nature neuroscience

Fast adaptation of mechanoelectrical transducer channels in mammalian cochlear hair cells

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Outer hair cells are centrally involved in the amplification and frequency tuning of the mammalian cochlea, but evidence about their transducing properties in animals with fully developed hearing is lacking. Here we describe measurements of mechanoelectrical transducer currents in outer hair cells of rats between postnatal days 5 and 18, before and after the onset of hearing. Deflection of hair bundles using a new rapid piezoelectric stimulator evoked transducer currents with ultra-fast activation and adaptation kinetics. Fast adaptation resembled the same process in turtle hair cells, where it is regulated by changes in stereociliary calcium. It is argued that sub-millisecond transducer adaptation can operate in outer hair cells under the ionic, driving force and temperature conditions that prevail in the intact mammalian cochlea.

Hair cells, the sensory receptors of the inner ear, convert sound-evoked vibrations of the cochlear partition into electrical signals encoding the frequency and intensity of the acoustic stimulus. Mechanoelectrical transduction is accomplished in the stereociliary bundle, which when deflected opens mechanically sensitive ion channels at the tips of the stereocilia[1]. Although these transducer channels have not yet been molecularly characterized, their functional properties have been extensively documented in some vertebrates[2,3]. One notable feature is regulation by intracellular calcium, which when elevated leads to channel closure[4,5]. Calcium enters the stereocilia through cation-selective transducer channels already opened by the mechanical stimulus and acts in a negative feedback manner to reset the channel's operating range. This process is known as adaptation[6,7], and it involves multiple mechanisms with distinct kinetics and dynamic ranges[8,7]. One component, fast adaptation, occurs in milliseconds and may require direct interaction of Ca^{2+} with the channel[3,9]. Under some conditions, the adaptive decline in current can generate oscillations at a frequency in the animal's auditory range, suggesting that it contributes to cochlear frequency tuning[9].

Experiments on hair cells in early neonatal culture have shown that mechanoelectrical transduction in mammals possesses many of the same features seen in non-mammalian vertebrates[10–13]. Nevertheless, little is known about transduction in cochlear hair cells in animals with fully developed hearing[14,15], about the speed or extent of adaptation, or about modifications in transducer channels that occur with hair-cell maturation at the onset of hearing. Indeed, it is unclear whether fast adaptation exists in the mammalian cochlea. Without such information, it is difficult to define the contribution of the hair cells to cochlear mechanics and frequency selectivity[16–18]. This is especially important for the outer hair cells, whose electromechanical behavior[19] driven by the voltage-dependent reorganization in the membrane protein prestin[20], is thought to be the lynchpin of the cochlear amplifier[21]. Here we describe measurements of mechanotransducer currents displaying fast, calcium-dependent adaptation in outer hair cells of rats both before and after the onset of hearing.

RESULTS

Activation and adaptation of transducer currents

Mechanotransducer currents in response to rapid deflections of the hair bundle, achieved with a new piezoelectric stimulator (see Methods), were recorded from outer hair cells in rats both before and after the onset of hearing. Currents increased in amplitude with displacements up to 0.5 μm, but even for the smallest stimulus they had a swift onset (**Fig. 1**). The activation time constant for small stimuli was 50 ± 10 μs in five cells with a recording time constant of 46 ± 8 μs. The activation time constant did not accelerate with current amplitude, but was limited by the stimulation and recording speeds. The rate limit suggests that mechanotransducer channels in mammalian hair cells, even at room temperature, have unusually fast kinetics compared to those in lower vertebrate hair cells[4,22]. For stimuli producing less than ten percent of the maximum response amplitude, adaptation of the current was fast and often nearly 100% complete. In the five cells quoted above, the adaptation time constant was 154 ± 31 μs with 1.5 mM Ca^{2+}. There was a significant variation in the maximum amplitude of the transducer current among the cells studied, perhaps due to variable damage to the hair bundle and loss of channels. Cells with the largest transducer currents, which may have suffered least injury, had the fastest adaptation rates (**Fig. 2**).

Calcium effects on transduction

Lowering extracellular calcium to 50 μM (similar to the concentration (30 μM) in endolymph[23]) increased the maximum current by a factor of 1.58 ± 0.19 (n = 4) and slowed the adaptation time constant by 2.37 ± 0.99 (**Fig. 1**). The time constant in low calcium remained fast (390 ± 190 μs), indicating that when mammalian hair bundles are exposed to calcium in the physiological range, mechanotransduction still

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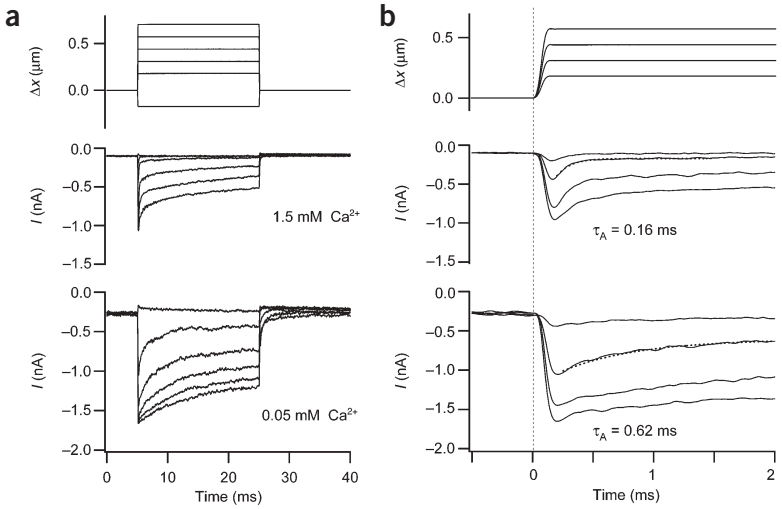

Figure 1 Ca^{2+} modulation of mechanotransducer currents in outer hair cells. (**a**) Reducing Ca^{2+} concentration in the hair bundle perfusate from 1.5 mM (middle) to 0.05 mM (bottom) increased the peak amplitude of the mechanotransducer current (*I*) and slowed the rate of adaptation. The stimulus (Δ*x*) is shown at the top. In this and subsequent figures, the stimulus monitor is the voltage delivered to the piezoelectric device, delayed by 30 μs to allow for the lag in the probe motion (see Methods). (**b**) Response onsets show a four-fold slowing of the time constant of fast adaptation (τ_A) in reduced Ca^{2+}. The changes were fully reversible. In this and subsequent figures, the adaptation time constant was obtained from fits to low-level linear responses (dashed lines). At all levels, the current developed as fast as the bundle changed position. Each response is the average of ten presentations. Holding potential, –84 mV; $T = 20$ °C; P8 rat.

displays substantial adaptation. Two effects of calcium on transduction have been previously observed in hair cells of turtles[24,25] and frogs[23]. One of these is an external block of the channel, and the relief of block on reducing calcium increases the current amplitude; the other is an intracellular action to promote adaptation, whereby calcium entering through the transducer channels open during an excitatory stimulus acts at an intracellular site to counteract the stimulus and close the channels.

It was previously shown in turtle hair cells that the rate of fast adaptation is proportional to the calcium influx into the stereocilia, which was quantified by loading the cells with a fluorescent calcium indicator[25]. Calcium influx can be altered in several ways. Lowering extracellular calcium concentration reduces the influx and hence slows adaptation (**Fig. 1**). Conversely, if a hair cell possesses more mechanotransducer channels per stereocilium, there is a larger calcium entry to increase its stereociliary concentration. This may partly account for the finding that cells with larger mechanotransducer currents have faster rates of adaptation (**Fig. 2**), a relationship that has also been observed in turtle hair cells[26], but other mechanisms may also contribute to this relationship. A third method for changing calcium influx is to alter the membrane potential. Hyperpolarization increased the peak current and accelerated the adaptation rate (**Fig. 3**). In two experiments, one using postnatal day 8 (P8) animal before the onset of hearing and the other a P13 hearing animal, the holding potential was increased from –84 mV to –134 mV. The larger driving force increased the maximum current by a mean factor of 1.9 ($n = 2$) and the adaptation rate by a factor of 1.8. These values are sufficiently similar to argue that adaptation was accelerated proportionally with the size of calcium influx[25].

Changes in outer hair cells with maturation

Being altricial mammals, rats are immature, blind and deaf at birth and develop their sensory capacities during the second and third week of life. There have been several reports that electrical responses to sound cannot be recorded in rats until the second postnatal week[27–29]. As indicators of functional cochlear output, the auditory nerve compound action potential[28] and the acoustic brainstem response[29] are not measurable until about P12. This timing agrees with our limited behavioral test showing no startle responses to sound until P11–P12. During development, a series of changes occur in the rat cochlea including modifications to the outer hair cells[30]. We observed an increase in outer hair-cell linear capacitance with age: 7.1 ± 1.4 pF ($n = 9$; P3–P7); 10.7 ± 2.1 pF ($n = 18$; P8–P11); 14.4 ± 3.7 pF ($n = 17$; P12–P18). The third group comprised animals that by behavioral assessment were judged to be hearing. The increase in linear capacitance may largely reflect the elongation of the outer hair cells, and along with expression of the motor protein prestin, may be linked to the appearance of the contractility[31,32]. Such contractility is thought to be essential for the high sensitivity and sharp frequency selectivity of the mammalian cochlea[17,19].

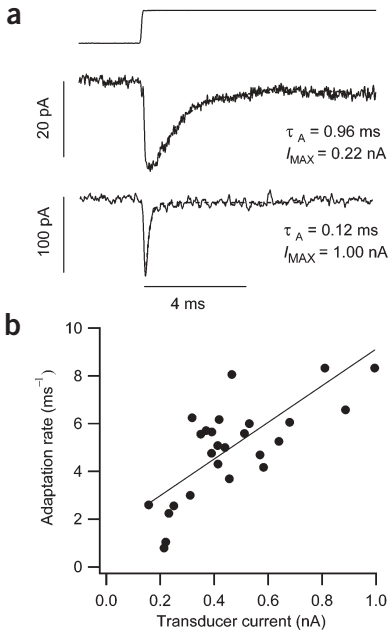

Figure 2 Adaptation rate depends on the size of the transducer current. (**a**) Examples of low-level responses in two outer hair cells with different maximum transducer currents (I_{max}) of 0.22 nA (P7) and 1.00 nA (P8). For the same size of bundle displacement (0.18 μm), the adaptation time constant was 8 times faster in the cell with the larger maximum current. Adaptation time constants (τ_A) derived from single exponential fits (dashed lines). (**b**) Plot of adaptation rates ($1/\tau_A$) versus maximum current in 26 outer hair cells, P5–P18. Holding potential –84 mV, $T = 18$–22 °C. Line is least-squares fit, $r = 0.77$.

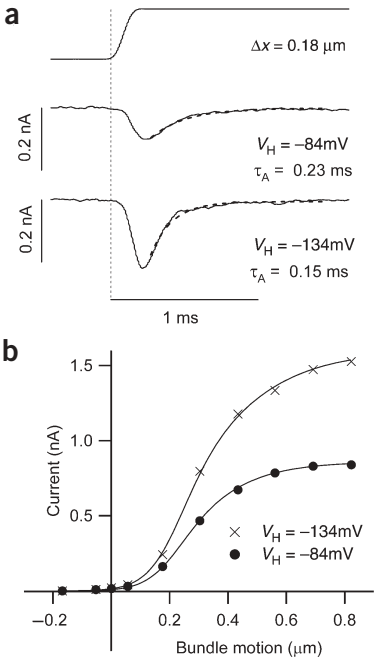

Figure 3 Adaptation rate increases with holding potential. (**a**) Increasing the holding potential (V_H) from –84 to –134 mV decreased the adaptation time constant from 0.23 to 0.15 ms. Adaptation to small stimuli was fitted with single exponential decays (dashed lines). (**b**) Current displacement relationships show that increasing the holding potential approximately doubled the peak current. Each filled symbol at –84 mV is the mean of values before and after the measurement at –134 mV. The increase in holding potential simulated the effect of adding the endolymphatic potential. P8 rat, $T = 22$ °C.

Because the cochlea becomes more difficult to isolate with postnatal age, fewer measurements were obtained in the older hearing animals. Although rats are hearing at P12, their auditory sensitivity continues to improve until at least P30 (ref. 28), and at P18 (the oldest animal from which we have results) the compound action potential threshold is still 14 dB above the adult value. Nevertheless, our limited results gave no evidence of a large change in the number or properties of mechanotransducer channels with the onset of hearing (**Fig. 4**). The peak amplitude of the transducer current was 0.530 ± 0.208 nA (mean ± s.d.) in 14 non-hearing animals in the age range P5–P12 and 0.504 ± 0.153 nA in seven hearing animals in the age range P11–P18. These mean values do not differ significantly. There was also no significant change in fast adaptation between these two populations with mean time constants of 193 ± 59 μs in non-hearing animals and 183 ± 27 μs in hearing animals.

In contrast to the mechanotransducer channels, clear alterations in the voltage-dependent channels of rat outer hair cells (**Fig. 5**) similar to those seen in mouse[33] were observed. There was an increase in both the size and activation rate of the outward current turned on by depolarization. Moreover, an inward current, turned off by hyperpolarization from –84 mV to –144 mV was seen in all six outer hair cells for hearing animals (mean inward current, 368 ± 79 pA; P13–P16) but did not occur in five cells from immature animals (4 ± 13 pA; P3–P5). This probably reflects deactivation of the $I_{K,n}$ current flowing through K^+ channels[34,35] that are thought to contain the KCNQ4 subunit[33,36]. The appearance of this current may largely account for the increase in the resting potential of the outer hair cells from –58 ± 4 mV (P3–P5) to –73 ± 8 mV (P12–P16).

DISCUSSION

Fast adaptation is common to all hair cells

There are two components of transducer adaptation in inner-ear hair cells, distinguished by their kinetics and mechanism[8,37]. One occurs on a millisecond time scale and probably involves calcium binding at or near the mechanotransducer channel. The other takes place over tens or hundreds of milliseconds and influences mechanical input to the channel through operation of an unconventional myosin (*e.g.*, myosin 1c[12]). The properties of fast adaptation in outer hair cells—submillisecond time course and dependence on calcium and current amplitude—are similar to those seen in the turtle cochlea[25,26]. The properties suggest that fast adaptation in mammals, as in the turtle, may result from direct interaction of calcium with the transducer channel[4,24] rather than through the action of a myosin (1c or 7a). The time constants of transducer-channel activation and adaptation are even faster than the equivalent turtle values[4,38]. This argues for expression of a different isoform of the mechanotransducer channel in mammalian outer hair cells to cope with higher sound frequencies that must be transduced. The adaptation we measured is more pronounced and its time constant faster than previously seen in neonatal outer hair cells (4 ms)[11]. One explanation for this discrepancy is slower bundle displacement achieved by the water-jet method of stimulation compared to the fast piezoelectric stack actuator used here. Slowing the displacement step has been shown to attenuate fast adaptation[8].

What is the role of fast adaptation? In non-mammalian vertebrates, it is thought to drive

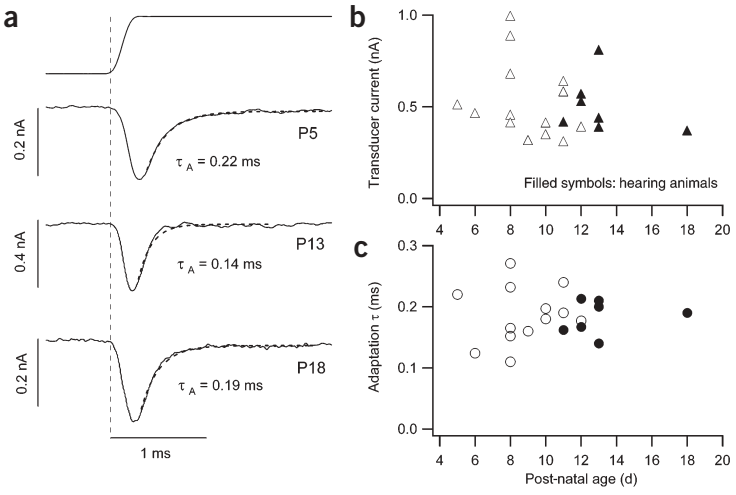

Figure 4 Properties of mechanotransduction during cochlear maturation. (**a**) Low-level transducer currents in animals of three different postnatal ages, including two 'hearing' animals (P13 and P18). The adaptation time constant was similar at the three ages. Maximum current: 0.51 nA (P5), 0.81 nA (P13), 0.37 nA (P18). (**b**) Maximum transducer current plotted against postnatal age. (**c**) Adaptation time constant versus postnatal age. Filled symbols in **b** and **c** denote 'hearing' animals. $T = 18$–22 °C.

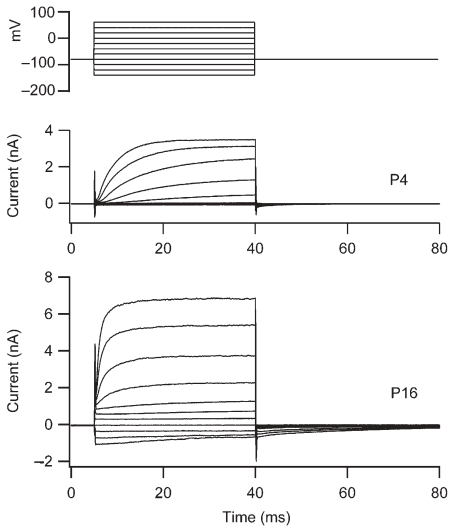

Figure 5 Outer hair cell voltage-dependent currents change with maturation. Outward currents were smaller and slower in a P4 (non-hearing) compared to a P16 (hearing) rat. Currents with similar size and kinetics to those illustrated were seen in recordings from five other 'non-hearing' and four 'hearing' animals. The inward current, $I_{K,n}$, was present at P16 but not at P4.

active motion of the stereociliary bundle[39,40], which can sum with and hence amplify the acoustic stimulus[41]. Furthermore, the time course of fast adaptation and active bundle motion vary systematically with the hair cell's characteristic frequency, suggesting their participation in cochlear frequency tuning[3,40]. In contrast, somatic electromotility of the outer hair cells is currently the most popular mechanism for the mechanical amplification that enhances frequency selectivity in the mammalian cochlea[17–19]. Our observation of fast Ca^{2+} -dependent adaptation in outer hair cells raises the possibility that a bundle amplifier may also exist in mammals[42].

Mechanotransducer channels *in vivo*

We measured the properties of mechanotransducer channels in isolated preparations exposed to different conditions from those in the intact cochlea. To extrapolate to the *in vivo* performance of the outer hair cells, it is necessary to take account of several factors that affect the values. These include the endolymphatic potential and the higher body temperature, both of which increase the amplitude of the transducer current and adaptation rate, as well as the endolymphatic calcium concentration, which augments the current but slows the adaptation rate. The presence of K^+ as the major monovalent cation in endolymph will also produce a ~25% increase in the amplitude of the transducer current and adaptation rate over that measured in the experimental Na^+ -based solution[24,25]. Scaling factors for the effects of endolymphatic potential and calcium concentration were available from the experiments: to extrapolate the results from 20 °C to 37 °C, a Q_{10} of 2 was used for the channel kinetics[4,22] and a Q_{10} of 1.3 for the channel conductance (Ref. 43 gives a Q_{10} range of 1.2–1.5 for the temperature dependence of open channel conductances). After correction for an increased driving force of 150 mV across the transducer channel (80 mV endolymphatic potential summed with –70 mV resting potential) and the differences in endolymphatic cation (K^+ instead of Na^+) and temperature, the adaptation time constant, τ_A, of 390 μs in 50 μM calcium, is reduced to 49 μs *in vivo*. This is equivalent to a half-power frequency ($1/2\pi\tau_A$) of 3.2 kHz, approximately two-thirds of the characteristic frequency at the cochlea location where the recordings were made (5 kHz; Methods). A similar ratio exists between the adaptation time constant and characteristic frequency in the turtle cochlea[9], where the adaptation rate changes in parallel to the characteristic frequency. Corrections for the same factors can be applied to the transducer current to estimate its *in vivo* amplitude. Starting with 1.6 nA, the largest value measured experimentally in 50 μM Ca^{2+} (**Fig. 1**), a transducer current of at least 6 nA is predicted at the 5 kHz place in the intact cochlea.

Hair-cell maturation

The size of the transducer current *in vivo* is increased by the endolymphatic potential which sums with hair cell resting potential to approximately double the electrical driving force across the mechanotransducer channels in the stereociliary membrane. Although no evidence was found for a significant up-regulation of these channels at the onset of hearing, the transducer current will grow around the onset of hearing because of the development of the endolymphatic potential, as it increases[44] between P8 and P16. This period coincides with the emergence of the potassium-selective current, $I_{K,n}$, which flows through channels activated at the resting potential and thus behaves like a leak current. One role for $I_{K,n}$ may be as part of a K^+ recirculation pathway, allowing efflux into the perilymph of K^+ ions that have entered through the mechanotransducer channels[45]. Is $I_{K,n}$ sufficiently large to fulfill this role? If we assume a maximum mechanotransducer current *in vivo* of 6 nA, about 10% activated at the resting position of the bundle would require a current return of 0.5–1 nA for moderate but continuous acoustic stimulation, comparable to the measured $I_{K,n}$ (refs. 33,35; **Fig. 5**). An implication is that the larger $I_{K,n}$ in more basal outer hair cells[46] may reflect an increase in transducer current in high-frequency hair cells[47] as found in the turtle[26]. An important future experiment will be to record from outer hair cells at other cochlear locations to determine if there is a tonotopic variation in the size or kinetics of the transducer current.

METHODS

Experiments were performed on outer hair cells in the apical turn of the organ of Corti isolated from Sprague-Dawley rats[48] at P3–P18. Rats were killed by cervical dislocation, decapitated and the temporal bone removed using procedures approved by the Animal Care Committee of the University of Wisconsin. After shaving the bone from the apical and middle turns, the stria vascularis was unpeeled and, following a 15-min incubation in saline containing 50 μg/ml of bacterial subtilisin proteinase (Sigma type XXIV), the tectorial membrane was removed. Excised apical and middle turns were fixed in the experimental chamber with strands of dental floss[26] and viewed through a 40× LWD water-immersion objective on a Zeiss Axioskop FS microscope. The experimental chamber was perfused with artificial perilymph (150 mM NaCl, 6 mM KCl, 1.5 mM $CaCl_2$, 1 mM $MgCl_2$, 2 mM Na–pyruvate, 10 mM glucose and 10 mM Na–HEPES, pH 7.4). The apical surface of the organ of Corti was separately perfused through a 100-μm pipette with a perilymph-like solution lacking $MgCl_2$ and with normal (1.5 mM) or reduced (50 μM) $CaCl_2$. The free Ca^{2+} concentration in the apical perfusate was measured with a Ca^{2+} electrode (MI-600, Microelectrodes Inc.) previously calibrated in a series of calcium buffer solutions (World Precision Instruments). To determine whether animals were hearing at the time of the experiment, they were tested for a startle response to a 'kissing' sound presented at 50–60 dB sound pressure level.

Hair cells were whole-cell voltage clamped at room temperature (18–22 °C) with borosilicate patch electrodes connected to an Axopatch 200A amplifier (Axon Instruments). Membrane potentials were corrected for a 4 mV junction potential. Unless otherwise stated, the holding potential was –84 mV. The patch electrode was introduced into the organ of Corti along the longitudinal axis of the cochlea through a small hole made in the reticular lamina. Most recordings were made from first or second row outer hair cells at the beginning of the apical turn, about 0.75 of the distance along the basilar membrane from

the base. The recording site corresponded to a characteristic frequency of ~5 kHz[49]. Recording from high-frequency cells located more basally was not attempted because of increased damage to the organ of Corti incurred by bone removal. Patch pipettes were filled with an intracellular solution (150 mM KCl, 3.5 mM $MgCl_2$, 1 mM EGTA, 5 mM Na_2ATP, 0.5 mM Na_2GTP and 10 mM K–HEPES, pH 7.2). The time constant of the recording system, with up to 70% series-resistance compensation, was between 40 and 80 μs. Data were filtered at 10 kHz, sampled at 100 kHz with a CED Power1401 (Cambridge Electronic Design) and stored on computer for subsequent analysis (Igor Pro v4.0; WaveMetrics). Each transducer current illustrated is the average response to ten presentations. Values are given as mean ± s.d.

Hair bundles were mechanically deflected by axial motion of a glass pipette driven by a piezoelectric stack actuator (PA8/12; Piezosystem Jena)[38]. The tip of the pipette was fire-polished so that its tip (~3 μm diameter) fit into the V-shaped stereociliary bundle of the outer hair cell (**Supplementary Fig. 1**); probes with different diameters were used to accommodate the change in bundle size with development. The actuator had a resonant frequency of 50 kHz and was driven with voltage steps filtered with an 8-pole Bessel filter set at 5–10 kHz. The time course of the glass probe was calibrated by projecting its image onto a pair of photodiodes as previously described[40]. When the voltage step to the piezoelectric device was filtered at 5–10 kHz, the mechanical displacement had a rise time of 100–50 μs, and was delayed by ~30 μs with respect to the voltage. The glass probe was cleaned in chromic acid before an experiment to help it adhere to the bundle. The fraction of current turned on at the resting position of the bundle may have been underestimated, as this was obtained by pulling the bundle in the negative direction, which tends to part probe and bundle. Nevertheless, the fraction of current on at rest was similar to values reported for outer hair cells in other isolated cochleas (<0.1)[11] but smaller than the 0.2–0.5 measured *in vivo*[10,50]. Both the sudden appearance of the transducer current as the probe was advanced onto the bundle, and the negligible delay between the onset of the stimulus and the current indicate that for positive stimuli, there was good contact between probe and bundle.

Note: Supplementary information is available on the Nature Neuroscience website.

ACKNOWLEDGMENTS

This work was supported by grant RO1 DC 01362 to R.F. from the National Institutes on Deafness and other Communicative Disorders (NIH) and travel grants to M.G.E. and H.J.K. from the Wellcome Trust.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

Received 14 March; accepted 5 June 2003
Published online 29 June 2003; doi:10.1038/nn1089

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