The breaking of cochlear scaling symmetry in human newborns and adults

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Scaling symmetry appears to be a fundamental property of the cochlea as evidenced by invariant distortion product otoacoustic emission (DPOAE) phase above ~ 1 – 1.5 kHz when using frequency-scaled stimuli. Below this frequency demarcation, phase steepens. Cochlear scaling and its breaking have been described in the adult cochlea but have not been studied in newborns. It is not clear whether immaturities in cochlear mechanics exist at birth in the human neonate. In this study, DPOAE phase was recorded with a swept-tone protocol in three, octave-wide segments from 0.5 to 4 kHz. The lowest-frequency octave was targeted with increased signal averaging to enhance signal-to-noise ratio (SNR) and focus on the apical half of the newborn cochlea where breaks from scaling have been observed. The results show: (1) the ear canal DPOAE phase was dominated by the distortion-source component in the low frequencies; thus, the reflection component cannot explain the steeper slope of phase; (2) DPOAE phase-frequency functions from adults and infants showed an unambiguous discontinuity around 1.4 and 1 kHz when described using two- and three-segment fits, respectively, and (3) newborns had a significantly steeper slope of phase in the low-frequency portion of the function which may suggest residual immaturities in the apical half of the newborn cochlea. © 2011 Acoustical Society of America. [DOI: 10.1121/1.3569737]

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I. INTRODUCTION

The question of whether the human cochlea exhibits functional immaturity at birth is an intriguing and challenging one. Sensory cells seem to be morphologically mature by the end of the second fetal trimester. However, supernumerary hair cells have been reported around the time of birth, and outer hair cell (OHC) innervation by medial efferent fibers occurs relatively late into the third trimester, possibly into the perinatal period (Bredberg, 1968; Pujol *et al.*, 1998; Tanaka *et al.*, 1979). Functionally, the newborn cochlea shows nonlinear distortion and strong cochlear amplification inferred from robust cochlear reflection (Abdala 2000; Abdala and Dhar, 2010a). However, non-adult-like features of distortion product otoacoustic emission (DPOAE) phase have also been reported in neonates, in particular for low-frequency signals. The glaring lacuna in this developmental literature is the dearth of measurements from the apical half of the cochlea.

A. Cochlear scaling in the apex

In mammals, it appears that apical function differs from basal function in various ways (Cooper and Rhode, 1995; Nowotny and Gummer, 2006). A distinct boundary between the features of apical and basal otoacoustic emissions (OAEs) has been reported in human (adults and newborns), chinchilla, guinea pig and cat, intimating a *functional* demarcation (Shera *et al.*, 2000; Shera and Guinan, 2003; Abdala and Dhar, 2010a; Shera *et al.*, 2010). This apical-basal demarcation presumably reflects differences in micromechanics in the apical and basal halves of the cochlea and may signal a gradual break in cochlear scaling symmetry. At the simplest level, scaling symmetry implies that a signal will accumulate the same phase regardless of where it peaks along the cochlear partition; thus, when the cochlear tonotopic map is exponential, the traveling wave envelope is simply transposed along the basilar membrane, producing shift similarity (Zweig, 1976).

Distortion product (DP) OAE phase recorded using a fixed f_2/f_1 ratio can be used to assay cochlear scaling and its breaking (Shera *et al.*, 2000). The DPOAE is a dual-source response comprised of components with distinct generation mechanisms: a nonlinear distortion (or wave-fixed) source generated at the overlap between f_1, f_2 traveling waves, and a predominantly linear reflection (or place-fixed) source

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produced around the DP region ($2f_1 - f_2$) (Kim, 1980; Shera and Guinan, 1999; Knight and Kemp, 2000, 2001; Talmadge *et al.*, 2000). DPOAE phase measured in ear canal reflects the characteristics of the dominant of these two components, which, in the general population overall, is the distortion source. Since the distortion component is approximately invariant over most of the frequency range so, too, is the measured phase of the DPOAE recorded in the ear canal (Kemp and Brown, 1983; Shera and Guinan, 1999; Shera *et al.*, 2000).

The invariance of DPOAE phase across frequency has been attributed to cochlear scaling (Zweig, 1976; Zweig and Shera, 1995). The phase of the DPOAE is referenced to the phases of the stimulus tones. As long as the relative distance between the stimulus tones is maintained on the basilar membrane as they are swept in frequency, their phases do not vary at each characteristic frequency (CF) region; nor does the phase of the ensuing DPOAE. In this way, DPOAE phase serves as a metric of the phase relationship between the primary tones and mirrors the scaling properties along the basilar membrane. If the cochlea is scaled, as long as the distortion component is dominant in the ear canal measure, a fixed relationship between stimulus tones ensures approximately invariant DPOAE phase for most of the frequency range. However, below a low- to mid-frequency boundary, there is a break from scaling as noted by more rapidly accumulating DPOAE phase. The origin of this break in scale invariance at the apex of the cochlea is not clear, although possible sources have been posited (Shera *et al.*, 2000).

B. Cochlear scaling in newborns

A recent study reported immature DPOAE phase in human newborns (Abdala and Dhar, 2010a). Both adults and newborns exhibited near-zero DPOAE phase-gradient delays above 2 kHz and prolonged delays below; however, newborns exhibited more prolonged delays than adults for the two lowest frequency bands (0.5–1 and 1–1.5 kHz), suggesting steeper phase slope in the apical half of the infant cochlea. Although an intriguing finding, the data in the low-frequency bands included reduced numbers due to an inadequate signal-to-noise ratio (SNR). Additionally, the frequency at which the phase pattern transitioned from steep to invariant could not be well delineated because the data were binned into 500-Hz intervals to compensate for reduced observations. This initial finding could have implications for the maturation of cochlear scaling in the newborn cochlea but requires further scrutiny. One of the most daunting tasks in studying the apical half of the newborn cochlea is the implementation of effective OAE paradigms that overcome the intrinsically high noise floor at frequencies below ~ 1.5 kHz.

In this experiment, we implemented a modified DPOAE swept-tone protocol to target the low-frequency apical regions of the infant cochlea while measuring DPOAE phase. This study was conducted to describe the apical-basal transition frequency, to characterize age differences in the break from local cochlear scaling symmetry, and to consider the results relative to maturation of cochlear mechanics overall.

II. METHODS

A. Subjects

Fifteen healthy term newborns and 13 normal-hearing adults participated in this study. Ten male and five female newborns were tested at a mean age of 45.8 h after birth (range = 23–70 h). Data were collected from 12 right and three left ears. Only right ears were tested in seven female and six male adults with a mean age of 30.6 yr (range = 20–42 yr). Newborns passed a hearing screening conducted with a click-evoked auditory brainstem response at 35 dB hearing level (HL). Although some DPOAE experiments with newborns allow for relatively liberal SNR criteria and unfiltered recruitment of subjects, for this targeted low-frequency protocol, only neonates in an optimal, quiet testing state were included. DPOAE data were collected from newborns in the Infant Auditory Research Laboratory on the third floor of the Neonatal Intensive Care Unit, LAC+USC Medical Center, Inpatient Tower. During testing, infants slept comfortably within a sound-attenuating isolette (Eckels Industries, Ontario, Canada) that provided 25 dB of attenuation from 0.5 to 2 kHz and up to 45 dB of attenuation between 2 and 8 kHz.

B. Instrumentation

Signal generation and recording were controlled using custom software run on an Apple MacBook Pro computer via a MOTU (Mark of the Unicorn, Cambridge MA) 828 Mk II audio interface (24 bits/44 100 Hz). Stimulus tones were presented to the subjects' ear canal via ER2 insert transducers as part of an ER10B+ probe microphone assembly. The output of the microphone was pre-amplified and then passed through an analog high-pass filter with 300-Hz cutoff frequency before being digitized by the MOTU and stored on disk. Primary tones were presented at 65 (L1)–55 (L2) dB sound pressure level (SPL) and swept at 8 s/octave using a constant stimulus-frequency ratio of $f_2/f_1 = 1.22$.

DPOAE level and phase estimates were obtained using a least-squares-fit algorithm (LSF) as described by Long and colleagues (2008) and yielded estimates every 2–4 Hz around 0.5–1 kHz and every 12 Hz around 4 kHz. The noise floor was estimated similarly after phase-inverting every alternate sweep window. The phase was “unwrapped” by sequentially subtracting 360° from all points beyond identifiable discontinuities. Additionally, the computed phases of the stimulus tones, $2\phi_1 - \phi_2$, where $\phi_{1,2}$ are phases of the lower and higher frequency stimulus tones, respectively, were subtracted from the estimated phase of the DPOAE.

C. Protocol

1. General DPOAE sweep protocol

An initial screening DP-gram was conducted to ensure present DPOAEs and to select the optimal ear for testing (i.e., generally the ear with highest DPOAE levels). Subjects were tested using a DPOAE swept-tone paradigm whereby data were collected in three separate octave-wide frequency segments: 0.5–1 kHz (octave 1), 1–2 kHz (octave 2), and 2–4 kHz (octave 3). The order of presentation was counter-

balanced. Data were collected in octave-wide segments to optimize SNR in the low-frequency ranges which required the greatest amount of signal averaging. Octave 1 was of primary interest because the objective of this study was to define deviations from scaling in the apical half of the human cochlea. At higher frequencies, where adequate SNR was more easily achieved, it was not necessary to average extensively.

For newborn testing in octave 1, the experimenter began with a default of 20 sweeps. Beyond this baseline, the tester continued to average until he/she assessed SNR to approximate 10 dB by visual online assessment. At that point, the test was paused and DPOAE data were examined. If the SNR was inadequate by the tester's assessment, additional averaging was conducted. In newborns, between 20 and 46 primary tone sweeps were presented to obtain a high-quality DPOAE average in octave 1. This is in marked contrast to the eight-sweep routine typically implemented with newborns. Data in octave 2 were collected with between 12 and 20 sweeps, and eight sweeps were presented for data collection in octave 3. In adults, 14 sweeps were averaged in octave 1 and eight sweeps were averaged in octaves 2 and 3.

2. DPOAE suppression protocol

Five of the 15 newborn subjects were tested with the inclusion of a 55 dB SPL ipsilateral suppressor tone presented simultaneously with the stimulus tones 0.2 octave below $2f_1 - f_2$, which will be referred to as the DP region. A companion paper describes results of a more extensive suppression experiment conducted with a group of 10 normal-hearing adults. In the adult experiment, suppressors were presented to reduce contributions from the reflection source and to test the assumption that ear canal DPOAE phase was dominated by the distortion component from the generator region around f_2 in the typical adult ear. A suppression protocol was implemented here with five of the newborn subjects to similarly ensure that, overall, the dominant source driving the phase-frequency function in newborns was from the generator region.

D. Calibration

Stimulus tones approximately calibrated at the plane of the eardrum were delivered by compensating for the depth of probe insertion in adult subjects (Siegel, 2009). Insertion depth in adult subjects was estimated by normalizing the slow chirp response between 0.2 and 20 kHz to that recorded in a 50 ft long copper tube with an internal diameter of 7.9 mm (approximately that of the adult ear canal). The pressure response recorded for a similar insertion depth in a standard ear simulator (Bruel and Kjaer, Naerum, Denmark, 4157) was used to compensate the driving voltage to the earphones in order to deliver the desired SPL at the plane of the eardrum. For newborns, pilot testing in 20 ears ascertained the $1/2$ wave resonance frequency most typically associated with the newborn ear canal was ~ 9600 Hz. To simplify testing in this population, the corresponding correction filter for this depth insertion was applied to all newborn subjects.

E. Analysis

Since the phase recordings were obtained in three different octave-wide intervals to most effectively enhance SNR, the individual segments were linked together to form a continuous phase-frequency function by aligning segments with a phase shift of ± 1 full cycle if necessary.

1. Component dominance

To determine the dominant component driving ear canal phase, an inverse fast Fourier transform (IFFT) was conducted on DPOAE recordings from each octave-wide frequency segment using MATLAB-based analysis software (NIPR) (Talmadge *et al.*, 1999). DPOAE distortion- and reflection-source components were separated based on their respective group delays. During IFFT, DPOAE complex pressure measured in the frequency domain was multiplied by a moving Hann window in overlapping 50 Hz steps. The length of the Hann window was adjusted on a logarithmic scale in accordance with the cochlear frequency map and ranged from 400 Hz at the lowest DPOAE frequency to 930 Hz at the highest. Impulse response (IPR) functions were determined for each window and rectangular time-domain filters applied to each IPR to extract the desired delay. The filtered windows of data were then transformed back to the frequency domain by fast Fourier transform (FFT) and the level and phase of the distortion and reflection component reconstructed.

2. DPOAE phase with suppressor

In addition to the IFFT analysis described in the previous paragraph, a suppression paradigm was conducted with five infants to determine contribution from the DP region. The slope of DPOAE phase for octave 1 and octave 2 was measured by fitting a linear regression equation to each phase-frequency function recorded without a suppressor and then to the corresponding function recorded with a suppressor tone presented at 55 dB SPL around the DP region. The slope values in no-suppressor and +suppressor conditions were compared to determine whether the reflection component was driving the DPOAE ear canal phase.

3. Spline regression modeling

Spline modeling was used to fit the phase versus frequency functions from 13 individual adults and ten newborn subjects (SPSS ver 18 for OS X). This type of modeling attempts to approximate a curvilinear relationship with a series of linear fits. The spline model looks for junctions in the data set that indicate significant changes; these points are referred to as knots. The frequency of the knot is determined computationally by the best fit to the data and not specified *a priori*. The analysis then calculates the slope of each segment between knots with a linear fit and provides an estimate of the square of the sample correlation coefficient or the coefficient of determination (R^2) to characterize the amount of systematic variation captured by the model. With the objective of defining the frequency at which DPOAE phase

changed from relatively flat to non-zero phase, spline analyses with one- and two-knots were attempted.

Goodness of fit was examined using R^2 and residuals to determine whether the one- or two-knot solution captured maximum variance. Both solutions generated strong fits to the adult and infant data. Mean R^2 values calculated from the individual fits to adult and infant functions were 0.982 and 0.990, respectively, for the one-knot solution and 0.983 and 0.995 for the two-knot solution. Normal Q-Q (quantile-quantile) plots showed conformity of residuals to expected values based on normal distribution for both age groups. Because the two-knot solution did not explain additional variance, the one-knot fit was used to model DPOAE phase-frequency functions. The knot frequency and slope of phase values below and above the knot frequency were extracted from this solution and statistically tested for age differences.

III. RESULTS

A. Signal-to-noise ratio (SNR)

The present experiment targeted the apical half of the neonatal cochlea, thus, extensive signal averaging was implemented to enhance SNR while recording low-frequency DPOAE phase. Establishing excellent SNR was fundamental to the objectives of this experiment because no off-line algorithm was implemented to eliminate noisy data points as is typically conducted with infants (Abdala and Dhar, 2010a,b). This study sought to characterize the trajectory and slope of phase with high precision; therefore, it was important to avoid “gaps” in the phase versus frequency function that require interpolation of data and consequently might contaminate estimates of phase slope.

Although minimum SNR criteria were not employed (for reasons explicated in the preceding paragraph), measured SNR provided high-quality estimates of DPOAE phase in both adults and infants as shown in Fig. 1. This figure displays the individual SNR for each newborn and adult subject in octaves 1, 2, and 3 as well as the mean SNR in red (adult) and blue (newborn) superimposed. Due to minima in DPOAE fine structure, the SNR showed associated, local oscillations; however, overall SNR for octave 1 was 24 dB in newborns [standard deviation (SD)=7.56] and 27 dB in adults (SD=5.4 dB); 88% of all newborn data points in this low-frequency octave exhibited SNR of >10 dB. In octave 2, newborns showed mean SNR of 27 dB (SD=11.9 dB) and adults of 29 dB (SD=7.8 dB). Data from octave 3 showed mean SNR of >30 dB for both infants and adults. These data suggest that the DPOAE phase estimates were minimally influenced by the noise floor and were of sufficient quality to characterize the slope of phase across frequency with adequate resolution.

B. Inverse FFT

Figure 2 shows the mean component magnitude of both distortion- and reflection-source components for each octave-wide segment. Although the mean value necessarily dilutes the variability among individuals and across frequency, the overall robust trend is that the distortion source

FIG. 1. Individual DPOAE SNR in each octave segment for all adult (left column) and newborn (right column) subjects. The mean SNR for each condition is shown in red for adults and blue for newborns.

magnitude is dominant in the group of newborns tested here. (It was previously shown in a companion paper that the same is true in adults.) This suggests that in the unsuppressed condition, the distortion source determined the phase of the ear canal DPOAE.

C. Suppressor tone

An assumption was made that if ear canal phase was determined by the distortion emission in the unsuppressed (i.e., typical) condition, then including a suppressor around the DP region to further reduce reflection contribution would not alter DPOAE phase slope. Results show that an ipsilateral suppressor tone presented near the DP region simultaneously with the primary tones did not alter the slope of

FIG. 2. IFFT-derived DPOAE component magnitude for the distortion- and reflection-source components in three, octave-wide intervals: 0.5–1 kHz, 1–2 kHz, and 2–4 kHz. The black lines display distortion-component levels and the gray lines, reflection. Dashed lines represent ± 1 SD.

DPOAE phase in five newborn subjects. The suppressor did act to smooth local oscillations (i.e., the “saw-tooth” pattern) in the phase-frequency functions, confirming its effectiveness in reducing contributions from the reflection component. Figure 3 includes representative phase data from two newborns, with and without a suppressor tone near the DP region for octaves 1 and 2. Table I provides the mean phase slope (cyc/kHz) for the five subjects and both frequency segments. Results suggest that DPOAE phase measured in the newborn ear canal with moderate-level stimulus tones is dominated by the distortion source generator region near f_2 , even in the low-frequency segment of the phase-frequency function where more rapid phase accumulation is observed. A companion paper reported the same finding in normal-hearing adults, confirming that the steepened phase slope below 1.5 kHz cannot be explained by contamination from the reflection component from the DP region.

It is noteworthy that a phase advance of ~ 0.1 cycles was produced in several of the subjects when a suppressor tone was added (see phase-frequency functions from two such subjects displayed in Fig. 3). This shift was not present in all subjects and, when present, not always evident across the entire frequency range. When considering a separate group of adults tested with a similar suppression paradigm, variability was also noted with respect to presence of this phase lead. The source of this effect is not clear. It is not likely an artifact of the LSF analysis method applied to estimate DPOAE phase/level because the suppressor tone (presented 0.2 octave $< 2f_1 - f_2$) was well outside of the LSF analysis window of 500 ms. It is possible that the suppressor tone (55 dB SPL) impacted primary tone level and, in doing so, altered level separation between tones somewhat; however, a careful examination of DPOAE amplitude in octaves

TABLE I. Slope of DPOAE phase (cyc/kHz) in five individual newborns for octave 1 (0.5–1 kHz) and octave 2 (1–2 kHz) with suppressor around the DP region (+S) and without suppressor (NoS).

Newborn	Octave 1 + S	Octave 1 – NoS	Octave 2 + S	Octave 2 – NoS
168	–2.22	–2.82	—	—
169	–3.31	–3.06	—	—
170	–1.93	–1.91	–1.13	–1.15
171	–2.80	–2.73	–1.19	–1.60
172	–1.89	–1.78	–1.60	–1.56
Mean	–2.45	–2.46	–1.31	–1.44

1 and 2 showed little change in DPOAE level when the suppressor was presented. Thus, this slight phase lead is not likely to be an artifact of methodology but may reflect a physiological response to the suppressor tone, although it is not well understood at present.

D. DPOAE phase-frequency functions

Figure 4 shows phase versus frequency functions for ten newborns and 13 adults. As noted, there were neither abrupt discontinuities indicating substantial component-interference nor excessive noise that might have adversely affected estimates of phase slope. The functions did show small, local oscillations in some subjects consistent with contribution from the reflection source. To estimate both the frequency at which DPOAE phase transitioned from approximately invariant to relatively steep and at the slope of phase (cyc/kHz) for each frequency segment, a constrained nonlinear regression spline model with one knot was applied to each subject’s data (see Sec. II). The spline model estimated the knot frequency at the intercept of the two linear segments and

FIG. 3. DPOAE phase in octave 1 (0.5–1 kHz) and octave 2 (1–2 kHz) is shown for two newborn subjects (out of five) tested with a suppressor tone. The gray line shows phase recorded with an ipsilateral suppressor tone presented 0.1 octave below f_{dp} . The black line shows unsuppressed DPOAE phase.

FIG. 4. DPOAE phase versus frequency functions for 13 adult (gray) and ten newborn (black) subjects.

calculated slope of phase for both the low- and high-frequency lines. To illustrate the variability among functions and the accuracy of the spline models in fitting these functions, Figs. 5 and 6 include the raw phase data and regression fits for all 23 subjects in the age comparison.

1. Knot frequency

Table II provides the mean knot frequencies and low- and high-frequency phase slope derived from the spline modeling for each group. There was no effect of age on knot frequency which was centered around 1.4 kHz for both adults and newborns applying the one-knot model. The phase-frequency functions sometimes had an intermediate segment which was more notably present in adult data. The two-knot solution appeared to model these functions better and the corresponding knot and slope values are also presented in Table II. With the two-knot solution, the mean apical-basal transition frequency defined by the lower-frequency knot was ~ 1 kHz in adults and ~ 1.2 kHz in newborns. The differential presence of an intermediate segment in adults and newborns may reveal an age effect not adequately captured with statistical analysis. This is further considered in Sec. IV.

2. Slope of phase

There was an effect of age on slope of phase ($F = 4.70$; $p = 0.021$). When low- and high-frequency segments were analyzed independently, newborns had a significantly steeper slope of phase than adults only for the low-frequency segment from 0.5 to 1.4 kHz ($F = 8.32$; $p = 0.009$). The slope of phase (cyc/kHz) for octave 1 was -1.76 ($SD = 0.3$) in adults and -2.53 ($SD = 0.9$) in newborns. As noted by the SDs and confidence intervals, estimates of phase slope are substantially more variable in newborns compared to adults in the low-frequency segment of the phase-frequency function.

The slope of the invariant high-frequency segment was similar between age groups. Figure 7 displays the adult and newborn mean, SD and individual values of knot frequency, and phase slope for the low-frequency segment. The corresponding phase-gradient delay estimates (1.76 and 2.53 ms) in adults and newborns, respectively, are similar to the mean delays reported in an earlier study with independent groups of adult and newborn subjects (1.74 versus 2.35 ms; Abdala and Dhar, 2010a).

To summarize the results, the knot or bend in the phase-frequency function was similar in adults and infants and centered near 1.4 kHz if modeled with two segments and closer to 1 kHz if modeled with three. Anecdotal observations suggest a difference in the configuration of newborn and adult functions with a more prominent intermediate segment noted in adult data. Slope of phase was steeper in newborns compared to adults only for the low-frequency segment below the bend in the phase-frequency function.

IV. DISCUSSION

This report describes DPOAE phase recorded using a modified data collection protocol with a targeted low-frequency emphasis in adults and newborns. The enhanced SNR achieved in the frequency region between 0.5 and 1 kHz allowed for precise characterization of DPOAE phase-frequency function in the apical half of the human cochlea.

TABLE II. Mean knot frequency and DPOAE slope of phase (cyc/kHz) for each segment of the phase-frequency functions measured in 13 adult (A) and ten newborn (NB) subjects. Values for both the one-knot and two-knot solutions modeled with a spline analysis are shown. The SDs and 95th % confidence intervals are also provided. LF, low-frequency; MF, intermediate frequency; HF, high frequency.

One-knot	Age	Knot frequency (Hz)		LF phase slope	HF phase slope	
Mean	A	1415	—	-1.76	-0.18	—
SD	A	165.6	—	0.29	0.16	—
CI(95%)	A	86	—	0.16	0.09	—
Mean	NB	1439	—	-2.53	-0.15	—
s.d.	NB	251	—	0.90	0.13	—
CI(95%)	NB	156	—	0.56	0.08	—
Two-knot		Knot 1 frequency (Hz)	Knot 2 frequency (Hz)	LF phase slope	MF phase slope	HF phase slope
Mean	A	1046	1765	-2.09	-0.95	-0.14
SD	A	186.6	295	0.44	0.40	0.21
CI(95%)	A	101.5	160	0.24	0.22	0.11
Mean	NB	1189	1912	-2.56	-0.91	-0.08
s.d.	NB	223	298	1.04	0.51	0.13
CI(95%)	NB	138	185	0.64	0.32	0.08

FIG. 5. The DPOAE phase versus frequency functions for 13 adult subjects are shown in gray and the one-knot spline fit to model the data is superimposed as a black line.

Prior to characterizing the salient features of the phase-frequency functions, initial measurements were undertaken in a small group of newborns to determine that the ear canal phase was dominated by the distortion component and not the reflection component from the DP region. Although this had been confirmed in adults, it was important to establish component dominance in newborns because the relative contribution from the reflection source appears to be more prominent in this population (Abdala and Dhar, 2010a).

The results shown in Figs. 2 and 3 as well as Table I suggest that the distortion source was driving ear canal DPOAE phase in newborns, and hence, determining the trajectory of phase-frequency function described in this experiment. One

caveat to consider is the effect of suppression in the apical region of the cochlea. If the apex is, in fact, more weakly nonlinear or demonstrates a different form of nonlinearity than the basal cochlea, the effectiveness of a suppressor tone in reducing contributions from DP region for frequencies <1 kHz is not certain (cf. Kalluri and Shera, 2001; Shera *et al.*, 2008). This caveat notwithstanding the smoothing of local level oscillations (i.e., saw-tooth pattern) by the suppressor tone suggests the paradigm was effective in reducing contributions from the reflection source.

Phase versus frequency functions indicate an unambiguous change in DPOAE phase trajectory approximately mid-way along the human cochlear map (~ 1.4 kHz) in both

FIG. 6. The DPOAE phase versus frequency functions for ten newborn subjects is shown in gray and the one-knot spline fit applied to model the data is superimposed as a black line.

newborns and adults. When applying a model that specifies three segments in the function, the initial transition frequency is closer to 1 kHz. At frequencies below the bend in this function, DPOAE phase accumulates relatively rapidly (~ 2 cyc/oct), with a significantly steeper phase slope exhibited by the newborn group. Because frequency-scaled primary tones were presented, this steeply rotating portion of the phase-frequency function exemplifies a break from cochlear scaling.

Although the portion of the phase-frequency function below 1.4 kHz is clearly different in phase slope from the portion above, it was not zero as one would expect for a per-

FIG. 7. The upper panel shows the mean slope of phase (cyc/kHz) modeled by the one-knot spline analysis for the low-frequency segment of the phase-frequency function, below the estimated knot frequency. Individual phase slopes for each subject are also shown (small gray x). The lower panel displays the mean knot frequency modeled by a one-knot spline analysis as well as the individual knot values (small gray circles).

fectly scaled cochlea. Considering delays imposed by the middle ear may help understand this result. [Puria \(2003\)](#) provided a mean estimate of the human middle ear round-trip delay of 0.2 ms for the mid- to high-frequencies, very close to the observed delays above 1.4 kHz in the present work. If the middle ear can account for this phase slope in the flattened portion of the function, cochlear scaling is perhaps better than the measured slope here suggests and close to zero once the middle ear delay is subtracted.

A. Apical-basal transition frequency

In this report, we did not characterize the episodic breaking from scale invariance across small intervals as noted in phase fine structure, most often corresponding to component-interference as described earlier by [Shera and colleagues \(2000\)](#). Neither were we focused on describing discontinuities reflecting whole integers of a cycle that also correspond to component mixing or measurement ambiguities. The moderate stimulus level applied here produced minimal interference from reflection source emissions, and thus, it is unlikely that component mixing contributed greatly to the characterization of the phase gradient. Our objective in this paper was to describe the secular breaking of OAE phase previously reported as occurring around 1 kHz in humans and 3–4 kHz in guinea pig and chinchilla ([Shera et al., 2000; 2010; Shera and](#)

Guinan, 2003). This break is hypothesized to have functional correlates and has been speculatively equated with a transition from basal to apical micromechanical motion in the cochlea.

Evidence has accumulated suggesting that the apex behaves differently than the basal cochlea in significant ways. Cooper and Rhode (1995) reported weaker nonlinearities in the guinea pig apical cochlea. The observed nonlinearity was not focused at CF and thus was not effective at modifying auditory sensitivity or frequency tuning in the same manner observed at basal and mid-basal regions of the cochlea. Nowotny and Gummer (2006) reported counter-phasic motion of sensory hair cells relative to tectorial membrane motion in the apical half of the chinchilla cochlea only. This action is thought to compress and extend the sub-tectorial space and possibly provide a means of direct fluid coupling between OHCs and inner hair cells in the apex. At the neural level, in chinchilla, there may be a more gradual transition in the morphology of auditory nerve fiber tuning curves, occurring first in the low frequencies around 1 kHz with a second more abrupt change between 3 and 4 kHz (Temchin *et al.*, 2008). Changes in cochlear gain and wave propagation estimated by neural data in chinchilla also show a demarcation around 4 kHz (Shera, 2007). Clearly, although traditional traveling wave motion as described by Bekesy half a century earlier may define the dominant motion of the basilar membrane in the base and mid-basal region, alternative modalities of motion may more effectively describe apical cochlear mechanics (Karavitsaki and Mountain, 2007a,b; Guinan *et al.*, 2005).

B. Cochlear scaling symmetry

1. DPOAE phase

The breakdown of cochlear scaling appears to characterize mammalian apical function. DPOAE phase across frequency elucidates cochlear scaling symmetry and its breaking because it is referenced to the phase relationship between the stimulus tones, which is invariant over most of the frequency range. As reviewed in Sec. I, the breaking of this DPOAE phase invariance suggests that the two primary tones no longer sustain a fixed relationship in the apex and the exponential relationship between place and frequency along the basilar membrane has been disrupted.

Because the DPOAE is a mixed-type OAE, derived from at least two distinct mechanisms, it was initially plausible to consider that contribution from the long-latency, reflection source from the DP region might influence the rapid accumulation of phase in the apex. The suppression portion of the present experiment and the IFFT analysis addressed this possibility by ensuring that ear canal DPOAE phase was dominated by the distortion source generated near f_2 . The objective of suppression was not to eliminate the contribution from the reflection component altogether, but to at least establish that ear canal phase would be dominated by wave-fixed emissions from the generator region near the overlap of f_1 and f_2 . With a suppressor near the DP region, the DPOAE phase gradient remained equally steep in the low frequencies suggesting the noted “break” was unrelated to shifts in component contribution.

2. DPOAE and SFOAE: Same or different?

In humans, both DPOAE phase-frequency functions and stimulus frequency (SF) OAE measures of phase-gradient delay show a discontinuity somewhere around ~ 1 – 1.5 kHz that has been associated with a break in cochlear scaling (see Fig. 1 in Shera and Guinan, 2003 for SFOAE); however, the correspondence between these two OAE phase measures is unclear. In chinchilla, SFOAEs, generally considered long-latency, place-fixed emissions, appear to include a short-latency component that becomes more prominent in the apical half of the cochlea where the break in scaling is observed (Shera *et al.*, 2008, 2010). It is not known whether a similar short-latency SFOAE component is present in humans.

An intriguing incongruity between DPOAE and SFOAE measures of scaling is noted in the *direction* of phase change signaling the break. DPOAE phase shifts from near-zero across the mid- and high-frequency range to more steeply sloping at low-frequencies. In contrast, the SFOAE-based phase discontinuity exemplifies the apical-basal transition with a shift from relatively steep phase slope for most of the frequency range to a more shallow phase slope at low frequencies. Can discontinuities in phase behavior that show opposite trends, reflect the same underlying feature of cochlear mechanics, and is this common feature responsible for the disruption of scaling in the apical half of the mammalian cochlea? Clearly, in the cochlear apex, the tenets and assumptions providing the basis for traditional theories of OAE generation seem to be violated, apparently blurring the distinction among OAE types. A detailed comparison of different OAE-types and the break in cochlear scaling they elucidate, is warranted.

C. Developmental differences in cochlear scaling

The apical-basal transition frequency appears to be adult-like in newborns when the phase-frequency function is measured as two segments. However, anecdotal observation suggests that a more gradual intermediate segment is variably present in newborns and more regularly observed in adult functions. If this intermediate segment is considered and the phase-frequency function is defined with three segments (two knots: one at the initial low-frequency bend and another between intermediate and flat segments), the apical-basal transition frequency shows a mean difference between newborns and adults of ~ 200 Hz, still well within the normal variability established by the 95th percentile confidence intervals.

It is of interest to note that adding a third segment to model the phase-frequency function, steepens the mean low-frequency slope estimate for adults, presumably by eliminating the shallow, intermediate segment between 1 and 1.4 kHz. However, the mean low-frequency slope of phase estimate for newborns is unchanged if the fit encompasses the range between 0.5 and 1.4 kHz (one knot) or between 0.5 and 1.2 kHz (two knot). This suggests the absence of a distinct, intermediate segment in newborns and indicates that any movement toward shallow phase-slope is initiated beyond 1.4 kHz in this age group.

As noted in Table II, there was increased scatter of knot frequency and phase slope estimates in newborns compared to adults. This variability is not likely to reflect a noisier newborn data set; but rather, variability intrinsic to the dynamic process of maturation. Figure 1 confirms that phase-frequency functions from newborns were not characterized by noisiness or irregularities and showed a systematic phase trajectory. The mean newborn SNR in octave 2 (where the bend in the function has been defined) was 27 dB, clearly sufficient for high-quality DPOAE measurement. Thus, the increased inter-subject variability in newborns is likely to reflect somewhat idiosyncratic rates of maturation for peripheral auditory system function in humans around the time of birth.

The most marked difference between adults and newborns was observed in the slope of phase on the low-frequency side of the apical-basal transition frequency; that is, the rate at which cochlear scaling breaks. Newborns manifest a steeper slope of phase than adults in the apical region from 0.5 to 1.4 kHz. The steeper neonatal phase gradient corresponds to a mean phase-gradient delay prolonged by 0.8 ms in newborns. This is similar to the age difference reported in an earlier, less detailed study of cochlear maturation (Abdala and Dhar, 2010a). It is not clear what the more rapid phase accumulation in the apical half of the newborn cochlea indicates. A conventional interpretation would suggest a more marked deviation from the exponential relationship between place and frequency in the newborn cochlea. Although speculative, this would suggest that apical basilar membrane motion is not yet fully mature at birth.

There is a dearth of information about the physical properties, the mass and stiffness gradient, of the human basilar membrane around the time of birth. Even more notably lacking is information about the apical cochlea, although it has been reported that this region shows developmental peculiarities relative to the base. Apical OHCs show less regimented organization, sparse efferent innervation, supernumerary cells around the time of birth (Bredberg, 1968; Pujol *et al.*, 1998), and apical hair cells exit the cell cycle earlier than basal hair cells even though they differentiate later than their basal counterparts (Matei *et al.*, 2005). If immaturities in cochlear mechanics are, in fact, present in the strongly precocial human auditory periphery, the later developing apex seems to be the logical site for these immaturities.

D. The immature middle ear

It has been estimated that immaturities in middle ear transmission attenuate sound through the newborn middle ear by approximately 15 dB in the high-frequencies (Abdala and Keefe, 2006; Keefe and Abdala, 2007). A comparable measure of middle ear transmission in neonatal ears for low-frequency signals is not available. In cases where calibration is conducted in an adult ear coupler, much of this inefficiency in middle ear transmission is counteracted by the functional “boost” in SPL that reduced neonatal ear canal area provides when sound is presented via insert phone (Keefe and Abdala, 2007). However, in the current experiment, the ear canal effect was ameliorated by the depth-compensated calibration protocol (see Sec. II). A custom equalization filter was applied to newborn ears to compensate the driving voltage to the earphones in

order to deliver the desired SPL at the plane of the tympanic membrane. Thus, the primary conductive immaturity potentially impacting the phase results presented here is the inefficient forward transmission through the immature middle ear.

If reduced levels are driving the newborn cochlea due to middle ear inefficiencies, one could argue that it might explain a steeper phase slope. SFOAE phase, for example, is dependent on stimulus level and latencies increase as level decreases (Schairer *et al.*, 2006). However, studies exploring the level dependence of DPOAE phase have found the distortion component is not significantly affected by level (Abdala *et al.*, 2011; Konrad-Martin and Keefe, 2005). Recall that ear canal DPOAE phase is driven by the phase of the dominant component, determined here to be the distortion-source component; thus, attenuated levels driving the newborn cochlea cannot easily explain the excessive steepness of phase noted in newborns in the apical half of the cochlea.

Another possibility is that a frequency-dependent delay through the infant middle ear is non-adult-like in newborns thereby producing DPOAE phase irregularities. However, the mean newborn phase-gradient delay is 2.5 ms (nearly 1 ms longer than the adult delay); whereas phase values extrapolated from human middle ear cadaver data (Puria, 2003) suggest a round-trip delay of approximately 0.46 for frequencies below 1.4 kHz and 0.2 ms at higher frequencies. Certainly, a more exhaustive exploration of the level dependence of DPOAE phase may be warranted to further scrutinize the impact of inefficient transmission through the neonatal middle ear on measures of DPOAE phase.

V. CONCLUSIONS

The mammalian cochlea exhibits a discontinuity in OAE phase behavior at a putative juncture between its apical and basal halves. This break has been assayed using both DPOAEs and SFOAEs although the relationship between these two metrics of scaling is unclear at present. Suppression experiments conducted here suggest that the break in DPOAE phase invariance is not produced by the reflection component or by shifts in component contribution.

Human newborns show a break from cochlear scaling symmetry at frequencies below 1.4 kHz, as do adults, although the configuration of newborn phase-frequency functions shows some non-adult-like features. Additionally, newborns present a significantly steeper phase slope than adults below the apical-basal transition frequency, resulting in a phase-gradient delay prolonged by a mean of 0.8 ms (relative to adult measures). This indicates a more marked break in scaling, possibly suggesting immaturity in the apical half of the cochlea at birth. The age difference cannot be easily explained by a simple middle ear attenuation of primary tones. The impact of immature middle ear transmission in newborns will need to be further explored to more firmly associate this immaturity in low-frequency DPOAE phase with cochlear scaling.

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