deflections that evoked $P_{\text{Open}} = 0.5$. Bottom pair were evoked by supersaturating bundle deflections, $P_{\text{Open}} = 1.0$. Note that the inferred-shift analysis reveals the presence of fast adaptation even for the large bundle deflections. Bottom pair of traces show the stimulus protocols. Vertical scale bars indicate current (black lines) and extent of adaptation (symbols). Horizontal scale bars = 10 ms.

FIG. 5.

Analysis of inferred-shift data allows for separation of fast and slow adaptation in inner and outer hair cells. *A* and *B*: extent of adaptation as a function of time [$X_e(t)$], generated with the inferred-shift method from a representative inner (*A*) and outer hair cell (*B*). Data (symbols) were fit with a double-exponential function (lines, Eq. 2). *C–F*: time constants and extent parameters for slow (circles) and fast (squares) adaptation extracted from fits of Eq. 2 to inferred-shift data from 12 inner hair cells (*C* and *E*) and 9 outer hair cells (*D* and *F*). For inner hair cells (*C*), slow extent was fit with a line that revealed the extent of slow adaptation was 29% ($r = 0.989$). Extent of fast adaptation was 41% ($r = 0.996$). Total extent (gray line) was 69% ($r = 0.999$). For outer hair cells (*D*), the slow extent was fit with a line that had a slope of

31% ($r = 0.991$). Fast extent had a slope of 43% ($r = 0.997$). Total extent (gray line) was 73% ($r = 0.999$). *E* and *F*: average time constants for adaptation in inner and outer hair cells. Time constants were fit with linear regressions. For inner hair cells (*E*) τ_{fast} was fit with a flat line with a y-intercept of 1.6 ms ($r = 0.73$) and τ_{slow} was fit with a line that had a y-intercept of 12.2 ms and a slope of 14.2 ms/ μm ($r = 0.80$). Time constants for outer hair cells (*G*) were fit with flat lines with y-intercepts of $\tau_{\text{fast}} = 1.8$ ms ($r = 0.62$) and $\tau_{\text{slow}} = 20$ ms ($r = 0.40$).

FIG. 6.

Comparison of the rate and extent of fast and slow adaptation. *A* and *B*: average initial rates of adaptation (X_0/τ) for inner and outer hair cells, respectively, generated from the data in Fig. 5. Left vertical scale applies to the fast component of adaptation (squares) and the right vertical scale applies to the slow component. *C*: fast rate constants were plotted as a function of slow rate constants for individual inner (squares) and outer (circles) hair cells. Rate constants were derived from the slope of linear fits to rate-deflection data (i.e., similar to that plotted in *A* and *B* for 15 inner and 9 outer hair cells). Rate constant data were fit with linear regressions (not shown) that revealed no significant difference between inner and outer hair cells. Furthermore, there was no correlation between the rates of fast and slow adaptation measured in individual cells. Data were pooled and fit with a line (not shown) that had a correlation coefficient of $R = 0.08$, $P > 0.5$. Legend in *D* applies to *C*. *D*: inverse correlation between the extent of fast and slow adaptation in individual cells. For each cell, fast and slow extents were determined over a range of step sizes; the resultant slow extent-deflection and fast extent-deflection plots were fit with separate linear equations; the percentage adaptations (slopes) are plotted here. Linear fits to the inner and outer hair cell data revealed no significant difference, and thus the data

were pooled. A linear fit to the pooled data is shown: slope, -0.41 ; y-intercept, 0.54 ; $R = 0.48$, $P < 0.05$.

Properties measured from confocal images of FM1-43-stained hair bundles from inner (IHC) and outer hair cells (OHC) excised from the apical end of P7 mouse cochlea

Hair Cell	Height, μm	Interstereocilia Distance, μm	Gamma, s/h	n Stereocilia	n Putative Tip-Link Sites	Angle, deg; Radius, μm
IHC						
Mean	4.6 $\pm$ 0.3 (5)	0.66 $\pm$ 0.07 (112)	0.14	48 $\pm$ 3 (7)	30 $\pm$ 5 (7)	79 $\pm$ 18 (11); $r = 3.4 \pm 0.8$
Range	4.3–5.0	0.48–0.86		44–53	28–33	50–100 $r = 2\text{--}5$
OHC						
Mean	3.7 $\pm$ 0.4 (15)	0.44 $\pm$ 0.07 (117)	0.12	81 $\pm$ 4.9 (7)	51 $\pm$ 4 (7)	73 $\pm$ 10 (33);
Range	3.0–4.2	0.24–0.62		71–86	44–56	56.7–93.8

Values are means $\pm$ SD (number of measurements). Gamma was estimated from mean interstereocilia distance/mean bundle height.

TABLE 2
Electrophysiological properties of transduction and adaptation in inner (IHC) and outer hair cells (OHC) from the apical end of P7 mouse cochlea

Hair Cell	$I_{\max}$, pA	Operating Range 10–90%, μm	τ_{fast} , ms	τ_{slow} , ms
IHC				
Mean	324 $\pm$ 118 (16)	0.86 $\pm$ 0.17	1.47 $\pm$ 1.26 (12)	21.5 $\pm$ 15.3 (12)
Range	195–580	0.53–1.15	0.66–5.28	9.4–54.3
OHC				
Mean	311 $\pm$ 69 (11)	0.44 $\pm$ 0.12	1.75 $\pm$ 0.93 (11)	15.4 $\pm$ 9.4 (11)
Range	200–418	0.29–0.66	0.46–3.17	8.6–36.4

Values are means $\pm$ SD (number of measurements). Fast and slow adaptation time constants (τ_{fast} and τ_{slow}) were calculated from double-exponential fits to inferred-shift data extracted from transduction currents at $P_0 \sim 0.5$.