Research Article

Distortion-Product Otoacoustic Emission Measured Below 300 Hz in Normal-Hearing Human Subjects

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ABSTRACT

Physiological noise levels in the human ear canal often exceed naturally low levels of otoacoustic emissions (OAEs) near the threshold of hearing. Low-frequency noise, and electronic filtering to cope with it, has effectively limited the study of OAE to frequencies above about 500 Hz. Presently, a custom-built low-frequency acoustic probe was put to use in 21 normal-hearing human subjects (of 34 recruited). Distortion-product otoacoustic emission (DPOAE) was measured in the enclosed ear canal volume as the response to two simultaneously presented tones with frequencies f_1 and f_2 . The stimulus–frequency ratio f_2/f_1 was varied systematically to find the “optimal” ratio evoking the largest level at $2 f_1 - f_2$ frequencies 87.9, 176, and 264 Hz. No reference data exist in this frequency region. Results show that DPOAE exists down to at least 87.9 Hz, maintaining the bell-shaped dependence on the f_2/f_1 ratio known from higher frequencies. Toward low frequencies, however, the bell broadens and the optimal ratio increases proportionally to the bandwidth of an auditory filter as defined by the equivalent rectangular bandwidth. The DPOAE phase rotates monotonously as a function of the stimulus ratio, and its slope trend supports the notion of a lack of scaling symmetry in the apex of the cochlea.

Results were preliminarily presented at the Audio Engineering Society 58th International Conference: Music Induced Hearing Disorders, June 2015 and at the International Symposium on Auditory and Audiological Research, August 2015.

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INTRODUCTION

Human ears do not just receive acoustic signals; they also emit sound at sound pressure levels near the threshold of hearing spontaneously and in response to sound (Kemp 1978). Otoacoustic emission (OAE) can be recorded in the normal ears with a sensitive microphone coupled into the ear canal. It arises within an active cochlear mechanism that amplifies and sharpens waves traveling on the basilar membrane and shapes the excitation of the auditory nerve fibers. OAE is thus a valuable, yet not fully understood, source of information about how the ear works in individuals and in general.

Distortion-product otoacoustic emissions (DPOAEs) are the ear canal acoustic pressure responses at distortion frequencies of two simultaneously presented tones with frequencies f_1 and f_2 ($f_1 < f_2$) (Kemp 1979). The wave traveling on the basilar membrane as a result of the f_1 stimulus tone travels further towards the apex than the wave traveling as a result of the f_2 stimulus tone. Up until after the characteristic place of the f_2 wave, the basilar membrane and coupled constituents of the organ of Corti, such as the outer hair cells, are stimulated by two frequencies simultaneously. DPOAE is fundamentally evidence that two tones presented simultaneously are not processed by these structures as if they were presented separately. DPOAEs are thus said to be generated by nonlinearity, primarily near the f_2 place on the basilar membrane where interaction of the f_2 wave with the f_1 wave is maximum (Kim 1980). There is also

evidence suggesting significant contributions from the region basal to the f_2 place (Harris 1990; Martin et al. 2003, 2013). Generated distortion components interact with the characteristic places of their frequencies. The 2 f_1 - f_2 DPOAE in particular has been studied extensively because of its dominating prevalence in humans (Lonsbury-Martin and Martin 2007), and the consensual theory is that it propagates from its f_2 -related region of generation to its characteristic place, apical to the characteristic place of both f_1 and f_2 , where it undergoes partial reflection (Zweig and Shera 1995). The DPOAE measured in the ear canal is believed to be the sum of a contribution propagating directly from the f_2 -related region of nonlinear generation and another contribution propagating from the 2 f_1 - f_2 region after linear reflection (Stover et al. 1996b; Mauermann et al. 1999; Shera and Guinan 1999; Talmadge et al. 1999).

The DPOAE component at the 2 f_1 - f_2 frequency is the subject of the present study. This component is systematically dependent on the levels and frequencies of the stimulus tones. The standard measurement uses L_1 and L_2 levels of 65 and 55 dB SPL (sound pressure level) for the f_1 and f_2 tone, respectively, as these levels have been shown to separate clinically normal from impaired ears well (Stover et al. 1996a; Lonsbury-Martin and Martin 2007). The DPOAE level is also systematically dependent on the frequency separation between f_1 and f_2 , typically specified as the ratio f_2/f_1 . Studies of this dependence show that it is bell-shaped with a 3-dB bandwidth of about 0.1 f_2/f_1 . They define the “optimal” stimulus ratio as that which, for a given reference frequency, f_1 , f_2 , or the DPOAE frequency, evokes the largest DPOAE level. The standard measurement keeps the ratio fixed between 1.20 and 1.25 (typically at 1.22) as the reference frequency is swept.

DPOAEs have been studied extensively at f_2 frequencies above 1000 Hz in humans. Measurements in the range from 500 to 1000 Hz are sometimes included, but only as a small part of wider band measurements with the ratio fixed near 1.22. In an early study, Probst and Hauser (1990) followed the recommendation by Harris et al. (1989) and measured DPOAEs at geometric-mean frequencies from 500 to 8000 Hz with stimulus-frequency ratios that decreased from 1.35 to 1.15, respectively. The resulting lowest 2 f_1 - f_2 frequency at 280 Hz is perhaps the lowest seen in the literature, aside from a few examples in individuals, e.g., by Martin et al. (2009). No effort was made to equate the noise floor across frequency and the low-frequency results discouraged further study. Similar conclusions were drawn in later, relatively large-scale studies of the clinical relevance of DPOAEs at f_2 frequencies between 500 and 8000 Hz (Gorga et al. 1993), where the ratio had been fixed at 1.2 (2 f_1 - f_2 between 333 and 5333 Hz). Efforts to

equate the noise floor across frequency by extending the time spent measuring, varying the high-pass cutoff frequency with the f_2 frequency, and changing the way of calculating the noise floor were only partially successful (Gorga et al. 1994; Stover et al. 1996a). Traditional measurement methods rely on certain assumptions about the nature of the noise which increases steeply toward low frequencies. The problem of low-frequency noise specifically has been solved effectively by high-pass filtering the output of the microphone. Kemp (1978) set the cutoff frequency at 400 Hz and, more broadly, one finds both fixed and variable with f_2 cutoffs between 250 and 750 Hz in the literature. High-pass filtering in both commercial and custom probe systems has, even in the absence of lacking motivation, inhibited the systematic study of OAEs at low frequencies. It can be stated fairly confidently that no measurements of DPOAE in humans have been reported for 2 f_1 - f_2 frequencies below 300 Hz.

There are however certain, reported trends of the DPOAE toward the lowest frequencies studied. The range of response levels evoked by a wide range of stimulus levels is smaller for f_2 at 500 Hz than at 4000 Hz when the stimulus ratio is fixed at 1.22 (Gorga et al. 2007). The fixed ratio DPOAE is also more broadly tuned at the low frequency (Gorga et al. 2008). Perhaps as a result of that broader tuning, the basal skirt of the f_2 wave on the basilar membrane appears to play a different, if not more significant, role in the generation of low-frequency DPOAE compared to that of higher-frequency DPOAE (Martin et al. 2003). Further indication of basal sources of DPOAE have been found in their phase responses which typically have a rather abrupt change in the slope in the octave between 1000 and 2000 Hz (Shera et al. 2000; Abdala et al. 2011). Suppressing the contribution from reflection at the 2 f_1 - f_2 place on the basilar membrane does not alter the change in the phase slope (Dhar et al. 2011). Applying the same suppression strategy one-third octave basal to the f_2 place, however, does alter the change in the phase slope (Martin et al. 2010).

Aside from the observations that DPOAEs are broader tuned, supposedly with larger contributions from the regions of the basilar membrane significantly basal to the f_2 place of maximum vibration, fundamentally different mechanisms at play in the apical cochlea associated with low-frequency hearing have been suggested (Nowotny and Gummer 2006; Reichenbach and Hudspeth 2010; Shera et al. 2013). Models of low-frequency hearing cannot easily be tested because physiological data by surgery into the apex of the live cochlea are hardly accessible without compromising its delicate anatomical structures (Cooper and Rhode 1997; Dong and Cooper 2006).

In general, the extent to which models derived from data related to high-frequency hearing apply to low-frequency hearing is not known.

With suitably designed instrumentation, that is, a probe system and software designed for measurements at low frequencies, the purpose of the present work was to extend the systematic study of DPOAE dependence on the stimulus ratio f_2/f_1 to lower frequencies, particularly to 2 f_1 - f_2 frequencies 87.9, 176, and 264 Hz. Although the dependence on stimulus levels and the influence of the middle ear remain to be tested, this first very low-frequency study might provide a basis for DPOAE to noninvasively probe the active mechanisms of the apical cochlea associated with low-frequency hearing in humans and other animals.

METHODS

Subjects

Thirty-four human subjects were recruited mainly from the campus of Aalborg University on the criteria that they were between 18 and 30 years of age and did not have a cold or similar. No subjects were excluded on the basis of an initial questionnaire about their hearing history, music- and noise-exposure history, and listening habits in the everyday. Seven subjects were excluded because the eardrum could not be seen for ear wax deep in the ear canal. Three subjects were excluded because they had one or more hearing thresholds above 20 dB hearing level (HL) as measured by pure-tone audiometry in octave steps from 125 to 8000 Hz and half-octave steps where neighboring octave thresholds were more than 5 dB apart. Even though low frequencies are the focus of the present study, normal high-frequency thresholds were required as they may affect lower-frequency emissions (Arnold et al. 1999). No subject with normal thresholds had abnormal tympanometry. In three subjects, the custom-built acoustic probe used for the DPOAE measurements could not be fitted properly. These criteria left 21 audiometrically normal-hearing subjects in the DPOAE part of the experiment. One female subject included also participated in our previous study (Christensen et al. 2015a).

Experimental Protocol

The subject was seated in a reclining chair and given time to calm down and breathe slowly. During the experiment, he or she was asked to breathe mainly through the mouth as it was found in pilot experiments that nose breathing resulted in slightly higher noise in the frequency band where measurements were made. The subject was instructed to sit as quietly as possible but allowed to read on a tablet computer, and every 10–

15 min, the subject had a break to clear the throat, sip water, or similar. It took approximately 10 min to fit the probe and wait for its yellow foam tip to expand and settle to give a good seal of the ear canal. The seal was deemed stable once the stimulus tones reached a stable level between 60 and 65 dB SPL for the f_1 stimulus tone and between 50 and 55 dB SPL for the f_2 stimulus tone. The DPOAE was measured within about 40 min as a function of the f_2/f_1 ratio with 2 f_1 - f_2 fixed at 87.9, 176, and 264 Hz for ratios below 1.5 and with f_2 - f_1 fixed at the same frequencies for ratios above 1.5. The f_2 - f_1 measurements were included to explore the coinciding 2 f_1 - f_2 and f_2 - f_1 frequencies when $f_2 = f_1$ is 1.5. They are however not discussed further in this paper. All measurements were monitored for noise on a periodically updating fast Fourier transform (FFT) plot of the recorded signal, and if the subject was generally quiet with DPOAEs above the noise floor at 87.9 or 176 Hz or both, another 40 min of measurements were carried out. These explored further the interaction of 2 f_1 - f_2 and f_2 - f_1 in the frequency range where 1.5 was expected to be the optimal ratio from about 70 to 180 Hz. Measurements were carried out in this frequency range with ratios fixed at 1.4, 1.5, and 1.6, but a report of those measurements are outside the scope of this paper. All subjects signed a consent form upon initial instructions and were compensated monetarily on an hourly basis for their participation. The experiment was set up in the audiometry room (ISO:8253-1 2010) at the acoustics laboratory of Aalborg University in Denmark.

Instrumentation

A custom acoustic probe was designed and built with higher signal-to-noise ratio and less harmonic distortion than commercial alternatives below 500 Hz. For instance, whereas the microphone sensitivity of a probe system like the ER10C from Etymotic Research Inc. (Elk Grove Village, IL) rolls off below about 100 Hz, the sensitivity of the custom probe does not roll off significantly until about 10 Hz. The mechanoacoustic details were described by Christensen et al. (2015c) and the performance of the probe is summarized in Figures 1 and 2.

Briefly, the probe has two dynamic Kingstate KDM13008L-02 (Kingstate Electronics Corporation, Suzhou, China) loudspeakers with 12 mm membranes coupled into the ear via a narrow stainless steel tube. Two Knowles NR-23158-000 (Knowles Corporation, Itasca, IL) electret pressure-gradient microphones connected in parallel and coupled into the ear via another narrow stainless steel tube records the response. The transducers have separate preamplifier circuits to minimize crosstalk between the channels. The loudspeaker preamplifiers have -10 dB gain and appropriately low-output impedances to cope with the

FIG. 1. Acoustic probe for low-frequency measurements in its final stage of assembly and in a subject during testing.

low-impedance loudspeakers. Microphone preamplifier has +40 dB gain; high-input impedance, two analog second-order Butterworth high-pass filters with cutoff 30 Hz; and a passive resistor-capacitor high-pass filter at 12 Hz. A Roland (Roland Corporation, Hamamatsu, Japan) UA-55 Quad-Capture 24-bit USB soundcard provides the interface to a laptop running Windows 7 (Microsoft Corporation, Redmond, WA) and custom measurement software written in MATLAB (Mathworks, Inc., Natick, MA) with the Playrec audio input/output library (Humphrey 2007). The software interface allows the experimenter to monitor the measurements and pause in case of brief excessive noise. A Madsens Orbiter 922 clinical audiometer (GN Otometrics A/S, Taastrup, Denmark) with Sennheiser HDA 200 headphones (Sennheiser electronic GmbH & Co. KG, Wedemark, Germany) and an Interacoustics tympanometer AT235 (Interacoustics A/S, Middelfart, Denmark) were used in the initial screening of the subjects.

Stimulus Parameters

Stimulus levels were calibrated and equalized across frequency in a Brüel & Kjær (B&K) 4157 (Sound and Vibration Measurement A/S, Nærum, Denmark) ear simulator (IEC:60318-4 2010). The calibration was within 1 dB when checked for every five subjects. The microphone transfer function was measured in a

FIG. 2. Transducer distortion and noise of the ER10C probe system (black curves) and the custom low-frequency probe (gray curves) during constant pressure stimulation with 65 and 55 dB SPL tones through the probe loudspeakers into a B&K 4157 ear simulator. The recordings are made with **a** the ear simulator microphone and **b** the probe microphone. The distortion curves shown are comprised of several significant components, namely $f_2 - f_1$, $2 f_1 - f_2$, $2 f_1$, $2 f_2$, $3 f_1$, and $3 f_2$, where f_1 and f_2 are the frequencies of the high- and low-level stimulus tone, respectively. The ER10C distortion is mostly third harmonic from the loudspeakers while the low-frequency probe has mostly second harmonics. Below 300 Hz both systems have the $2 f_1 - f_2$ distortion component at -20 dB SPL or below. The noise in the two systems is similar in quiet, but the low-frequency probe appears from experience to be more prone to noise from, e.g., subject movement.

custom cavity with reference to a B&K 4182 probe microphone. The transfer function of the probe microphone itself was measured in a short tube with reference to a B&K 4134 1/8" microphone. It is noted that at the low frequencies tested in this experiment, the B&K ear simulator microphone and the microphone in the custom-built acoustic probe measure the same sound pressure because the wavelengths are significantly larger than the largest dimensions of the enclosed volume. No in situ calibration was carried out. This made sure that the transducers were driven by the same voltage and low amount of transducer distortion in all subjects at the cost of a 5-dB variation in the stimulus level across subjects. The resulting level of the low-frequency stimulus tone measured at the probe position in the ear canal was between 60 and 65 dB SPL, while the level of the high-frequency stimulus tone was between 50 and 55 dB SPL. Time available in the experiment did not allow for further exploration of level effects. The evoked DPOAE level generally increases with the stimulus level, but effects on the optimal ratio are subtle Christensen et al. (2015a). Standard levels

were selected for the present experiment to see if they would also evoke DPOAEs in the low-frequency region. It was a major objective of the experiment to investigate how the optimal ratio changes toward low frequencies, and, therefore, measurements were taken at a wide range of ratios, shown in Figure 3. Less than half of the measurements, shown with black symbols, are reported in the present paper as the rest require further analysis before a detailed account can be given. By current standards in research laboratories, the resolution of the ratios measured is relatively low and it does not allow for extraction of contributions from distinct sources by, e.g., inverse FFT. It follows that some distinct features and variation in the data may, unfortunately, not directly reflect features of the sources in the cochlea but rather the interaction between them. Such distinct features appear however to occur at randomly distributed frequencies across subjects (Reuter and Hammershøi 2006), meaning that they will cancel out on average. There are two reasons why the measurements were not made with a higher resolution. First, the measurement of each point in the curves is relatively time consuming, more so the lower the frequency. Second, spectral leakage in the FFT analysis of discrete tone measurements is increasingly difficult to avoid toward low frequencies without sacrificing accuracy of ratios, $2f_1-f_2$ frequencies and bandwidths involved. The specific frequencies and ratios were selected carefully according to the paradigm proposed by Christensen et al. (2015b).

Data Analysis

In-house developed software stimulated, recorded, and analyzed measurements in blocks of 32,768 samples at a sampling rate of 48,000 Hz. All stimulus

FIG. 3. Overview of measurements made. The ones shown with black symbols are reported here. Note that at the ratio of 14/9, $2f_1-f_2$ was 70.3 Hz instead of 88 Hz because f_2-f_1 was held fixed for ratios above 1.5.

and response frequencies were selected to exactly complete an integer number of cycles within the analysis window to avoid spectral leakage in the FFT analysis with 1.46 Hz resolution (Christensen et al. 2015b). Individual blocks were rejected automatically, if the crest factor was higher than 2, or if the pressure difference between the first and the last sample was excessive. This latter criterion was implemented to cope with nonstationary noise and frequency content below what could be represented unambiguously within the analysis window. Typically, 10% of measurements were rejected and the averaging duration was extended accordingly to end with the specified number of time blocks. No further post-processing or offline noise rejection on individual measurements was found necessary. To compensate for the increasing noise floor below 300 Hz, which we estimated in pilot testing to be 40 dB/decade in the ear canal, the averaging duration was set to 8.19 s at 300 Hz and then increased a factor 4 per octave (100 per decade). The durations were then 95.7, 24.7, and 9.6 s for measurements at 87.9, 176, and 264 Hz. The DPOAE level was estimated from the power at the 1.46-Hz wide FFT bin centered at the $2f_1-f_2$ frequency. The noise level at the $2f_1-f_2$ frequency was estimated as the root-mean-square power of the FFT bins within the upper and lower third of the ERB at $2f_1-f_2$. This frequency-dependent strategy shifts the sense of which frequencies are “near to” the distortion frequency according to perceptual frequency selectivity. The DPOAE phase was calculated by subtracting from the measured phase at that frequency $2\phi_1-\phi_2+\phi_{\text{inst}}$. Here, ϕ_1 and ϕ_2 are the phases measured at the stimulus frequencies and ϕ_{inst} is the linear phase contribution from the instrumental delay, estimated from a measurement of the impulse response between the loudspeakers and the microphone when the probe was placed in free air.

RESULTS

Individual Results

Figure 4 shows DPOAE level and noise as a function of f_2/f_1 for each individual subject. In line with data at higher frequencies, the DPOAE level curves at 176 and 264 Hz are typically bell shaped with a single maximum that defines the optimal ratio. The bell can also be inferred at 87.9 Hz, but it is broader and only one side of it is convincingly present within the range of ratios measured. The maximum level is similar at the three frequencies. Several subjects, such as f, k, and s, have the highest maximum at 176 Hz, showing that at these low frequencies, the maximum DPOAE level does not necessarily decrease monotonously with decreasing frequency. Some curves appear distinctly

FIG. 4. DPOAE level and noise as a function of the stimulus ratio in 20 out of 21 subjects who had at least 3/7 points with a signal-to-noise ratio better than 3 dB. Measurements with signal-to-noise ratios better than 3 dB are shown with *solid symbols*, and those with less are shown with *hollow symbols*. Results at 87.9 Hz are shown in

green with triangle symbols, 176 Hz in *red with circles*, and 264 Hz in *blue with squares*. Noise curves have the *same colors but no marker symbols*. The *symbols immediately above the horizontal axes* signify the individual optimal ratios as defined by the ratio evoking the maximum DPOAE level.

notched, such as those for subjects a and l, rather than bell shaped or just noisy. The noise is similar across ratios, or independent of the ratio, but it increases with decreasing frequency even though more time was spent measuring at lower frequencies.

Figure 5 shows the DPOAE phase which rotates monotonously as a function of f_2/f_1 , except when thrown off by single or several adjacent measurements with low signal-to-noise ratio. The phase polarity has been set in the figure to signify a consumption of phase as the f_1 and f_2 stimulus waves approach the base of the basilar membrane by increments of f_2/f_1 . The phase decreases 0.5π to 1.5π radians of the $2f_1-f_2$ frequency within the bell of the level curve. The slope

is shallower at lower frequencies as the bell broadens. The phase curves are also linked to the level curves in that they appear to cross 0 , π , and 0 radians near the maximum level observed, or optimal ratio, for 87.9, 176, and 264 Hz, respectively.

Collective Results

Figure 6 shows the measurements from all subjects at each of the three $2f_1-f_2$ frequencies tested. Shown for context is also data from Christensen et al. (2015a). The present and the previous studies included different subjects, except one, and the phase data from the previous study were not reported earlier.

FIG. 5. DPOAE phase as a function of the stimulus ratio, corresponding to the level curves in Figure 4.

Twenty out of 21 subjects (11 males, 9 females, aged 18 to 28 years, median 23) included in the study has at least 3/7 DPOAE data points with more than 3 dB signal-to-noise ratio at 264 Hz. The prevalence as determined by this criterion then declines toward lower frequencies. Fifteen subjects (eight males, seven females) remains at 176 Hz and eight (three males, five females) at 87.9 Hz. Increased noise levels toward low frequencies effectively harden the prevalence criterion and account for the observed decrease (see Discussion).

The optimal ratios are different at the three frequencies. They are calculated as the average of the ratios exhibiting the maximum level in each level-ratio curve where at least 3/7 points had a signal-to-noise ratio higher than 3 dB. The optimal ratios are then 1.461, 1.376, and 1.310 at 87.9, 176, and 264 Hz, respectively, with standard deviations 0.052, 0.037, and 0.044. They are shown with triangles in Figures 6 and 8. Note that they differ slightly from the maxima of the averaged DPOAE level curves as these are “locked” to one of the seven measurement points. Note also that the standard deviations are, in part, determined by the ratio resolution. The $2f_1-f_2$ frequency and the found optimal ratios may be referred to the corresponding f_1 frequencies 163, 282, and 382 Hz and f_2 frequencies 238, 388, and 501 Hz. Christensen et al. (2015a) found that it did

not make a difference on the optimal ratio to fix f_2 instead of $2f_1-f_2$ around 246 and 1231 Hz, but those data are arguably not sufficient to justify extrapolation of optimal fixed f_2 data from optimal fixed $2f_1-f_2$ data.

The slope of the phase as a function of f_2/f_1 decreases with decreasing $2f_1-f_2$ frequency. The slopes are -2.8 , -3.2 , and -4.4 cycles, respectively, of the $2f_1-f_2$ frequency per increment of 1 of the ratio, for instance, as f_2 goes from f_1 to $2f_1$. The standard deviations are 1.1, 1.0, and 1.0. Statistical significance for the average optimal ratios and phase slopes were made using pair-wise comparisons for each pair of $2f_1-f_2$ frequencies. The ratio and slope data are summarized in Table 1, and the results of the pair-wise comparisons are summarized in Table 2.

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FIG. 6. All individual DPOAE level, phase, and noise curves from Figures 4 and 5 presented on top of each other to show the collective tendencies. Comparable measures taken at 246 and 1231 Hz in different subjects (except one) are also shown (Christensen et al. 2015a). The *lightest-gray* curves in the background are the actual measurements whereas the ones in foreground have been shifted to

zero by their own average and then to the grand average of all curves. The phase curves have been shifted by multiples of 2π to better indicate the decreasing trend in the slope toward low frequencies. *Triangles at the bottom* are optimal ratios calculated as the average of the ratio with the highest DPOAE level in each curve and the *error bars* signify one standard deviation of those ratios.

DISCUSSION

Our data show that DPOAEs exist at $2 f_1 - f_2$ frequencies down to at least 87.9 Hz in humans with a systematic dependence on the stimulus–frequency ratio. Low-frequency DPOAE levels exhibit a bell-shaped dependence on the stimulus ratio, as known from DPOAEs at higher frequencies, but a stimulus ratio of 1.22 is not optimal for their measurement. At low frequencies, the bell-shaped level–ratio dependence broadens, and the ratio evoking the largest DPOAE level increases, from 1.310 at 264 Hz to 1.461 at 87.9 Hz. Keeping the ratio fixed at the suboptimal 1.22 results in an average reduction of the DPOAE level of about 8 dB. This dB reduction would directly affect the observed prevalence of DPOAE.

DPOAE phase is expected to rotate when the stimulus waves, interacting on the basilar membrane, move relative to each other (Zweig and Shera 1995). It is a consequence of scaling symmetry known from the basal turns of the cochlea that the phase is roughly invariant for fixed ratio frequency sweeps (Shera et al. 2000). All other measurement paradigms, the fixed f_1 , fixed f_2 , as well as the one employed here, fixed $2 f_1 - f_2$, shift the spatial phase relationship between the stimulus waves interacting on the basilar membrane, while the reference phase ($2\phi_1 - \phi_2$) measured in the ear canal does not change. The result is, as observed here at low frequencies, phase rotation as a function of the stimulus ratio.

The data show specifically that the phase rotates progressively slower as a function of the ratio (i.e.,

TABLE 1

Optimal ratios estimated as the average of the ratios exhibiting the largest DPOAE level in each individual level–ratio curve. The phase slopes were estimated by least-squares fitting of straight lines to the phase–ratio curves

$2 f_1 - f_2$ frequency		87.9 Hz	176 Hz	246 Hz	264 Hz	1231 Hz
Optimal f_2/f_1	Average	1.461	1.376	1.328	1.310	1.226
	Std. dev.	0.052	0.037	0.097	0.044	0.050
Phase slope (cycles/°)	Average	−2.794	−3.155	−3.943	−4.425	−5.300
	Std. dev.	1.070	1.009	0.987	0.994	2.0224

TABLE 2

Results from a pair-wise comparisons using *t* tests with the Bonferroni correction for family-wise errors (*p* values shown)

Comparison (Hz)	Optimal f_2/f_1	Phase slope
87.9–176	0.0001**	0.2161
87.9–246	0.0008**	0.0079
87.9–264	1.4246×10^{-8} ***	0.0004*
87.9–1231	4.1978×10^{-11} ***	0.0016*
176–246	0.0414	0.0181
176–264	2.2742×10^{-5} ***	0.0004*
176–1231	4.0089×10^{-11} ***	0.0004*
246–264	0.2316	0.0781
246–1231	0.0002*	0.0103
264–1231	1.5737×10^{-6} ***	0.0470

Statistical significance is shown by *5 %, **1 %, and ***<1 %

shallower slope) toward low frequencies. One possible explanation in line with the work by Martin et al. (2003) is that low-frequency DPOAEs arise further into the basal skirts of the stimulus waves where a change in the stimulus ratio causes a smaller relative change between the stimulus waves. This reflects a deviation from scaling symmetry at low frequencies, a gradual change, so to speak, that waves undergo as they peak further toward the apex. Another explanation is rooted in the possible impact of the $2 f_1$ – f_2 place. Here, the progressively smaller phase slope toward low frequencies may result from a corresponding trend in the internal stimulus which reaches the $2 f_1$ – f_2 place from the generation region, at and basal to the f_2 place. Smaller slopes would be associated with less nonlinear amplification (Ruggero and Rich 1991).

The unwrapped phase curves cross 0, π , and 0 radians at the optimal ratio of 87.9, 176, and 264 Hz, respectively. The fact that the stimulus frequencies overlap partially for these fixed distortion frequencies rules out whole-cycle unwrapping errors between 176 and 264 Hz and 87.9 and 176 Hz, but not 87.9 and 264 Hz. Supported by the maximum level often seen at 176 Hz, the π -radian phase shift bears resemblance to that of a second-order resonance due to two interacting components. Consensual DPOAE theory does hold that multiple components of the $2 f_1$ – f_2 frequency interact (Shera and Guinan 1999), but this is derived from data at higher frequencies, and phase shifts due to this interaction are typically only a half cycle, if they occur at all. Alternatively, the f_2 – f_1 and $2 f_1$ – f_2 frequencies are equal when f_2/f_1 is 1.5. These two distinct components may interact when the measurement follows the increasing optimal ratio toward low frequencies.

The data solidify the trends observed by Christensen et al. (2015a) at 246 and 1231 Hz. The optimal ratio and phase slope are, plausibly, not significantly different at 246 and 264 Hz even though the data stem from different subjects (except one). The 1231-Hz data are significantly different from the data at the other frequencies in a way that extends the trends observed at them up to the well-

known frequency range above 500 Hz. There is still however a potentially significant gap between the new low-frequency data reported here and the low- and mid-frequency data reported in the previous study. Below, a f_1 – f_2 frequency of 100 Hz DPOAE data are still sparse, but there is not an indication so far that the DPOAE itself is limited toward low frequencies. As the optimal ratio approaches and exceeds 1.5, however, composite analysis of the $2 f_1$ – f_2 , and the f_2 – f_1 DPOAE components is increasingly relevant in probing the active mechanisms in the apical cochlea.

Prevalence of DPOAE at Low Frequencies

Less than half of our subjects had DPOAEs at the lowest frequency tested. That these subjects have DPOAEs at similarly high levels pose the question whether the observed decrease in prevalence toward low frequencies is caused by the noise which increased despite the efforts made to equalize it across frequency. Figure 7 shows the prevalence of all recorded data as a function of the signal-to-noise ratio. The prevalences at 87.9, 176, and 264 Hz line up closely when shifted horizontally by the differences in average noise levels. The prevalence is nearly unchanged when shifted horizontally by the average or maximum DPOAE levels, indicating that the observed decrease in the prevalence is indeed caused mainly by increasing noise and not generally decreasing DPOAE levels. In comparison with data from Christensen et al. (2015a) at 1231 Hz, the prevalence appears even to be slightly overestimated by the prevalence at lower frequencies shifted by the average noise levels. Broader bell-shaped level–ratio curves lead to a higher such prevalence across ratios if the overall level is not reduced.

Insofar as the indications in Figure 7 hold true in less noisy low-frequency data, it can be said that the DPOAE level reached at the optimal level does not change with the frequency. The restoring forces of the middle ear which work against the transmission of low frequencies must somehow be countered inside the cochlea. It is possible that the stimulus to the DPOAE source exhibits a gradual increase because the source itself is sensitive to displacement under constant pressure stimulation (Salt and Hullar 2010). Alternatively, the increased spread of excitation on the basilar membrane of low-frequency stimulation includes more generating hair cells and results in an increased strength of the source as a whole.

Relation to Equivalent Rectangular Bandwidth

The increasing optimal ratio toward low frequencies probably reflects the increased spread of excitation by the stimulus waves on the basilar membrane. An indirect measure of the spread is the equivalent rectangular bandwidth (ERB), as derived from psychophysical tuning curves (Glasberg and Moore 1990). Specifying the stimu-

FIG. 7. Points on the level-ratio curve as a function of the signal-to-noise ratio. The percentage on the ordinate indicates how many points have at least the signal-to-noise ratio on the abscissa. The calculation of a single curve in the *bottom panel* is exemplified in the *upper panel* where the gray curves from individual subjects average to the curve with symbols. Signal-to-noise ratios at different frequencies are compared in the *bottom panel* where data from Christensen et al. (2015a) are also included. The curves with large symbols are the same as the curves with small symbols with the same color but shifted by the average noise floor at the frequency. The 87.9- and 176-Hz measurements are shifted by their respective noise level relative to the noise level at 264 Hz. The 246- and 264-Hz measurements are shifted in the same way by their noise levels relative to the noise level at 1231 Hz.

lus-frequency separation in a DPOAE measurement as a difference instead of a ratio allows for fitting of the ERB model to experimental optimal ratio data (Christensen et al. 2015a). Scaling the ERB by a parameter λ to minimize the squared distance to the data gives,

$$f_2 - f_1 = \lambda \cdot \text{ERB}(f_2), \quad \text{or} \quad (1)$$

$$f_1 - (2f_1 - f_2) = \frac{\lambda}{1 - 2\alpha\lambda} \text{ERB}(2f_1 - f_2), \quad (2)$$

where $\text{ERB}(f) = \alpha f + \beta [\text{Hz}]$, $\alpha = 24.7 \cdot 4.37/1000 \approx 0.108$, and $\beta = 24.7$. Optimal ratio data from eight previous studies and the present study were each fitted to the model, weighted by the number of

subjects they included (158 unique subjects in total), and averaged to yield a combined one value of 1.48, as opposed to 1.52 reported earlier (Christensen et al. 2015a). The fit is shown in Figure 8. Given f_2 , the optimal stimulus frequency difference is,

$$f_1 = f_2 - 1.48 \cdot \text{ERB}(f_2) \quad (3)$$

$$= 0.840 \cdot f_2 - 36.5 \quad (4)$$

And with $2f_1 - f_2$ given,

$$f_1 = 2f_1 - f_2 + \frac{1.48}{1 - 2\alpha \cdot 1.48} \text{ERB}(2f_1 - f_2) \quad (5)$$

$$= 1.23 \cdot (2f_1 - f_2) + 53.7 \quad (6)$$

By prediction of the ERB model, a stimulus ratio of 1.22 is only optimal around a $2f_1 - f_2$ frequency of 2000 Hz, but above 1000 Hz measurements with 1.22 are well within the bandwidth of the level-ratio curves. Using the 1.48 ERB (f_2), optimal separation allows for DPOAE measurements below 300 Hz and may reduce intersubject variability in general. Subjects with reduced DPOAE level or phase slope because of a shifted optimal stimulus ratio may also exhibit shifted tonotopic organization (Greenwood 1990),

FIG. 8. Stimulus-frequency ratios f_2/f_1 evoking the largest $2f_1 - f_2$ as found by eight independent studies, including the present one. The points comprise data from 158 subjects. The black line is the average of least-squares fits of the ERB model to each data set weighted by the number of subjects they include. This line is proposed as model for the optimal frequency separation in $2f_1 - f_2$ DPOAE measurements.

abnormal cochlear scaling symmetry (Shera et al. 2000; Dhar et al. 2011) (possibly related to the observed near-zero phase accumulation at the optimal ratio), or abnormal psychophysical tuning curves (Glasberg and Moore 1990).

Any linear model could in principle be scaled to fit the optimal ratio data as well as the ERB model. The ERB result is sensible in that (1) at more than 1 ERB(f_2), the traveling f_2 wave on the basilar membrane would not be expected to be suppressed by the f_1 wave; (2) at less than 2 ERB (f_2), the peaks of the traveling waves are still close enough to interact strongly (especially with the 10-dB difference in the L_1 and L_2 stimulus levels); and (3) at 1.48 ERB (f_2), the model breaks down when f_1 reaches 54 Hz ($f_2 = 2 f_1$ and $2 f_1 - f_2 = 0$).

This frequency is close to where the most apical auditory filter is found between 40 and 50 Hz in measurements of low-frequency psychophysical tuning curves at 15 dB sensation level (SL) (Jurado and Moore 2010). Studies of low-frequency DPOAEs in other mammals might indicate more convincingly if the optimal stimulus ratio has in fact a relation to the lowest auditory filter.

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