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Otoacoustic emissions without somatic motility: Can stereocilia mechanics drive the mammalian cochlea?

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Abstract

Distortion product otoacoustic emissions (DPOAEs) evoked by low-level tones are a sensitive indicator of outer hair cell (OHC) function. High-level DPOAEs are less vulnerable to cochlear insult, and their dependence on the OHC function is more controversial. Here, the mechanism underlying high-level DPOAE generation is addressed using a mutant mouse line lacking prestin, the molecular motor driving OHC somatic motility, required for cochlear amplification. With prestin deletion, attenuated DPOAEs were measurable at high sound levels. DPOAE thresholds were shifted by ~50 dB, matching the loss of cochlear amplifier gain measured in compound action potentials. In contrast, at high sound levels, distortion products in the cochlear microphonic (CM) of mutants were not decreased *re* wildtypes (expressed *re* CM at the primaries). Distortion products in both CM and otoacoustic emissions disappeared rapidly after death. The results show that OHC somatic motility is not necessary for the production of DPOAEs at high SPLs. They also suggest that the small, physiologically vulnerable DPOAE that remains without prestin-based motility is due directly to the mechanical nonlinearity associated with stereociliary transduction, and that this stereocilia mechanical nonlinearity is robustly coupled to the motion of the cochlear partition to the extent that it can drive the middle ear.

I. INTRODUCTION

When presented with two tones, a listener hears combination tones at mathematical combinations of the input frequencies, f_1 and f_2 , the most prominent of which is at $2f_1 - f_2$. The nonlinearity responsible for these combination tones originates in the auditory periphery: when two tones are presented, response components at $2f_1 - f_2$ can be measured in auditory nerve discharge (Goldstein and Kiang, 1968), in ear-canal sound pressure (e.g., Mountain, 1980) and in basilar membrane motion (Robles et al., 1997). These nonlinear responses, termed distortion product otoacoustic emissions (DPOAEs) when measured in the ear canal, are extremely labile: i.e., they are suppressed by manipulations which reduce cochlear sensitivity (e.g., Mountain,

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1980; Siegel et al., 1982). For this reason, they are widely used as a noninvasive assay of cochlear function (Lonsbury-Martin and Martin, 1990).

Somatic electromotility of outer hair cells (OHCs), which can be demonstrated *in vitro* (Brownell et al., 1985), drives an amplification process, *in vivo*, which is required for mechanical sensitivity in the mammalian cochlea (Liberman et al., 2002). Thus OHCs, and their feedback enhancement of cochlear vibration, must contribute to the amplification of DPOAEs. However, the source of the nonlinearity which produces DPOAEs is less clear. Indeed, different sources may contribute at high vs low sound levels (Mills and Rubel, 1994). Candidate sources in the cochlear transduction process include nonlinearities in (1) mechano-electric transduction by stereocilia (Corey and Hudspeth, 1979), or (2) electro-mechanical transduction by somatic motility (Santos-Sacchi, 1991). Alternatively, distortion may arise, not through transduction *per se*, but via the mechanical nonlinearities associated with the gating of transduction channels (Jaramillo et al., 1993), which could be mechanically coupled to the cochlear partition such as to make the entire vibration system nonlinear.

A direct test of the source(s) of nonlinearity generating DPOAEs is available via the mouse line with targeted deletion of prestin, a member of the sulfate transporter family expressed in OHCs, and proposed as the molecular motor driving cochlear amplification (Zheng et al., 2000). Prestin deletion reduces cochlear sensitivity *in vivo* by 35–60 dB, as it eliminates OHC electromotility *in vitro* (Liberman et al., 2002). A recent study of prestin mutants did not focus on DPOAEs, because DPOAE thresholds were close to the level of system distortion. With an improved acoustic delivery system, we now revisit the question of DPOAE generation without prestin, and show that distortion components at $2f_1-f_2$, although attenuated, remain in both cochlear microphonic potentials and ear-canal sound pressure.

These observations are not inconsistent with the conventional view that, in the normal ear, mammalian DPOAEs originate when stereocilia-based nonlinearities in forward transduction create distortion components in OHC receptor potentials, which are then reverse-transduced into mechanical distortions via prestin-mediated somatic motility. However, the persistence of ear-canal DPOAEs in the absence of OHC somatic motility indicates that a source of mechanical nonlinearity must remain. We suggest that this source is the mechanical manifestation of the nonlinearities in forward transduction, i.e., the nonlinear mechanics of the stereocilia bundle. If true, mammalian stereocilia must be sufficiently well coupled to the motion of the cochlear partition that they can drive the middle ear to produce DPOAEs in the ear canal.

II. METHODS

A. Animals, experimental groups, anesthesia and surgery

The creation of the mouse line with targeted deletion of the prestin gene, has been described elsewhere (Liberman et al., 2002). In the present study we used mice from a subsequent generation (*F3-F5*) of this line, propagated by matings of heterozygous carriers of the deletion. Animals were studied at 1–2 months of age. Groups of wildtype, heterozygous and homozygous littermates were studied during the same experimental session. All procedures were approved by the IACAUC of the Massachusetts Eye and Ear Infirmary.

Animals were anesthetized with ketamine (100 mg/kg i.p.) and xylazine (20 mg/kg i.p.) with booster injections (1/2 original dose) as needed. Surgical preparation involved removal of the right pinna, followed by exposure of the right bulla, which was opened with a #11 scalpel for the recording of cochlear potentials.

B. Cochlear potentials

The compound action potential (CAP) and cochlear microphonic (CM) were recorded from the right ear of each mouse via a fine, Teflon-coated silver wire on the round window membrane referred to the tongue. The response was amplified (10,000 X), filtered (100 Hz–30 kHz), and averaged with an A–D board (National Instruments, 6052) in a LabVIEW-driven data acquisition system. For CAP, thresholds were measured under computer control in response to 5-ms tone pips (0.5-ms rise–fall with a $\cos^2$ onset envelope, delivered at 20/sec). At each SPL, 32 responses were averaged (with stimulus polarity flipped on half of the presentations to remove microphonic potentials). Threshold was defined as the sound pressure required to produce a peak-to-peak neural response of 3 μ V. For CM measurement, continuous tones were presented and the voltage from the round-window electrode digitized, averaged and analyzed as described for DPOAEs.

C. Distortion product otoacoustic emissions (DPOAEs)

The DPOAE at $2f_1$ – f_2 was recorded with a custom acoustic assembly consisting of two electrostatic drivers (ES-1; Tucker Davis Technologies) to generate primary tones (f_1 and f_2 with $f_2/f_1 = 1.2$ and f_2 level 10 dB, < f_1 level) and a Knowles miniature microphone (EK3103) to record ear-canal sound pressure via a probe tube concentrically situated within the common sound-delivery tube. The sensitivity of the probe-tube microphone (volts/dB SPL at the tip of the probe tube) was measured in a small coupler using a calibrated 1/4" condenser microphone (Bruel and Kjaer). Stimuli were generated digitally and presented to the drivers via custom-built low-distortion attenuators. Ear-canal sound pressure was amplified and digitally sampled at 4 μ s with sampling locked to the digital stimulus generation. FFTs were computed on waveform averages (25 repetitions of a 8192-point buffer) to lower the noise floor, and 5 spectra were averaged, from which the $2f_1$ – f_2 DPOAE amplitude and surrounding noise floor were extracted.

III. RESULTS

A. Cochlear threshold sensitivity and loss of gain of the cochlear amplifier

A previous report on cochlear sensitivity in mice with targeted deletion of the prestin gene (Liberman et al., 2002) reported threshold elevation in homozygous mutants ranging from ~40 dB at 5.6 kHz to 60 dB at 22.6 kHz. Thresholds in mice heterozygous for the deletion were surprisingly close to the wildtypes, i.e., within 3–6 dB. These estimates of gain loss due to the elimination of the putative motor protein of the cochlear amplifier were based on measures of auditory brainstem responses (ABRs), the far-field potentials extracted from needle electrodes in the scalp in response to tone pips at different test frequencies.

In the present study, the loss of gain in each mutant animal was estimated by measuring compound action potentials (CAPs) from the round window membrane in response to tone pips. These potentials represent the summed soundevoked activity of the auditory nerve. As shown in Fig. 1, CAP results are similar to those reported with ABRs. The mean threshold difference between the most sensitive homozygous mutants [black open circles in Fig. 1(a)] and the most sensitive wildtypes [black filled circles in Fig. 1(a)] ranged from ~35 dB at 3.2 kHz to ~55 dB at 16 kHz [Fig. 1(b)]. As reported previously (Liberman et al., 2002), heterozygous prestin mutants showed cochlear sensitivity very close to that of the wildtypes. The small (~3 dB), but statistically significant, decrease in sensitivity for heterozygotes *re* wildtypes reported previously using ABR measures was not replicated in the present study using CAP [although it has recently been verified in a second group of animals studied via ABR (Wu et al., 2004)]. The lack of difference in the present study may be explained by the smaller sample size and the larger intra-group variance in the present study. The increased

variance arises because measurement of CAP involves an invasive surgical procedure which must produce varying degrees of threshold shifts.

B. Effects of gain loss on distortion products: Ear canal vs cochlear microphonic

In the present study, distortion products were measured in every animal in two different ways: (1) as acoustic signals, by an analysis of the ear-canal sound pressure waveform, and (2) as electric signals, by analysis of the cochlear microphonic (CM) potentials recorded from the round window, which represent the summed transduction currents through cochlear hair cells (e.g., Dallos and Cheatham, 1976).

Distortion product otoacoustic emissions (DPOAEs) at $2f_1-f_2$ were measurable in all wildtypes and heterozygous mutants, and DPOAE amplitudes in heterozygotes were similar to those seen in wildtypes. Data are shown for $f_2 = 22.6$ in Fig. 2(a); similar results were obtained at other frequencies tested (5.6 to 45.2 kHz in half-octave steps).

DPOAEs were measurable in roughly half of the homozygous prestin mutants. The strongest responses were seen for $f_2 = 22.6$ and 8.0 kHz, frequencies close to the regions of maximum sensitivity in these mutants [Fig. 1(a)]. Those homozygous animals in which DPOAEs were measurable [black open circles in Fig. 2(a)] corresponded to cases with the lowest CAP thresholds [black open circles in Fig. 1(a)]. DPOAE amplitudes in the homozygotes, when present, were clearly lower than those in either wildtypes or heterozygotes. As illustrated in Fig. 2(a), the horizontal shift in the DPOAE growth functions in mutants *re* wildtypes was about 50 dB, i.e., similar to the threshold shift (i.e., “loss of gain”) measured by the CAP.

Cochlear distortion at $2f_1-f_2$ was also measured in the round-window CM in all animals [Fig. 2(b)]. As seen in DPOAE measurements, distortion product (DP)CM amplitudes were similar in wildtypes and heterozygotes, whereas those in homozygous mutants were greatly depressed, especially at low stimulus levels. Note that homozygotes showing measurable DPOAEs (black unfilled circles) had larger DPCM than those for which the DPOAEs were below the noise floor (gray unfilled circles). Note also that the horizontal shift in DPCM growth functions for homozygotes is smaller than the loss of gain measured via CAP [Fig. 1(a)] or via DPOAEs [Fig. 2(a)].

The primary components (f_1 and f_2) in the CM response were also measured in each animal. As shown in Fig. 2(c), the CM amplitudes were, in general, lower in homozygotes than in wildtypes and heterozygotes. Among homozygotes, amplitudes of the primaries in the CM tended to be larger in the animals with measurable ear canal DPOAEs (black unfilled circles) than in those for which the DPOAEs were in the noise (gray unfilled circles). To assess possible changes in the operating point of mechano-electric transduction in the prestin null mice, CM was also measured in response to a low-frequency tone (2 kHz) at 90 dB SPL and compared to the input waveform as Lissajous patterns: no differences were seen between homozygotes and wildtypes (data not shown).

The decreased CM amplitudes in homozygotes [Figs. 2(b), 2(c)] must arise, in part, because of basal-turn OHC loss in these ears (Liberman et al., 2002), and, in part, because of the loss of cochlear amplification. However, the horizontal shift in DPCM growth functions [Fig. 2(b)] is less than the loss of gain measured via CAP (Fig. 1), because the cochlear amplifier is less important to round-window CM than CAP. CM arises from hair cells distributed throughout the basal turn (Kletsky and Zwislocki, 1980), and, for many of these generators, the primaries are well below their best frequency. In contrast, the CAP threshold is dominated by responses generated near the best frequency and is thus more dependent on the cochlear amplifier.

At high stimulus levels, the DPCM in homozygotes was almost as large as that in wildtypes, when viewed relative to the CM amplitudes to the primaries [Fig. 2(d)]. Since there is loss of cochlear amplification, and there may be variable degrees of hair-cell loss in the prestin null mouse (Liberman et al., 2002), the absolute values of the CM and the DPCM are difficult to interpret. A more relevant measure is the ratio of DPCM to CM produced by the primaries. As shown in Fig. 2(d), the DPCM amplitudes at high stimulus levels are roughly 20 dB down from the amplitudes of the primary components in the same ears, and there are almost no differences between wildtypes and homozygotes in this measure.

C. Loss of ear-canal DPOAEs post-mortem

The stimulus range over which DPOAE measurements can be made is limited by nonlinearities in the stimulus delivery and measurement systems, which produce distortion components at $2f_1$ - f_2 at high stimulus levels. Given the small size and high evoking levels for the DPOAEs measured in the homozygotes, it is critical to demonstrate that they do not arise from system distortion.

The fact that those mutant animals with the highest CAP thresholds showed no measurable ear canal distortions, even at the highest SPLs, suggests that distortion artifacts arising from acoustic delivery or signal measurement are not generating the DPOAEs in the more sensitive mutants. Further evidence for a biological origin for the attenuated DPOAEs in homozygotes is seen in Fig. 3(a), where DPOAE amplitudes evoked by 80 dB SPL primaries are tracked over time, before and after death and/or cochlear partition destruction. In all cases, DPOAE amplitudes fell precipitously within a few minutes after death, clearly demonstrating a biological origin. For those few cases in which a measurable DPOAE lingered post-mortem, subsequent cochlear partition destruction (accomplished by the insertion of a small right-angle hook through the round window without moving the acoustic assembly or disarticulating the stapes) eliminated the responses. Note that the time course for the disappearance of the high-level DPOAEs closely mirrors the time course for post-mortem decay of the cochlear microphonics [Fig. 3(b)].

IV. DISCUSSION

A. Existing ideas about mammalian DPOAE generation: High vs low-level responses

Studies in animal models show the vulnerability of DPOAEs, at low stimulus levels, to manipulations which damage OHCs, e.g., ototoxic drugs (Henley et al., 1996), acoustic overstimulation (Mills, 2003) or decreased endocochlear potential (Avan et al., 2003), and their lack of vulnerability to loss of inner hair cells (e.g., Liberman et al., 1997). The prevailing view of DPOAE generation is that (1) nonlinearities in mechano-electric transduction in OHCs produce distortion components in the OHC receptor potential when two tones are present; that (2) these electrical distortions drive the electro-mechanical transducer, i.e., prestin-mediated electromotility in OHCs; and that (3) this OHC-based somatic motility at the distortion frequency is coupled to motions of the cochlear partition which propagate from the site of generation (near the cochlear region tuned to f_2) both apically to the cochlear region tuned to $2f_1$ - f_2 and basally to stapes, where the energy is transmitted to tympanic membrane motion and sound generation in the ear canal at $2f_1$ - f_2 (Zheng et al., 1996; Lukashkin and Russell, 1998; Withnell and Yates, 1998; Kalluri and Shera, 2001; Mom et al., 2001).

It has been suggested that different mechanisms underlie DPOAE generation at low vs high stimulus levels (e.g., Mills and Rubel, 1994). These speculations derived from two observations: (1) that the growth of DPOAE amplitude with primary level can show prominent nonmonotonicities (i.e., dips) associated with phase shifts, at primary SPLs of 60–70 dB SPL; (2) that a number of manipulations, including i.v. diuretics, cochlear ischemia and death, reduce

DPOAE amplitudes for low-level primaries (below the dip), with little effect on DPOAE amplitudes for high-level primaries (above the dip). These observations, and the idea that the effects of OHC somatic motility on basilar membrane motion are large only at low SPLs (e.g., Ruggero and Rich, 1991), led Mills and Rubel (1994) to postulate two DPOAE sources: one “active” (dominating at low SPLs) and one “passive” (dominating at high SPLs), with opposite phase such that they cancel at about 65 dB SPL (and produce the dip in the amplitude vs level function). They speculated that the two processes might arise from different structures and/or different cochlear locations, and that only the “active” low-level process required OHC amplification deriving from somatic electromotility.

Recently, this two-source model has been criticized on both theoretical and empirical grounds. Frolenkov *et al.*, (Frolenkov *et al.*, 1998) perfused *in vitro* blockers of OHC electromotility, through an *in vivo* preparation and found that DPOAEs were abolished at both high and low primary levels. Avan and colleagues (Mom *et al.*, 2001) provided indirect evidence for an OHC role at both high and low SPLs, showing that the DPOAEs remaining after cochlear ischemia were highly vulnerable to acoustic overstimulation, and arguing that this temporary acoustic injury targets mainly OHC stereocilia. Russell and colleagues (Lukashkin *et al.*, 2002) showed empirically that furosemide delivery, designed to selectively reduce the low-level DPOAE, shifted the growthfunction dip to higher input SPLs, i.e., the opposite direction predicted from a simple two-component, anti-phase model. They also showed mathematically that a single nonlinearity, appropriately configured, can reproduce the nonmonotonicities and phase shifts seen in the DPOAE data (Lukashkin and Russell, 1998). Finally, Mills (Mills, 2002) reported that the regularity of the growth function notches revealed by an exhaustive examination of the 4-parameter space defined by the frequency and level of the primaries is more easily explained by a one-component model deriving from a single nonlinearity.

B. DPOAEs without prestin: Can mammalian stereocilia drive the cochlear partition?

The prestin null mouse provides a powerful tool in studying the source(s) of DPOAEs. The prestin gene, expressed only in OHCs, codes for a key component of the molecular motor underlying cochlear amplification (Zheng *et al.*, 2000). The loss of prestin expression leads to loss of *in vitro* electromotility (Liberman *et al.*, 2002) and a 40–60 dB loss of cochlear sensitivity *in vivo* (Fig. 1). Cochlear effects of prestin elimination are arguably simpler to interpret than those of other manipulations used to reduce cochlear amplifier gain, such as furosemide injection, perfusion of electromotility blocking agents, acoustic overstimulation, ototoxic drugs, cochlear ischemia or death. These chemical and mechanical manipulations likely have multiple sites of action, and thus other effects beyond reducing the gain of the cochlear amplifier. Such additional sites of action may explain why the electromotility blocker studied by Frolenkov *et al.*, (1998) eliminated high-level DPOAEs in their study, whereas prestin deletion in our study did not.

Several lines of evidence suggest that OHC stereocilia transduction is normal in prestin-null ears. The roundwindow CM data (Fig. 2) show that, in the absence of prestin, nonlinearities in OHC stereocilia transduction are still producing a distortion component at $2f_1-f_2$ in the OHC receptor currents. DPCM at $2f_1-f_2$ was ~20 dB down from the primaries, at high SPLs, in both mutants and wildtypes [Fig. 2(d)]. These data argue that the $2f_1-f_2$ distortion source is normal in the absence of prestin and that the set point of the transduction channels (i.e., the open probability at rest) has not been changed by prestin deletion. The normality of forward transduction in the mutants is further corroborated by (1) the lack of difference in our CM Lissajous patterns for wildtypes vs prestin nulls and (2) CM measurements of Cheatham and colleagues who measured “pseudotransducer functions” (peak positive and negative CM as a function of peak pressure, normalized to the maximum response in each animal) and found no differences between prestin knockouts and wildtypes (Cheatham *et al.*, 2004).

The persistence of high-level DPOAEs in prestin-null mice shows that OHC somatic motility is not required for their production. Furthermore, DPOAEs without prestin cannot arise according to the conventional explanation, i.e., that nonlinearities in forward transduction in OHC stereocilia produce a distortion-frequency component in receptor current which is then reverse-transduced and amplified via OHC somatic motility into distortion-frequency vibrations of the organ of Corti.

Where is the nonlinearity that produces DPOAEs in prestin-null mice? Nonlinearities in prestin-based motility have been eliminated by the targeted deletion (Liberman et al., 2002). Although motion of the basilar membrane is normally nonlinear, it is likely that the basilar membrane, in isolation, is linear and that its motion nonlinearities originate elsewhere in the feedback system of the cochlear amplifier (e.g., Kanis and de Boer, 1993), which has been eliminated in these prestin-null mice. Thus, the only remaining known nonlinearities are in OHC stereocilia. Although these nonlinearities can no longer produce mechanical motion via distortions in receptor currents that are reverse transduced by OHC somatic motility, they could directly generate mechanical distortions, because (1) they are manifest in the mechanical properties of the OHC stereocilia and (2) the OHC stereocilia are mechanically coupled to the organ of Corti. Thus, a simple view of the persistent DPOAEs in the prestin-null mouse is that distortions in the organ of Corti motion, a requisite for DPOAE generation, arise from the direct coupling of the mechanical nonlinearities of OHC stereocilia bundles. Such nonlinearities have been described in hair cells from nonmammalian vertebrates which lack somatic motility (e.g., Ricci et al., 2002). With tone-pair stimuli, such nonlinearities produce $2f_1$ - f_2 distortion in hair bundle motion (Jaramillo et al., 1993).

The finding that DPOAEs in prestin-null mice disappear rapidly after death (Fig. 3) suggests that these DPOAEs are produced by “active” processes in OHC stereocilia. The mechanical nonlinearity of hair bundles is strongly affected by fast adaptation due to calcium-gated closure of the transduction channel (Hudspeth et al., 2000). Mammalian OHCs also display a fast calcium-dependent adaptation (Kennedy et al., 2003). Thus, in the mammalian ear, bundle stiffness nonlinearities should be highly vulnerable to ischemia/death, because (1) the normal endocochlear potential declines dramatically after death (Sadanaga and Morimitsu, 1995) and (2) this +80 mV DC potential is a large part of the driving force for calcium entry through the transduction channel. Indeed, in the prestin null mice, the high-level DPOAEs decayed rapidly after death, with a similar post-mortem time course as the CM (Fig. 3). Thus, the high-level DPOAEs must arise from an “active” (i.e., energy dependent) process that depends on endocochlear potential. This strongly suggests that the DPOAE-generating processes is affected by stereocilia fast adaptation, as opposed to the stereocilia nonlinearity that remains without fast adaptation.

If stereocilia mechanical nonlinearities can directly drive cochlear mechanics and produce DPOAEs in the absence of prestin, the contribution of this direct mechanical coupling to normal DPOAE generation must be considered. In other words, has the loss of reverse transduction unmasked a phenomenon which is normally too small to play an important role, or does the direct coupling of stereocilia mechanical nonlinearities normally make a significant (or even dominant) contribution to DPOAE generation? There are several ways of looking at this issue. In prestin-null mice DPOAE “thresholds” were shifted by an amount matching the loss of gain extracted from cochlear neural responses [Fig. 2(a)]. At first, this seems consistent with the idea that, at threshold, the same distortion generators are operating in normal and prestin-null mice, and that the lack of a cochlear amplifier has been compensated for by raising sound level. However, the full cochlear-amplifier gain is present only at low levels, near CF; off CF, and at higher levels, the gain is less. Thus, increasing primary levels by an amount equal to the gain reduction returns BM motion to threshold levels near CF; but away from CF, it produces BM motions greater than normal at the DPOAE threshold. Thus, in prestin-null mice at the DPOAE

threshold, increased BM motion away from the f_2 place might produce DPOAEs not present at threshold levels in normal mice. Another approach to this question is to compare the decrement in high-level DPOAE amplitudes associated with a lack of prestin [~ 35 dB *re* wildtypes: Fig. 2(a)] to that expected by the elimination of the cochlear amplifier at these high stimulus levels. At 70–80 dB SPL, cochlear amplifier gain is reduced to about 1/3 of its value (in dB) at low-levels, according to basilar membrane iso-response contours (Ruggero, 1992). According to our mouse data, normal low-level amplifier gain is -55 dB [Fig. 1(b)]. Thus, in prestin-null mice, the estimated loss of gain at high levels (15–20 dB) is smaller than the 35 dB reduction in DPOAE amplitude. Two other factors complicate this comparison, with opposing influences on the size of the discrepancy: (1) at high levels, DPOAE generation sites spread basally from the f_2 place, recruiting cochlear loci stimulated below the best frequency where amplifier gain is less even in normal ears (thereby increasing the size of the discrepancy); however, (2) there is basal-turn OHC loss in prestin null mice (Liberman et al., 2002), thus the number of generation sites will be smaller than in wildtypes. Uncertainties as to precise values for all contributing factors make a definitive conclusion difficult. Nevertheless, these rough calculations suggest that direct coupling of stereocilia mechanical nonlinearities into motion of the cochlear partition could, with prestin-based mechanical amplification, produce DPOAEs within 10–20 dB of those seen in normal ears.

In summary, the presence of DPOAEs in prestin-null mice indicates that the only remaining nonlinearity, the stereocilia mechanical nonlinearity, can drive the motion of the cochlear partition and that this phenomenon needs to be explicitly considered in models of cochlear mechanics. The results further suggest that, although prestin is required for cochlear amplification in mammals (Liberman et al., 2002), “active processes” in the stereocilia bundle could also contribute, as seen for hair cell systems of nonmammalian vertebrates (Jaramillo et al., 1993).

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FIG. 1.

Loss of cochlear amplifier gain associated with loss of prestin, as seen in cochlear potentials from wildtype and mutant mice. (a) CAP thresholds for all of the wildtype (filled circles), heterozygous (filled triangles) and homozygous (open circles) mutant mice studied. For each genotype, animals are separated into those with better thresholds (black) and worse thresholds (gray) based on the mean values at the three test frequencies closest to those evaluated in the DPOAE testing when $f_2 = 22.6$ ($f_1 = 18.8$ and $2f_1 - f_2 = 15.06$). (b) Loss of gain computed by subtracting mean CAP thresholds for the best wildtype and heterozygote ears from the means for the best homozygote ears.

FIG. 2.

Distortion products at $2f_1-f_2$, although attenuated, remain in the most sensitive homozygous mutant animals, in both the ear-canal sound pressure [panel (a)] and in the cochlear microphonic (CM) recorded from the round window [panels (b) and (d)]. The key for panel (a) applies to all panels. For all panels, data are shown for $f_2=22.6$ kHz from the most sensitive wildtypes and heterozygotes (as defined in Fig. 1) as well as for all the homozygotes. For homozygotes, those with the best CAP thresholds (Fig. 1) are shown in black, while those with worse thresholds are shown in gray. The gray band indicates the noise floor for these measurements. Panel (a): Amplitude vs level functions for DPOAE at $2f_1-f_2$ recorded in the ear canal. Panel (b): Amplitude vs level functions for the $2f_1-f_2$ component measured in CM. Panel (c): Amplitude vs level functions for the CM response at the lower of the two primary frequencies (f_1). Panel (d): Normalized amplitude vs level curves for the $2f_1-f_2$ component measured in the CM, referred to the appropriate f_1 amplitude measured at the same level in the same ear.

FIG. 3.

Vulnerability of the high-level DPOAEs measured in the most sensitive prestin $-/-$ ears demonstrates that these signals are generated by “active” processes in the animal, not artifacts of the acoustic system. Panel (a): The amplitude of the DPOAE at $2f_1$ - f_2 in response to the primaries with $f_2=8.0$ kHz and 80 dB SPL. Each connected set of symbols shows data from a different case: genotypes are shown in the key. Panel (b): Shows the time course of the post-mortem decrease in CM responses to the primary tone (f_1 for $f_2=8$ kHz) for the same cases illustrated in panel (a). Death was caused by intracardiac anesthetic injection and defined as the time at which respiration ceased.