Research Article

Estimation of Round-Trip Outer-Middle Ear Gain Using DPOAEs

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ABSTRACT

The reported research introduces a noninvasive approach to estimate round-trip outer-middle ear pressure gain using distortion product otoacoustic emissions (DPOAEs). Our ability to hear depends primarily on sound waves traveling through the outer and middle ear toward the inner ear. The role of the outer and middle ear in sound transmission is particularly important for otoacoustic emissions (OAEs), which are sound signals generated in a healthy cochlea and recorded by a sensitive microphone placed in the ear canal. OAEs are used to evaluate the health and function of the cochlea; however, they are also affected by outer and middle ear characteristics. To better assess cochlear health using OAEs, it is critical to quantify the effect of the outer and middle ear on sound transmission. DPOAEs were obtained in two conditions: (i) two-tone and (ii) three-tone. In the two-tone condition, DPOAEs were generated by presenting two primary tones in the ear canal. In the three-tone condition, DPOAEs at the same frequencies (as in the two-tone condition) were generated by the interaction of the lower frequency primary tone in the two-tone condition with a distortion product generated by the interaction of two other external tones. Considering how the primary tones and DPOAEs of the aforementioned conditions were affected by the forward and reverse outer-middle ear transmission, an estimate of the round-trip outer-middle ear pressure gain was obtained. The round-trip outer-middle ear gain estimates ranged from -39 to -17 dB between 1 and 3.3 kHz.

Keywords: distortion product otoacoustic emissions, middle ear, outer-middle ear gain, forward transmission, reverse transmission

Nomenclature: OAE – Otoacoustic emission; DPOAE – Distortion product otoacoustic emission; f_1 – Primary tone with frequency f_1 ; f_2 – Primary tone with frequency f_2 ; I/O – Input/output; DP – Distortion product; f_a – Primary tone with frequency f_a ; f_b – Primary tone with frequency f_b ; OMEG – Outer-middle ear gain; DPOAE_{2T} – DPOAE generated by the interaction of primary tones f_1 and f_2 in the two-tone condition; L_2 – Level of the primary tone with frequency f_2 ; G_{rt} – Forward outer-middle ear transmission; L_{2C} – L_2 Level in the cochlea; G_{rc} – Forward cochlear gain from base to the f_2 place; L_1 – Level of the primary tone with frequency f_1 ; L_a – Level of the primary tone with frequency f_a ; L_b – Level of the primary tone with frequency f_b ; DPOAE_{3T} – DPOAE generated by the interaction of primary tones f_a and f_b in the three-tone condition; L_{abC} – Distortion product generated by L_a and L_b ; L'_{2C} – Best place wave for the distortion product L_{abC} ; DPOAE_{3T} – DPOAE generated by the interaction of f_1 and L'_{2C} in the three-tone condition; G'_{rc} – Forward cochlear gain from L_{abC} place to $2f_a$ – f_b place; Gen'_{3T} – DPOAE_{3T} generator component; G_{rc} – Cochlear gain from the f_2 place to the base; G_{rt} – Reverse outer-middle ear transmission; LSF – Least squares fit

INTRODUCTION

Otoacoustic emissions (OAEs) are sounds generated in the cochlea due to an active cochlear mechanism (Kemp 1978) and are widely used to assess the health and function of the cochlea. The outer and middle ear affect

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sound transmission to/from the inner ear; hence, they affect OAEs and the primaries. Investigating how the outer and middle ear shape the transmitted sound would benefit OAEs as a research and diagnostic tool. Efforts to determine sound transmission characteristics of the middle ear invasively were made on living human ears (Huber et al. 2001; Chien et al. 2009), human cadaveric temporal bones (Puria and Rosowski 1996; Puria et al. 1997; Voss et al. 2000; Aibara et al. 2001; Puria 2003; Nakajima et al. 2009), gerbils (Dong and Olson 2006; Ravicz et al. 2008; Dong et al. 2012), cats (Voss and Shera 2004), chinchillas (Songer and Rosowski 2007; Ravicz et al. 2010; Ravicz and Rosowski 2013), and guinea pigs (Nuttall 1974; Magnan et al. 1997). Huber et al. (2001) measured stapes displacement during surgery in patients who were going under cochlear implantation. While such measurements were very informative, they were limited to a specific pathological population and cannot be performed in normal hearing individuals. Although other invasive techniques provide useful tools to estimate middle ear transmission characteristics in humans' cadaveric data, a noninvasive approach is needed to understand individual differences.

There are not many noninvasive methods (Zwicker and Harris 1990; Keefe 2002; Shera and Miller 2002) for estimating middle ear transmission characteristics. Zwicker and Harris used a cancellation tone to cancel the distortion product otoacoustic emission (DPOAE) at $2f_1 - f_2$ in two conditions. In the first condition, the level and phase of the cancellation tone was adjusted by the investigator by means of visual inspection of the DPOAE spectrum. In the second condition, the participant adjusted the phase and level of the cancellation tone until he/she could not hear the distortion product at $2f_1 - f_2$. The level difference between the two cancellation tones in the two conditions was used as an estimate of the reverse middle ear pressure transfer function (Zwicker and Harris 1990). The resulting reverse transfer function had poor frequency resolution. Furthermore, the addition of the cancellation tone may have suppressed the OAE leading to contamination of the results.

Another noninvasive technique was developed by Keefe (2001) to estimate the forward and reverse middle ear pressure transfer functions using input/output (I/O) functions of DPOAEs (Keefe 2001, 2002). The idea was based on cochlear scaling symmetry, which implies that the shapes of the I/O functions of DPOAE are assumed to stay the same across frequency, and the difference between the I/O functions is a result of middle ear effects (Keefe 2002). Accordingly, the L_2 primary tone level change and the DPOAE level change (i.e., horizontal and vertical translations of the I/O functions; see Fig. 2 in (Keefe 2002)) across frequency were used as an estimate of the middle ear forward and reverse transfer function spectrums shapes, respectively (Keefe 2001, 2002). Shera and Miller (2002) improved Keefe's method and solved

some consistency issues with the Keefe's technique. The cochlear scaling symmetry assumption, which implies that phase accumulation of a signal is constant along the cochlea, might not be valid at all frequencies (Keefe 2001, 2002; Shera and Miller 2002). Another issue with Keefe (2001, 2002) and also with Zwicker and Harris' method is that they assumed the DPOAE came from a single source in a region of the cochlea (Zwicker and Harris 1990; Keefe 2001, 2002). Although Shera and Miller's approach (Shera and Miller 2002) was beneficial, it only provided estimates of the forward and reverse transmission spectrum shapes. Therefore, there is still a need to determine middle ear transmission values noninvasively.

This study aims to provide an estimate of round-trip outer-middle ear pressure gain (OMEG) noninvasively. Outer-middle ear gain estimation is potentially helpful to separate the impact of the cochlea from the outer and middle ear on OAEs. In addition, the gain estimation can be used to develop models of the ear, which can help in better understanding of the outer and middle ear function. In this study, a method was developed to estimate the round-trip outer-middle ear gain. DPOAEs I/O functions were used in a different sense than in Keefe's method to provide such estimation. In addition to generating DPOAEs using the standard two external primaries f_1 and f_2 (two-tone condition), DPOAEs were generated using a three-tone condition; a three-tone condition was employed previously by Shera and Guinan (2007) and Martin et al. (2016). In the three-tone condition, f_1 from the two-tone condition was kept and instead of presenting f_2 , primary tones f_a and f_b were presented. The distortion product (DP) produced by f_a and f_b interacted with f_1 to generate DPOAE at the same frequency as the DPOAE generated in the two-tone condition. The comparison between the I/O functions of the two conditions provided an estimate of the round-trip outer-middle ear gain (this is explained in more details in "Theoretical Framework" section). In order to provide the gain estimates, the DPOAE components were extracted (see "Data Analysis" section).

DPOAEs are believed to come from two different regions/sources in the cochlea (e.g., Kemp 1986; Talmadge et al. 1998; Mauermann et al. 1999) and based on two different mechanisms: nonlinear distortion and linear reflection (Shera and Guinan 1999). If the frequencies of the two primary tones to generate DPOAE are close enough, they will overlap on the basilar membrane and intermodulation distortion will be generated (Kummer et al. 1995; Brown et al. 1996; Gaskill and Brown 1996; Talmadge et al. 1997, 1998, 1999; Mauermann et al. 1999). The acoustic energy generated by the intermodulation distortion travels both basally and apically. The backward (i.e., basally) traveling wave is recorded in the ear canal and often called the generator component (Talmadge et al.

1998; 1999; Dhar et al. 2002; Shaffer et al. 2003). The generator component (also called the overlap or nonlinear-region component), generated due to nonlinear interaction between the two tones in the maximum overlap region, has a short latency and slow phase change with DPOAE frequency. The forward traveling wave travels to its best place (i.e., characteristic frequency place) on the basilar membrane, where it is partially reflected back due to linear reflection from cochlear micromechanical perturbations and is called the reflection component (Talmadge et al. 1998, 1999; Dhar et al. 2002; Shaffer et al. 2003). The reflection component has a long latency with rapid phase change (Talmadge et al. 1998, 1999; Kalluri and Shera 2001). The DPOAE, which is recorded in the ear canal, is mainly the sum of the two components (Kemp and Brown 1983; Brown et al. 1996; Talmadge et al. 1997).

The DPOAE recorded in presence of the two-tone or the three-tone stimuli is called the composite DPOAE. The generator and reflection components were estimated from the composite DPOAEs; and the OMEG, sum of forward and reverse transmission, was estimated using the composite DPOAEs and the generator components. Furthermore, an interaction-control condition was introduced to evaluate impact of the primaries on the DPOAE in the three-tone condition.

METHODS

Theoretical Framework

In the two-tone condition, the interaction between f_1 and f_2 primaries ($f_2 > f_1$) in the cochlea generated DPOAE_{2T} (see Fig. 1a). It was anticipated that primary tone L_2 in the ear canal is affected by the forward outer-middle ear transmission gain and the cochlear amplification gain before reaching its best place in the cochlea (L_2 in the cochlea is named L_{2C}). Therefore, L_{2C} at f_2 place on the basilar membrane is proportional to L_2 plus the forward outer-middle ear transmission gain, i.e., G_{ft} , plus the cochlear gain in the forward direction from the base to the f_2 place, i.e., G_{fc} (see Fig. 1b). This is translated into Eq. 1.

$$L_{2C}(f) \propto L_2(f) + G_{ft}(f) + G_{fc}(f) \quad (1)$$

It should be noted that the gains are expressed in dB. In the three-tone condition, we kept L_1 from the two-tone condition and added the auxiliary tones L_a and L_b ($f_b > f_a > f_1$). These extra tones resulted in a distortion product that induced vibration of the basilar membrane at the distortion product place (i.e., $2f_a - f_b$). The DPOAE generated by L_a and L_b

primaries was recorded as DPOAE_{3T} in the ear canal. The distortion product generated by f_a and f_b primaries close to the f_b place on the basilar membrane (called L_{abC}) traveled to its best place at $2f_a - f_b = f_2$ (called L'_{2C}), where the interaction of L'_{2C} with f_1 resulted in generation of DPOAE_{3T} in the ear canal (see Fig. 1a). L_{abC} is amplified by the cochlear gain from the L_{abC} generation site close to the f_b place to the L_{abC} best frequency place at $2f_a - f_b$, where it generated L'_{2C} wave (see Fig. 1b). This is shown in Eq. 2 in which the aforementioned cochlear gain is represented by G'_{fc} .

$$L'_{2C}(f) \propto L_{abC}(f) + G'_{fc}(f) \quad (2)$$

The source of the DPOAE_{3T} generator component is L_{abC} in the cochlea (L_{abC} is the DP generated by f_a and f_b). L_{abC} is affected by both the cochlear gain from the L_{abC} generation site close to the f_b place to the base and the reverse outer-middle ear transmission before reaching the ear canal and being recorded as the generator component. Accordingly, Eq. 3 can be derived in which the DPOAE_{3T} generator component is represented by $Gen'_{3T}(f)$, the reverse cochlear gain is shown by G_{rc} , and the reverse outer-middle ear transmission is represented by G_{rt} (see Fig. 1b).

$$Gen'_{3T}(f) \approx L_{abC}(f) + G_{rc}(f) + G_{rt}(f). \quad (3)$$

When DPOAE_{3T} and DPOAE_{2T} were similar, L'_{2C} (in the three-tone condition) and L_{2C} (in the two-tone condition) are anticipated to be similar since L_1 is the same for the two- and three-tone conditions. Therefore, from Eq. 1 and Eq. 2, we can obtain that

$$L_2(f) + G_{ft}(f) + G_{fc}(f) \approx L_{abC}(f) + G'_{fc}(f) \quad (4)$$

Finding L_{abC} from Eq. 3 and plugging it into Eq. 4, the following equation can be used:

$$Gen'_{3T}(f) - G_{rc}(f) - G_{rt}(f) + G'_{fc}(f) \approx L_2(f) + G_{ft}(f) + G_{fc}(f), \quad (5)$$

and therefore

$$Gen'_{3T}(f) - L_2(f) \approx G_{ft}(f) + G_{rt}(f) + [G_{rc}(f) - G'_{fc}(f) + G_{fc}(f)]. \quad (6)$$

As seen in Eq. 6, the difference between the DPOAE_{3T} generator component (i.e., Gen'_{3T}) and L_2

FIG. 1. Schematic of the two- and three-tone conditions. (a) Two-tone condition: DPOAE_{2T} was generated by the interaction between f_1 and f_2 in the cochlea. Three-tone condition: f_a and f_b interactions generated DPOAE'_{3T} at $2f_a - f_b$ in the ear canal; the distortion product,

generated by f_a and f_b interaction inside the cochlea, interacted with f_1 in the cochlea and generated DPOAE_{3T} at $2f_1 - f_2$ frequency. (b) Schematic of the two- and three-tone conditions with parameters that affect the forward and reverse transmissions.

provides an estimate of the sum of forward and reverse outer-middle ear transmission (i.e., round-trip outer-middle ear gain). It should be noted that this estimate is affected by the cochlear gain as well. If we consider the I/O function in the two-tone condition as the DPOAE_{2T} as a function of L_2 , and the I/O function in the three-tone condition as the DPOAE_{3T} as a function of Gen'_{3T} , then the level shift between the two I/O functions (i.e., $Gen'_{3T} - L_2$) would provide an estimate of the round-trip outer-middle ear gain (called OMEG). This approach permitted us to indirectly modify the stimulus of level L'_{2C} in the cochlea by varying L_b and comparing the I/O functions of the two- and three-tone conditions to estimate OMEG.

In order to evaluate the impact of f_a on the DPOAE level at $2f_1 - f_2$ in the three-tone condition, an interaction-control condition was developed. In the interaction-control condition, external tones f_1 , f_2 , and f_a were presented and the DPOAE generated at $2f_1 - f_2$ was compared to the $2f_1 - f_2$ DPOAE level in the two-tone condition. The level difference between the DPOAE in the two-tone condition and the DPOAE in the interaction-control condition determined the amount of reduction/enhancement due to presence of f_a .

Participants

Twelve normal hearing adults, 7 females and 5 males, between 22 and 37 years old were recruited for the

study. All participants passed an initial hearing evaluation, which included otoscopy, audiometry, and tympanometry. Otoscopy was done to ensure that there was nothing blocking the ear canal. All participants had hearing thresholds of lower than 15 dB HL at half-octave frequencies between 250 and 8000 Hz. Participants' middle ear/eardrum function was evaluated using 226 Hz Tympanometry (Grason Stadler GSI 33 Middle ear analyzer) to ensure that TPP (tympanometric peak pressure) was less than 50 daPa. DPOAE data were obtained from all participants; however, only 7 of the participants (4 females and 3 males) were included in this study because more complete data sets were obtained from them.

Stimuli and Procedure

The level and frequency ranges used for each of the participants are shown in Table 1. Continuous logarithmically sweeping tones (going up in frequency, 1 s per octave) were used as the stimuli. L_a was set to 75 dB SPL (or 65 dB SPL for NDP2) to increase the SNR. If L_1 was fixed and L_2 varied, the DPOAE level would saturate and start to decrease at higher L_2 s (Brown and Gaskill 1990; Whitehead et al. 1995). Therefore, DPOAE data were not collected for L_2 above 55 dB SPL for most participants.

In the three-tone condition, interactions between different distortion products and the primaries may affect the DPOAE level at $2f_1-f_2$. Frequency ratios $f_b/f_a = 1.25$ and $f_2/f_1 = 1.25$ were considered (for NDP1, NDP3, NDP11, and NDP12 participants) to minimize the impacts of such interactions. The ratio $f_2/f_1 = f_b/f_a = 1.225$ was also acceptable and because this frequency ratio yields the largest distortion product, $f_2/f_1 = f_b/f_a = 1.225$ was used for obtaining DPOAE from two participants, NDP5 and NDP6. The ratios $f_2/f_1 = 1.2$ and $f_b/f_a = 1.25$ were used to collect data from NDP2 before we checked the interactions between the distortion products. Since in the three-tone condition $2f_2-f_a$ was equal to $2f_1-f_2$ ($f_2 = 2f_a-f_b$) when $f_b/f_a = 1.25$ and $f_2/f_1 = 1.2$ for NDP2, the distortion product at $2f_2-f_a$ might have affected the DPOAE

estimates at $2f_1-f_2$. To check whether $2f_2-f_a$ had such reduction/enhancement effects on the $2f_1-f_2$ DPOAE in the three-tone condition, the interaction-control condition was used for NDP2.

At each level, the ear canal recordings were saved alternately into two buffers to provide two independent data sets for each level. Interleaving the data into two buffers permits good indication of the reliability of the data while minimizing contamination of the data by probe slippage. Therefore, similarities of the data collected by the two buffers indicate reliability of the measurements. The number of sweeps for each buffer was 240 at $L_b = 35$ and 40 dB SPL; and 180 at $L_b = 45$ and 50 dB SPL; and 120 at $L_b = 55$ dB SPL for the first four participants in Table 1 (i.e., NDP1, NDP3, NDP11, and NDP12). The number of sweeps in the three-tone condition for NDP5 was higher than that for the first four participants in the table, and the number of sweeps for NDP2 was fewer than that for all the other participants in the three-tone condition. A higher number of runs was used to optimize the SNR. Consequently, a larger number of runs was used in the three-tone condition in comparison with the two-tone condition for NDP5, NDP6, and NDP3. In the interaction-control condition (for NDP2), the level and frequency ranges of f_1 and f_2 , and also the number of sweeps were the same as those in the two-tone condition for this participant; the level and frequency range of f_a were the same as the three-tone condition.

Data Collection. DPOAE data were obtained from the right ear of each participant while they were sitting quietly in a recliner within a double-walled IAC sound-treated booth. Stimuli were generated and the ear canal response recorded using custom Mac software (OSX) interfaced with a MOTU 828 Firewire audio interface. After passing the signals through a Tucker Davis Technology headphone buffer (TDT HB6), the signals were delivered to the ear canal using two or three Etymotic ER-2 insert earphones (for the two- or the three-tone conditions) coupled to the OAE probe. The ER-2 insert earphones were designed to provide flat frequency responses at the tympanic membrane (e.g.,

TABLE 1

DPOAE level and frequency ranges for all participants

Participant	L_1	L_2	L_a	L_b	f_1	f_2	f_a	f_b	f_2/f_1	f_b/f_a
NDP1	65	25:10:65	75	40:5:55	1200–4800	1500–6000	2000–8000	2500–10,000	1.25	1.25
NDP3	65	25:10:65	75	35:5:50	1200–4800	1500–6000	2000–8000	2500–10,000	1.25	1.25
NDP11	65	25:10:65	75	35:5:55	1200–4800	1500–6000	2000–8000	2500–10,000	1.25	1.25
NDP12	65	25:10:65	75	35:5:55	1200–4800	1500–6000	2000–8000	2500–10,000	1.25	1.25
NDP5	65	25:10:65	75	45:5:65	1224–2449	1500–3000	1936–3872	2372–4743	1.225	1.225
NDP6	65	30:5:65	75	45:5:65	1224–2449	1500–3000	1936–3872	2372–4743	1.225	1.225
NDP2	65	20:5:65	65	20:5:65	1250–5000	1500–6000	2000–7998	2449–9998	1.2	1.25

All the levels are in dB SPL and the frequencies are in Hz

Chen et al. 2014). The ear canal response was recorded with an Etymotic three-port ER-10A microphone/preamplifier system connected to a battery-operated Stanford Research Systems SR560 low-noise preamplifier. The output of the SR560 was connected to the MOTU, which digitized the signal at a sampling rate of 44.1 kHz, before it was stored on the Mac computer for offline analysis. An appropriate size GSI eartip was placed on the ER-10A probe before putting the probe into the participants' ear canals. The probe was placed near the entrance of the ear canal with shallow insertion. It should be noted that the distance between the probe microphone and the tympanic membrane was around 10–20 mm and was smaller than the whole ear canal length. The position of the OAE probe (probe fit) was checked by evaluating the spectrum of broadband noise, presented through the three ER-2s, before and after DPOAE recordings at each level. The ear canal recordings in response to broadband noise were called ear canal calibrations and were obtained to ensure the reliability of the OAE probe assembly throughout data collection.

Data Analysis

Prior to extracting the DPOAE levels from the ear canal recordings, visual inspection of the spectrograms of the responses along with an artefact rejection algorithm were used to exclude noisy data. The artefact rejection algorithm downweighted noisy segments of the recorded ear canal response to reduce the impact of noise on the averages of the sweeps from each buffer.

Overlapping Hann-windowed segments of the data were analyzed using a least squares fit (LSF) procedure in the time-frequency domain (Long and Talmadge 1997) for estimating the DPOAE, primaries, the noise floor, and the generator and reflection components. Using the LSF technique, the difference between the predicted waveform and the ear canal recording was minimized by adjusting the phase and amplitude of the expected components (Long and Talmadge 1997; Talmadge et al. 1997, 1999; Long et al. 2008). The Hann-window bandwidth was fixed to 8 Hz for estimating the composite DPOAE. Due to the latency differences of the two components, they have different frequencies at any time point. The chosen bandwidth of 8 Hz included both components making it possible to estimate the composite DPOAE. The center frequency of the filter changed depending on the DPOAE frequency; the DPOAE phase and level were estimated at each frequency. A narrow-band filter with 2 Hz bandwidth was used to estimate the generator and reflection components. Since there is a delay in generation of the reflection component after the generator component, the reflection component was modeled as a time-latency component of the generator component (Long et al. 2008). When the narrow-band

filter with a fixed latency is used, only the generator component falls within the filter since there is a delay in generation of the reflection component (Long et al. 2008). When the narrow-band filter with a frequency-dependent latency function is used, the generator component would fall out of the window permitting estimation of the reflection component (Long et al. 2009). The I/O functions for the two- and three-tone conditions were calculated at frequencies with SNR of larger than 6 dB and level difference of smaller than 4 dB between the two buffers. These criteria were considered to ensure reliability of the DPOAEs. The level shift between the two I/O functions provided an estimate for the round-trip outer-middle ear pressure gain.

Since the primary tone levels L_1 , L_a , and the maximum of L_b were 65, 75, and 55 dB SPL, respectively, they may have evoked middle ear reflex. The middle ear acts linearly unless it receives feedback from the central nervous system. In humans, the stapedius muscle contracts in response to moderate sound levels of approximately 65 dB SPL (Feeney et al. 2004). Activation of the middle ear reflex changes the impedance of the middle ear, thereby, affecting the primary tones measured in the ear canal (Henin et al. 2014). The changes in primary levels and phases were evaluated to check for middle ear muscle activation during data collection.

RESULTS

The $2f_1$ – f_2 composite DPOAEs, generator components, and reflection components for the three-tone condition for participant NDP12 are shown in Figure 2. The solid and dashed lines show data extracted from buffer 1 and buffer 2, respectively. The two buffers are displayed to show the reliability of the measurements. As can be seen in panel a, the estimated DPOAE levels approach the noise at low frequencies, leading to an increase in the discrepancies between the two buffers at these frequencies. The generator components (as seen in panel b) are well above the noise floor for most frequencies except at very low frequencies. Consequently, the SNR is smaller at low frequencies, which is reflected in the discrepancies between the two buffers. Comparing panels a and b reveals that, as expected, the generator components are less variable across frequency and less affected by noise than the composite DPOAEs. As seen in panel c, the reflection components are lower in level and closer to the noise than the generator components (panel b) in this participant, leading to lower SNRs and more contamination of the reflection component estimates by noise. The in-phase and out-of-phase interaction of the reflec-

FIG. 2. [NDP12] $2f_1-f_2$ composite DPOAEs (a), generator components (b), and reflection components (c) at different L_b s (shown in the legend) of the three-tone condition. Buffer 1 and buffer 2 are depicted by *solid* and *dashed* lines, respectively. The noise levels are shown by *red dashed lines*.

tion and generator components results in the composite DPOAE, which is not stable across frequency (see panel a).

The $2f_1-f_2$ generator components for the two- and three-tone conditions for NDP12 are shown in Figure 3. The $2f_1-f_2$ generator components for the

FIG. 3. [NDP12] $2f_1-f_2$ generator components for the two- and three-tone conditions shown by *dashed* and *solid* lines, respectively, at different L_b s (as seen in the legend) for the buffer 1. The *red dashed lines* show the beginning and end of the frequency range for which $2f_1-f_2$ generator components for both conditions were available.

two-tone condition (dashed lines) are higher in level than those of the three-tone condition (solid lines). The I/O functions for NDP12 were calculated between 1638 and 3271 Hz because $2f_1-f_2$ generator components were available for both conditions for these frequencies (marked by red dashed lines in Figure 3). For all participants, the I/O functions were extracted for buffer 1 in the frequency ranges for which the generator components were available for both two- and three-tone conditions. The I/O functions of the generator components of the two- and three-tone conditions for NDP12 at an arbitrary frequency $2f_1-f_2 = 3001$ Hz are shown by red and blue stars, respectively, in Figure 4.

The level shift between the two- and three-tone conditions I/O functions was considered as the OMEG estimate. To find the horizontal level shift between the two I/O functions, a line was fitted to each I/O function using a LSF technique (dashed lines in Figure 4). The magenta and cyan dashed lines in Figure 4 represent the lines fitted on the I/O functions for the two-tone condition (shown in red circles) and the three-tone condition (shown in blue circles), respectively. It should be noted that the slopes of the fitted lines could be different. Since the two I/O functions might not be completely parallel, the level shift between the two I/O functions was estimated as the mean of $a_{\max}$ and $a_{\min}$ (orange arrows in Figure 4). Here, $a_{\max}$ denotes the level shift between the $DPOAE'_{3T}$ generator components and L_2 both associated with the maximum value of $2f_1-f_2$ generator component in the three-tone condition. In addition, $a_{\min}$ represents the level shift between the $DPOAE'_{3T}$ generator component and L_2 both associated with the minimum value of $2f_1-f_2$ generator component in the two-tone condition. The mean of $a_{\min}$ and $a_{\max}$ was considered as an estimate of the OMEG.

The OMEG estimates were determined at frequencies where the data fitted our inclusion criteria and are plotted as a function of frequency for all participants in Figure 5. In addition to the estimation of the OMEG from the generator components, shown by the green circles in Figure 5, the OMEG was also estimated using the composite DPOAEs, shown by black crosses. The inclusion criteria for estimating OMEG at each frequency were SNR of higher than 6 dB and difference of smaller than 4 dB between the two buffers. Therefore, OMEG could be estimated for fewer frequencies when the generator components (or the composite DPOAEs) of either the two-tone or the three-tone conditions were closer to the noise floor and the discrepancies between the two buffers were larger. As seen in Figure 5, more gaps exist in OMEG estimates using the composite DPOAEs in comparison with the OMEG estimates using the

generator components in most of the participants. This was due to the lower SNR and higher discrepancies between the two buffers in the composite DPOAEs in comparison with the generator components. Furthermore, the OMEG estimated by the generator components looks like a lowpass-filtered version of the OMEG estimated using the composite DPOAEs. The OMEG estimated using the composite DPOAEs has many dips and peaks, while the OMEG estimated using only the generator components is more smooth across frequency.

The data obtained at higher levels (e.g., above $L_b = 55$ dB SPL) were not included in OMEG estimation because the I/O functions were beginning to saturate and decrease at higher levels. The I/O functions of the two- and three-tone conditions for NDP2 at an arbitrary frequency $2f_1-f_2 = 1144$ Hz are shown in Figure 6. As seen in the two-tone condition, DPOAE increases as L_2 increases and at L_2 s of higher than 55 dB SPL, DPOAE starts to decrease. In the three-tone condition, as L_b becomes larger than 50 dB SPL, $DPOAE'_{3T}$ (on the x-axis) decreases and therefore, the I/O function is inclined toward left. The data that were included in OMEG estimation at 1144 Hz for NDP2 are shown in darker color circles in Figure 6. For NDP11, DPOAE data obtained at levels for which probe-fit measurements changed significantly were not included in OMEG estimations (some of the probe-fit measurements are shown in Appendix B for participant NDP12).

To evaluate potential reduction/enhancement by $2f_2-f_a$ on $2f_1-f_2$ ($f_2 = 2f_a-f_b$) DPOAE in the three-tone condition (for NDP2), the $2f_1-f_2$ levels in the two-tone condition were compared to those in the interaction-control condition. Such comparison shows how $2f_1-f_2$ DPOAE ($f_2 = 2f_a-f_b$) was affected by the presence of f_a in the three-tone condition. Comparison between the $2f_1-f_2$ generator components levels in the two-tone and the interaction-control conditions are shown in Figure 7. As seen, the generator components levels are contaminated by noise at most frequencies when $L_2 = 25$ dB SPL. To quantify the amount of reduction/enhancement, the $2f_1-f_2$ generator components levels in the interaction-control conditions were subtracted from those in the two-tone condition and are shown in Figure 8. The difference between the $2f_1-f_2$ generator components levels in the two-tone and the interaction-control conditions was calculated for frequencies with SNR of greater than 6 dB and when the difference between the two buffers was smaller than 4 dB. As can be seen in Figure 8, few points are shown at $L_b = 25$ dB SPL because the data was near the noise floor (see Fig. 7). As observed in Figure 7, the impact of $2f_2-f_a$ on $2f_1-f_2$ was to reduce the generator components levels for L_b s of 30, 35, 40, 45, and 50 dB SPL. The changes in levels were smaller than

FIG. 4. [NDP12] Two- and three-tone I/O functions (red and blue stars) along with fitted LSF lines for the two- and three-tone conditions (magenta and cyan dashed lines) at $2f_1-f_2 = 3001$ Hz. The level shift between the two I/O functions was estimated as the mean of $a_{\max}$ and $a_{\min}$.

11 dB at all levels and across all frequencies except at several frequencies around 3 kHz at $L_b = 30$ dB SPL, where the level changes approached 13.5 dB because the generator components were closer to the noise floor and could be contaminated by noise; these frequencies (at this level) were not included in the OMEG estimates. Therefore, the presence of f_a resulted in reduction of $2f_1-f_2$ level in the three-tone condition because of the interaction between $2f_2-f_a$ and $2f_1-f_2$. The amount of such reduction, estimated by comparison of the $2f_1-f_2$ levels of the two-tone and the interaction-control conditions, should be compensated for. The level changes at different frequencies were added to the generator components levels corresponding to those frequencies in the three-tone

condition for NDP2 to compensate for the effect of the interaction of $2f_2-f_a$ and $2f_1-f_2$ on OMEG estimation. The OMEG estimation for NDP2 after compensating for the reduction effects is shown in Figure 9. Mean of the differences between the original OMEG estimates and the OMEG estimates after removing interaction effects is 4.8 dB.

To check the middle ear muscle activation during data collection, levels and phases of the primaries f_1 and f_a were estimated at the entrance of the ear canal. Because the primaries f_1 and f_a were constant for all L_b levels in the three-tone condition, their estimated levels and phases in the ear canal should stay the same at different L_b levels. The changes in the phases of f_1 and f_a primaries did not exceed 0.1 and 0.3 Rad,

FIG. 5. OMEG estimates using the composite DPOAEs (black crosses) and the generator components (green circles) for all participants.

FIG. 6. [NDP2] The I/O functions of the generator components for the two-tone (red circles) and the three-tone (blue circles) conditions at $2f_1 - f_2 = 1144$ Hz; $L_1 = 65$, $L_a = 65$, $L_2 = 30-65$ dB SPL (in 5 dB steps), and $L_b = 40-65$ dB SPL (in 5 dB steps). The arrows show the progression direction with L_2 and L_b levels in the two- and three-tone condition. Darker circles indicate the points that were included for OMEG estimation.

respectively, which were negligible and within the measurement error. The L_1 and L_a changes at different L_b s did not exceed 5 dB. Such differences between the primary levels at different L_b s might be due to probe slippage during DPOAE data collection. To check the probe fit during data collection, the ear canal responses to broadband noise generated by the ER-2s used to generate L_1 and L_a (called ear canal calibrations) before and after obtaining DPOAE recordings at each level were recorded. Changes in the levels of primary tones f_1 and f_a across L_b were highly correlated and similar to the changes in the levels of L_1 and L_a ear canal calibrations (see Appendix B).

DISCUSSION

A noninvasive approach was used to estimate the round-trip outer-middle ear gain using DPOAE ob-

tained in two conditions; in the first condition (i.e., two-tone condition), the DPOAE was generated by the interaction of two external tones f_1 and f_2 (Fig. 1). In the second condition (i.e., three-tone condition), the DPOAE was generated by the interaction of one of these external tones (f_1) along with a distortion product generated by the interaction of two other external tones (f_a and f_b). When DPOAE in these two conditions were similar, L_2 in the cochlea (in the two-tone condition) and the distortion product at $2f_a - f_b$ (in the three-tone condition) were expected to be similar. Since L_2 was mainly affected by forward transmission (in the two-tone condition) and DPOAE generated by f_a and f_b (in the three-tone condition) was affected by reverse transmission, the difference between L_2 and the generator components of the DPOAE by f_a and f_b was considered as an estimate of the round-trip outer-middle ear pressure gain (called OMEG). Such difference was the level shift between the I/O functions of the two conditions ($2f_1 - f_2$ DPOAE as a function of L_2 in the two-tone condition

FIG. 7. [NDP2] $2f_1 - f_2$ generator components in the two-tone and interaction-control conditions, shown by solid and dashed lines, respectively. For the interaction-control condition, f_a was added to f_1 and f_2 to evaluate potential interaction of $2f_2 - f_a$ and $2f_1 - f_2$ distortion product, shown by solid and dashed lines, respectively. Noise levels are shown by red solid and dashed lines accordingly.

FIG. 8. [NDP2] Difference between $2f_1-f_2$ generator components in the two-tone and the interaction-control conditions. The black dashed lines show zero level difference between the two conditions.

or as a function of DPOAE generated by f_a and f_b interaction in the three-tone condition; Fig. 4). To evaluate the reduction/enhancement of $2f_1-f_2$ DPOAE by $2f_2-f_a$ ($f_2 = 2f_a-f_b$) in the three-tone condition, an interaction-control condition was introduced in which f_a was added to f_1 and f_2 of the two-tone condition. Comparison between $2f_1-f_2$ levels in the two-tone and interaction-control conditions provided an estimate of the amount of reduction/enhancement of $2f_1-f_2$ DPOAE by $2f_2-f_a$ (see Fig. 8).

The OMEG estimated using DPOAE generator components ranged from -39 to -17 dB between 1 and 3.3 kHz for seven participants (Fig. 5). It should be noted that the OMEG estimated here is slightly affected by forward and reverse cochlear gain (see “Theoretical Framework” section). We lack measurement/estimation of the cochlear gain in humans. In vivo studies done in mammals provided estimates of cochlear gain for several species mainly at the cochlear base (see Robles and Ruggero (2001) for a review) but no measurements were made in humans to determine cochlear gain in different places along the cochlea. It is known that the cochlea provides a frequency-dependent amplification to the traveling wave toward the f_2 place (DeBoer and Nuttall 2001;

Olson 2001; Shera and Guinan 2007) but the amount is not known in humans. Further research has to be done to determine the amount of cochlear gain from one place to another along the basilar membrane in humans. How the OMEG estimates were affected by the cochlear gain is partly explained in Appendix A.

The interaction-control condition was performed on one of the participants (NDP2) in which the $2f_2-f_a$ distortion product occurred at the place of $2f_1-f_2$ distortion product. The effect of $2f_2-f_a$ on $2f_1-f_2$ distortion product was to reduce the generator components levels when $L_b = 30, 35, 40, 45$, and 50 dB SPL at frequencies when the SNR was larger than 6 dB and when the difference between the two buffers was smaller than 4 dB. The amount of reduction only exceeded 13.5 dB when the data was not reliable. To determine the effect of such reduction on OMEG estimation, the interaction impact on the DPOAEs generator components were compensated for. The comparison between the estimated OMEG with and without interaction compensation showed differences up to 7 dB. The OMEG estimated after compensating for the aforementioned interaction was larger in value (see Fig. 9). The interactive effects of $2f_2-f_a$ on $2f_1-f_2$ are not expected to be major

FIG. 9. [NDP2] OMEG estimates using the DPOAE generator components (green circles); OMEG estimates after removing the reduction impact of $2f_2-f_a$ on $2f_1-f_2$ DPOAE generator components in the three-tone condition (black crosses).

in the other participants and conditions because $2f_2 - f_a$ was far enough from $2f_1 - f_2$ (more than 100 Hz apart). However, the interaction may have introduced small errors in the estimated OMEG.

Middle ear muscle activation during DPOAE recordings might affect the DPOAE levels and hence the OMEG estimation. To investigate middle ear muscle activation, the levels and phases of f_1 and f_a were extracted from averaged ear canal recordings collected at each L_b . Since the levels of stimuli f_1 and f_a were constant for all L_b s, the estimated levels and phases in the ear canal should have not changed unless the middle ear muscle was activated or the position of the probe changed. The L_1 differences and L_a differences at different L_1 s did not exceed 5 dB in the frequency range that the OMEG was estimated. The comparison of the estimates of probe fit before and after data collection at each L_b suggested that there was probe slippage during the recordings because such changes were highly correlated to the L_1 and L_a changes (see Appendix B). Since the primary level changes due to middle ear muscle activation can be smaller than 1 dB (Henin et al. 2014), the probe slippage may have prevented us from detecting the middle ear muscle activation through observing primary level changes. Because the middle ear muscle activation changes the impedance of the middle ear, the primary tones phases should also be affected if the middle ear muscle is activated. The f_1 phase differences and f_a phase differences at different L_b s did not exceed 0.1 and 0.3 Rad, respectively, in the frequency range that the OMEG was estimated. The amount of phase change in our data is negligible in comparison with what was observed by Henin et al. (2014) due to middle ear muscle activation. Such small phase changes are within the errors of measurement and were probably not a result of middle ear muscle activation. Although it looked like the largest difference was coming from probe slippage and minimal effect was coming from the middle ear muscle activation, the presence of the middle ear muscle activation should be further investigated by keeping the position of the probe constant during the recordings and looking at the primary level and phase changes.

Another issue that should be considered in our estimation of the OMEG is the impact of the efferents. Efferent activation affects the outer hair cells amplification and therefore the DPOAE level; hence, it might impact the OMEG estimates. It has been shown that the impact of efferents is minimal on the generator components in comparison with their effects on the composite DPOAEs (Abdala et al. 2009; Henin et al. 2011, 2014). Furthermore, the efferents impact on the generator components is reduction of the level by upto 1.5 dB, while the

efferent effect on the composite DPOAE is less systematic and can lead to enhancement or reduction of the level by about 5 dB (Henin et al. 2011). Although the efferent effect on OMEG estimation is expected to be minimal, more research needs to be done to determine the effect of efferents on OMEG estimation.

The individual OMEG estimates from our participants are shown in Figure 10 using different symbols. The estimated round-trip middle ear pressure gain by Puria and Rosowski (1996) from one cadaver is also shown in Figure 10 (red dotted line). The round-trip middle ear pressure gain, ranged between -27 and -5 dB with one global minimum between 1 and 3.3 kHz (Puria and Rosowski 1996). The round-trip middle ear gain was estimated in five temporal bones in a later study by Puria (2003), which ranged between -34 and 0 dB. It should be noted that Puria (2003) used forward transmission estimates from Aibara et al. (2001) to estimate the round-trip gain because a less invasive cochlear preparation was performed in Aibara et al. (2001). The OMEG estimates are also compared with the round-trip estimates from a model of human middle ear developed by Keefe (2015) based on the cadaveric data collected by Nakajima et al. (2009) as shown by a dotted blue line in Figure 10. The phase of the round-trip outer-middle ear transmission is not reported in the present study. The OMEGs estimated for our participants ranged between -39 and -17 dB. The gain estimates range was smaller in our estimates than those of Puria and Rosowski (1996) and Puria (2003) but was similar to the range of the gain estimated by Keefe (2015). It should be noted that the OMEG estimates were not available for all frequencies between 1 and 3.3 kHz in our participants so the gain range might be larger than found here. The mean middle ear gain estimate for the five participants in Puria (2003) was -9 dB (std: 4 dB) and -12 dB (std: 2 dB) in Puria and Rosowski (1996) between 1 and 2 kHz which were quite larger than the mean OMEG estimate for our participants, which was -27 (std: 4 dB). The mean gain estimates were comparable and more similar between 2 and 3.3 kHz; the mean gain estimate in Puria (2003) was -21 dB (std: 5 dB), was -20 dB (std: 3 dB) in Puria and Rosowski (1996), and was -27 dB (std: 5 dB) in our participants between 2 and 3.3 kHz. This can be partly explained by the differences in the impedance of the sound sources that the ear canal was terminated with.

The probe-microphone that the ear canal was terminated with in our study was a three-port ER-10A, which was different than the probe-microphone used by Puria and Rosowski (1996) (i.e., ER-10C, Etymotic Research Inc., with a foam earplug) and Puria (2003) and Nakajima et al. (2009) (i.e., ER7C, Etymotic Research Inc.). The extent to which the gain

FIG. 10. Comparison of the estimated OMEG (shown by different symbols for different participants) with round-trip middle ear pressure gain estimates using an ER-10C (red dotted line), taken from Puria and Rosowski (1996), with round-trip middle ear pressure gain

estimates using an ER-7C with a foam earplug (dashed lines with different colors for different participants), taken from Puria (2003), and with round-trip middle ear gain estimates using an ER-7C (dotted blue line), taken from Keefe (2015).

estimates could be affected by the probe-microphone should be investigated further by comparing the impedances of the aforementioned probe-microphones paired with the eartips that were used. The impedance of the probe-microphones mainly affect the reverse sound transmission through the outer-middle ear. Puria and Rosowski (1996) showed that a theoretically perfect occlusion (infinite impedance) would change the round-trip middle ear gain by up to ~4 dB between 1 and 2 kHz and reduce the gain by about 13 dB at higher frequencies. They also showed that for reverse middle ear gain, which is the component that the acoustic source impedance affects, the gain was much lower (upto 15 dB) at most frequencies when the ear canal was terminated with their lower-impedance custom-built acoustic source than when it was terminated with an ER-10C and an eartip. This finding highlights the importance of the ear canal termination during reverse transmission and could partly explain the differences between their estimations of middle ear gain and our estimations of OMEG. Comparison between the impedances of ER-10C (with a foam earplug), ER-7C, and three-port ER-10A can shed light on the source of differences in middle ear gain estimates.

Although the differences between the probe-microphone characteristics could account for some of the differences between our OMEG estimates with others, the level differences in the gain estimates could have other sources. As explained in the previous paragraph and as can be seen in Figure 10, the levels of the gain estimates are higher in Puria and Rosowski (1996) and Puria (2003) in comparison with our estimates at frequencies lower than 2 kHz. It has to be noted that the reverse stimulus by Puria and Rosowski (1996) and Puria (2003) was introduced at a location closer to the base than the site of DPOAE generation in our estimates. Therefore, at these lower frequencies, the cumulative cochlea gain effect on the OMEG estimates could be larger. This can explain some of the differences

between our gain estimates and those of Puria and Rosowski (1996) and Puria (2003), which were mainly observed at lower frequencies. Estimate of round-trip gain by Keefe (2015) was closer in level to our estimates of OMEG. The difference between Keefe (2015) and Puria and Rosowski (1996) and Puria (2003) could be due to the differences in the experimental setup or individual differences in the temporal bones in addition to the usage of different probe-microphones.

Although the estimated OMEG for three of our participants had a minimum close in frequency to the minimum in gain estimates by Puria and Rosowski (1996), we observed more minima and maxima in OMEG estimates in our participants than in the other study. The dependency of the minima and maxima in OMEG estimates to the DPOAE generator components are discussed in Appendix A. The OMEG estimates for our participants all had one to three local minima and one to three local maxima in the frequency range between 1 and 3.3 kHz. The number of maxima and minima might be underestimated, because OMEG estimates were not available at all frequencies between 1.3 and 3.3 kHz due to low SNRs or discrepancies between the two buffers. There are several possible interpretations of the presence of minima and maxima in OMEG: (i) Resonances of the outer ear: the OMEG maximum at 3.1 kHz for NDP3 could be as a result of this resonance. The OMEG for NDP2 might also have a maximum around 3 kHz. The outer ear was not included in gain estimates by Puria and Rosowski (1996) and Puria (2003). (ii) Middle ear resonances and antiresonances: e.g., a minimum was observed for NDP2, NDP3, and NDP12 around 2.7 kHz that was around the deep minimum in Puria and Rosowski (1996) estimate, which could be an antiresonance of the middle ear. (iii) Ear canal termination impedance: the impedance of the ER-10A microphone has a minimum around 1.75 kHz. A maximum is observed for all participants around 1.75 kHz except for NDP2 and NDP6 for which the

data around this frequency range is not available. (iv) Cochlear impact: since the basal part of the cochlea also affects the estimates of the OMEG (see Eq. 6), the round-trip gain of the cochlea might result for minima and maxima pattern in OMEG estimates (explained in [Appendix A](#)). This has to be further investigated to determine which extrema points are associated with the round-trip gain in the cochlea.

All of Puria and Rosowski (1996), Puria (2003), and Keefe (2015) used invasive measurements in the vestibule and at the tympanic membrane. The invasive measurements meant that a hole was drilled in the vestibule to place a hydropressure transducer to measure the pressure and another hole was drilled into the scala tympani anterior to the round window to place the inner ear sound source (Puria and Rosowski 1996; Puria 2003). Such invasive procedures may have changed the cochlear impedance (Allen 1986) and affect estimations of middle ear gain. If the holes drilled in the cochlea are not completely sealed, the cochlea impedance would decrease mainly at lower frequencies (Slama et al. 2010) and the middle ear pressure gain would be underestimated by a few dB. This could be another source for the differences observed between our gain estimates and those of Puria and Rosowski (1996) and Puria (2003) at frequencies below 2 kHz. For better comparison of the OMEG estimates with the findings in the literature, OMEG estimates should be provided at more frequencies and a wider frequency range. This can be done by having subjects with higher DPOAE levels or by developing more sophisticated noise rejection algorithms. Both of these will result in higher SNR, and therefore, OMEG estimates can be provided at more frequencies and at higher frequencies where the SNR is lower.

CONCLUSIONS

The round-trip outer-middle ear pressure gain was estimated noninvasively using DPOAE generator components in humans. The estimated round-trip outer-middle ear gain was negative with attenuations ranging from -39 to -17 dB between 1 and 3.3 kHz. The gain estimates in this study were in similar range as the gain estimates in cadaveric ears at frequencies between 2 and 3.3 kHz. Differences between the gain estimates level between 1 and 2 kHz and also in the patterns of the gains were observed. Such differences in the gain estimates were mainly due to differences in the impedances of the sound sources, differences in the position of the sound sources in the ear canal, the individual differences, and the cochlear impact on our gain estimates. Because estimating outer-middle ear gain using DPOAEs was limited to DPOAE levels with SNR of larger than 6 dB, therefore, it may not be

possible to obtain estimates from individuals with sensorineural or conductive hearing loss for whom DPOAE is in the noise floor. This noninvasive approach is potentially helpful to separate the impact of outer and middle ear on sound transmission when using OAEs. Furthermore, the OMEG estimates provided here can be used to design and develop individual models of living human ear. Transmission characteristics of the outer and middle ear and the impedances of different parts of the ear can be estimated by developing a model using the OMEG estimates (Naghbolhosseini 2015; Naghibolhosseini and Long 2016).

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest The authors declare that they have no conflict of interest.

APPENDIX

OMEG Estimates and Generator Components Levels

Since the OMEG estimates were computed from the generator components levels, they were affected by the cochlear function. This section explains how the generator components levels for the two- and the three-tone conditions affect the OMEG estimates. The OMEG estimates are shown next to the two- and three-tone generator components levels in Figure 11. Each row of Figure 11 belongs to a different participant (participants' identifiers are denoted in the left panels). The frequencies corresponding to the local maxima and minima of the OMEG estimates are marked by the vertical red and black dashed lines, respectively (Figure 11). Since the OMEG estimates are negative, minima in OMEG estimates are associated with more attenuation and maxima are associated with less attenuation. As can be observed in Figure 11, frequencies of minima and maxima in OMEG estimates (see left panels) mainly occur in close proximities of frequencies of maxima and minima in the generator components levels (solid lines) of the three-tone condition (see right panels) for all participants. The pattern of the maxima and minima in the generator components levels can affect the OMEG estimates (see "Discussion" section). As seen in Eq. 6, the OMEG estimates are affected by the basal cochlear gain. Removing such effects from the OMEG estimates should be further investigated in future.

FIG. 11. Left panels: OMEG estimates using the generator components. Right panels: $2f_1$ – f_2 generator components levels for the two- and three-tone conditions, shown by dashed and solid lines, respectively. The frequencies of the maxima and minima of the

OMEG estimates are marked by the vertical red and black dashed lines, respectively, in all graphs. The participants' identifiers are shown in left panels; the levels are shown in the legend.

Middle Ear Muscle Activation and Ear Canal Calibration

Middle ear muscle activation during data collection can affect the OMEG estimates. The activation of the middle ear muscle can change the levels and phases of the primary tones measured in the ear canal and therefore, the estimates of the OMEG. To check for middle ear muscle activation, the levels and phases of the primary tones were estimated. It should be noted that the probe slippage can also result in changes of the estimated primary tones in the ear canal. Using the LSF technique, explained in “Data Analysis” section, the levels and phases of the

primary tones f_1 and f_a (in the three-tone condition) were estimated at each L_b at the microphone in the ear canal. Since L_1 and L_a primary tone levels presented in the ear canal were the same across different L_b s, the estimated levels and phases of f_1 and f_a in the ear canal should stay the same at different L_b s unless the middle ear muscle was activated or the probe position changed during the data collection. The difference between the level of f_1 primary tone at $L_b = 55$ dB SPL and the levels of f_1 primary tone at $L_b = 35, 40, 45$, and 50 dB SPL are displayed in Figure 12a for subject NDP12; same differences were calculated for f_a primary tone levels and are shown in Figure 12c. The differences between the phases of f_1 primary tone, and also the

FIG. 12. [NDP12] The patterns observed when subtracting L_1 or L_a at L_b s of 35, 40, 45, and 50 dB SPL from L_1 or L_a when $L_b = 55$ dB SPL (panels **a** and **c**). Panels **b** and **d** show same differences for f_1 and f_a phases, respectively. The vertical dashed lines show the beginning and end of the frequency range for which $2f_1-f_2$ generator components for both conditions were available.

phases of f_a primary tone are shown in panels **b** and **d** (Fig. 12), respectively. The corresponding levels of L_b for calculating the level and phase differences are shown in the legend of Figure 12. As shown in panels **b** and **d**, the phase differences for both f_1 and f_a for all L_b levels are smaller than 0.08 Rad for subject NDP12; the phase differences for other participants did not exceed 0.1 and 0.3 Rad for f_1 and f_a , respectively, which are negligible and within the measurement error. L_1 and L_a differences (Fig. 12a, c) have similar patterns across frequency and they do not exceed 5 dB for NDP12; the level difference was smaller than 5 dB for other participants as well.

As explained in the previous paragraph, the estimated primary tone levels f_1 and f_a can change at different L_b s due to the change in the probe position in the ear canal. The f_1 and f_a primary level changes are shown in Figure 12a, c). As explained in “Data Collection” section, changes in the position of the probe were checked by recording the ear canal responses to broadband noise (i.e., ear canal calibrations) before and after DPOAE recordings at each L_b level. If the probe remained stable during the data collection, the

L_1 and L_a ear canal calibration would remain the same at different recordings. Otherwise, the ear canal calibration would change as a result of probe slippage in the ear canal. In order to relate the primary level changes to the changes in the ear canal calibrations, the difference between the L_1 ear canal calibrations along with the L_1 change between $L_b = 55$ and 35 dB SPL are shown in Figure 13a; the comparison when $L_b = 55$ and 40 dB SPL can be seen in Figure 13b (same graphs for L_a are shown in Figure 14a, b). If the primary level changes were due to probe slippage, then high correlations between the primary level changes and the ear canal calibration changes were expected. The difference between the mean of the ear canal calibrations obtained before and after DPOAE recording at $L_b = 55$ dB SPL and the mean of the calibrations at $L_b = 35$ dB SPL (or at $L_b = 40$ dB SPL) is shown in gray lines in Figures 13 and 14. The difference between the mean ear canal calibrations at $L_b = 55$ dB SPL and the calibrations done before the DPOAE recordings at $L_b = 35$ dB SPL (or $L_b = 40$ dB SPL) are shown in light green. The difference between the mean

FIG. 13. [NDP12] Differences between L_1 ear canal calibrations when $L_b = 55$ and $L_b = 35$ (a) or 40 dB SPL (b). The difference between ear canal calibrations mean at $L_b = 55$ and before 35 or 40 dB SPL are shown by light green lines; the difference with after 35 or 40 dB SPL are plotted by dark green lines (mean calibration level

differences are depicted by gray lines). The differences between L_1 for $L_b = 55$ and $L_b = 35$ or 40 dB SPL are shown by the blue lines. The vertical dashed lines show the beginning and end of the frequency range for which $2f_1-f_2$ generator components for both conditions were available.

FIG. 14. [NDP12] Differences between L_a ear canal calibrations when $L_b = 55$ and $L_b = 35$ (a) or 40 dB SPL (b). The difference between ear canal calibrations mean at $L_b = 55$ and before 35 or 40 dB SPL are shown by light green lines; the difference with after 35 or 40 dB SPL are plotted by dark green lines (mean calibration level

differences are depicted by gray lines). The differences between L_1 s for $L_b = 55$ and $L_b = 35$ or 40 dB SPL are shown by the blue lines. The vertical dashed lines show the beginning and end of the frequency range for which $2f_1-f_2$ generator components for both conditions were available.

ear canal calibrations at $L_b = 55$ dB SPL and the calibrations collected after the DPOAE recording at $L_b = 35$ dB SPL (or $L_b = 40$ dB SPL) are shown in dark green curves. As seen in panel a in Figure 13, the L_1 change (between $L_b = 35$ and 55 dB SPL), shown by blue line, is highly correlated with the difference in the mean ear canal calibration at $L_b = 55$ and the calibrations after $L_b = 35$ dB SPL, shown by the dark green line. The difference between the mean L_1 ear canal calibration at $L_b = 55$ dB SPL and the calibration before 35 dB SPL is spiky (Fig. 13a), shown by the light green curve, which is due to the presence of noise during the calibrations. As seen in Figure 13b, the difference between mean L_1 ear canal calibrations at $L_b = 55$ dB SPL and the calibration before and after $L_b = 40$ dB SPL (shown by the light and dark green lines, respectively), and the L_1 and L_a differences (shown by the blue lines) follow the same pattern and are highly correlated. The difference between the mean L_1 ear canal calibrations at $L_b = 55$ dB SPL and the mean ear canal calibrations at $L_b = 40$ dB SPL (shown by the gray line) is very similar to the L_1 change (see Fig. 13b). The amount of L_a ear canal calibration changes and L_1 differences (Fig. 14a, b) are correlated and consistent with the findings for L_1 in Figure 13a, b. Therefore, due to high correlations between the primary level changes and the ear canal calibration changes and also phase changes that were within the measurement error, the probe slippage was the main source for changes in the primary tone levels and phases. However, to determine how much of the change was due to middle ear muscle activation should be further investigated.

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