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Received: 2011.02.10 Accepted: 2011.06.12 Published: 2011.10.01	Contralateral suppression of otoacoustic emissions: Input-Output functions in neonates						
Authors' Contribution: A Study Design D Data Collection C Statistical Analysis	Ualace De Paula Campos ¹ ^(EOO) , Stavros Hatzopoulos ² ^(OO) , Krzysztof Kochanek ³ , Lech Sliwa ³ , Henryk Skarzynski ³ , Renata Mota Mamede Carvallo ¹⁰ ¹ University of São Paulo, São Paulo, Brazil ² Department of Audiology, University of Ferrara, Ferrara, Italy ³ Institute of Physiology and Pathology, Warsaw, Poland						
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	Summary						
Background:	The literature suggests that contralateral acoustic stimulation (CAS) alters the amplitude of the distortion product otoacoustic emissions (DPOAEs), but it is still unknown whether the DPOAE Input/Output (I/O) functions are also affected. To elucidate this aspect of the DPOAEs, the present study assessed the effects of CAS on DPOAE I/O functions at the frequencies of 2 kHz and 4 kHz, in a sample of term neonatal subjects.						
Material/Methods:	Sixty randomly selected neonates were included in the study. The DPOAE I/O functions were obtained at 2 kHz and 4 kHz, in the presence of a 60 dB SPL broad band-contralateral white noise, using the TDH39 headphones contralaterally. DPOAEs were recorded up to a stimulus level of L_2 =35 dB peSPL.						
Results:	Significant DPOAE amplitude suppression effects were observed at various L_2 stimulus levels for both tested frequencies at 2 and 4 kHz. In contrast, the corresponding DPOAE slopes showed various alterations that were not statistically significant.						
Conclusions:	The data from the present study show that contralateral acoustic stimulation significantly affects only the amplitude of the DPOAE I/O functions; the slope is affected, but not significantly. This observation can shed light on the nature of CAS, suggesting that the latter is primarily a linear phenomenon without the cochlear compression and non-linear components seen in the healthy cochlea. From the available data it is not possible to infer whether the sample size has influenced the obtained results and the study should be repeated with a larger sample size and assessing more frequencies.						
key words:	Contralateral Acoustic Suppression $ \cdot $ Otoacoustic emissions $ \cdot $ DPOAE I/O functions $ \cdot $ newborn $ \cdot $ cochlear function						
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Author's address:	Stavros Hatzopoulos, Department of Audiology, Ferrara University, Corso Giovecca 203, Ferrara, 44100 Italy, e-mail: sdh1@unife						

BACKGROUND

Since Rasmussen [1], it has been known that the organ the Corti receives efferent innervations from olivocochlear neurons. The medial olivocochlear system (MOC) has mainly contralateral connections with the outer hair cells (OHC) [2,3]. The majority of the information on the MOC system comes from electrical stimulation in animals. Rajan and Johnstone [4] have shown that contralateral acoustic stimulation (CAS) generates similar effects as those of the electrical stimulation, at the floor of the fourth ventricle.

Otoacoustic emissions (OAEs) reflect the mechanical properties of the OHCs. The OAE responses have been used extensively as a probing tool in the assessment of the cochlear function [5–10]. They can be categorized by the invoking stimulus in 2 classes — the evoked and the spontaneous OAEs. A sub-category of the first class is called distortion product OAEs (DPOAEs) and refers to cochlear responses evoked by 2 pure tones – fl and f2 [11]. To obtain a DPOAE response, one can either vary the amplitude of the stimulus at a fixed frequency (Input-Output – I/O-function) or fix the amplitude and vary the frequency (DP-Gram). The I/O function provides information about the hearing threshold and the associated nonlinear OAE behavior [12].

In the analysis of DPOAE I/O functions, a number of variables are of critical importance, including: (i) the DPOAE amplitude; (ii) the DPOAE slope; and (iii) the DPOAE threshold. The latter is used in the estimation of the "hearing threshold" [10-13]. The DPOAE slope is the growth rate of the DPOAE response. The slope value decreases at higher stimulus intensities, especially in the range from 50 to 80 dB peSPL, where the cochlear compression occurs [14]. Cochlear compression decreases with the increased severity of cochlear lesions, so the DPOAE slope can represent a variable with high specificity and low sensitivity [8,15]. Gehr et al. [16] studied DPOAE I/O functions in pigmented guinea pigs with induced middle and inner ear alterations and reported that animals exposed to noise presented steeper DPOAE slopes, suggesting a loss of cochlear compression. Animals characterized by middle ear alterations did not show any changes in the DPOAE slope values. In this context, the authors suggested that the DPOAE I/O functions and the value of the I/O slope could be useful indices in distinguishing conductive from sensorial hearing loss.

In humans, when the MOC pathway is activated by noise in the contralateral ear, changes in the DPOAE amplitude level can be recorded in the ipsilateral ear [17]. The broad-band-noise and the white noise are frequently used as contralateral acoustic stimuli [9,18]. Moulin et al. [18] found that contralateral broad-band noise (BBN) has a suppressive effect on DPOAEs recorded from 0.5 kHz to 5 kHz. This effect was not caused by cross-hearing due to the usage of earphones and no change in the noise floor occurred under increasing contralateral stimulation. Deeter et al. [19] also studied the contralateral stimulation with BBN levels of 60, 70 and 80 dB SPL in adults. The contralateral stimulation reduced the amplitudes of the DPOAEs and the suppression effects were more pronounced as the level of the contralateral noise increased. Ugur et al. [20] found that contralateral stimulation with

white noise resulted in a significant suppression of TEOAEs amplitudes in healthy controls. Quantitatively, Bassim et al. [21] concluded that after contralateral stimulation with a BBN of 60 dB SPL, the average DPOAE suppression was 1.1 dB (0.3–2.7 dB).

Atcherson et al. [22] affirmed that, in general, the broader the bandwidth of the noise stimulation, the greater the suppressive effect. Harrison et al. [23] suggested that otoacoustic emissions (DPOAEs) can be both enhanced and suppressed. DPOAE amplitude is suppressed by an increase in contralateral stimulation and enhanced by a decrease in the CAS.

Chéry-Croze et al. [24] concluded that the suppression is frequency-dependent, at least for the middle frequencies of 1 and 2 kHz. Sun [25] confirmed these findings, reporting that, at the middle frequency peaks the suppression effect on DPOAEs was larger. Similarly, Ibargüen et al. [26], reported that white noise and pure-tones at 1, 1.5, and 2 kHz had the greatest suppressor effects on the TEOAE amplitude with suppression effects varying from 0.5 to 2.5 dB.

The data in the literature suggest that contralateral acoustic stimulation alters the DP-gram amplitudes of neonatal subjects [9,18–21,27], but it is still unknown whether the DPOAE I/O functions are also altered. If an inhibitory efferent effect significantly reduces the DPOAE amplitude, it should also affect (partially or fully) the DPOAE I/O parameters such as the amplitude and the slope. To elucidate this hypothesis the present study assessed the effects of contralateral acoustic stimulation on DPOAE Input/Output functions in a sample of term neonatal subjects.

MATERIAL AND METHODS

Subjects

Sixty randomly selected term neonates (32 males and 28 females) from the hospital of Ferrara University participated in the study. The inclusion criteria were: (i) an age between 36 and 60 hours; (ii) lack of any risk indicators associated with permanent congenital or progressive hearing loss (JCIH, 2007); (iii) TEOAEs (elicited by nonlinear clicks of 80±3dB SPL) with an overall reproducibility level of at least 75% and a signal-to-noise ratio at 2, 3 and 4 kHz exceeding 6 dB [28].

Procedures and data-collection

The DPOAE data were recorded with an ILO-292 apparatus (version 5.6, Ilodynamics) and DPOAE I/O functions were obtained only at 2 kHz and 4 kHz, in the presence of a 60 dB SPL broad band-contralateral white noise, contralaterally using the TDH39 headphones. The study's initial design intended the evaluation of more frequency points, but difficulties in obtaining valid responses at 1 or 1.5 kHz made us restrict testing to 2 frequency points.

Noise was generated by a dedicated software program (Tone Gen, NCH software). The levels of noise were calibrated with a Bruel and Kjaer impulse precision sound level meter type 2209, coupled with a 1-inch condenser microphone type 4145 for free field use. The latter has a normal incidence free field response, which is linear from 1 to 2 kHz

Table 1. Means and p-values of the DPOAE I/O functions levels at all stimulus intensities – with and without CAS. The legend Level refers to the L,	
stimulus values (dB SPL). The values of the DPOAE responses are expressed in dB SPL.	

2 kHz		All Neonates		4 kHz		All Neonates	
Level	w/o CAS	With CAS	p-value	Level	w/o CAS	With CAS	p-value
70	13.88	11.17	0.13	70	11.61	8.48	0.08
65	10.71	9.11	0.32	65	8.41	5.38	0.13
60	6.05	4.66	0.40	60	5.82	2.43	0.05*
55	4.96	1.75	0.07	55	2.65	-1.04	0.06
50	2.98	-0.7	0.03*	50	-1.18	-5.88	0.01*
45	-0.27	-1.28	0.49	45	-5.64	-7.04	0.39
40	-2.42	-3.68	0.38	40	-6.03	-8.39	0.38
35	-4.2	-6.71	0.10	35	-6.63	-8.33	0.26

* Means: statistically significant at 0.05 level.

(±3 dB), 2–18 kHz (±1.5 dB) and meets the requirement of the ANSI (American National Standards Institute) for a laboratory standard type L microphone.

DPOAEs were recorded from term neonates in a quite room. Responses were acquired at stimulus intensities between 35 and 70 dB peSPL, at 2 and 4 kHz. Each response was an average of 8 responses (higher averages would excessively prolong the test sequence). The stimulus paradigm proposed by Kummer et al [8,10,30] was used, in which the primary tone stimulus was set to $L_1 = (0.4 * L_2) + 39 \text{ dB peSPL}$. The I/O datasets were obtained by decreasing L₂ in 5 dB steps. The DPOAE slope values were estimated from 2 sets of data: (i) from all tested stimulus intensities, measuring the inclination of the DPOAE response-growth from 35 to 70 dB peSPL; (ii) from a sub-set of data referring to L₉ levels between 40 and 60 dB peSPL. Linear trend modeling was used for fitting the data with linear functions and subsequent estimation of the DPOAE I/O slopes (see the Appendix for additional details).

The testing time requirements per subject were approximately 30 minutes. Only the data from the right ear were considered in this study.

All test procedures were explained to the parents of the subjects, who provided written informed consent.

Statistical analysis

Descriptive and comparative methods were applied to the data analysis. ANOVA analyses were performed to compare the values of the DPOAE slopes. Values of $p \le 0.05$ were considered statistically significant. SPSS version 16 was used for all statistical procedures.

RESULTS

In the first phase of the analyses all data from all stimulus levels (ie, 35-70 dB peSPL) were included. The DPOAE responses were considered as valid when the corresponding S/N ratio >0 dB. This criterion is low for high-level DPOAE responses (ie, 65–55 dB SPL) [28], but it is adequate for responses elicited by low-level stimuli (<40 dB). One way to control the validity of this criterion is to consider that, theoretically, the amplitudes of the DPOAE I/O functions decrease in a monotonic manner from higher to lower eliciting stimuli. In this context, physiological processes should generate responses that decrease in amplitude according to the eliciting stimulus. Since no cases were observed with low S/N values at higher stimuli and with correspondingly higher S/N values at lower stimuli, the chosen criterion was considered appropriate.

The amplitudes of the DPOAE I/O functions were altered after contralateral acoustic stimulation at all L_2 levels and in both 2 and 4 kHz tested frequencies. The mean suppression was 1.74 dB (sd=0.67) and 1.87 dB (sd=0.67) at 2 and 4 kHz, respectively.

Significant suppression was observed only at a few stimulus levels. Specifically at 2 kHz, the DPOAE responses elicited by a L₂=50 dB peSPL stimulus presented significant suppression (p=0.034). Similarly, at 4 kHz the suppression was significant at L₂=50 and 60 dB peSPL. Table 1 summarizes the findings from the first phase.

The second phase of analysis used a sub-set of the original dataset. Subjects who did not present an S/N >3 dB at L₂=60 dB peSPL were excluded. This criterion reduced the number of cases for subsequent analyses. Forty-five cases presented S/Ns >3 dB SPL at 2 kHz and 46 at 4 kHz.

At 2 kHz, suppression effects were observed at L_2 =35, 50, 55, 60 and 70 dB peSPL. At 4 kHz, suppression effects were observed at all L_2 stimulus levels from 50–70 dB peSPL. For this frequency, suppression effects were also observed at lower L_2 levels (35–45 dB SPL), but were not significant. The data from the second phase are summarized in Table 2.

Statistical analyses on the DPOAE slope did not reveal a significant CAS effect. The analyses were conducted on 2 datasets. In the first set, all DPOAE amplitude data were included ($L_y=35-70$ dB peSPL), using an S/N >0 dB response Table 2. Means and p-values of the DPOAE I/O functions levels at all stimulus intensities and at 2 and 4 kHz – with and without CAS. This table shows data from neonates who presented SNR ≥3 dB SPL at stimulus level L₂=60 dB SPL. The number of neonates who reached this criterion was 45 at 2 kHz and 46 at 4 kHz. The legends follow the connotation of Table 1.

2 kHz						4 kHz					
Level	w/o CAS		with CAS				w/o CAS		with CAS		
	Mean	SD	Mean	SD	– p-value	Level	Mean	SD	Mean	SD	- p-value
70	16.03	4.76	13.13	5.93	0.05*	70	14.51	5.20	10.79	7.96	0.05*
65	13.09	4.55	10.92	5.34	0.12	65	12.7	5.37	8.21	7.66	0.01*
60	10.24	4.27	6.16	6.81	0.01*	60	10.12	5.06	4.54	7.15	0.01*
55	7.68	6.21	3.51	7.26	0.03*	55	5.56	6.50	1.32	6.20	0.01*
50	4.60	6.61	0.64	7.41	0.05*	50	1.79	6.00	-2.09	6.80	0.03*
45	0.52	6.10	-0.88	6.16	0.52	45	-3.93	6.16	-5.96	8.00	0.29
40	-1.14	6.85	-3.43	5.32	0.19	40	-5.46	5.44	-6.94	4.59	0.29
35	-3.26	5.26	-6.63	5.43	0.03*	35	-7.17	4.15	-9.43	6.97	0.16

* Means: statistically significant at 0.05 level.



Figure 1. DPOAE I/O functions at 2 and 4 kHz with and without contralateral acoustic stimulation and considering all tested stimulus intensities (35 to 75 dB peSPL). The y-axis depicts stimulus intensity and the x-axis DPOAE amplitude. The growth/pattern of the curves is not significantly modified, after CAS at 2 or at 4 kHz.

criterion. In the second dataset only data from stimulus levels L₂=40–60 dB peSPL were included, using an S/N >3 dB response criterion.

In the first set, the slope of the DPOAE I/O function presented means of 2.84 and 2.79 at 2 kHz and 2.84 and 2.21 kHz at 4 kHz, without and with CAS, respectively. After contralateral acoustic stimulation, the slopes became slightly flatter, but the induced alteration was not significant either at 2 or at 4 kHz (p=0.83 and 0.37, respectively).

In the second set, the slope of the DPOAE I/O function presented means of 2.99 and 2.36 at 2 kHz and 3.63 and 2.46 at 4 kHz, without and with CAS, respectively. The suppression effect on the DPOAE slopes was higher than in the first group, but the estimated differences were not statistically significant (p=0.16 and 0.28). Figure 1 summarizes these findings.

DISCUSSION

The objective of this study was to investigate the possible alteration of the DPOAE I/O parameters in term neonates, after a 60 dB SPL contralateral acoustic stimulation. From the available test frequencies, data were assessed only at 2 and 4 kHz, because the DPOAE responses at the lower frequencies (1–1.5 kHz) presented very poor S/N ratios even at moderate to high stimuli (50–60 dB peSPL). Significant suppression effects were observed at various L₂ stimulus levels for both 2 and 4 kHz, but the corresponding DPOAE slope values did not show significant alterations.

The suppression of the DPOAE amplitudes after CAS, observed in this study, corroborate the data presented previously by other authors. Chèry-Croze et al. [24] studied the suppression with narrow band noise and concluded that the CAS was frequency-specific.

Bassim et al. [21] evaluated the CAS in DP-grams with Narrow Band Noise (NBN) at 60 dB SPL and found that the average suppression was 1.1 dB (range of 0.3–2.7). Zhang et al. [31] affirmed the importance of DPOAE for the evaluation of the efferent system, but the observed suppression was approximately 0.92dB (sd=0.71). Significant differences between the tested frequencies were not reported. In the present study, the average suppression at 2 and 4 kHz was 1.74 and 1.87 dB (sd=0.67 and 0.67), respectively, and significant suppression effects were restricted to moderate-to-high L2 stimuli (50 and 60 dB peSPL). When the available data were filtered with a 3 dB criterion, broader suppression effects were observed at both tested frequencies. The different suppression patterns observed in the 2 sets of data (all cases and those exceeding the S/N 3 dB criterion) provide the grounds to assume that the detectability of suppression depends on the S/N of the DPOAE responses. Thus, different acquisition parameters (averages per sample, level of ambient noise) might be necessary for this type of DPOAE recording, rather than those established in various hearing screening studies [31].

The data show that contralateral acoustic stimulation does not significantly modify the slope of the DPOAE I/O functions. Data from previous studies [32-34] on adults with normal hearing or with varieties of hearing pathologies are available, but a direct comparison with the present data is not reliable. For example, Abdala et al. [32] evaluated 4 subjects with auditory neuropathy and found that they lacked efferent suppression of OAEs. Wang and Zhong [33] studied the medial olivocochlear system (MOCS) by the CAS in individuals with cochlear and retrocochlear hearing loss. While the CAS effect was less frequently present in individuals with cochlear hearing loss, the individuals with retrocochlear hearing impairment presented no suppression, and in some cases they presented increased DPOAE amplitudes. Interestingly, Abdala [34], in a study of the suppression of the DP-gram amplitude and tuning curves on premature newborns, reported no DPOAE suppression. The influence of the immature conductive pathways cannot be entirely ruled out as a factor contributing to these results.

CONCLUSIONS

The data from the present study show that in term neonates CAS significantly affects only the amplitude of the DPOAE I/O functions. The DPOAE slope is altered, but the induced changes are not significant. These findings can shred light on the nature of CAS, suggesting that it might be primarily a linear phenomenon, deprived of the cochlear compression and non-linear components seen in the healthy cochlea [35]. From the available data it is not possible to conclude whether the sample size influenced the obtained results, and the study should be replicated with a larger sample size and assessing a larger number of frequency points.

APPENDIX

Usually the DPOAE I/O functions show a non-linear behavior, especially at low and high stimuli, due to the nature of the cochlear amplifier. In a stimulus range from 40 to 55 dB peSPL the DPOAE responses show enhanced linearity. It is possible to represent the whole I/O curve with a slope estimation in its linear region as proposed by Gorga et al. [7] and by Janssen et al. [8]. Alternatively, it is possible to fit the non-linear data to a linear function and then estimate the slope of the fitted I/O function.

In this paper, the slopes of each DPOAE I/O function were estimated with a linear trend model. The later can be used to make and justify statements about tendencies in the data set. By using trend estimation it is possible to construct a





model that is independent of anything known about the nature of the process of an incompletely understood system. This model can then be used to describe the behavior of the observed data. In particular, it may be useful to determine if measurements exhibit an increasing or decreasing trend that is statistically distinguished from random behavior.

The DPOAE I/O data were fitted with linear functions for the stimulus range from 70 to 35 dB SPL. Once a linear fit was obtained, the slope was estimated at 2 points of the y coordinate equal with $y_2=70$ and $y_1=35$ dB SPL. Given the corresponding points of the DPOAE amplitude as x_2 and x_1 , the slope of the fitted linear function was defined as: $\beta=(y_2-y_1)/(x_2-x_1)$. Figure 2 demonstrates how a slope can be estimated from a set of I/O DPOAE data.

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