Role of the Tectorial Membrane Revealed by Otoacoustic Emissions Recorded From Wild-Type and Transgenic TectaΔENT/ΔENT Mice

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Lukashkin, Andrei N., Victoria A. Lukashkina, P. Kevin Legan, Guy P. Richardson, and Ian J. Russell. Role of the tectorial membrane revealed by otoacoustic emissions recorded from wild-type and transgenic TectaΔENT/ΔENT mice. J Neurophysiol 91: 163–171, 2004. First published October 1, 2003; 10.1152/jn.00680.2003. Distortion product otoacoustic emissions (DPOAE) were recorded from wild-type mice and mutant TectaΔENT/ΔENT mice with detached tectorial membranes (TM) under combined ketamine/xylaxine anesthesia. In TectaΔENT/ΔENT mice, DPOAEs could be detected above the noise floor only when the levels of the primary tones exceeded 65 dB SPL. DPOAE amplitude decreased with increasing frequency of the primaries in TectaΔENT/ΔENT mice. This was attributed to hair cell excitation via viscous coupling to the surrounding fluid and not by interaction with the TM as in the wild-type mice. Local minima and corresponding phase transitions in the DPOAE growth functions occurred at higher DPOAE levels in wild-type than in TectaΔENT/ΔENT mice. In less-sensitive TectaΔENT/ΔENT mice, the position of the local minima varied nonsystematically with frequency or no minima were observed. A bell-like dependence of the DPOAE amplitude on the ratio of the primaries was recorded in both wild-type and TectaΔENT/ΔENT mice. However, the pattern of this dependence was different in the wild-type and TectaΔENT/ΔENT mice, an indication that the bell-like shape of the DPOAE was produced by a combination of different mechanisms. A nonlinear low-frequency resonance, revealed by nonmonotonicity of the phase behavior, was seen in the wild-type but not in TectaΔENT/ΔENT mice.

INTRODUCTION

Sensory processing in the cochlea involves the amplification of low-level and compression of high-level acoustically driven basilar membrane (BM) responses. The source of amplification is attributed to the electro-motile outer hair cells (OHCs) (Brownell et al. 1985; Liberman et al. 2002), which exert their effects on the cochlear partition through their interaction between the BM and tectorial membrane (TM) (Gummer et al. 1996). For example, OHCs are unable to effectively boost the BM’s vibrations in the α-tectorin mutant (TectaΔENT/ΔENT) mice where the TM is detached from the otherwise normal organ of Corti (OC) and energy flow between the OHCs and BM vibrations is de-synchronized (Legan et al. 2000). The TectaΔENT/ΔENT mouse provides an opportunity to demonstrate how noninvasive distortion product otoacoustic emission (DPOAE) measurements can be used for assessing and understanding the role of the TM in cochlear sensory processing. DPOAEs can be recorded from the cochlea as a consequence of the nonlinearity of OHC electromechanical feedback. DPOAE generation is closely associated with OHC motility (Frenkel et al. 1998) at all primary tone levels (Lukashkin et al. 2002). The nonlinearity of the current flow through the mechanoelectrical transducer conductance is probably the major source of distortion in the OHC’s acoustically driven mechanical responses (Patuzzi et al. 1989; Santos-Sacchi 1993) and is therefore the dominant DPOAE producing nonlinearity. DPOAEs measured in response to low sound pressure levels (SPLs) are physiologically vulnerable and correlate with a healthy cochlea and effective OHC feedback. Therefore the same requirement for fine synchronization in the motion of the cochlear partition’s major elements that is essential for sensitive hearing is also necessary for successful DPOAE generation.

Legan et al. (2000) demonstrated that the TM imposes a static load on the OHC hair bundles so that in wild-type animals the operating point of the OHC mechanoelectrical transducer is biased into the most-sensitive region around of the point of inflection of the transfer function when ~50% of the transducer channels are open (Russell and Kössl 1992). Only ~10% of the OHC transducer channels are open at rest in freestanding hair bundles in the cochlea of TectaΔENT/ΔENT mice (Legan et al. 2000). Thus the OHCs of TectaΔENT/ΔENT mice operate in an insensitive region of their receptor-potential transfer function and are therefore unable to provide optimum feedback to the BM. The specific position of the operating point of the mechanoelectrical transducer in free-standing OHC bundles should also lead to specific modification of the harmonic and intermodulation components at the output of the transducer (Bian et al. 2002; Lukashkin and Russell 1998, 1999). Consequently the DPOAE amplitude and phase patterns (Bian et al. 2002; Frank and Kössl 1996, 1997) of TectaΔENT/ΔENT mice should also be altered. By comparing emissions from mutant and normal cochleae, we have the opportunity to assess the role of the TM in the generation and transmission of the DPOAEs including the possible filtering of DPOAEs (Allen and Fahey 1993; Brown et al. 1992). Thus the objective of this paper is to assess the role of the TM in cochlear signal processing and also in the generation and transmission of the DPOAEs.

METHODS

TectaΔENT/ΔENT mice 6–8 wk in age were prepared as described in Legan et al. (2000). Mice were anesthetized with a combination of ketamine (0.12 mg/g body wt ip) and xylaxine (0.01 mg/g body wt ip). The heart rate was monitored with a pair of skin electrodes placed on both sides of the thorax. A tracheal cannula was inserted, and the core...
temperature was maintained at 38°C with a heating blanket and heated head holder.

Sound was delivered to the tympanic membrane by a closed acoustic system comprising two Microtech Gefell MK 103.1 1-in microphones for delivering tones and a single Bruel and Kjaer 4135 1/4-in microphone for monitoring sound pressure at the tympanum. The microphones were coupled to the ear canal via 1-cm-long, 4-mm-diam tubes to a conical speculum, the 0.5-mm-diam opening of which was placed close to the tympanum. The closed sound system was calibrated in situ for frequencies between 1 and 50 kHz. Known sound pressure levels were expressed in dB SPL re 2×10−5 Pa. White noise for acoustical calibration and tone sequences for auditory stimulation were synthesized by a Data Translation 3010 board at 200 kHz and delivered to the microphones through low-pass filters (100-kHz cutoff frequency). Signals from the measuring amplifier were digitized at 200 kHz (sampling depth of 12 bits) using the same board and averaged in the time domain. Amplitudes and phase angles of the spectral peaks were obtained by performing an FFT on a time-domain averaged signal that was 4,096 points in length. Noise amplitude was calculated as an average of the fourth to the sixth points to the left of the DPOAE bin in the amplitude spectrum. The maximum level of the system distortion measured with an artificial ear cavity for the highest levels of primaries used in this study was 80 dB below the primary level. Experimental control, data acquisition and data analysis were performed using a PC with programs written in Testpoint (CEC).

The following experimental procedures were used: 1) DPOAE-grams (f2 sweeps, f2/f1 ratio is constant, L1 and L2 are constant, L2 is 10 dB SPL below L1) were recorded for different levels of primaries to study the frequency dependence of DPOAEs. DPOAE-grams for L1 = 40 dB SPL (wild-type) and for L1 > 70 dB SPL (TectaΔENT/ENT) mice were recorded on a regular basis during the experiments to determine cochlear sensitivity. Data collected from an animal were rejected if the DPOAE level changed by >5 dB at the f2 frequencies used for the procedures used in these experiments. 2) DPOAE growth functions were recorded during the simultaneous increase of L1 and L2 (L2 was 10 dB below L1). The growth functions were recorded for different values of f2 but for the same ratio: f2/f1 = 1.23. 3) DPOAE growth functions with increasing L1 were measured while keeping L2 and the frequencies of primaries constant. The growth functions were measured at different f2/f1 ratios but for the same f2. And 4) DPOAE ratio functions were measured during f1 sweeps with constant f2, L1 and L2 were constant during each sweep with L2 being 10 dB SPL below L1.

All procedures involving animals were performed in accordance with UK Home Office regulations.

RESULTS

2f1f2 frequency components of the DPOAE could be measured in the ear canal of every wild-type mouse that was used in the experiments reported in this paper but could be detected above the measurement noise floor in 17 of a total of 28 TectaΔENT/ENT mice used in these experiments, and only when L1 was above 65 dB SPL. In the remaining TectaΔENT/ENT mice tested, the highest levels of the primaries used (L2/L1 = 75/85 dB SPL) were not sufficient to evoke DPOAEs above the noise floor. All other frequency components of the DPOAEs (e.g., f2f1, 3f1−2f2, etc.) recorded from TectaΔENT/ENT mice had magnitudes that were close to the noise floor of the measuring system, so we limited our comparison of DPOAEs recorded from wild-type and TectaΔENT/ENT mice to the 2f1f2 frequency component.

Frequency dependence of the DPOAE

DPOAE-grams show the level of emission generated by different frequencies of the primaries. They therefore reflect the electromechanical activity that can be elicited at different places along the length of the cochlear partition (Gaskill and Brown 1990). DPOAE-grams were measured for both wild-type and TectaΔENT/ENT mice by increasing the f2 frequency in steps while maintaining the f2/f1 ratio and SPLs of the primaries constant. The f2 frequency ranged from 8 to 40 kHz, although the full range of frequencies was not explored in every animal. A few DPOAE-grams were measured in each experiment for different levels of the primaries.

The 2f1f2 DPOAE in wild-type mice could be recorded over a wide range of levels of the primary tones, from L2/L1 = 10/20 dB SPL. Figure 1A shows representative DPOAE-grams ob-

![Figure 1](http://jn.physiology.org/)

**FIG. 1.** Distortion product otoacoustic emission (DPOAE)-grams showing the level of 2f1f2 DPOAE frequency component as a function of f2 in wild-type (A) and TectaΔENT/ENT (B) mice. C, averaged plots (means ± SE) of the A and B, f2/f1 ratio is 1.23. The levels of primaries are given within each panel.
tained from five wild-type mice for levels of the primaries \( L_2/L_1 = 40/50 \) and 60/70 dB SPL. For both stimulus levels, emission is generated at each of the \( f_2 \) frequencies that were tested. Between 10 and 40 kHz, the magnitude of the DPOAE has an absolute maximum at \( f_2 = 21–22 \) kHz and a secondary maximum at 30–31 kHz and minima at 18–19 kHz (Fig. 1C). This level-dependent nonmonotonic pattern is similar among all animals tested, minima and maxima of the amplitude response occur at the same frequencies for the same SPLs of the primaries. It is unlikely that this pattern is a consequence of the physical properties of the acoustical system used in our study because the position of the peaks and troughs depends on the level of the primaries and is different for wild-type animals and \( Tecta^{ENT/ENT} \) mice (see following text).

In \( Tecta^{ENT/ENT} \) mice, \( 2f_2/f_1 \) DPOAEs can be recorded above the measurement noise floor when the primaries exceed \( L_2/L_1 = 60/70 \) dB SPL. Figure 1B shows DPOAE-grams obtained from seven \( Tecta^{ENT/ENT} \) mice with primaries of \( L_2/L_1 = 75/85 \) dB SPL. The DPOAE-grams from \( Tecta^{ENT/ENT} \) mice reach an absolute maximum at approximately \( f_2 = 27–28 \) kHz (Fig. 1B) as seen in the averaged DPOAEs shown in Fig. 1C. This shift in the maximum to higher frequencies correlates with the decrease of the DPOAE amplitude for \( f_2 < 20 \) kHz. The DPOAE-grams of \( Tecta^{ENT/ENT} \) mice also reveal a periodic pattern; there are peaks and troughs in the DPOAE-grams of \( Tecta^{ENT/ENT} \) animals. The high inter-subject variability observed for the DPOAE-grams of \( Tecta^{ENT/ENT} \) mice results in the SEs in the \( Tecta^{ENT/ENT} \) average plot being much larger than those in the wild-type average plots (Fig. 1C).

**DPOAE growth functions**

DPOAE growth functions represent the DPOAE amplitude as a function of the SPLs of the primaries. DPOAE growth functions were recorded for both the wild-type and \( Tecta^{ENT/ENT} \) mice at various \( f_2 \) frequencies. To measure the growth functions, SPLs of the primaries were incremented by 1-dB steps with \( L_2 \) being maintained 10 dB below \( L_1 \). When the \( f_2/f_1 \) ratio is set to 1.23, which usually produces maximum DPOAEs in wild-type mice, the DPOAE growth functions recorded from wild-type mice have an amplitude minimum and associated phase transition (Fig. 2A). The abruptness of the phase transition correlates with sharpness of the minimum. For an \( f_2/f_1 \) ratio of 1.23, this minimum appears when \( L_1 \) is \( \sim66 \) dB SPL \([66.01 \pm 0.32 \text{ (SD) dB SPL}]\) as measured from 71 level functions in five preparations for \( f_2 \) over the frequency range of 10–40 kHz. Amplitude minima accompanied by phase transitions can also be observed in \( Tecta^{ENT/ENT} \) mice at higher \( L_1 \) than those observed for wild-type mice (Fig. 2B). However, the amplitude minimum in this case occurs at much lower levels of DPOAE and is close to the measurement noise floor and is revealed by averaging responses across closely spaced frequencies (thick line, Fig. 2B). The level of \( L_1 \) at which the amplitude minimum and phase transition appeared was remarkably similar in four preparations, being \( 74.5 \pm 1.29 \) dB SPL from 57 level functions for \( f_2 \) over the frequency range of 10–40 kHz. However, there was considerable variation in the form and magnitude of the level functions. The other three preparations were less sensitive and generated smaller DPOAEs, and it was difficult to accurately detect the amplitude minimum and associated phase transition within the measurement noise floor. When an amplitude minimum was observed in these three preparations, it appeared over a much wider range of the primary amplitudes (Fig. 2C) than was observed for the wild-type and more-sensitive transgenic mice. Linear growth of the DPOAEs in \( Tecta^{ENT/ENT} \) mice was also frequently observed (Fig. 2C, \( f_2 = 17, 26 \) kHz).

DPOAE growth functions recorded from wild-type and

![Fig. 2](http://jn.physiology.org/DownloadedFrom/10.220.33.4)
**DPOAE dependence on the ratio of the primary frequencies**

When recorded from wild-type mice, the dependence of DPOAEs on the \( f_{2}/f_{1} \) ratio demonstrates the well-known band-pass characteristic (Allen and Fahey 1993; Brown et al. 1992; Harris et al. 1989). The band-pass characteristic is usually recorded using a specific experimental paradigm; namely, the levels of the primaries and the frequency of the high-frequency primary are kept constant and only the frequency of the low-frequency primary is changed. When this experimental paradigm is applied, the DPOAE amplitude grows when the frequency separation between the primaries is decreased (Fig. 3A). However, the amplitude declines again when the \( f_{2}/f_{1} \) ratio becomes smaller than an optimal frequency ratio.

DPOAEs with similar band-pass characteristics were measured in 8 of the 10 Tecta\(^{ENT/ENT}\) mice (Fig. 3B) for which the dependence of the emission on the \( f_{2}/f_{1} \) ratio was studied. However, in two cases, the DPOAE had a high-pass characteristic, i.e., its amplitude increased when the \( f_{2}/f_{1} \) ratio was decreased \((2f_{1}-f_{2} \text{ increased in this case}; \text{Fig. 3C})\). When the relationship between DPOAE magnitude and \( f_{2}/f_{1} \) ratio has band-pass characteristics, the low- as well as high-ratio cut-off of the DPOAE amplitude is significantly sharper for the Tecta\(^{ENT/ENT}\) mice than for the wild-type animals (compare Fig. 3, A and B). The gradients of the low and high cut-off slopes measured from 38 \( f_{2}/f_{1} \) ratio functions in five wild-type mice were 209.0 ± 9.16 and -182.6 ± 11.7 (SD) dB per octave, respectively. The gradients were measured as the mean of a 10-dB range of the steepest region of the low and high cut-off slopes. The gradients of the low and high cut-off slopes measured from 18 \( f_{2}/f_{1} \) ratio functions in seven Tecta\(^{ENT/ENT}\) mice were twice as steep as those from wild-type mice, being 430.4 ± 35.17 and -365.7 ± 15.6 dB per octave, respectively. The DPOAEs recorded from the Tecta\(^{ENT/ENT}\) mice look noisier (Fig. 3). However, in all experiments presented in Fig. 3, the noise level was around -10 dB SPL, which is considerably below the recorded DPOAE level. Therefore the apparent noisiness of the data are, in fact, an irregular frequency-dependent behavior of the DPOAE, which correlates with the higher variability of the DPOAE growth functions recorded for the different primary frequencies in the Tecta\(^{ENT/ENT}\) mice (Fig. 2, B and C).

**DPOAE phase changes in vicinity of the second resonance**

DPOAEs recorded from the wild-type mice show near-resonance behavior (Lukashkin and Russell 2003) when the DPOAE frequency is swept around a hypothetical low-frequency resonance about a half of an octave below \( f_{2} \). Namely, when \( f_{2} \) and \( L_{2} \) are constant and only \( L_{1} \) is changed, then the DPOAE phase behavior depends on the \( f_{2}/f_{1} \) ratio. If for a given \( f_{2}/f_{1} \) ratio, the frequency of the DPOAE is situated below or above the postulated nonlinear resonance about a half of an octave below \( f_{2} \), then the DPOAE phase lags or, respectively, leads when \( L_{1} \) increases (Fig. 4A). This inversion of the phase behavior is observed over a finite range of the DPOAE frequencies as \( f_{1} \) is moved further away from \( f_{2} \), then the DPOAE phase lags or, respectively, leads when \( L_{1} \) increases (Fig. 4A). This inversion of the phase behavior is observed over a finite range of the DPOAE frequencies as \( f_{1} \) is moved further away from \( f_{2} \), then the DPOAE phase lags or, respectively, leads when \( L_{1} \) increases (Fig. 4A). This inversion of the phase behavior is observed over a finite range of the DPOAE frequencies as \( f_{1} \) is moved further away from \( f_{2} \), then the DPOAE phase lags or, respectively, leads when \( L_{1} \) increases (Fig. 4A).
levels and on the properties of the particular nonlinear system. Accordingly, the phase pattern seen in Fig. 4A for the $2f_1$-$f_2$ DPOAE component would be expected if the resonance frequency becomes lower when the level of the stimulus ($L_1$) is increased. The TM may be an important component of a complex mechanical system with multiple degrees of freedom and associated resonances, one of which could be the nonlinear resonance revealed by the level dependent behavior of the DPOAE phase considered in this section. Hence, the DPOAE phase pattern may be significantly different when recorded from the Tecta$^{ENT/ENT}$ mice, which lack the TM. Indeed, the DPOAE phase does not show any dependence on $L_1$ (Fig. 4B) when recorded for the same experimental paradigm and for the same range of the $f_2/f_1$ ratios, which reveal the near-resonance phase behavior in the wild-type mice.

DISCUSSION

A protein, prestin, has been identified as the molecular driver of OHC somatic motility (Zheng et al. 2000) and cochlear amplification (Liberman et al. 2002). Low- and high-level DPOAEs disappear when OHC motility is blocked (Frolov et al. 1998) and are absent in homozygous prestin null mice and greatly reduced in heterozygous prestin mutants (Liberman et al. 2002). Thus the recording of DPOAEs from Tecta$^{ENT/ENT}$ mice indicates that their OHCs are motile. Accordingly, a simple scenario for DPOAE generation begins with nonlinear current flow through the transducer conductance, which is represented in fl DPOAE generation begins with nonlinear current OHCs are motile. Accordingly, a simple scenario for phase behavior in the wild-type mice.

## DISCUSSION

A protein, prestin, has been identified as the molecular driver of OHC somatic motility (Zheng et al. 2000) and cochlear amplification (Liberman et al. 2002). Low- and high-level DPOAEs disappear when OHC motility is blocked (Frolov et al. 1998) and are absent in homozygous prestin null mice and greatly reduced in heterozygous prestin mutants (Liberman et al. 2002). Thus the recording of DPOAEs from Tecta$^{ENT/ENT}$ mice indicates that their OHCs are motile. Accordingly, a simple scenario for DPOAE generation begins with nonlinear current flow through the transducer conductance, which is represented in changes of the cell’s membrane potential that drives the OHC motility to provide a nonlinear outflow of energy, thereby boosting the cochlear mechanical response to sound. In this scheme, the DPOAE is a direct reflection of the mechanical output from the OHCs (Bian et al. 2002; Fahey et al. 2000; Frank and Kössl 1996, 1997; Lukashkin and Russell 1998, 1999; Lukashkin et al. 2002; Santos-Sacchi 1993). Although much simplified, the main events in this description of DPOAE generation have been confirmed by many experimental observations.

### 2f1-f2 emissions are high-pass filtered in Tecta$^{ENT/ENT}$ mice

DPOAE-grams (Fig. 1) show that emissions in wild-type mice are generated over a wide frequency range. DPOAEs recorded from Tecta$^{ENT/ENT}$ animals are high-pass filtered in that the DPOAE amplitude decreases with decreasing frequency of the primary tones; a trend similar to that of the auditory nerve compound action potential (CAP) threshold (Legan et al. 2000). Legan et al. proposed that the CAP’s frequency dependence was due to excitation of the freestanding hair bundles in the Tecta$^{ENT/ENT}$ mice via viscous coupling to BM displacements. We suggest that the high-pass characteristics of DPOAE generation in Tecta$^{ENT/ENT}$ mice are due to the same mechanism. However, on the basis of data presented in this paper, it is not possible to rule out the hypothesis that the TM is involved in propagating energy from the site of DPOAE generation to the stapes. Hence, in the Tecta$^{ENT/ENT}$ mice, where the TM is not coupled to the organ of Corti, not only is the generation of distortion energy at the apical, low frequency region of the cochlea ineffective, but reverse propagation of the energy from this region may also be impaired.

### 2f1-f2 emission in Tecta$^{ENT/ENT}$ mice is generated at high SPLs

It is necessary to deliver primaries at higher SPLs in Tecta$^{ENT/ENT}$ than in wild-type mice to observe the same level of emission, even at high frequencies when the OHC hair bundles should be effectively coupled to BM vibrations through viscous coupling to the surrounding fluid. This could be because distortion energy propagation from the place of the
DPOAE generation to the stapes in TectaENT/ENT mice is impaired. However, there are two additional factors that could contribute to TectaENT/ENT mice emitting lower level DPOAEs, even at high frequencies, than wild-type mice. First the absence of the TM from the organ of Corti alters the operating point of the OHC transducer to a less-sensitive region of the transfer function (Legan et al. 2000). Second, the viscous rather than direct mechanical coupling of OHC hair bundles to BM displacement, as in wild-type mice, could alter the timing of feedback so that OHC forces are not delivered during maximum BM velocity, which is optimal for cochlear amplification (Geisler and Sang 1995; Gummer et al. 1996; Legan et al. 2000; Markin and Hudspeth 1995; Nilsen and Russell 1999). It would be expected that larger hair-bundle displacements, which are caused by higher SPLs, are necessary to compensate for the insensitive operating point and feedback de-synchronization in TectaENT/ENT mice to elicit DPOAEs that are comparable to those from wild-type mice.

Operating point of the OHC mechnoelectrical transducer is intrinsically controlled in TectaENT/ENT mice

The local amplitude-minimum and corresponding phase-transition, which are characteristics of DPOAE growth functions recorded from rodents, are suggested to be due to the nonmonotonic behavior of distortion components at the output of a single saturating nonlinearity (Lukashkin and Russell 1998, 1999; Weiss and Leong 1985). For a given nonlinear function, the location of the minimum or its absence depends on the position of the operating point of the distortion producing nonlinearity (Lukashkin and Russell 1999) and also on the gain of the cochlear amplifier (Lukashkin et al. 2002). The sharp minimum and associated phase transition are present in DPOAE growth functions recorded from TectaENT/ENT mice (Fig. 2B) although they occur at much lower levels of DPOAE that are close to the measurement noise floor. A simple model of distortion generation at the output of a single saturating nonlinearity with a positive feedback (Fig. 5) (see also Lukashkin and Russell 1999 for detailed description of the model) suggests an explanation for this phenomenon. In this model, the DPOAE producing nonlinearity is assumed to be the mechnoelectrical transducer of the OHCs. In the wild-type animals, the operating point of the transducer is situated slightly above the nonlinearity inflection point (Fig. 5, $x_{\text{set}} = 9$ nm) when $\sim 50\%$ of the transducer channels are open at rest. Calculations of the output distortion amplitude show that the notch in this case appears at relatively high levels of the distortion but at lower levels of the input signals. However, the notch is observed at much lower distortion levels but for higher levels of the input signals when feedback gain is set to zero and the operating point is shifted to a position when only 10% of the transducer channels are open (Fig. 5, $x_{\text{set}} = -21$ nm), i.e., when the TectaENT/ENT cochlea is simulated. The same model offers an explanation for much greater variability of the notch position and absence of the notch in less sensitive TectaENT/ENT animals (Fig. 2C). The amplitude notch has a distinctive pattern (Fig. 6) in two-dimensional space of the primary intensities and the position of the transducer operating point being almost parallel to the intensity axis at low-level primaries. Only growth functions (i.e., sections of the plane parallel to the intensity axis), which intersect the notch, show the local minima (Fig. 6A, vertical lines at $x_{\text{set}} = -22$ and $-21$ nm; Fig. 6B, vertical lines at $x_{\text{set}} = 9$ and 10 nm). It is worth noting that if the position, $x_{\text{set}}$, of the operating point is close to its value under which the notch is observed for low level primaries, then even slight variations in $x_{\text{set}}$ cause either a significant shift, $\Delta L$, of the amplitude minimum position in the distortion level functions (compare cross-sections at $x_{\text{set}} = -22$ and $-21$ nm in Fig. 6A) or a total disappearance of the minimum (cross-section at $x_{\text{set}} = -20$ nm in Fig. 6A). However, the same 1-nm bias of the operating point leads to a much smaller shift, $\Delta L$, of the notch position along the amplitude axis.
A shift of the operating point by 1 nm (cross-section at \(x_{\text{set}}\) amplitude that are used as input signals for the calculations. The amplitude of position of the operating point and input amplitudes of the 2 sinusoids of equal output of the mechanoelectrical transducer with 0 dB gain (Fig. 5) on the for the system with active feedback (Fig. 6B). Thus the system with active feedback is far more stable in its responses to small changes in the operating point, as is observed for the position of the notch measured in wild-type as compared with \(Tecta^{ENT/ENT} \) mice in real experiments (Fig. 2).

Thus our results confirm Legan et al.’s (2000) observations that the OHC hair bundles are free-standing and their resting position is controlled intrinsically probably as a balance between elastic forces developed within the bundle and tension of the mechanoelectrical transducer adaptation motor (Assad and Corey 1992) and are not due to interaction with the TM as in the cochlea of wild-type mice. Legan et al.’s observations were based on round window CM recordings, which are dominated by responses from basal turn OHCs (Patuzzi et al. 1989). DPOAEs are, however, generated mainly from the place of greatest overlap between primaries, which is close to the \(f_2\) CF place (Knight and Kemp 2000; Brown and Kemp 1984; Martin et al. 1987). Therefore the DPOAE growth functions presented in Fig. 2 for a wide range of \(f_3\) frequencies provide information about a more extended region of the cochlea.

Greater variability is also seen in \(Tecta^{ENT/ENT}\) than in wild-type mice of the amplitude DPOAEs generated at a single cochlear location when \(f_2\) is kept constant and only \(f_1\) is varied. This variability occurs despite the DPOAEs of \(Tecta^{ENT/ENT}\) (Fig. 3, B and C) and wild-type mice (Fig. 3A) being of similar magnitude and therefore a similar amount above the measurement noise floor. Hence, it appears that the TM in the normal cochlea acts as a spatial integrator that smooths out changes in the system properties either along the length of the cochlea or in the frequency domain.

**Band-pass characteristics of the DPOAEs have several origins**

Various hypotheses have been put forward to explain the band-pass characteristics of DPOAEs recorded from the mammalian cochlea, all of which assume a different role for the TM. The idea that the distortion product energy is directly filtered by the TM resonance tuned to about half of an octave below \(f_2\) (Allen and Fahey 1993; Brown et al. 1992) is attractive because it also explains why DPOAEs of different orders peak at about the same frequency. This suggestion is also supported by recent finding of a nonlinear resonance tuned to about half an octave below \(f_2\) in every frequency location along the length of the cochlea (Lukashkin and Russell 2003). However, the filter hypothesis fails to account for the band-pass characteristics of DPOAEs that have been recorded from species without a TM (Taschenberger et al. 1995). A mutual suppression between primaries and distortion products can lead to a reduction of the DPOAE amplitude when frequency separation between the primaries is decreased (Kanis and de Boer 1997). A similar suppression of the distortion product amplitude is observed at the output of a single saturating nonlinearity due to the re-distribution of the energy between higher-order distortion components (Lukashkin and Russell 1998, 2001). However, the suppression hypotheses fail to explain the appearance of the band-pass characteristics of DPOAEs at the lowest levels of the primaries when suppression is not effective (Kanis and de Boer 1997). For these levels of the primaries, a local minimum that is routinely observed in DPOAE level functions recorded from rodent and other nonprimate mammals shapes the emission (Fig. 2) (see also Lukashkin and Russell 2001). Similarly, the vector summation of DPOAEs from two sources (Stover et al. 1999) or from an extended region along the cochlea (Vetešník and Nobili 2003) could also create the band-pass like characteristics of the DPOAEs in response to changes in the ratio of the primaries.

Band-pass characteristics are seen in the DPOAEs recorded from \(Tecta^{ENT/ENT}\) mice (Fig. 3B), but they are clearly different from those of the wild-type animals (Fig. 3A), thereby implying different mechanisms being responsible for generating the DPOAE band-pass characteristics in \(Tecta^{ENT/ENT}\) and wild-type mice. Clearly the responses in the \(Tecta^{ENT/ENT}\) mice cannot be frequency filtered by the TM. It is also unlikely that the DPOAEs are filtered by other cochlear structures because DPOAEs from \(Tecta^{ENT/ENT}\) mice have steeper low- and high-ratio cut-offs (Fig. 3B) in spite of the broader mechanical tuning of the cochlea (Legan et al. 2000). It is also unlikely that the variable, and often
absent, local amplitude minimum in the DPOAE level functions (Fig. 2, B and C) shape the ratio responses. The sharp DPOAE cut-off and much steeper DPOAE growth functions in TectaΔENT/ΔENT animals indicate that a level-dependent mechanism, namely mutual suppression, is the basis for the generation of the DPOAE’s band-pass characteristics in the TectaΔENT/ΔENT mouse cochlea. The increase in the suppression at low \( f_2/f_1 \) ratios is likely to be due to growth of the effective level of the primaries at the place of DPOAE generation, when frequency separation between the primaries is decreased. In support of this, changes in DPOAE, which are usually observed with changes in primary levels, have also been recorded when the \( f_2/f_1 \) ratio is varied (Lukashkin and Russell 2001). The high-pass characteristics of the DPOAE that were recorded in two cases (Fig. 3C) can be explained by the augmentation of distortion products at the output of a saturating nonlinearity when its operating point is situated well below the point of inflection of the transfer function (Lukashkin and Russell 1998). That is exactly the case for the OHC mechanoelectrical transducer conductance in TectaΔENT/ΔENT mice (Legan et al. 2000). Therefore our results lead us to suggest that the band-pass characteristics of DPOAEs in both TectaΔENT/ΔENT and wild-type mice may be due to a combination of the mechanisms that have been described in the preceding text. For example, for wild-type animals, the amplitude of the DPOAEs induced by high-level primaries might be reduced largely by mutual suppression when the frequency separation between high-level primaries is small. On the other hand, frequency filtration and the minimum in the DPOAE level functions might shape low-level DPOAEs.

**Second resonance is absent in TectaΔENT/ΔENT mice**

A resonance tuned to a half-octave below the CF place, and which depends on the presence of an intact TM, has been measured in tone-induced BM vibrations (Legan et al. 2000) and DPOAEs (Fig. 4A) recorded from wild-type mice but not from TectaΔENT/ΔENT mice where the TM is detached from the OC (Fig. 4B). The resonance is an indication that the TM forms a multi-resonance complex with the OC so that, at each location along the cochlea’s length, the complex has two normal modes of vibration. One mode is tuned to the frequency of the low-frequency resonance and the other is tuned to a higher frequency, which is close to the CF of the location. Therefore it is plausible that, as suggested by Allen and Fahey (1993), the shear motion between the TM and reticular lamina occurs almost counter phase at the high-frequency resonance, which is optimal for stimulating both the OHCs and inner hair cells.

The major findings of this paper are consistent with accumulating evidence that the OHC transducer conductance is the cochlear nonlinearity responsible for generating DPOAEs in the mammalian cochlea (Bian et al. 2002; Fahey et al. 2000; Frank and Kössl 1996, 1997; Lukashkin and Russell 1998, 1999; Lukashkin et al. 2002; Santos-Sacchi 1993). Data presented in this paper confirm the reported functional characteristics of the TectaΔENT/ΔENT cochlea (Legan et al. 2000) and advance new ideas concerning the TM’s role in cochlear signal processing and DPOAE generation. The TM filters the cochlea response but is not essential for conducting DPOAEs from the cochlea. DPOAEs provide a noninvasive way of determining the operating characteristic of OHCs over a greater extent of the cochlea than is usually possible using invasive electrophysiological and micromechanical techniques.

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