Estimating the operating point of the cochlear transducer using low-frequency biased distortion products

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Distortion products in the cochlear microphonic (CM) and in the ear canal in the form of distortion product otoacoustic emissions (DPOAEs) are generated by nonlinear transduction in the cochlea and are related to the resting position of the organ of Corti (OC). A 4.8 Hz acoustic bias tone was used to displace the OC, while the relative amplitude and phase of distortion products evoked by a single tone [most often 500 Hz, 90 dB SPL (sound pressure level)] or two simultaneously presented tones (most often 4 kHz and 4.8 kHz, 80 dB SPL) were monitored. Electrical responses recorded from the round window, scala tympani and scala media of the basal turn, and acoustic emissions in the ear canal were simultaneously measured and compared during the bias. Bias-induced changes in the distortion products were similar to those predicted from computer models of a saturating transducer with a first-order Boltzmann distribution. Our results suggest that biased DPOAEs can be used to non-invasively estimate the OC displacement, producing a measurement equivalent to the transducer operating point obtained via Boltzmann analysis of the basal turn CM. Low-frequency biased DPOAEs might provide a diagnostic tool to objectively diagnose abnormal displacements of the OC, as might occur with endolymphatic hydrops.

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I. INTRODUCTION

There is a general consensus in the literature that tight regulation of the organ of Corti (OC) position and the related operating point (OP) of the cochlear transducer is necessary for the maintenance of hearing sensitivity, and modulation of the OP has been directly linked to auditory threshold fluctuations (Hirsh and Ward, 1952; Davis, 1983; Patuzzi *et al.*, 1984a, 1984b; Kim, 1986; Kemp, 1986; Dallos, 1992). Furthermore, it has been suggested that the hearing dysfunction in patients with Meniere's disease may be related to endolymphatic hydrops, which may involve an abnormal displacement of the OC (Valk *et al.*, 2004; Xenellis *et al.*, 2004). Presently there is no accurate, non-invasive objective measure of the OC position or OP. The main aim of the present study was to develop methods to estimate the OC position non-invasively using low-frequency biased distortion product otoacoustic emissions (DPOAEs). We also aimed to compare this estimate to measurements of the OP obtained through Boltzmann analysis of the cochlear microphonic (CM).

Researchers have previously estimated the OP using low-frequency tones to bias the OC position while measuring even-order distortion products in both the CM and DPOAEs (Frank and Kössl, 1996; Kirk *et al.*, 1997; Kirk and Patuzzi, 1997; Bian *et al.*, 2002; Bian, 2004; Sirjani *et al.*, 2004; Bian, 2006). When the OP of a nonlinear transducer with a first-order Boltzmann distribution is near zero (the symmetric point on the transfer curve), sinusoidal inputs produce

relatively symmetric distorted outputs, and the amplitude of the even-order distortions in the output signal are relatively small compared to situations when the OP is displaced from zero and the output is asymmetric. Conversely, the amplitudes of odd-order distortions are largest when the OP is near zero and decrease as the OP is displaced from zero (Weiss and Leong, 1985; Frank and Kössl, 1996).

Despite recent research into the use of DPOAEs for the estimation of the cochlear transducer OP, there are many potential sources of nonlinearity in the cochlea that may contribute to DPOAE generation. One possibility is that they are generated by distortion of the mechanical-to-electrical transduction in the cochlea via the mechano-electrical transduction (MET) channels on the hair bundles (Corey and Hudspeth, 1979), which is then reverse-transduced into mechanical vibration (Kim, 1986; Kirk and Yates, 1998; Yates and Kirk, 1998), producing distortion products in the ear canal (Lukashkin and Russell, 1997, 1998; Liberman *et al.*, 2004). Another possibility is that DPOAEs are predominantly generated by distortion in the electrical-to-mechanical process that underlies the active amplification of OC vibration (Santos-Sacchi, 1991) or nonlinear compliance of the MET channels (Howard and Hudspeth, 1988; Jaramillo *et al.*, 1993). Whatever the mechanism underlying distortion in the cochlea, if the sources of distortion products in both the ear canal and the electrical response in the cochlea were the same, both sets of distortion products would have similar characteristics and might be used to determine the properties of transduction in the cochlea.

Forward-transduction in the cochlea follows a Boltzmann distribution due to the distribution of MET channel conductance (Holton and Hudspeth, 1986; Kros, 1996). Re-

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searchers have used the CM as an approximation of the local MET channel conductance and have fitted a first-order Boltzmann function to the relationship between the CM and the acoustic stimulus (Corey and Hudspeth, 1983; Crawford *et al.*, 1989; Patuzzi and Rajan, 1990; Patuzzi and Moleirinho, 1998; Sirjani *et al.*, 2004), assuming that the CM is dominated by local hair cells (Dallos *et al.*, 1972; Patuzzi *et al.*, 1989) and that the MET channels are the dominant nonlinearity in the forward-transduction (Holton and Hudspeth, 1986; Kros, 1996). The three main parameters used to fit the Boltzmann function

$$y = V_{\text{off}} + \left(-P_{\text{sat}} + \frac{2 \cdot P_{\text{sat}}}{1 + \exp(2 \cdot S \cdot P_{\text{sat}}(\text{input} + \text{OP}))} \right)$$

are the OP, which is related to the asymmetry of transduction, the saturating voltage (P_{sat}), which is related to the potential between the open and closed states of the MET channels, and a sensitivity parameter (S), which represents the slope of the curve at the mid-point of the Boltzmann curve (Sirjani *et al.*, 2004). The OP is of particular interest because it is most likely a reasonable estimate of the resting conductance of the MET channels, which can be affected by a number of mechanical changes in the cochlea such as (1) displacements of the OC toward either scala vestibuli (SV) or scala tympani (ST), possibly due to a pressure differential across the partition (Nieder and Nieder, 1968a, 1968b; Patuzzi and Sellick, 1983; Russell and Kossel, 1992; Cheatham and Dallos, 1994), (2) hair cell length changes, which are a property of the outer hair cells (OHCs) (Brownell *et al.*, 1985; Zenner, 1986; Evans and Dallos, 1993), and (3) changes in the MET channel compliance (Howard and Hudspeth, 1988; Jaramillo *et al.*, 1993). Normally, the cochlea is relatively insensitive to dc pressures in the ear canal due to the acoustic high-pass filtering properties of the middle ear and helicotrema (Dallos, 1970; Ruggero *et al.*, 1986), and therefore the OP is most likely determined by cochlear factors, as mentioned above.

Despite the cochlear's insensitivity to dc pressures in the ear canal, we expected that low-frequency tones could be used to displace the OC position and transducer OP, thereby altering the phase and amplitudes of the distortion products in the CM and DPOAE evoked by a probe stimulus. We also predicted that properties of the transducer, such as its OP, could be indirectly determined from the amplitude and phase of the distortion products measured during low-frequency biasing. Presumably, the instantaneous pressure of a bias tone that causes even-order distortion products to be reduced to a minimum would represent the point where the transducer OP was displaced back to the mid-point of the Boltzmann transducer curve, which is what we define as zero OP. In this case, the applied bias pressure would be equal, but oppositely directed to the unbiased, innate OP of the transducer. This technique allows us to non-invasively probe the OP and monitor it during experimental manipulations.

II. METHODS

A. Animal preparation

Cochlear potentials and otoacoustic acoustic emissions (OAEs) were recorded in acute experiments in 19 NIH strain pigmented guinea pigs of either sex. Animals were anesthetized with an initial dose of 100 mg/kg sodium thiobutabarbital (Inactin, Sigma, St. Louis) and maintained in a state of deep anesthesia by periodic supplements via a cannula in the external jugular vein. Pancuronium bromide (2 mg/ml) was given intravenously as a muscle relaxant to reduce myogenic artifacts and to eliminate middle ear muscle contractions. Animals were artificially ventilated through a tracheal cannula, with end-tidal CO_2 maintained near 38 mm Hg (5%). Heart rate and vascular $p\text{O}_2$ were monitored with a pulse-oximeter (Surgivet, Waukesha, WI). Rectal temperature was maintained at 38 °C with a dc-powered, thermistor-controlled heating blanket. Animals were mounted in a head-holder, and the auditory bulla was exposed by a ventral approach, allowing access to the cochlea. The external ear canal was transected to allow placement of a hollow ear bar, through which acoustic signals were delivered.

The experimental protocols for this study were approved by the Animal Studies Committee of Washington University (Approval Nos. 20040209 and 20070147).

B. Acoustic stimulus generation, calibration, and acoustic emission recording

Tucker-Davis (TD) system-3 hardware was used to generate acoustic stimuli under the control of a custom-written Visual Basic (Microsoft) program. Three channels of sinusoidal stimuli were generated by two TD-RP2 modules, with each channel passed through a TD-PA5 attenuator to control level before being amplified by a headphone amplifier (TD-HB7). Two channels of probe stimuli (f_1 and f_2) were delivered to the ear canal by a modified Etymotic ER10C system coupled to the hollow ear bar. The third sound channel was used to generate and deliver 4.8 Hz bias tones. Bias tones were generated by a Sennheiser HD 580 or HD 265 driver, sealed into an acrylic coupler and connected to the ear bar with a short length of polyethylene tubing. All sound stimuli were delivered, and acoustic emissions were recorded within this closed sound system. The microphone within the ER10C system was used both for recording acoustic emissions and for the calibration of stimuli. In each animal, calibration curves were determined for all three transducers by tracking the attenuation required to generate a stimulus level of 70 dB SPL (sound pressure level) in the external canal as frequency was varied in $\frac{1}{4}$ octave steps. Calibration tables were generated, taking into account sensitivity changes of the microphone as a function of frequency. These calibration data were used by the software to deliver all subsequent stimuli calibrated on a dB SPL basis. Stimulus levels presented in this study were measured close to the tympanic membrane so they exclude the normal gain of 5–10 dB (from 2 to 10 kHz) provided by the ear canal (Sinyor and Laszlo, 1973). Calibrating at the tympanic membrane therefore means that the cochlea was exposed to stimulation levels of 5–10 dB lower than those experienced when stimuli are calibrated and pre-

TABLE I. Distortion levels measured in the ear bar, with and without an animal present. All levels are dB SPL, with standard deviation ($\pm$) and number of animals used (n) shown.

Stimulus	500 Hz, 90 dB SPL	500 Hz, 90 dB SPL	4 and 4.8 kHz, 80 dB SPL	4 and 4.8 kHz, 80 dB SPL
DPOAE:	2f1	3f1	f2-f1	2f1-f2
No animal (dB SPL)	35	18	-22	-4
Noise floor (dB SPL)	-15	-18	-27	-11
Animal average (dB SPL)	44 ($\pm 10, n=15$)	42 ($\pm 7, n=15$)	26 ($\pm 5, n=7$)	29 ($\pm 10, n=7$)
Noise floor (dB SPL)	-7 (± 8)	-14 (± 6)	-3 (± 8)	-13 (± 8)

sented free field. In order to calibrate the 4.8 Hz bias tones with the Etymotic ER10C microphone, it was necessary to extend the microphone response curve to lower frequencies, which was performed by comparing coupler responses from the ER10C with those of a Brüel & Kjær 4135 $\frac{1}{4}$ in. microphone as frequency was varied.

1. Transient hypoxia

Transient hypoxia was induced by coupling the ventilator output to the input through a container of CO₂ absorber (Sodalime, W.A. Grace, S.A.), so that the animal was re-breathing the same air, thereby slowly reducing the O₂ level, while the CO₂ was maintained at a constant low level to minimize vascular changes. The container incorporated a narrow vent line to maintain constant atmospheric pressure in the system. Gas sampling for CO₂ analysis was disabled during the procedure. Hypoxia was allowed to develop until endocochlear potential (EP) fell below approximately 50 mV, after which room-air ventilation was restored.

C. Cochlear potential recording (non-biased)

Cochlear potentials were recorded from a Ag/AgCl ball electrode placed on the bone at the margin of the round window (RW) membrane or from electrolyte-filled glass pipettes inserted into ST or scala media (SM). Signals from the RW electrode were recorded differentially with respect to a platinum needle electrode at the vertex using a TD HB4 optically coupled amplifier with a gain of 1000 $\times$ and high-pass filtered at 5 Hz. Glass pipettes with a 5 μ m tip diameter were filled with 500 mM NaCl for ST recordings or with 500 mM KCl for SM recordings. Responses from the glass pipettes were recorded through dc-coupled electrometer amplifiers with a gain of 10, referenced to the animal's earth electrode, which consisted of a salt-bridge AgCl electrode placed on the exposed muscles of the neck. EP was also recorded from pipettes inserted into SM, with the voltage measured prior to inserting the pipette into SM defining the zero level.

Cochlear potentials and acoustic emissions were collected simultaneously using the input channels of the TD-RP2 modules, sampling at 24 or 48 kHz. The software permitted data from four input channels to be collected and averaged simultaneously. In addition, two channels of data were also sampled at 44.1 kHz and streamed to disk using a PC equipped with an internal sound card. Time-averaged waveforms from the TD system were subjected to a number of analyses, including the following.

- (i) Fast Fourier transform (FFT) spectral analysis using National Instruments Component Works routines called by the Visual Basic software. For responses collected without low-frequency biasing, FFT spectra were typically obtained from non-windowed time waveforms collected as the average of ten phase-locked 8192-point, 168 ms duration epochs. Three FFT spectra, each derived from independent data collections, were then averaged to form a final spectrum from which measurements were made. Spectral phase data were not averaged but were measured from the last block of data collected. The frequencies of probe stimuli were adjusted to ensure that the collected waveform contained an integer number of complete cycles, allowing sharp spectra to be obtained without windowing the time waveforms. The level of distortion generated by the sound delivery system, in response to one or two probe tones, was measured with the speakers and microphone placed in the ear bar, identical to experimental testing, but with the ear bar sealed into a 0.3 ml cavity, partly filled with wire wool to diffuse standing waves. A summary of the distortion product levels with and without an animal present is presented below in Table I. It should be noted that DPOAE levels in the animal depend greatly on the position of the OP in the cochlea where they are generated, which can vary between normally hearing animals, so there is an inherent complexity of averaging DPOAEs across animals. Nonetheless, we have provided average distortion levels for the purposes of indicating that they were well above that generated by the sound delivery system and well above the noise floor measured 10 Hz on either side of the distortion product.
- (ii) A Boltzmann analysis of the CM waveform was performed using a 21 ms segment of the averaged time waveform described above. The waveform was transferred online to an Excel spreadsheet and fitted with a waveform synthesized from a Boltzmann curve relating voltage output to applied pressure input (Kirk *et al.*, 1997; Sirjani *et al.*, 2004). The parameters derived from this analysis were the following: P_{sat} : the saturation voltage of the curve, slope: the maximum steepness of the transducer function at zero input pressure, and OP_{Boltz} : the OP, which represents the position on the transducer curve at the zero crossings of the probe stimulus. Because the Boltzmann equation used in the present study multiplied the OP by the

FIG. 1. Schematic of the experimental protocol in which a 4.8 Hz biasing tone is utilized to modulate OP of the cochlea during the simultaneous presentation of a probe tone. During each 208 ms cycle of the bias tone, eight independent collections were performed, and results were averaged over multiple bias cycles. Each of the eight data blocks was subsequently analyzed separately.

sensitivity to the probe stimulus [i.e., $2 \cdot S/P_{\text{sat}}^*$ (input + OP)], the OP_{Boltz} estimate obtained from CM measurements was effectively scaled in terms of probe tone pressure. Those parameters providing the best fit to a specific CM waveform were transferred back to the control program for plotting and storage. Online analysis of CM waveforms was only performed for responses to single-tone stimuli. Acoustic stimuli were turned on 5–6 s before data collection commenced to avoid onset phenomena, which might be related to efferent effects (Lukashkin and Russell, 2002; Halsey *et al.*, 2005).

- (iii) The level of a tone-burst required to evoke a 10 μV compound action potential (CAP) waveform, averaged from ten consecutive responses evoked at a rate of 10.9/s, was measured for a range of tone-burst frequencies from 1 to 22 kHz. This allowed verification that the animal had normal sensitivity at the start of the experiment and that experimental protocols were not causing sensitivity changes.

D. Responses collected during low-frequency biasing

It has previously been shown that the guinea pig cochlea generates CM to stimuli with frequencies as low as 0.1 Hz (Salt and Demott, 1999), demonstrating that their OHCs are capable of transducing extremely low frequencies. The biasing protocol in the present study utilized a 4.8 Hz tone with a period of 208 ms, which allowed eight independent, equally-spaced, 21 ms data collections to be performed during each cycle of the bias (Fig. 1). The data buffers (1024 points with 48 kHz sampling or 512 points with 24 kHz sampling) were too short to obtain sharp spectra, so four independent data collections were made (32 buffers total),

and the four waveforms collected at the same point on the bias were concatenated, producing 2048 points at 24 kHz or 4096 points at 48 kHz (84 ms of data in both cases), which was subjected to spectral and Boltzmann analysis. Stimulus frequencies were optimized to ensure an integer number of cycles in the original buffers so that concatenation did not contribute noise to the spectra. In addition, signals from the microphone were digitally high-pass filtered below 100 Hz to minimize transitions at the concatenation points. The eight independent time waveforms collected during the bias cycle were subjected to spectral and Boltzmann analysis as described above.

E. Modeled distortion generation

Interpretation of changes in DPOAE levels due to displacements of the OP was simplified by considering what was theoretically expected. The cochlear transducer was represented by a first-order Boltzmann transducer driven by one or two probe sinusoids, with OP varied to represent displacements by the bias tone. The generation of distortion products by a nonlinear transducer and their amplitude and phase relationship to the transducer's OP have been previously described using slightly different transducer properties as those used in the present study (Weiss and Leong in 1985; Frank and Kössl, 1996; Kirk *et al.*, 1997; Sirjani *et al.*, 2004). The specific parameters of the Boltzmann function used to model distortion generation were $y = 1 - 2/[1 + \exp(-4 \cdot (x + \text{OP}))]$, which were chosen so that the output waveform represented a typical CM recorded from the basal turn [Fig. 2(A), middle row]. The transfer function of the input sinusoid to the output waveform, with the OP at -1.2 , zero, and $+1.2$, is shown in Fig. 2(B), where the input sinusoid plus the OP are plotted on the x -axis, and the output waveforms from Fig. 2(A) are plotted on the y -axis. The amplitude and phase of the distortion products measured from FFTs of the output waveform were then compared as OP was varied from one extreme ($+1.2$) to the other (-1.2) [Figs. 2(C)–2(J)].

Similar to the changes observed in previous studies, the amplitude of the 2f1 and f2-f1 distortion products (referenced to the amplitude of f1) was lowest when the OP was near zero [Figs. 2(C) and 2(G)], while the amplitude of the 3f1 and 2f1-f2 distortions was greatest when the OP was at zero [Figs. 2(E) and 2(I)]. The phase of the 2f1 and f2-f1 distortions flipped between 90° and 270° (relative to f1) as the OP changed from positive to negative values [Figs. 2(D) and 2(H)], while the phase of the 3f1 and 2f1-f2 distortion products was 0° or 180° depending on the OP value, flipping phase when the amplitude of these distortions was close to zero [Figs. 2(F) and 2(J)].

We also determined that when the transducer was driven by a probe sinusoid that produced substantial saturation of the transducer (i.e., when the amplitude of the output waveform was within 5% of maximum transducer output with the OP at zero), the 3f1 and 2f1-f2 distortions passed through a minimum as the OP was displaced. For one-tone probes, these minima occurred when the OP was near $\frac{1}{4}$ the peak-to-peak amplitude of the input sinusoid [Fig. 2(E)], while for two probe inputs these minima occurred near $\frac{1}{5}$ the peak-to-

FIG. 2. Modeled changes in the distortion products generated by a pressure transducer with a first-order Boltzmann distribution driven by one or two sinusoids, as the transducer OP was varied. (A) The modeled transducer output when the OP was -1.2 Pa (top), 0 Pa (middle), and 1.2 Pa (bottom waveform), and the transducer was driven with a single 3.2 Pa peak-to-peak sinusoid. (B) The Lissajous curves of the modeled outputs from (A) (thick black line) plotted as a function of the sinusoidal input displaced by the OP (open circle) and approximated with a Boltzmann curve (thin black line). [(C)–(F)] The amplitude and phase of $2f_1$ and $3f_1$ distortion products (relative to f_1) versus the OP, as the OP was biased from -1.2 to 1.2 Pa in 0.075 Pa steps. [(G)–(J)] The amplitude and phase of the f_2-f_1 and $2f_1-f_2$ distortion products produced when the same transducer was driven with two sinusoids with a peak-to-peak amplitude of 5 Pa and the OP was varied from -1.2 to 1.2 Pa.

peak amplitude of probe sinusoids [Fig. 2(I)]. We found this relationship to be important because it allowed our OP_{Bias} measurement, derived from DPOAE biasing, to be referenced to the transducer displacement produced by the probe tone. This meant we could describe our OP_{Bias} measurement as a pressure relative to the probe tone (in pascal), by scaling our OP_{Bias} values so that these notches occurred at the appropriate pressures (presented in more detail in Sec. II F).

F. OP_{Bias} derived from the amplitude of the biased distortion products

With a clear understanding of the relationship between the OP and the amplitude distortion products, we could then begin to derive an estimate of the OP from the modulation of the DPOAEs throughout a bias tone. That is, in addition to estimating the Boltzmann OP (OP_{Boltz}) derived by fitting a

synthesized probe stimulus to the CM waveforms, we could also derive an OP estimate from the bias-induced modulation of the distortion products measured from either the ear canal or CM, which we termed “ OP_{Bias} .” The OP_{Bias} estimate was derived by fitting a synthesized bias-induced modulation of distortion products generated by a first-order Boltzmann transducer (where the bias directly modulated OP of the transducer) to the measured distortion products in the ear canal or CM throughout the acoustic bias tone. While both the OP_{Boltz} and the OP_{Bias} are both estimates of the transducer OP and both are presented relative to the level of the probe tone(s), we have used different subscripts to clarify that the two measures come from completely different analytic techniques.

Figure 3 demonstrates how OP_{Bias} was derived from the eight measurements of f_2-f_1 and $2f_1-f_2$ DPOAE amplitude throughout the 4.8 Hz bias. In cases where only one probe

FIG. 3. (A) The eight FFT measures of f_2-f_1 throughout the bias (white circles), fitted with the absolute amplitude of a sinusoid plus a dc offset (thick black line). The dc offset was assumed to be proportional to the transducer OP without any bias (dashed line with arrowhead), and the sinusoid with the dc offset was assumed to be proportional to the modulation of the OP (dashed sinusoid). (B) The f_2-f_1 measurements and fit plotted against the sinusoid plus dc offset. (C) The eight FFT measurements of $2f_1-f_2$ during the bias (white squares), overlaying the f_2-f_1 modulation. (D) The $2f_1-f_2$ measurements plotted against the sinusoid plus dc offset fitted to the f_2-f_1 measurements, scaled so that the notches in the modulation (which was fitted with the absolute amplitude of an inverted parabola) occurred at $\frac{1}{5}$ of the peak-to-peak amplitude of the probe input (i.e., 5 Pa in this case).

FIG. 4. Effects of a 4.8 Hz bias tone on the CM recorded from SM in the basal turn of animal PH05. (A) CM waveforms plotted for the eight points in the bias cycle as described in the methods. (B) CM data from (A) (black, coursing lines) versus the sinusoidal input displaced by the fitted OP_{Boltz} (white circles), shown with the fitted Boltzmann transducer curve (extended black line). [(C)–(E)] The OP_{Boltz} and the amplitudes of 2f1 and 3f1 respectively plotted as a function of time during one bias cycle. The amplitudes of the distortion products (2f1 and 3f1) are presented as a ratio relative to f1. [(F)–(H)] OP, 2f1, and 3f1 respectively plotted as a function of OP_{Bias} . The near-linear relationship between the OP_{Boltz} and OP_{Bias} values throughout the bias suggests that OP_{Bias} was as accurate an estimate of the transducer OP as the OP_{Boltz} measure. Furthermore, there was a V-shaped relationship between 2f1 and the OP, and a W-shaped relationship between 3f1 and the OP [dashed lines in (G) and (H)], with 3f1 nearing zero as the OP neared $\frac{1}{4}$ the pressure of the probe tone (equal to 0.45 Pa for the 500 Hz, 90 dB SPL tone).

tone was used, the OP_{Bias} was derived from the modulation of 2f1 and 3f1 using the same method. The same technique was also used to derive OP_{Bias} from the bias-induced modulation distortion products in CM waveforms. In the figure, the absolute amplitude of a 4.8 Hz sinusoid with an added dc offset was fitted to the spectral amplitude of eight f2-f1 distortion product measurements (relative to the spectral amplitude of f1), which were modulated by the low-frequency bias [Fig. 3(A)]. The dc offset, amplitude, and phase of the sinusoid were adjusted to best fit the modulated amplitude of the f2-f1 distortion product. The instantaneous “sinusoid +dc offset” at each point on the bias waveform was theoretically proportional to the transducer OP throughout the bias, and the amplitude of f2-f1 could be plotted against it to produce a V-plot [Fig. 3(B)]. In order to directly compare the OP_{Bias} and OP_{Boltz} estimates, we transformed OP_{Bias} onto a scale related to the pressure of the probe tone. To scale OP_{Bias} , the amplitude of the biased 2f1-f2 (relative to f1) was plotted against the bias sinusoid +dc offset pressures, as was done for f2-f1 [Fig. 3(D)]. This resulted in a “W-shaped” curve, similar to an inverted parabola, with minima in the amplitude of the distortion products that occurred at specific bias values. From our model of distortion generated by a first-order Boltzmann function (Sec. II E), we determined that these minima theoretically occurred at $\frac{1}{4}$ of the peak-to-peak amplitude of the probe for single-tone inputs or $\frac{1}{5}$ peak-to-peak amplitude for two-tone inputs [Figs. 2(E) and 2(I)]. We could therefore scale our OP_{Bias} values so that notches in the modulated 2f1-f2 distortion product occurred at values which were equivalent to $\frac{1}{5}$ the peak-to-peak pressure of the probe. If there was insufficient modulation to produce a minimum in 2f1-f2, we extrapolated the modulation using an

inverted parabola fitted to the modulation pattern. The scaled dc offset [Fig. 3(A)] or the average of all OP_{Bias} points fitted throughout the bias cycle [Figs. 3(B) and 3(D), vertical dashed line] represented the transducer OP at the zero crossings of the bias, i.e., comparable to measurements without a bias present.

III. RESULTS

A. Low-frequency biasing of the cochlear microphonic

An example of the effect of a 4.8 Hz 115 dB SPL bias tone on the CM evoked by a 500 Hz 90 dB SPL tone, recorded from the SM in the basal turn, is shown in Fig. 4(A). The two heavy traces show the responses nearest the maximum and minimum bias pressures, in which considerable differences in CM waveshape are apparent. The thick coursing lines on the Lissajous plots [Fig. 4(B)] show the same CM waveforms (Y-axis) plotted relative to the 500 Hz sinusoidal input (X-axis) with the bias-induced OP changes added to it. The extended thin lines are the fitted Boltzmann functions, and the white circles indicate the OP_{Boltz} , which is the location on the Boltzmann curve at the zero crossings of the 500 Hz input sinusoid. The changes in the OP_{Boltz} produced by the bias tone almost entirely account for the bias-induced shape changes of the CM. The bias-induced changes in OP_{Boltz} , 2f1, and 3f1 for the eight time points during the bias are shown in Figs. 3(C), 3(E), and 3(D), respectively. Curves with symbols show changes during the 115 dB SPL bias tone, while thinner lines show the similar but progressively smaller modulations as the bias tone was reduced in 5 dB steps.

FIG. 5. (A) The CM waveforms from animal PH09, evoked by 70, 80, 90, and 100 dB SPL, 500 Hz tones, at the extremes of the 115 dB SPL, 4.8 Hz bias, with the level of the 500 Hz probe shown above each set of two CM waveforms. (B) The Lissajous curves of the CM waveforms from (A) (coursing black line) plotted as a function of the probe displaced by the fitted OP_{Boltz} (open circles) and approximated with a Boltzmann curve (extended black line). (C) The amplitude of the 2f1 distortion products (relative to f1) versus the OP_{Boltz} derived from the CM evoked by different level 500 Hz probes, recorded throughout 4.8 Hz bias tones at 100, 105, 110, and 115 dB SPL. The dashed lines in (C) is the V-shaped relationship between 2f1 and the OP_{Boltz} when the stimulus was 90 dB SPL. (D) The modeled 2f1 versus OP_{Boltz} plots when a modeled first-order Boltzmann transducer was driven with different level sinusoids, and the OP was varied from -0.6 to $+0.6$. The outputs of the modeled transducer were ac coupled before being re-fitted with a Boltzmann function to mimic the ac coupling of the CM responses. The extreme OP displacements are shown as white circles.

Changes in OP_{Boltz} approximately followed a sinusoidal modulation during the 4.8 Hz sinusoidal bias tone, demonstrating that OP_{Boltz} was a reliable indicator of the transducer input. The relative amplitude of the second harmonic (2f1, relative to the amplitude of f1) showed a bimodal modulation, with distortion minima occurring when OP_{Boltz} was near zero and maxima when OP_{Boltz} was furthest from zero. Conversely, the relative amplitude of the third harmonic (3f1, relative to f1) was minimal when OP_{Boltz} was biased from zero and maximal when OP_{Boltz} was at zero. The three measures plotted against OP_{Bias} are shown in Figs. 4(F)–4(H). OP_{Boltz} followed a near-linear relationship with OP_{Bias} [Fig. 4(F)], suggesting that both methods estimated the transducer OP similarly. The second harmonic showed substantial changes in amplitude as the OP was displaced (93% modulation), producing a V-shaped relationship when plotted against OP_{Bias} [Fig. 4(G)]. Changes in the 3f1 amplitude throughout the bias were far smaller (35% modulation), with the amplitude of 3f1 largest when OP_{Bias} was closest to zero and reaching a minimum as OP_{Bias} neared $\frac{1}{4}$ the peak-to-peak pressure of the probe (0.45 Pa).

B. Single-tone probe level and modulation of the OP

The probes used to evoke CM or DPOAEs in this study were high-level, sufficient to produce saturated CM waveforms without being traumatic. CAP thresholds and the EP, measured before and immediately after the presentation of our DPOAE probes, were unaltered by the presentation of

probes at levels lower than 100 dB SPL for 500 Hz tones and lower than 85 dB SPL for 4 and 4.8 kHz frequencies (Salt *et al.*, 2009).

Examples of biased CM waveforms produced by 500 Hz probe tones at 70, 80, 90, and 100 dB SPL, at the extreme bias points during a 115 dB SPL 4.8 Hz tone, are shown in Fig. 5(A). At each level of probe stimulus, CM waveforms for the positive and negative limits of the bias are plotted against time [Fig. 5(A)] or against the input stimulus [Fig. 5(B)]. On the Lissajous curves in Fig. 5(B), the output waveforms are indicated by thick dark lines and the OP is indicated by open circles. At each probe level, the difference between the two paired curves demonstrates the OP changes induced by the bias.

The amplitude of 2f1 from the CM evoked at each probe level used was measured during a range of 4.8 Hz bias tones at levels of 100, 105, 110, and 115 dB SPL and is plotted versus the OP_{Boltz} in Fig. 5(C) [i.e., each plot in Fig. 5(C) consists of a group of 2f1 versus OP_{Boltz} measurements, with the probe level indicated on the left, but with all bias tone levels]. Calculated changes in 2f1 and OP_{Boltz} fitted to the ac-coupled output of a first-order Boltzmann equation (using the same equation described in Sec. II E), driven by different level probes and with various OP displacements, are also shown in Fig. 5(D). Initially, it appeared that there was less modulation of the OP by the bias tone when the 500 Hz probe level was 70 dB SPL. However, we found the same result in both the CM and model-derived waveforms and demonstrated that it was due to errors in fitting a Boltzmann curve to a relatively undistorted waveform evoked by a low-

FIG. 6. [(A) and (B)] The OP_{Boltz} and 2f1 (white circles) simultaneously measured in animal PH10 from the RW CM (top row), ST CM of the basal turn (second row), SM CM of the basal turn (third row), and the OP_{Bias} and 2f1 from the ear canal OAE (bottom row) during simultaneous presentation of both 500 Hz, 90 dB SPL and 4.8 Hz, 115 dB SPL tone. The OP_{Boltz} from the CMs were derived from Boltzmann fits to the CM waveforms, while the OP_{Bias} was derived from the relative displacement of the DPOAEs throughout the 4.8 Hz bias tone, as described in Sec. II F. Thin black lines are similar modulations using lower sound-level bias tones from 95 to 110 dB SPL in 5 dB steps. [(C) and (D)] The amplitude and phase of 2f1 versus the OP_{Boltz} or OP_{Bias} for each recording location. The dashed lines in each plot are the modeled changes in the amplitude and phase of 2f1 for similar OP modulations.

level stimulus. To avoid inaccurate Boltzmann fits to the CM, we generally used probe levels that produced sufficiently saturated CM waveforms, without producing any temporary or permanent changes in cochlear function, which in the case of 500 Hz tones was around 90 dB SPL.

Apart from an underestimation of the OP with low-level tones, the slope of the V-shaped relationship between 2f1 and the OP_{Boltz} [Fig. 5(D), dashed lines] varied for different input levels, particularly for 100 dB SPL. We attributed this to a variation in the relative amplitude of 2f1 to f1 over the range of input levels that was also present in the modeled data. One main difference between the real [Fig. 5(C)] and modeled [Fig. 5(D)] changes in the relationship between 2f1 and the OP_{Boltz} was a substantial positive shift in the average OP_{Boltz} in the real data when the probe level was 100 dB SPL, representing a physiological adaptation of the OP toward ST during the high-level probe tone. This OP displacement with high-level probes has been reported in previous studies (Patuzzi and Moleirinho, 1998).

C. Simultaneous modulation of distortion products from different locations

In a number of experiments, we simultaneously estimated the OP from different recording locations during 4.8 Hz biasing. The estimated OP_{Boltz} derived from the CM recorded from the RW, ST of the basal turn, and SM of the basal turn and the estimated OP_{Bias} derived from the modulated DPOAE are shown throughout the low-frequency bias in Fig. 6(A). Symbols show the influence of the bias tone at 115 dB SPL with thin lines representing similar modulations at lower bias levels from 110 to 95 dB SPL in 5 dB steps. All

OP measures were modulated in-phase and by a similar amount, except for OP_{Bias} from the DPOAEs, which was modulated 40% more than any of the OP_{Boltz} measures. The average OP throughout the bias differed between recording locations [Fig. 6(C), vertical lines], although the average OP from ST and SM measurements, which were at the same region along the length of the cochlea, was similar. This resulted in different modulations of the second harmonic, e.g., 2f1 from the RW was modulated sinusoidally, while 2f1 from ST, SM, and the ear canal measurements was modulated bimodally. This was best explained when the amplitude of 2f1 was plotted against the modulated OP from each recording location [Fig. 6(C)]. In these plots there was a V-shaped relationship indicated by the dashed line in each plot. As the OP from the ST, SM CM, and the OAE recordings passed through zero during the bias, the 2f1 was bimodally modulated. In contrast, the mean OP estimated from the RW CM was at a more negative value and the displacement by the bias did not cause it to cross zero, so the 2f1 exhibited a simpler sinusoidal modulation. The phase of 2f1 from each location is plotted against the OP for each set of data in Fig. 6(D). As predicted from the model (see Fig. 2), 2f1 phase changed by $180^\circ \pm 40^\circ$ when the OP crossed zero for the ST and SM recordings. However, the same phase relationship was not observed in the 2f1 versus OP_{Bias} plot from DPOAE [Fig. 6(D), bottom plot] because the phase of f1 in the ear canal was dominated by the stimulus and therefore was unrelated to the phase of the distortion products generated within the cochlea.

FIG. 7. Animal PH13. Modulations of the OP_{Boltz} , OP_{Bias} , and $2f1$ with different levels of the 4.8 Hz tones ranging from 95 to 120 dB SPL (shown at the right of the figure). In each plot, $2f1$ (relative to $f1$) has been plotted versus the estimated OP for each of the bias tone levels (open circles), with each bias level then shifted vertically from the next. In each plot, all biases within that plot have been overlaid at the bottom (gray circles). [(A) and (B)] $2f1$ versus the OP_{Boltz} derived from the RW CM and SM CM evoked by a 500 Hz, 90 dB SPL tone during the 4.8 Hz bias. (C) $2f1$ DPOAE evoked by a 500 Hz, 90 dB SPL tone versus OP_{Bias} derived from the modulation of the DPOAEs throughout the 4.8 Hz bias.

D. Bias level with a single-tone probe

We intended to use the average OP_{Bias} value as an estimate of the resting OC displacement and transducer OP. Therefore, we investigated if the bias tone altered the *average* OP by measuring the OP_{Boltz} and OP_{Bias} during the bias tone at a range of bias levels from 95 to 115 dB SPL. The average OP throughout the bias only underwent minor changes as the level of the bias tone increased [e.g., 0.08 Pa, Fig. 7(A), dashed vertical lines]. Higher-level bias tones typically produced larger modulations of the OP and $2f1$, maintaining the V-shaped relationship, with minimal changes in the average OP values. In each plot of Fig. 7 the amplitude of $2f1$ versus the OP at each bias level has been shifted vertically for clearer visualization of the overall OP bias (Fig. 7, white circles), with all bias levels then overlaid at the bottom of each plot (gray circles). The level of bias for each plot is provided at the right of the figure. The plots of $2f1$ versus OP_{Boltz} derived from the RW and SM CM evoked by a 500 Hz, 90 dB SPL probe tone are shown in Figs. 7(A) and 7(B), while $2f1$ versus the OP_{Bias} from the simultaneously recorded DPOAE is shown in Fig. 7(C). In all three cases the average OP was negative, equivalent to a displacement of the OC toward SV. At lower bias levels the $2f1$ was modulated sinusoidally, while at higher bias levels (at 115 dB for the RW, 105 dB for SM, and 115 dB for the OAE), the $2f1$ was modulated bimodally.

As in Fig. 7, higher-level biases produced proportionally more modulation of the OP up to 120 dB SPL, which was the highest bias level used in this study. The average modulation of the OP_{Boltz} derived from the CM evoked by a 500 Hz, 90 dB SPL tone, for different bias levels, recorded from both the RW and SM is shown in Fig. 8. For bias levels above 80 dB SPL, modulation of OP_{Boltz} (on a logarithmic scale) increased in a linear fashion with increasing bias level, demonstrating that the displacements produced by the bias tones

were not saturating over this range for levels up to 120 dB SPL. Typically, levels of the 4.8 Hz bias lower than 80 dB SPL produced modulations of the OP that were below the normal variation in the OP_{Boltz} throughout the bias, i.e., representing the noise floor (Fig. 8, white circles).

E. Bias level with a two-tone probe

Figure 9 shows changes in $f2-f1$ distortion (upper row) and $2f1-f2$ distortion (lower row) measured at different sites,

FIG. 8. The average modulation of the OP_{Boltz} (peak-to-peak OP change) derived from the 500 Hz, 90 dB SPL evoked CM recorded from (A) the RW and (B) SM of the basal cochlear turn, with the level of the 4.8 Hz bias varied from 60 to 115 dB SPL. The number of animals used to obtain the average modulation is shown in each figure. White circles represent modulations that were below the average variation in the OP_{Boltz} throughout the bias (the noise floor).

FIG. 9. Animal PH13. Modulations of the OP_{Bias} and $f2-f1$ derived from the modulated RW CM, SM CM, and DPOAE responses evoked by 4 and 4.8 kHz, 80 dB SPL tones during different levels of a 4.8 Hz tone, ranging from 105 to 120 dB SPL (shown at the right of the figure). [(A)–(C)] $f2-f1$ (relative to $f1$) versus the OP_{Bias} for each of the bias tone levels (open circles), with each bias level shifted vertically from the next, and all biases within that plot overlaid at the bottom (gray circles). [(D)–(F)] $2f1-f2$ versus the OP_{Bias} derived from each recording, demonstrating that the amplitude of $2f1-f2$ neared zero when the OP_{Bias} was displaced to $\frac{1}{5}$ of the peak-to-peak pressure of the probe or that the difference between the OP_{Bias} values at which the notches occurred was $\frac{2}{5}$ the pressure of the probe.

as bias level was varied from 105 to 120 dB SPL. The probe stimuli in this case were tones at 4 and 4.8 kHz presented at 80 dB SPL. With the high-frequency probe stimuli, a reliable Boltzmann analysis of the CM was not possible [due to factors such as electrical low-pass filtering of the CM by the cochlear scalae; see Strelhoff (1973) and Patuzzi *et al.* (1989)], and therefore OP_{Boltz} could not be obtained. In contrast, our estimate of OP_{Bias} is unaltered by changes in the overall amplitude of the distortion products (as might occur with electrical or acoustical filtering) but depends upon their modulation and the appearance of notches or minima throughout the bias. This allowed us to estimate OP_{Bias} even though Boltzmann analysis of the CM was not possible. We have therefore plotted the distortion products from each of the RW, SM, and OAE recordings against OP_{Bias} for each location. The $f2-f1$ distortion product from each recording was modulated bimodally [Figs. 9(A)–9(C)], particularly at higher levels of bias pressure in a similar manner to the modulation of $2f1$ evoked by single tones (Fig. 7). The magnitude of OP_{Bias} derived from the ear canal was over twice that derived from the basal turn CM recordings evoked by the two-tone stimulus, suggesting that the bias produced more displacement of the OC at the region where the DPOAEs were generated in the cochlea than at the base of the cochlea where the local CM was recorded.

The amplitudes of the $2f1-f2$ distortion products are

shown in Figs. 9(D)–9(F), further illustrating the technique used to relate OP_{Bias} values to the probe. The OP_{Bias} values were scaled so that minima in the modulated $2f1-f2$ occurred 0.45 Pa apart (which was $\frac{2}{5}$ the peak-to-peak pressure of the probe stimulus in the ear canal). It was necessary to extrapolate the minima in the case of the RW and SM recordings [Figs. 10(D) and 10(E)], while the DPOAE $2f1-f2$ was modulated sufficiently to produce notches [Fig. 10(F)]. While our modeled data suggested that the amplitude of $2f1-f2$ should have been maximal when the amplitude of $f2-f1$ was close to zero, there was a slight difference between the OP_{Bias} values at the maximal $2f1-f2$ position and the minimal $f2-f1$ position, particularly for the RW recording. These responses are analogous to the bias-induced modulation of $3f1$ to a single tone [Fig. 4(H)] and confirm that the OP can be derived from ear canal DPOAE measurements.

F. Using distortion measures to track OP during hypoxia

One way of comparing OP_{Boltz} from CM and OP_{Bias} from DPOAEs is to compare the two measures during manipulations that are known to change OP. Figure 10 shows the results from an experiment in which OP_{Boltz} and OP_{Bias} measures from the CM and DPOAE were compared during hypoxic episodes. Hypoxia has been shown to produce

FIG. 10. [(A)–(E)] Repeated measurements in animal PH14 during two hypoxic events [rebreathing times indicated by dark bars in (A)], with a 20 min break between recordings (indicated as line-breaks). (A) EP. (B) OP_{Boltz} obtained from 500 Hz, 90 dB SPL evoked CM. (C) The average OP_{Bias} obtained from biased CM and DPOAE responses evoked by 4 and 4.8 kHz 80 dB SPL tones during a 4.8 Hz, 115 dB SPL tone. (D) The relative amplitude of 2f1 from the CM and DPOAE evoked by a 500 Hz, 90 dB SPL tone. (E) The relative amplitude of f2-f1 from the CM and DPOAE evoked by 4 and 4.8 kHz, 80 dB SPL tones with no bias. Black circles represent measures from the CM, and white circles those from the ear canal. (F) A V-plot of the data in (B) versus the data in (D). [(G) and (H)] V-plots of the data from (E) versus the data in (C). Note that there are eight times more points in (G) and (H) than in (F) because there were eight measures of the distortion products and OP_{Bias} during the low-frequency bias.

changes in the EP, DPOAEs, and Boltzmann parameters (Rebillard and Lavigne-Rebillard 1992; Patuzzi and Moleirinho, 1998; Olzowy *et al.*, 2008). Responses were repeatedly

evoked by successive probes consisting of a 500 Hz, 90 dB SPL tone, simultaneously presented at 4 and 4.8 kHz, 80 dB SPL tones with a 115 dB SPL, 4.8 Hz bias tone and at 4 and

4.8 kHz, 80 dB SPL tones without a bias tone, with approximately 10 s of silence separating each probe set. Biased responses were used to derive the average OP_{Bias} from both the ear canal and the CM, while responses without the bias provided the unbiased two-tone distortion products.

Around 3–5 min after hypoxia was initiated, the EP began to decline gradually [Fig. 10(A)]. Concurrently, the OP_{Boltz} and OP_{Bias} values shifted toward SV [Figs. 10(B) and 10(C)]. When EP reached 48 mV normal ventilation was restored and the EP rapidly increased with an over-shoot before recovering to around 85 mV. As the EP recovered, OP_{Boltz} and OP_{Bias} shifted to around +0.2 Pa, equivalent to a shift toward ST, before decreasing with a slight over-shoot toward negative values. After a 20 min break in recordings, a second hypoxia was induced, producing similar but slightly larger changes in each measurement. During this second hypoxia CM, EP, and distortion products were monitored more frequently.

Prior to the hypoxic events, the OP_{Bias} was closer to zero than OP_{Boltz} . This difference was most likely due to differences in probe stimuli rather than being a difference in the method of estimating the OP *per se*. Regardless of this initial difference, the induced changes in OP_{Bias} and OP_{Boltz} were similar throughout both hypoxic events.

The amplitudes of the unbiased 2f1 and f2-f1 distortion products from the 500 Hz, 4 kHz, and 4.8 kHz probes throughout the recordings are presented in Figs. 10(D) and 10(E). While there were differences in the amplitudes of the unbiased 2f1 distortion product between CM and OAE recordings, this can be explained on the basis of a different initial OP of the transducer responses dominating the CM and the DPOAEs. The amplitudes of the unbiased f2-f1 distortion product from the CM and DPOAE were similar throughout the experiment, suggesting that the transducer generating f2-f1 in the CM and DPOAE had similar initial OPs. Induced distortion changes are more easily explained using plots of 2f1 or f2-f1 versus the OP_{Boltz} or OP_{Bias} , as shown in Figs. 10(F)–10(H). In all cases, distortion was reduced as the OP measure moved closer to zero.

IV. DISCUSSION

The present study outlined the dependence of DPOAE amplitude on the OP of the transducer and demonstrated that with a clear understanding of this relationship, DPOAE measurements can be used to non-invasively monitor cochlear function. In particular, low-frequency biasing of DPOAEs could be used to non-invasively estimate the resting position of the OC, which might provide a clinical tool for diagnosing disorders that perturb the normal transducer position, such as endolymphatic hydrops. Previous studies have attempted to use the amplitude of the DPOAEs as indicators of cochlear function (Rebillard and Lavigne-Rebillard 1992; Kossowski *et al.*, 2001; Kujawa and Liberman, 2001; Garner *et al.*, 2008; Olzowy *et al.*, 2008), reporting complex changes in the DPOAEs that were difficult to interpret, particularly as changes in the OP were not taken into consideration. Furthermore, other studies have taken similar approaches to investigate hearing function in humans (Mrowinski *et al.*, 1996;

Hensel *et al.*, 2007; Bian and Scherrer, 2007) and animals (Frank and Kössl, 1996; Kirk *et al.*, 1997; Lukashkin and Russell, 2002; Bian, 2004; Sirjani *et al.*, 2004; Salt *et al.*, 2005) and have reported similar relationships between pressure in the ear canal, the OP, and the amplitude of the DPOAEs as those reported here. However, this study is the first to directly relate the OP obtained from CM recordings to the OP derived solely from simultaneously recorded DPOAEs.

A. Differences between the OP_{Boltz} derived from CM and the OP_{Bias} derived from DPOAEs

While both OP_{Boltz} derived from the CM and OP_{Bias} derived from the DPOAEs were modulated sinusoidally during the low-frequency bias and both changed in a similar manner during the hypoxia experiments in the present study, often there were differences between the average OP_{Boltz} and average OP_{Bias} . As discussed by Kirk *et al.* (1997), who directly compared the Boltzmann OP from basal CM recordings to the biased DPOAEs, there are several possible reasons why there might be differences between the OP_{Boltz} and the minimal point of the f2-f1 DPOAE throughout the bias (equivalent to the average OP_{Bias} value in our study). To briefly summarize the points made by Kirk *et al.* (1997); first, the nonlinear transducer generating the DPOAEs might be better described by a *second-order* Boltzmann distribution, resulting in differences between the OP_{Boltz} (which was estimated using a *first-order* Boltzmann function) and our measurement of the OP_{Bias} . Second, there might be differences in adaptation to the probe tone between sources underlying the generation of OP_{Boltz} and OP_{Bias} . We present another explanation here, that the OP_{Boltz} derived from the basal turn CM is an estimate of the OC position at the base of the cochlea, while the OP_{Bias} derived from the DPOAEs is an estimate of the OC position at the overlapping region stimulated by the probe tone(s), more apical in the cochlea, and that there were differences between the resting OC position at either location. Therefore, it would be unreasonable to directly relate the amplitude of the DPOAEs to the OP_{Boltz} or distortion products from basal turn CM recordings, and it is essential to apply an individual OP estimate to the transducer generating the DPOAE.

The portion of the cochlea the OP_{Boltz} and OP_{Bias} measurements represent is most likely a weighted sum of the regions stimulated by the probe tones contributing to the CM and DPOAE measurements and most likely increases with the level of the stimulus as broader regions of the cochlea are recruited. That the OP_{Boltz} derived from the RW CM was often different from the OP_{Boltz} derived from ST or SM recordings in the present study (Fig. 6), which were as little as 4 mm apical from the RW, further suggests that the OC position can differ throughout the cochlea and that the OP_{Boltz} estimate is a relatively local measure of OC position. Furthermore, that the estimates of OP derived from ST and SM measurements made at the same region along the length of the cochlea were similar, even though their CM amplitudes differed almost tenfold, and their polarities were opposite,

indicates that the OP differences between locations throughout the cochlea were not due to the analysis technique.

Previous studies of DPOAEs suggest they are generated at both the “overlapping” region of the cochlea stimulated by the two probe tones (near the characteristic region of the probes) and at the characteristic region of the DPOAE itself (Talmadge *et al.*, 1999; Shera and Guinan, 1999). However it has been suggested that the presence of a low-frequency bias tone can suppress the component generated at the characteristic region of the DPOAE (Talmadge *et al.*, 1999; Bian and Scherrer, 2007), simplifying the regions of the cochlea contributing to the DPOAEs. Presumably, the OP_{Bias} derived from DPOAEs is primarily a measure of the OC position at the overlapping region and would be dependent on the probe frequency, which could be varied to investigate the resting position of the OC at different regions throughout the cochlea.

B. The OP_{Boltz} is a measure of local transduction

One motive for simultaneously measuring the OP_{Boltz} derived from ST and SM at the same region along the length of the cochlea in the present study was to demonstrate that Boltzmann analysis of CM is an accurate measure of local transduction. It had recently been suggested that distortion products in the CM were generated at distal regions within the cochlea, particularly for low-level stimuli (Zheng *et al.*, 2008). For stimulus levels that permitted reliable Boltzmann fits (above 80 dB SPL, Fig. 5), our OP_{Boltz} measurements derived from ST and SM CM were modulated in-phase and by a similar amount by the low-frequency bias, even though the ST and SM CM amplitudes differed by a factor of around 10, and the CM waveforms were of opposite polarity. If electrical distortion components from CM generated at more apical regions in the cochlea summated with CM generated in the basal turn where our measurements of ST and SM CM were made, our ST and SM OP_{Boltz} measurements would have been modulated 180 degrees out-of-phase during low-frequency biasing because remote potentials spread electrotonically to the basal turn would not invert polarity across the cochlear partition, and our analysis incorporated the inversion of the locally generated CM. Our data suggest that distortion in the CM is generated at the cochlear partition and that Boltzmann analysis of this CM is an accurate measure of local transduction, at least for 500 Hz stimulus levels above 80 dB SPL.

Similar changes in the amplitude and phase of the distortion products have been observed using the whole-cell voltage clamp technique to monitor the receptor current of OHCs (Takahashi and Santos-Sacchi, 1999), and Olson (2004) demonstrated that distortion products in fluid pressure measurements made close to the basilar membrane were generated locally, rather than at regions distal to the recording location. These studies provide further evidence that the dominant nonlinearity underlying distortion generation in the CM is produced by local OHC electromotility and that the distortion products in the CM can be used to estimate the OP of local OHC transduction.

C. Cochlear distortion products behave as predicted from a first-order Boltzmann function

Similar to results from previous studies (Kirk *et al.*, 1997; Sirjani *et al.*, 2004), the present study found that the amplitudes of the even-order distortion products from the CM and ear canal were minimal when the OP measure (OP_{Boltz} or OP_{Bias}) was near zero and maximal when the OP measure was displaced from zero. Conversely, the odd-order distortion products were maximal when the OP was near zero and minimal when the OP was displaced by $\frac{1}{4}$ to $\frac{1}{5}$ the peak-to-peak pressure of the probe. These changes suggest that the transducer generating distortion in the cochlea can be modeled with a first-order Boltzmann distribution function. However, there were several differences between the modeled and measured distortion products that suggest that slightly more accurate assessment of cochlear transduction might be achieved with more complex analysis methods, as discussed previously in detail by Patuzzi and Moleirinho (1998) with regard to Boltzmann analysis of the CM.

With the OP of a first-order transducer at zero, there should have been no even-order distortion products generated; however, often the even-order distortion products in the CM and DPOAE were not reduced to zero when the OP measure was near zero [Fig. 6(C)]. Furthermore, the relative phase of the even-order distortion products generated by such a transducer should have been either 90° or 270° (depending on the polarity of the OP and with the phase of f1 at zero), although often their phases were as much as 15° different from this when the OP was maximally displaced by the bias tone and as much as 90° different when the OP was near zero [Fig. 6(D)]. One likely explanation for the differences between the modeled and the measured distortion products is that there were additional non-dominant sources of distortion. Another possible explanation is that the CM and DPOAE distortion products are generated by a region of the cochlea along which the transducer properties change slightly, and therefore the distortion products are a slightly complex weighted sum of the contributing region, producing differences between our measured data and our estimates based on a single transducer.

In the case of the CM recorded from the base of the cochlea, which is predominantly generated by local hair cells (Dallos *et al.*, 1972; Patuzzi *et al.*, 1989), the other sources might have been either (1) other sources of nonlinearity in the local transduction, which also produced distortion of the receptor current but only dominated when the OP of the foremost nonlinearity was near zero, (2) distortion products generated by OHC at other regions of the cochlea, which spread electrotonically to the recording location and had different characteristics (phase and amplitude) than those generated by basal hair cells, (3) distorted OC vibration generated at other regions in the cochlea, which then produced additional vibration at the base of the cochlea, or (4) low-level distortion of the acoustic stimulus resulting in a non-sinusoidal stimulus. Whatever the other source(s) of distortion in the CM were, it resulted in only minor deviations from the values predicted by a first-order Boltzmann equation.

In the case of the DPOAEs, many previous studies have

researched the sources of DPOAEs in the cochlea, with the general consensus that there are two main contributors, a linear reflection component and a nonlinear distortion component (Talmadge *et al.*, 1999; Shera and Guinan, 1999). Multiple contributions to the DPOAEs might result in complex modulations of the DPOAE amplitudes throughout the bias that could not be modeled as a single transducer with a Boltzmann distribution. However, in the present study the DPOAEs often followed a bimodal modulation, suggesting either that one of the components typically dominated the DPOAE generation or that both components contributed equally, producing similar DPOAE modulations. We did not attempt to investigate the contribution from either component, and most likely there was some complex summation of the two sources, which decreased the accuracy of our OP_{Bias} measure slightly. Given the similarity between the OP_{Boltz} measure from basal turn CM, which is a more local measure and the OP_{Bias} from the DPOAEs measured in the present study, this inaccuracy is most likely small.

It is worth noting here that in the present study our Boltzmann function expressed OP_{Boltz} in terms of a static pressure in the ear canal [using $S^*P_{sat}^*$ (input+OP) in the equation], rather than as a measure unrelated to ear canal pressure but still proportional to the asymmetry of transduction [using $(S^*P_{sat}) + OP$ in the equation]. Patuzzi and Moleirinho (1998) used the latter method as they assumed that some OP changes might have been due to slight micromechanical changes in the position of the OC, unrelated to the ear canal pressure. While there are several situations that might lead to a difference between the OP estimated using these different methods, such as changes in middle ear conductance or a change in the OC compliance during a low-frequency bias, we routinely compared our OP measure to that obtained using the method employed by Patuzzi and Moleirinho (1998) and found the differences to be minimal, only differing substantially for changes in probe level, for which it was not possible to determine the preferable method as OP changes with stimulus level might have been produced by OC compliance changes *or* micromechanical changes in the OC position.

D. Probe and bias level effects on OP estimate

Ideally, we would want to use probe levels that generate distortion products in the ear canal or CM that are well above the noise floor of the recording and that generate CM waveforms that are sufficiently saturated to allow accurate Boltzmann analysis. However, relatively high probe levels (e.g., above 100 dB SPL for 500 Hz tones) often caused unwanted adaptation of the OP measure [Fig. 5(C)] and might lead to temporary threshold shifts particularly in the case of high-frequency probe tones (at levels above approximately 85 dB SPL for continuous stimulation; Chang and Norton, 1996). Therefore, the ideal range of probe levels used for the present techniques is most likely limited to around 65–80 dB SPL for high-frequency probe tones (i.e., around 4 kHz) and around 80–95 dB SPL for low-frequency tones (i.e., around 500 Hz). These probe levels did not alter the CAP thresholds or EP measurements before or after presentation of the

probes, suggesting that they did not cause any temporary or permanent changes in cochlear function the guinea pigs used in this study, other than a rapid slight adaptation which occurred at the immediate onset of the probe with a time constant of around 1 s (which was not included in our analysis of the responses), similar to that observed by Kujawa and Liberman (2001), and which was most likely related to efferent activity. As stimulus levels were measured close to the tympanic membrane, they were effectively 5–10 dB lower than if they had been presented and calibrated free field since no ear canal gain is available in this preparation.

Such levels are on the upper limit of what would be acceptable for human DPOAE testing (although levels as high as 85 dB SPL at 4 kHz have been used; Lonsbury-Martin *et al.*, 1990). We are currently evaluating whether OP_{Bias} can be determined from DPOAEs that does not require the transducer to be saturated.

While the slope of the relationship between the amplitude of the even-order distortion products and the OP changed over a range of sound levels (Fig. 5), this did not affect our estimation of the OP_{Bias} because it was not based solely on the amplitude of the distortion products. Rather, it was based on notches in the amplitude of the modulated distortion products during the bias, as outlined in Sec. II F, and the overall ratio of the distortion product to f1 (i.e., the y-axis of our V-plots) was not a key factor in our analysis.

Generally, different bias tone levels (from 95 to 115 dB SPL) simply produced different levels of OP modulation (Fig. 8), producing only a slight change in the average OP measure [Fig. 7(A)], which most likely reflects a small amount of OP adaptation to the bias tone. It was often necessary to use bias levels of 110 dB SPL in order to produce bimodal modulation of the even-order distortion products and a modulation of the odd-order distortion products that allowed estimation of notches in its amplitude, which was a limitation of the technique used to estimate OP_{Bias} . However, it is important to note that the cochlea is generally insensitive to such low-frequency stimuli due to the high-pass filtering characteristics of the helicotrema, as described below, and following exposure to the highest level bias tones used in the present study, cochlea sensitivity was unchanged. While the presence of the bias might have been responsible for the slight changes in the OP measure over the range of bias levels, these changes were negligible in comparison to the experimentally induced changes in OP such as that produced by hypoxia.

E. Referencing OP_{Bias} to the probe level

In order to compare the OP_{Boltz} and OP_{Bias} measures, it was necessary to reference the OP_{Bias} values to the level of the probe stimulus in the ear canal using characteristic changes in the distortion products as outlined in Sec. II F of the present study. This allowed estimation of the relative displacement of the OP at the region where the DPOAEs were generated in the cochlea, and it overcame the problem that the level of the distortion products in the ear canal could

change due to factors unrelated to the OP, such as conductive losses as the DPOAEs were transmitted from the cochlea to the ear canal.

Similar to results published in previous studies (Takahashi and Santos-Sacchi, 1999; Bian and Scherrer, 2007), we found amplitude minima or “notches” in the odd-order distortion products in the CM and DPOAEs during modulations of the OP. From our model of distortion generated by a first-order Boltzmann function, we found that for inputs which sufficiently saturated the transducer, these notches occurred at certain OP values that were related to the probe level. With the assumption that the modulated odd-order distortion products adhered to the relationship between the OP and notches in the modulated odd-order distortion products, we were able to relate the OP_{Bias} values to the probe based on the notches in the modulated odd-order distortion products. The values of the OP_{Boltz} and the corrected OP_{Bias} derived from the CM were similar [Fig. 4(F)], as was the magnitude of the overall changes in the OP_{Bias} and OP_{Boltz} during induced hypoxias, suggesting that the method for relating the OP_{Bias} to the probe level in the ear canal was viable.

The finding that the OP_{Bias} derived from the DPOAEs was modulated two to three times more than the OP_{Bias} derived from the basal turn CM during the low-frequency bias (Fig. 9) suggested that the bias produced a larger displacement of the OC near the 4–4.8 kHz characteristic frequency region than at the base of the cochlea. Presumably, this reflects the mechanical impedance change to low frequencies along the length of the cochlea (von Békésy, 1960; Patuzzi, 1996). Previous studies have used low-frequency modulation of odd-order DPOAEs to non-invasively estimate the sensitivity of the OC displacement to a low-frequency bias tone in humans (Bian and Scherrer, 2007; Marquardt *et al.*, 2007), changing the bias tone frequency between 15 and 480 Hz and examining the differences in the notches of the odd-order distortion products.

It is worth noting here that the same technique might be used to examine sensitivity of the OC displacement to the high-frequency probe tone(s) because the notches in the odd-order DPOAEs are a function of the sensitivity to both the bias tone and the probe tone(s) (i.e., notches in the biased odd-order distortions occur at $\frac{1}{4}$ or $\frac{1}{5}$ the pressure of the probe tone, which depends on the sensitivity to the probe tone). Interestingly, this might provide a non-invasive objective measure of the OC displacement and sensitivity throughout the entire cochlea (i.e., if the bias frequency is held constant while the probe frequencies are swept).

F. Estimating the transducer OP

This study did not attempt to determine the dominant nonlinearity underlying distortion in the ear canal and cochlea, be it nonlinearity of MET channel compliance (Howard and Hudspeth, 1988; Jaramillo *et al.*, 1993), nonlinearity of MET channel conductance (Corey and Hudspeth, 1979) that produces mechanical distortion via reverse-transduction (Kirk and Yates, 1998), or nonlinearity in the active process itself, whatever that process may be (see Hudspeth, 2008 for recent review). Rather, we suggest that the

dominant nonlinearity underlying distortion in both the ear canal and the CM is similar and that this distortion is related to the displacement of the OC.

Because we did not have a direct measure of the OC displacement, we estimated the OP as a measure that was equivalent to displacements of the OC produced by an acoustic stimulus in the ear canal. Measuring the OP relative to the probe stimulus allowed us to compare the OC displacement produced by the high-frequency probe tones (500 Hz or 4 and 4.8 kHz) to that produced by the low-frequency bias tone (4.8 Hz).

The 115 dB SPL, 4.8 Hz tone was equivalent to 32 Pa peak-to-peak pressure in the ear canal but, relative to the 500 Hz, 90 dB SPL probe tone (which was 1.79 Pa peak-to-peak), only produced a 0.19 Pa modulation of the OP_{Boltz} (Figs. 7 and 8). This suggests that the 4.8 Hz tone was 45 dB less effective in displacing the OC than the 500 Hz tone. This difference is most likely due to the acoustic high-pass filtering of the helicotrema, which attenuates frequencies lower than 100 Hz (Dallos, 1970; Ruggero *et al.*, 1986; Marquardt *et al.*, 2007). Presumably, higher-frequency bias tones would have produced a relatively larger displacement of the OC and greater modulation of the OP and would explain the more sensitive modulation of the DPOAEs reported by Bian and Scherrer (2007) who used 25–100 Hz bias tones.

We have recent evidence that suggests that the sensitivity differences between the high-frequency and bias tones are abnormal with auditory disorders such as endolymphatic hydrops (Marquardt *et al.*, 2007; Hensel *et al.*, 2007). If the helicotrema were partially or fully blocked off due to distension of Reissner’s membrane with endolymphatic hydrops, we might expect to see a larger sensitivity to the low-frequency bias tone due to the reduced effectiveness of the helicotrema as a low-frequency shunt. This has been confirmed experimentally in recent experiments where gel was injected into the cochlear apex (Salt *et al.*, 2009).

Finally, low-frequency biasing of DPOAEs might provide a twofold indication of hydrops. First, if endolymphatic hydrops generates an over-pressure in SM, displacing the OC toward ST, the OP_{Bias} estimate obtained using low-frequency DPOAE biasing would be abnormally positive. Second, if endolymphatic hydrops resulted in distension of Reissner’s membrane and blockage of the helicotrema, we might expect to find an abnormally high sensitivity to the low-frequency bias tone, as evident in the level of the bias required to produce notches in the odd-order distortion product.

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