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Distortion Product Otoacoustic Emissions to Single and Simultaneous Tone Pairs

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Abstract

Distortion product otoacoustic emissions (DPOAEs) evoked by single tone pairs and three simultaneous tone pairs were recorded in 60 normal-hearing adult ears. The purpose was to replicate a previous study using the commercially available probe assembly of the Grason Stadler GSI 60 and including ear of presentation in the statistical analysis along with frequency and condition. DPOAE levels were comparable between ears and conditions, although differences among frequencies were found. Noise levels were comparable between ears and tended to increase with increases in frequency for both conditions. The latter trend was not noted with the previous study and may be due to differences in the probe assembly and/or test environment. Further, noise levels were significantly greater at 2000 and 8000 Hz in the simultaneous condition. Caution should be exercised when interpreting results in the simultaneous condition because not all frequencies may have optimal signal-to-noise ratios when the test is terminated.

Key Words: Adults, distortion product otoacoustic emissions, normals, simultaneous tone pairs, single tone pairs

Abbreviations: ANOVA = analysis of variance, DPOAE = distortion product otoacoustic emission, OHC = outer hair cell

Human evoked distortion product otoacoustic emissions (DPOAEs) were first described by Kemp in 1979. Since that time, the number of DPOAE studies appearing in the literature has grown substantially, and the response has become a research focus of varied professionals, from clinicians to biochemists. The DPOAE is generated by presenting two frequencies (f_1 and f_2), referred to as primaries, simultaneously to the ear. Nonlinear properties of the cochlea generate distortion products at frequencies mathematically related to the primary tone pair (Kemp, 1998). The strongest DPOAE, or the one most often studied and used for clinical purposes in humans, is located at the frequency of $2f_1$-f_2 (Lafreniere et al, 1991; Lonsbury-Martin et al, 1993; Kemp, 1998; Salata et al, 1998).

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OAEs in general probably represent the process that produces the sharp frequency specificity in a healthy cochlea (Ohlms et al, 1990). Kim (1980) studied the $2f_1$-f_2 and f_2-f_1 distortions in animal models and concluded that DPOAEs are generated from the region of the cochlea corresponding to the primary frequencies. DPOAEs are generated by the outer hair cells (OHCs) of the cochlea (Siegel and Kim, 1982; Ohlms et al, 1990). Brownell (1990) concluded that the electromotility of the OHCs in particular is most likely responsible for generating the energy that is transmitted back toward the ear canal. Thus, for those interested in cochlear physiology, DPOAEs provide evidence of and a way to study the active processes of the cochlea.

DPOAEs have also found a place in clinical evaluation by providing the clinician with information regarding the status of the inner ear in a variety of patient populations. DPOAE testing has been shown to be useful in demonstrating evidence of abnormal cochlear function, for example, in noise-induced hearing loss (Lonsbury-Martin and Martin, 1990). When used in a test battery,

DPOAEs may be helpful in distinguishing between cochlear and retrocochlear sites of lesion (Lonsbury-Martin and Martin, 1990) such as for auditory neuropathy. It has been demonstrated that DPOAEs are useful in monitoring changes in Meniere's disease (Lonsbury-Martin et al, 1993; Kusuki et al, 1998) and in distinguishing between sensory and neural factors in sudden hearing loss or Meniere's disease (Ohlms et al, 1990). DPOAEs can be detected in newborns in the well-baby nursery (Lafreniere et al, 1991; Lasky et al, 1992; Smurzynski et al, 1993; Marco et al, 1995) and in the neonatal intensive care unit (Ochi et al, 1998; Rhodes et al, 1999), and it has been suggested that they be used for screening in both populations. It has been suggested that DPOAEs are feasible for use as a first-stage screen (Salata et al, 1998) or for use in conjunction with other tests such as auditory brainstem response in evaluating high-risk infants (Lafreniere et al, 1993). Further, DPOAEs may be useful in monitoring cochlear function in critically ill patients taking ototoxic medications (Lonsbury-Martin et al, 1993; Littman et al, 1998). These patients are often too ill to complete a behavioral test. DPOAEs may be used to monitor cochlear function in critically ill patients and possibly identify early manifestations of cochlear impairment due to ototoxic medications (Littman et al, 1998). Finally, it has been suggested that DPOAEs may be used in monitoring possible progressive hearing losses, such as hereditary hearing loss (Lonsbury-Martin and Martin, 1990; Ohlms et al, 1990).

It is apparent that DPOAEs will continue to be important in research and clinical environments. Decreased test time might be attractive to those using DPOAEs in either environment. A shorter test time would be beneficial from a monetary standpoint, for example, decreased test time for newborn hearing screenings. It would also be beneficial for patient comfort, such as for critically ill patients who may not wish to be disturbed for any length of time. Further, it may be beneficial for clinical monitoring of difficult-to-test patients who cannot maintain the appropriate state for long periods of time, such as newborns and patients with mental retardation. Finally, a shortened test time may allow for more data to be collected from sedated animals for animal model studies.

One way to shorten the test time is to present more than one tone pair at a time to elicit more than one DPOAE simultaneously. For example, Zapala found in newborns that DPOAEs elicited in the simultaneous condition were comparable to those elicited in the single condition (D. Zapala, personal communication, 1999). However, this information was presented in a poster and has not appeared in the literature to date. One study is available in the literature that demonstrated that DPOAEs can be reliably recorded in adults in response to three tone pairs presented simultaneously with results comparable to those obtained with single presentation of tone pairs (Kim et al, 1997). Both hearing-impaired and normal-hearing adults were tested, and it was observed that the methods were similar in their ability to distinguish between the two groups. Kim et al (1997) used an Etymotic Research ER-10B microphone in the GSI 60 probe assembly for recording DPOAEs in the ear canal. However, the Knowles Electronics EK3024 microphone is standard in the probe assembly of the GSI 60 unit.

Although a straightforward replication of the Kim et al (1997) results may provide little advancement of knowledge in this area if the same equipment was used, it would be valuable to investigate the feasibility of obtaining these results using a different system or microphone with different characteristics. This is particularly important with DPOAEs because a variety of systems is available for diagnostic and screening purposes, and discrepancies in results may be obtained due to differences in the equipment components (Christensen, 2000). Christensen examined the pass/refer criteria and normative data published for several commercially available systems and noted differences in DPOAE and noise levels among devices. She attributed the differences in part to differences in microphone characteristics and differences in subjects. In the Hornsby et al (1996) normative study, the CUBDIS and GSI 60 systems were compared among other devices. The CUBDIS system includes the ER-10B microphone that was used in probe assembly of the Kim et al (1997) study. This comparison is not emphasized here with the assumption that the ER-10B would behave similarly in the CUBDIS and GSI 60 systems. It is only included to illustrate the possibility of obtaining different results with microphones similar to those used in the Kim et al (1997) and the current study. In Figure 1 of the Hornsby et al (1996) study, it is apparent that the GSI 60 noise floor is greater in the high frequencies than the CUBDIS system by 10 dB or more. This would have implications, for example, if the criteria for determining DPOAE presence is signal-to-noise ratio (SNR). In this case, it appears that it would

be inappropriate to use the same criteria for both systems. However, comparison across devices and studies should be made with caution because, as Hornsby et al (1996) stated, different algorithms are employed in the different devices, for example, to determine the noise floor. Further, different parameters were used to determine test acceptance and rejection among systems. Thus, the differences observed may have been due to variation in mathematical treatment of the data rather than just the physical characteristics of the systems' components. Despite these concerns, the message is still the same: it cannot be assumed that the results obtained with one piece of equipment can be extrapolated to another device or to a device with different components.

It was also noted that the effect of presentation ear as an independent variable was not included in the analysis along with the method of presentation and frequency in the Kim et al (1997) study (i.e., is there a difference between ears for either single or simultaneous tone pair presentation?). Thus, the purpose of the current study was to replicate the Kim et al (1997) study with normal-hearing subjects using the standard GSI 60 probe assembly and taking presentation ear into consideration. The specific experimental questions were (1) Does method of tone pair presentation (single vs simultaneous), f_2 frequency, or presentation ear have an effect on the DPOAE level? and (2) Does method of tone pair presentation (single vs simultaneous), f_2 frequency, or presentation ear have an effect on the noise level?

METHODS

Participants

Fifteen males and 15 females between the ages of 21 and 35 years (mean = 26.6, ± 4.6) volunteered to participate in the current study. Each participant had hearing levels at or better than 20 dB HL for audiometric frequencies of 250 to 8000 Hz and Type A tympanograms, bilaterally, at the time of the DPOAE test. All participants were in general good health at the time of the evaluation. Participants were assessed in an awake but quiet state in a sound-treated booth. This study was approved by The University of Memphis Human Subjects Institutional Review Board. Each subject was informed of the experiment's goals and procedures, had any questions answered, and signed a written consent form prior to participation.

Equipment and Experimental Conditions

With the exception of the difference in probe microphones, the equipment and stimuli were the same for the current study as for the Kim et al (1997) study in order to facilitate comparison of results. The GSI 60 Distortion Product Otoacoustic Emissions System (Grason-Stadler, Inc.) was used for all DPOAE measurements. The Fast-Fourier Transform used to create the spectrum had 512 bins, and with a sampling rate of 32,000 Hz, each bin represented 62.50 Hz. The noise was calculated as the root mean square of the values of two bins of data on each side of the DPOAE frequency bin in the single condition. The values of two bins on each side of the lowest DPOAE frequency were used for noise estimation in the simultaneous condition.

The ratio of f_2/f_1 was 1.2. The f_1 stimulus was presented at 65 dB SPL (L1) and f_2 at 50 dB SPL (L2). Data were displayed in a DP-gram with DPOAE and noise levels plotted as a function of each f_2 frequency. All DPOAEs were analyzed at $2f_1$-f_2. There were two experimental conditions, the single condition and the simultaneous condition. In the single condition, one set of primary frequencies (or one tone pair) was presented at a time at f_2 frequencies of 1500, 2000, 3000, 4000, 6000, and 8000 Hz. In the simultaneous condition, three tone pairs were presented simultaneously. The first set of three tone pairs included those with f_2 frequencies of 1500, 3000, and 6000 Hz (set A). The second set of three tone pairs included those with f_2 frequencies of 2000, 4000, and 8000 Hz (set B). The custom stimulus protocol of the GSI 60 DPOAE software was used to create the stimulus files for both single and simultaneous conditions. Simultaneous option was selected for the simultaneous condition stimulus file. Set A was presented first in all runs for all participants. Each condition was recorded twice for each participant. Both ears of each participant were tested for a total of 60 ears (30 right, 30 left). The frame rejection criteria, test acceptance criteria, and test rejection criteria (i.e., the test configuration protocol) used in this study were identical to those used in the Kim et al (1997) study. Frames were rejected if the noise level was greater than 15 dB SPL or if L1 or L2 was out of tolerance by ±5 dB. The test was accepted with a minimum of 240 frames and average noise levels ≤ –15 dB SPL. Finally, the test was rejected if 1500 or more frames were presented, the acceptable noise level was exceeded in 750 or more frames, or if L1 or L2 was out of tolerance in 30 or more frames.

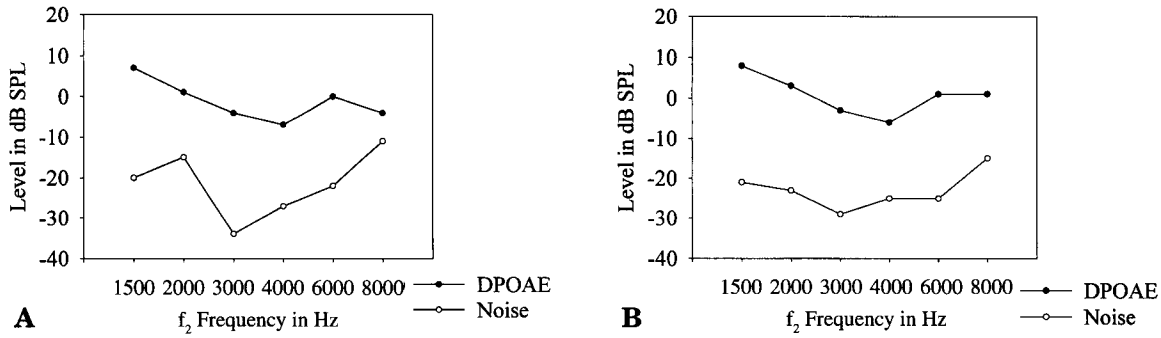

Figure 1 DP-grams of single and simultaneous evoked distortion product otoacoustic emissions (DPOAEs) from one participant. The graphs show DPOAE levels in dB SPL as a function of f_2 frequency in Hz. *A* shows the evoked responses in the simultaneous condition; *B* shows the evoked responses in the single condition.

Analysis

The DPOAE level and noise level values (all in dB SPL) for each f_2 frequency in each condition and for each ear were recorded in a database for statistical analysis. For this study, DPOAE level in all analyses refers to the $2f_1$-f_2 distortion product. All analyses were completed using SPSS Version 7.5.1 (SPSS Inc., 1998) statistical analysis software package.

Pearson product-moment correlation coefficients were calculated separately for single and simultaneous conditions to compare DPOAE levels for run one versus run two for each f_2 frequency to determine if the measures were stable at least across two replications. A three-way analysis of variance (ANOVA) was completed with DPOAE level as the dependent variable and method of presentation, frequency, and presentation ear as independent variables. A second three-way ANOVA was completed with noise amplitude as the dependent variable and the above-mentioned independent variables. Mean levels and standard deviations for DPOAEs and noise for each presentation method were plotted separately as a function of f_2 frequency. Finally, correlations comparing conditions (single vs simultaneous) for all f_2 frequencies were completed to observe the overall relationship of DPOAE level between conditions at each frequency. An alpha level of < 0.05 was considered significant for all analyses.

RESULTS

DPOAE Level

Example DP-grams for both conditions from one participant are displayed in Figure 1. The Pearson product-moment correlation coefficients comparing DPOAE levels in run one to those in run two for each f_2 frequency in each condition were all significant ($p < .05$) (Table 1). The r values ranged from .80 (8000-Hz single condition) to .99 (2000-Hz single condition). This indicated that the measures in both conditions were stable at least across two runs for all f_2 frequencies. Because there was no significant difference between runs, the values from the first run for each subject were arbitrarily chosen for the remaining analyses for both DPOAE and noise levels.

The first experimental question was Does method of tone pair presentation (single vs simultaneous), f_2 frequency, or presentation ear have an effect on the DPOAE level? Results of the three-way ANOVA indicated a significant main effect only for frequency ($p < .05$). There were no significant interactions. A Tukey HSD post hoc analysis revealed that the DPOAE levels for 1500 and 4000 Hz were significantly different from 6000 Hz ($p < .05$). Also, the DPOAE levels for 1500, 2000, 3000, 4000, and 6000 Hz were significantly different from 8000 Hz ($p < .05$). Because no difference between ears was

Table 1 Correlations of Distortion Product Otoacoustic Emission Level for Run One versus Run Two

f_2 Frequency (Hz)	*Single Condition*	*Simultaneous Condition*
1500	.986*	.947*
2000	.992*	.988*
3000	.981*	.957*
4000	.982*	.991*
6000	.932*	.824*
8000	.800*	.904*

*Significant at $p < .05$.

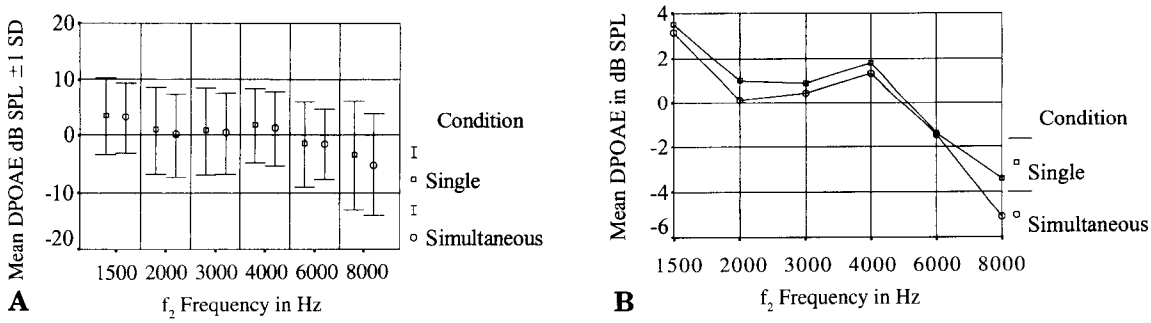

Figure 2 *A*, mean distortion product otoacoustic emission (DPOAE) levels in dB SPL ± 1 SD as a function of f_2 frequency for both single and simultaneous conditions. The DPOAE levels appear to be comparable between methods across frequency. Levels in both conditions decrease at 8000 Hz. Although there was a significant main effect for frequency, there was no main effect for method and no interaction between method and frequency (*B*).

indicated, the remaining analyses focus on results across ears. Mean DPOAE levels ± 1 SD are depicted in Figure 2A. The mean DPOAE levels at each f_2 frequency in the single tone pair condition are represented by squares, with ± 1 SD represented by the bars. Mean levels in the simultaneous tone pair condition are represented by the circles, with ± 1 SD represented by the bars. Levels in both conditions appear to decrease at 8000 Hz, reflecting the significant differences between this and the remaining frequencies. Figure 2B depicts the interaction plot of f_2 frequency and method of presentation. Although it appears that there is a difference between methods at 8000 Hz, a significant interaction was not revealed in the ANOVA. Finally, Pearson product-moment correlation coefficients comparing DPOAE levels between conditions for all f_2 frequencies are displayed in Table 2. All correlations were significant. The lowest r value occurred at 8000 Hz (.693), whereas the highest r value occurred for 2000 Hz (.970). Again, although it appears that there is some difference between the method means at 8000 Hz, they are at least significantly correlated. Figure 3 shows a sample scatterplot of DPOAE levels for the single versus simultaneous conditions at 2000 Hz. It appears that the values are more dispersed in the lower levels; however, it is still apparent that as the DPOAE level increases in the single tone pair condition, it also increases in the simultaneous tone pair condition.

Table 2 Correlations of Distortion Product Otoacoustic Emission Level for Single versus Simultaneous Conditions

f_2 Frequency (Hz)	*r Value*
1500	.942*
2000	.970*
3000	.894*
4000	.939*
6000	.760*
8000	.693*

*Significant at $p < .05$.

Noise Level

The second experimental question was Does method of tone pair presentation (single vs simultaneous), f_2 frequency, or presentation ear have an effect on the noise level? The three-way ANOVA revealed significant main effects for frequency and for method ($p < .05$). A significant interaction was identified for frequency with

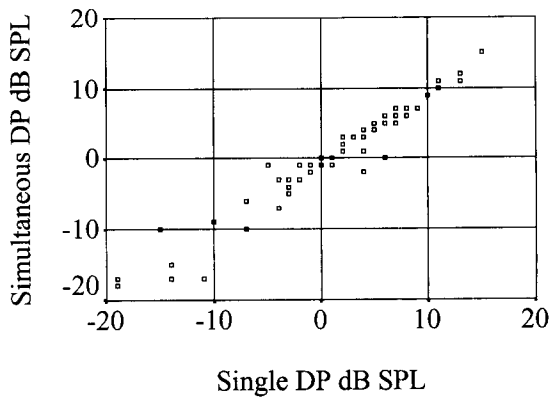

Figure 3 Scatterplot of distortion product otoacoustic emission (DPOAE) levels in dB SPL for single versus simultaneous conditions at 2000 Hz. The correlation of .970 was significant at $p < .01$. It appears that the values are more dispersed in the lower levels; however, it is still apparent that as the DPOAE level increases in the single tone pair condition, it also increases in the simultaneous tone pair condition.

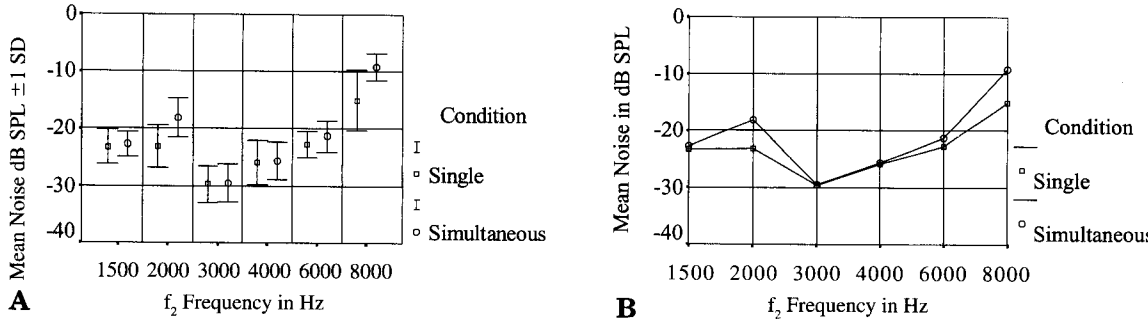

Figure 4 *A*, mean noise levels in dB SPL ± 1 SD as a function of f_2 frequency for both single and simultaneous conditions. Noise levels in both conditions appear to increase from 3000 to 8000 Hz. Levels also appear to differ between conditions at 2000 and 8000 Hz more than at the other frequencies. Main effects for frequency and method were found and a significant interaction was identified for frequency with method (*B*).

method ($p < .05$). Because no difference between ears was indicated, the remaining analyses focus on results across ears. Mean noise levels ± 1 SD are depicted in Figure 4A. The mean noise levels at each f_2 frequency in the single tone pair condition are represented by squares, with ± 1 SD represented by the bars. Mean levels in the simultaneous tone pair condition are represented by the circles, with± 1 SD represented by the bars. It appears that for both conditions, the noise level increases with increases in frequency from 3000 to 8000 Hz. Tests of simple main effects revealed that the noise levels were significantly different between methods at 2000 and 8000 Hz ($p < .05$). This is also observed in the interaction plot of frequency with method (Fig. 4B).

DISCUSSION

We determined that no significant differences existed between presentation ears for DPOAE or noise levels. Thus, it would not be necessary to collect individual normative data for each ear. Similar to Kim et al (1997), we found that different f_2 frequencies generated significantly different DPOAE levels. However, the strong correlations between DPOAE levels for single and simultaneous conditions at all f_2 frequencies indicated that the two modes of presentation were comparable across frequencies. Thus, in general, we concluded, as in the Kim et al (1997) study, that use of the simultaneous method for DPOAE collection is an acceptable method for evaluation of cochlear function.

In contrast to the Kim et al (1997) study, we found that noise levels increased with an increase in frequency. Consequently, the SNRs in the higher frequencies in the current study were less than those observed in the Kim et al (1997) study. This would have implications, for example, if SNR is used as criterion for DPOAE presence. If SNR is used, then the results of the Kim et al study (1997), or any study using a different probe microphone assembly, should not be used with the standard GSI 60 system, and that separate normative data should be established. This conclusion is in agreement with previous investigators who have advocated the development of separate normative data for different systems (Hornsby et al, 1996; Christensen, 2000). It does not imply that any system is inferior, only that the differences necessitate individual normative data.

Another difference between the current study and the Kim et al (1997) study was that noise levels at 2000 and 8000 Hz were found to be significantly elevated in the simultaneous condition as compared to the single condition. The small differences in signal levels (4.95 and 5.85 dB SPL) between the two conditions may cause minimal problems clinically in most situations. The finding of increased noise level may be attributable to differences in probe tone assembly, differences in room background noise, or other unidentified factors. A caution in using the simultaneous tone pair condition is that all frequencies may not have optimal SNRs at the same time. As the GSI 60 unit terminates the test once the first tone pair in the set reaches criterion, it may result in poorer recordings for the remaining frequencies. The increased noise level at 8000 Hz in the simultaneous condition along with the lower DPOAE level observed at that frequency could lead to false-positive results as the signal variance and noise variance may overlap. If the clinical situation relies on information at 8000 Hz, such as monitoring effects of ototoxic medications or possible high-frequency progressive hearing loss, this frequency should be

retested using a single tone pair condition if a DPOAE is not demonstrated.

One of the weaknesses of the current study is that data were not collected from individuals with sensorineural hearing loss. Therefore, sensitivity and specificity information cannot be determined with this study as it was for the Kim et al (1997) study. However, their study did find that simultaneously evoked DPOAEs were adequate in separating normal from abnormal ears. Because there are now at least two studies available demonstrating the feasibility of obtaining simultaneously evoked DPOAEs in adults with commercially available equipment, the next step should be to study the effects of presentation condition in, for example, newborns and other populations for whom decreased test time would be beneficial. Finally, the simultaneous condition should be collected with different f_2 frequencies specified as the first frequency in the set (i.e., the frequency at which the noise criteria is determined). Differences in noise and DPOAE levels may then be compared to determine if there is a significant effect when each serves as the first frequency in the set or as one of the remaining frequencies in the set.

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REFERENCES

Brownell WE. (1990). Outer hair cell electromotility and otoacoustic emissions. *Ear Hear* 11:82–92.

Christensen LD. (2000). A universal pass/refer criterion for DPOAEs: is it possible? *Hear Rev* 7:22–30.

Hornsby B, Kelly T, Hall JW III. (1996). Normative data for five FDA-approved distortion product OAE systems. *Hear J* 49:39–46.

Kemp DT. (1979). Evidence of mechanical nonlinearity and frequency selective wave amplification in the cochlea. *Arch Otorhinolaryngol* 224:37–45.

Kemp DT. (1998). Otoacoustic emissions: distorted echos of the cochlea's traveling wave. In: Berlin CI, ed. *Otoacoustic Emissions: Basic Science and Clinical Applications*. San Diego: Singular, 1–59.

Kim DO. (1980). Cochlear mechanics: implications of electrophysiological and acoustical observations. *Hear Res* 2:297–317.

Kim DO, Sun XM, Jung MD, Leonard G. (1997). New method of measuring distortion product otoacoustic emissions using multiple tone pairs: study of human adults. *Ear Hear* 18:227–285.

Kusuki M, Sakashita T, Kubo T, Kyunai K, Ueno K, Hikawa C, Wada T, Nakai Y. (1998). Changes in distortion product otoacoustic emissions from ears with Meniere's disease. *Acta Otolaryngol Suppl (Stockh)* 538:78–89.

Lafreniere D, Jung ME, Smurzynski J, Leonard G, Kim DO, Sasek J. (1991). Distortion-product and click-evoked otoacoustic emissions in healthy newborns. *Arch Otolaryngol Head Neck Surg* 117:1382–1389.

Lafreniere D, Smurzynski J, Jung M, Leonard G, Kim DO. (1993). Otoacoustic emissions in full-term newborns at risk for hearing loss. *Laryngoscope* 103:1334–1341.

Lasky R, Perlman J, Hecox K. (1992). Distortion-product otoacoustic emissions in human newborns and adults. *Ear Hear* 13:430–441.

Littman TA, Magruder A, Strother DR. (1998). Monitoring and predicting ototoxic damage using distortion-product otoacoustic emissions: pediatric case study. *J Am Acad Audiol* 9:257–262.

Lonsbury-Martin BL, Martin GK. (1990). The clinical utility of distortion-product otoacoustic emissions. *Ear Hear* 11:144–154.

Lonsbury-Martin BL, McCoy MJ, Whitehead ML, Martin GK. (1993). Clinical testing of distortion-product otoacoustic emissions. *Ear Hear* 1:11–22.

Marco J, Morant A, Caballero J, Ortells I, Paredes C, Brines J. (1995). Distortion product otoacoustic emissions in healthy newborns: normative data. *Acta Otolaryngol (Stockh)* 115:187–189.

Ochi A, Yasuhara A, Kobayashi Y. (1998). Comparison of distortion product otoacoustic emissions with auditory brain-stem response for clinical use in neonatal intensive care unit. *Electroencephalogr Clin Neurophysiol* 108:577–583.

Ohlms LA, Lonsbury-Martin BL, Martin GK. (1990). The clinical application of acoustic distortion products. *Otolaryngol Head Neck Surg* 103:52–59.

Rhodes MC, Margolis RH, Hirsch JE, Napp AP. (1999). Hearing screening in the newborn intensive care nursery: comparison of methods. *Otolaryngol Head Neck Surg* 120:799–808.

Salata JA, Jacobson JT, Strasnick B. (1998). Distortion-product otoacoustic emissions hearing screening in high-risk newborns. *Otolaryngol Head Neck Surg* 118:37–43.

Siegel JH, Kim DO. (1982). Efferent neural control of cochlear mechanics? Olivocochlear bundle stimulation affects cochlear biomechanical nonlinearity. *Hear Res* 6:171–182.

Smurzynski J, Jung MD, Lafreniere D, Kim DO, Kamath MV, Rowe JC, Holman MC, Leonard G. (1993). Distortion-product and click-evoked otoacoustic emissions of preterm and full-term infants. *Ear Hear* 14:258–274.