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Evaluation of anesthesia effects in a rat animal model using otoacoustic emission protocols[1]

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Abstract

Anesthesia effects on otoacoustic emission (OAE) recordings were evaluated in a group of 72 Sprague–Dawley rats (mean weight 225 ± 20 gr). Two anesthesia dosages (high and normal) and two anesthetic protocols (ketamine–xylazine, ketamine–xylazine–atropine) were tested. Transient evoked OAE (TEOAE) and distortion product OAE (DPOAE) responses were recorded in 10 min intervals, for a total period of 60 min. Analyses of the data with repeated measure models indicated the following: (1) The animals receiving a high dose of anesthesia (cumulative dose 66.6 mg of ketamine and 13.2 mg of xylazine/kg of body weight) presented significant alterations of the TEOAE response level and the signal to noise ratio at 3.0 kHz; (2) the animals receiving a normal dose of ketamine–xylazine anesthesia (cumulative dose 50 mg of ketamine and 10 mg of xylazine/kg of body weight) presented TEOAE and DPOAE responses invariant in terms of time; (3) significant differences were observed in the DPOAE responses from animals anesthetized with ketamine–xylazine and ketamine–xylazine–atropine. The data support the hypothesis that the ketamine anesthesia OAE suppressing mechanism is related to middle-ear mechanics.

Key words: Distortion product otoacoustic emission; Transient evoked otoacoustic emission; Anesthetic; Ketamine; Atropine; Sprague–Dawley rat

1. Introduction

Otoacoustic emission (OAE) responses are non-linear signals generated in the cochlea, upon its stimulation by an acoustic click (Kemp et al., 1990; Zweig and Shera, 1995; Shera and Guinan, 1999) or a pair of pure tones (Horner and Cazals, 1989; Hauser and Probst, 1991; Mills et al., 1993; Whitehead et al., 1992, 1995a,b). A direct relationship between the OAEs and the outer hair cells (OHCs) of the organ of Corti has been well established (Brownell et al., 1985; Brownell, 1990). Otoacoustic emissions are of great research and clinical interest especially for a number of animal models, where the OAEs are used to monitor the cochlear functionality from an OHC perspective. In clinical setups, otoacoustic emissions are used to detect OHC lesions.

Transient evoked OAEs (TEOAEs) and distortion product OAEs (DPOAEs) from a rat animal model have been reported recently in the literature (Khvoles et al., 1996, 1998; Hatzopoulos et al., 1999; Sochalingam et al., 2000). Traditionally the TEOAEs are evoked by click stimuli, while the intermodulation distortion products are generated when two simultaneous pure tones are presented to the cochlea with frequencies f_1 and f_2. The most prominent distortion product is the

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Abbreviations: DPOAE, distortion product otoacoustic emission; MAP, mean arterial pressure; OAE, otoacoustic emission; S/N, signal to noise ratio; TEOAE, transient evoked otoacoustic emission

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$2f_1-f_2$, called also cubic difference tone. In general in the rat, the DPOAE responses are characterized by large signal to noise ratios (S/N) in the order of 40 to 45 dB and the TEOAE responses show short latencies (2.5 ms) and average amplitudes (200–300 μPa). During experiments studying the ototoxic effects of cisplatin on the Sprague–Dawley rat strain (Hatzopoulos et al., 1999, 2001), it was observed that animals that were treated twice with a subcutaneous administration of ketamine presented the highest amplitude suppression of the OAE response (i.e. the response amplitude was significantly reduced). Significant post-anesthesia effects on TEOAEs and DPOAEs were also shown.

Considering that the major application of OAEs is the monitoring of the cochlear functionality and the detection and monitoring of inner ear lesions, it is important to determine if the process of anesthesia contributes to a significant alteration of the OAE responses (i.e. a decrease of the level of OAE response). Recent studies using different animal models have reported equivocal anesthesia-induced effects. A study conducted on chinchillas (Harel et al., 1997) has reported that ketamine anesthesia significantly increases the level of the OAE responses. A study on the gerbil (Zheng et al., 1997) has reported that ketamine anesthesia induces very small pressure changes in the middle ear and the suppression of the level of the DPOAE responses is minimal. The objective of this study was to evaluate the anesthesia effects of two widely used anesthetic protocols, the ketamine–xylazine and the ketamine–xylazine–atropine, on the OAE responses from Sprague–Dawley rats.

2. Materials and methods

2.1. Animals

Seventy-two male Sprague–Dawley rats obtained from Charles River, Italy (mean weight 225 ± 20 gr) were divided randomly into four groups. Due to the short duration of the experiments each animal served as its own control. Details of the anesthesia protocols are presented in Section 2.2.

1. **Normal-dose group** (h = 28). Each animal was treated with a standard dose of ketamine–xylazine anesthesia, administered in two consecutive phases, first i.p. and then subcutaneously.
2. **High-dose group** ($n = 34$). Each animal was treated as the animals of the normal-dose group, with the exception that the subcutaneous dose was doubled.
3. **Tympanic perforation** (TM) group (h = 5). Each animal was given ketamine anesthesia (as in the normal group) and underwent a tympanic membrane perforation. The TMs were perforated approximately at the division between postero-superior and inferior quadrant with the aid of an optical microscope. The diameter of the perforation was on the average 500 nm.
4. **Atropine group** ($n5$). Each animal was given ketamine–xylazine–atropine anesthesia administered in two consecutive phases, first i.p. and then subcutaneously.

The animals were treated according to the Italian guidelines DL 116/92 with reference to EEC directive No. 86-609.

2.2. Anesthesia

For the ketamine-anesthesia we have used an equal-volume combination of ketamine hydrochloride (Ketavet, Farmaceutici Gellini, Italy), xylazine (Rombun: Bayer, Italy) and saline in dosages of 1 ml/kg of body weight (b.w.). Each ml of anesthesia contained 33.3 mg of ketamine and 6.6 mg of xylazine. The anesthetic was administered in two consecutive phases. In phase one, the animal received a 1 ml/kg b.w. intra-peritoneal dose and upon the first signs of muscular relaxation (phase two) a second volume of 1.0 ml/kg (high-dose group) or 0.5 ml/kg (normal-dose and TM group) was administered subcutaneously. Normally rats are given a 0.5 ml/kg of subcutaneous anesthesia. In this context, the high-dose group received a total of 66.6 mg of ketamine and 13.2 mg of xylazine/kg. The normal-dose group received a total of 50 mg of ketamine and 10 mg of xylazine/kg.

For the ketamine–atropine anesthesia experiments, we used an equal-volume solution of ketamine hydrochloride (Ketavet, Farmaceutici Gellini, Italy), xylazine (Rombun: Bayer, Italy) and atropine (Atropina Solfato: Jacopo Monico, Venezia, Italy). The anesthesia was used in dosages of 1 ml/kg b.w. Each ml of anesthesia contained 33.3 mg of ketamine, 6.6 mg of xylazine and 0.16 mg of atropine. The anesthesia was administered in two consecutive phases with a halved subcutaneous dosage of 0.5 ml/kg, as described in the previous paragraph. The atropine group received a total of 50 mg of ketamine, 13.2 mg of xylazine and 0.24 mg of atropine/kg.

It should be noted that the employed anesthetics, resulted in a light anesthesia level, necessary to test the immobilized animal for approximately 60–70 min. This level of anesthesia did not lead to mechanical ventilation and all animals tested were spontaneously breathing.

2.3. OAE recording methods

2.3.1. Transient evoked otoacoustic emissions

The TEOAE responses were recorded in a soundproof cabin by the ILO-292 (Otodynamics Ltd) appa-

ratus. The TEOAEs were evoked by a 80 μs click stimulus of 63 ± 2 dB p.e. SPL, following a non-linear protocol (Kemp et al., 1990) – a stimulus train composed of four clicks: three positive and one negative with an amplitude 9.5 dB higher than the positive clicks. Details of the experimental protocol have been presented in a previous publication (Hatzopoulos et al., 1999). For the analyses of the TEOAE data we have considered only the recording segment from 1.5 to 5 ms. The TEOAE responses were transformed by a fast Fourier algorithm and S/N estimates were calculated at 1.5, 2.0, 3.0, 4.0, and 5.0 kHz.

In order to record the TEOAE response, the anesthetized animal was placed under a stereotaxic device holding securely a neonatal ILO probe. The latter was connected to the external meatus of the right ear of each animal by a thin tube having a length of 35 mm and a diameter of 3 mm. A tight seal was possible when the tube was inserted on the average 5 mm into the external auditory meatus. The tube connecting the probe and the acoustic meatus of the animal is considered an acoustic filter attenuating high frequencies. In our experimental context this was not a problem, because the ILO apparatus already attenuates the TEOAE frequencies >5 kHz by 80 dB/decade. The reader should note though that according to previous studies (Withnell and Yates, 1998; Withnell et al., 1998) the high TEOAE frequencies might be expressed as low-frequency intermodulation distortion products, which lie into the passband of the ILO hardware.

Throughout the recordings the body temperature of each animal was maintained at 37 ± 0.5°C by a Harvard Apparatus homeothermic blanket.

For the TEOAE data visualization a special in-house software package was developed[2]. For the data analysis we used ILO software version 5.6.

2.3.2. Distortion product otoacoustic emissions

The DPOAEs were recorded in a sound-treated cabin with Starkey 2000 equipment. The bandwidth of the cubic DPOAE responses ($2f_1-f_2$) was set to a frequency range from 4.0 to 16.0 kHz (referenced to f_2) and 12 points were sampled per octave. In order to avoid errors introduced by the presence of standing waves in the external meatus, the analyzed DPOAE data were restricted to a frequency range from 4.0 to 10 kHz. The primary tone ratio f_2/f_1 was set to 1.21. Each record was the average of 4 s of sampling with a noise tolerance of −15 dB SPL. The responses were evoked by a non-symmetrical DPOAE protocol, using unequal primary tone stimulus intensities, i.e. $L_1 > L_2$. Such protocols are generally considered a better choice for the identification of cochlear dysfunction (Whitehead et al., 1995a,b, 1992). The protocols were defined as: high level ($L_1 = 60$ and $L_2 = 50$ dB SPL); and medium level ($L_1 = 50$ and $L_2 = 40$ dB SPL). DPOAE measurements were conducted only on the right ear of each tested animal.

Throughout the recordings the body temperature of each animal was maintained at a 37 ± 0.5°C by a Harvard Apparatus homeothermic blanket.

2.4. Blood pressure recording methods

Eight animals (four plus four) randomly selected from high- and normal-dose groups were monitored for blood pressure alterations (mean arterial pressure – MAP), during the anesthesia sessions. The animals were first anesthetized with the two-phase procedure described in a previous section. The right carotid artery of each animal was catheterized (polyethylene cannule PE-20). The cannule was connected to a pressure transducer (TNF-R from Ohmeda). The blood pressure and the cardiac frequency were monitored using a six-channel polygraph (Gould RS3600). For technical reasons the recording of OAEs and the MAP started at $T_0 = 15$ min.

2.5. Statistical analysis

To statistically model the data, two separate repeated measure mixed effect models were explored, one for the TEOAE and one for the DPOAE data sets. Repeated measure models were chosen in preference to models assuming a linear trend in time because initial data exploration indicated that the responses did not follow such a trend. Proc mixed in SAS statistical software was used to fit each model to the appropriate data using the restricted maximum likelihood method. (Laird and Ware, 1982). The AIC and BIC model selection criteria were used to choose the most appropriate within-animal correlation structure from among unstructured, generalized autocorrelation and compound symmetry structures. Residual analysis was performed to validate model assumptions. To identify significant differences among groups, *F*-tests were performed at the 0.05 significance level, with follow-up multiple comparisons conducted where needed using the Tukey-Kramer multiple comparison procedure, also at the 0.05 level.

The TEOAE model was defined as:

$$TE_{xjk} = \mu + A_x + S_{(x)j} + \beta W_{(x)j} + T_k + AT_{xk} + \varepsilon_{xjk}$$

[2] The TEOAE visualization software used in the present study (see Fig. 1) was developed by a scientific collaboration between the technical University of Warsaw, Poland, and the Department of Audiology of Ferrara University, Italy. The viewer uses the data already stored by the ILO software in the dta ILO files. The program can be downloaded for free, from the OAE Portal site address: http// www.oae.it/

where TE_{xjk} is the TEOAE measurement on animal j in animal type x at time point k; μ is the overall mean; A_x is the effect of animal type x; $S_{(x)j}$ is the effect of animal j in animal type x (random); $W_{(x)j}$ is the weight of animal j in animal type x (covariate); T_k is the effect of time interval k; AT_{xk} is the interaction of animal type x and time interval k; ε_{xjk} is the random error associated with observation TE_{xjk}. The ε_{xjk} are assumed to be independent, normally distributed random variables with mean 0 and variance δ^2.

For the DPOAE data the following model was used:

$$DP_{xjk} = \mu + A_x + S_{(x)j} + T_k + AT_{xk} + \varepsilon_{xjk} \quad (1)$$

where DP_{xjk} is the DPOAE measurement on animal j in anesthesia x at time point k; μ is the overall mean; A_x is the effect of anesthesia x; $S_{(x)j}$ is the effect of animal j in anesthesia x (random); T_k is the effect of time interval k; AT_{xk} is the interaction of anesthesia x and time interval k; $\varepsilon_{\mathrm{xjk}}$ is the random error associated with observation DP_{xjk}. The ε_{xjk} are assumed to be independent normally distributed random variables with mean 0 and variance σ^2.

2.6. Experimental design

To investigate the effects of anesthesia on the Sprague–Dawley rat, four main experiments were conducted:

1. **High-dose group** (Exp. 1): This experiment was conducted in order to validate previous experiences on ototoxicity studies, testing the hypothesis that additional doses of ketamine–xylazine anesthesia (which are frequently used in order to maintain the anesthesia level of a tested animal) could affect the TEOAE recordings. For this group the TEOAE measurements started at $T_{0=}5$ min after the first dose of anesthesia and then were recorded at 10, 20, 30, 40, 50 and 60 min post T_0.
2. **Normal-dose group** (Exp. 2): The goal of this experiment was the evaluation of whether the standard ketamine–xylazine dose alters significantly the DPOAE and TEOAE responses. OAE measurements were recorded in 10 min intervals, starting at $T_0 = 5$ min after the first anesthesia dose and ending at 60 min post T_0. The order of OAE acquisition (first DPOAEs or TEOAEs) was also randomized.
3. **TM group** (Exp. 3): The goal of the experiment was to provide additional information on a middle-ear mechanism, possibly involved with OAE alterations triggered by the administration of ketamine anesthesia (Zheng et al., 1997). It was expected that animals with perforated TMs should not exhibit any suppression of the DPOAEs due to a hypothetical increase of the negative middle-ear pressure. For this experiment we excluded data from the DPOAE frequency of 4.0 kHz, because according to previous studies by (Zhao et al., 2000; Voss et al., 2001), this frequency would have been resulted as the most affected by the TM perforation. The DPOAEs were recorded in 10 min intervals, starting at $T_0 = 5$ min after the first anesthesia dose and ending at 60 min post T_0.
4. **Atropine group** (Exp. 4): The goal of this experiment was the investigation of whether the ketamine–xylazine–atropine anesthesia alters significantly the DPOAE responses. Cochlear functionality was assessed by DPOAE recordings. The measurements were recorded in 10 min intervals, starting at $T_0 = 5$ min after the first anesthesia dose and ending at 60 min post T_0.

3. Results

3.1. High-dose group (Exp. 1)

Fig. 1 shows typical TEOAE responses from the high-dose group. The top panel A, shows the TEOAE response, stimulus waveform and TEOAE spectrum at time $T_0 = 5$ min. The bottom panel B shows the same data at a time $T_0 = 20$ min. To quantify the changes in the spectral structure of the TEOAEs we conducted a point by point correlation of the cross-spectrum FFT values for the times $T_0 = 5$ and 20 min. The overall correlation value was high (86%) but data from the frequency band between 1.5 and 3.5 kHz suggested a considerable spectral disagreement between the two TEOAE recordings (band correlation = 57%).

The effects of weight and time-under-anesthesia on the TEOAE S/N ratios at 1.5, 4.0 and 5.0 kHz were not significant, therefore these variables were not analyzed further. For the S/N at 2.0 kHz, the effect of time-under-anesthesia was close to significant ($P = 0.0528$). Significant time-under-anesthesia effects were observed on the mean TEOAE response, S/N ratio at 3.0 kHz and TEOAE correlation, with corresponding P-values 0.002, 0.009 and 0.019 respectively. In this context, the TEOAE response and the TEOAE S/N ratio at 3.0 kHz were the variables most affected by the high-dose anesthesia. Fig. 2 shows the profile of these two variables in the 60 min observation window. The TEOAE response (Fig. 2, top panel) reaches a minimum value at 20 min and then slowly recovers. We have coined this pattern as a 'decay and recovery' profile. The profile of the S/N ratio at 3.0 kHz (Fig. 2, bottom panel) also shows a 'decay' behavior approximately at $T_0 = 20$ min, but the recovery process appears to be much slower.

In terms of differences between means at different anesthesia times, the following results were obtained:

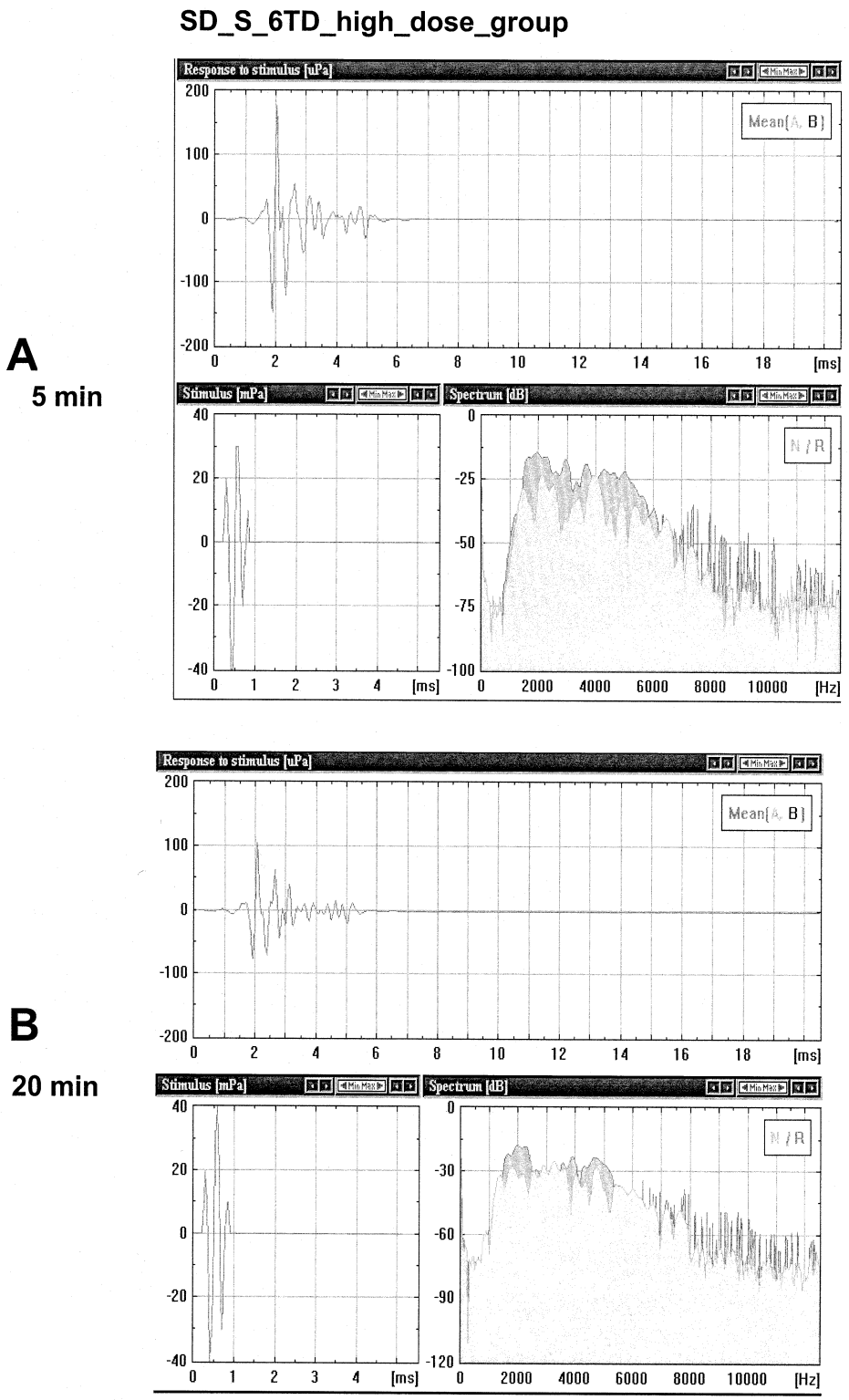

Fig. 1. TEOAE responses from animal SG_S_6TD (high-dose group). The top panel A shows (i) the TEOAE response; (ii) the stimulus waveform; and (iii) the response cross-spectrum at time $T_0 = 5$ min. In the cross-spectrum graph, the area presented with a dark color represents the TEOAE signal and the area presented with a light color represents the TEOAE noise. The bottom panel B shows data from a recording at $T_0 = 20$ min. Although the shape of the TEOAE response remains approximately unaltered in these two recordings, the magnitude of the signal at 2 ms shows a considerable reduction.

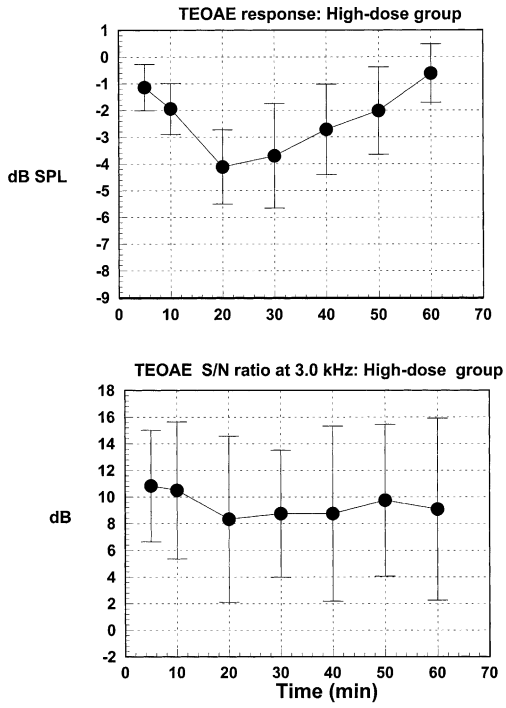

Fig. 2. Data from the high-dose anesthesia group, showing alterations of the mean TEOAE estimates in the time interval from 5 to 60 min. The vertical bars, over and below the data points, indicate 1 S.E.M. The top panel shows the means of the TEOAE responses and the bottom panel the means of the S/N ratio at 3.0 kHz. A number of outliers were identified in the residuals of the model fitting. In order to check the stability of the correlation model, the outliers were removed and the models were refit. As none of the results changed significantly, the reported results refer to the whole TEOAE data set.

(1) For the TEOAE response, the mean at anesthesia time $T_0 = 5$ min was greater than the means at time $T_0 = 20$ min (P-value = 0.041), 30 min (P-value = 0.006) and 40 min (P-value = 0.028); (2) for the S/N ratio at 3.0 kHz the mean at anesthesia time $T_0 = 5$ min was greater than the mean at time $T_0 = 40$ min (P-value 0.032).

3.2. Normal-dose group (Exp. 2)

The results from fitting a repeated measurement model on the DPOAE and TEOAE data sets of this group, failed to identify any significant anesthesia-time effects on the OAE variables. As it was expected, the means of the DPOAE variables from the higher stimulus protocol (60–50 dB SPL) were significantly greater than the means from the lower stimulus protocol (50–40) but none were sensitive to any time trends.

Fig. 3 shows the mean profiles of the TEOAE response and S/N ratio at 3.0 kHz from the normal-dose group. Decay and recovery profiles were also observed. The top panel of Fig. 3 shows that the mean TEOAE response reaches a minimum at 30 min and then slowly recovers. The mean profile of the S/N ratio at 3.0 kHz (bottom panel), shows a similar pattern reaching a minimum at 20 min and then slowly recovering. None of these variations were found to be statistically significant.

Fig. 4 shows DPOAE responses from a Sprague–Dawley rat, elicited from the 60–50 and 50–40 dB SPL protocols. The data refer to responses from the right ear of the animal, in the frequency range 4.0–11.0 kHz. In each panel the top traces represent the DPOAE levels at times equal to 5, 20 and 60 min post T_0 and the bottom traces to the corresponding levels of noise. For both protocols the growth of the top trace (DPOAE response at time = 60 min) indicates a recovery pattern. It should be noted that the responses at $f_2 = 11$ kHz were not used in the statistical analysis. They were included in the graphs in order to demonstrate more clearly, the recovery pattern of the DPOAE response at 60 min.

3.3. TM group (Exp. 3)

The DPOAE model was used to compare the DPOAE responses between the normal-dose and the TM group. For responses from the 60–50 stimulus: (1) At 4.0, 5.0, and 6.0 kHz, the profiles of the two groups are statistically coincident. The upper pair of

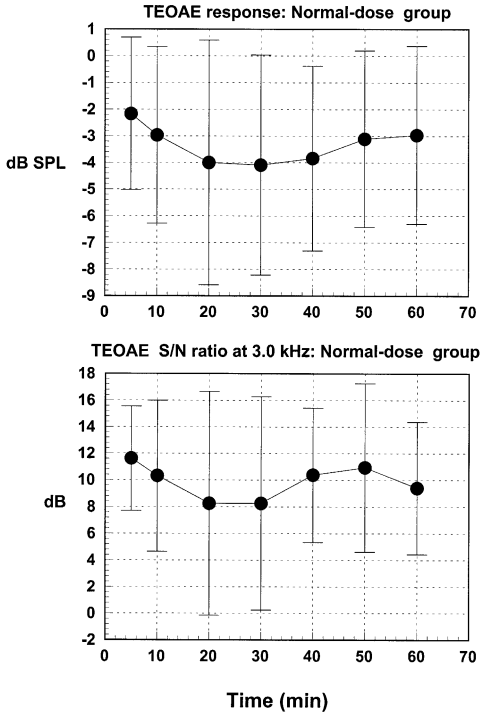

Fig. 3. Data from the normal-dose anesthesia group. The format follows the one shown in Fig. 2. The top panel shows the mean profile of the TEOAE response and the bottom panel the mean profile of the TEOAE S/N ratio at 3.0 kHz.

graphs in Fig. 5, which presents the mean profiles (±1 S.E.M.), illustrate this case. (2) At 8.0 and 10.0 kHz, the profiles exhibit a statistically significant interaction (i.e. are not parallel) due to greater differentials between the mean at time 60 and those at times 40 and 50 for the TM group, compared with the corresponding differentials for the normal group. In this context the 'recovery' portion of the 'decay and recovery' profile is stronger in the normal group than in the TM group. The lower two pairs of graphs in Fig. 5 illustrate this situation. For responses from the 50–40 stimulus the profiles of the two groups are statistically coincident at all frequencies.

3.4. Atropine group (Exp. 4)

The DPOAE model was also used to compare the DPOAE responses between the normal-dose and the atropine group. For responses from the 60–50 stimulus: (1) At 3.0 and 4.0 kHz, the profiles of the two groups are statistically coincident. (2) At 5.0 and 6.0 kHz, the profiles are statistically parallel, with a significant upward shift in the atropine group compared with the normal-dose group. The upper pair of graphs in Fig. 6 illustrate this case. (3) At 8.0 and 10.0 kHz, the profiles exhibit a statistically significant interaction due to greater differentials between the mean at time 60 and those at times 20, 30, 40, and 50 for the atropine group compared with the corresponding differentials for the normal group. As was the case for the TM group, the 'recovery' is stronger in the normal group than in the atropine group, as the lower two pairs of graphs in gr 6 illustrate. For responses from the 50–40 stimulus the profiles of the two groups are statistically coincident at all frequencies.

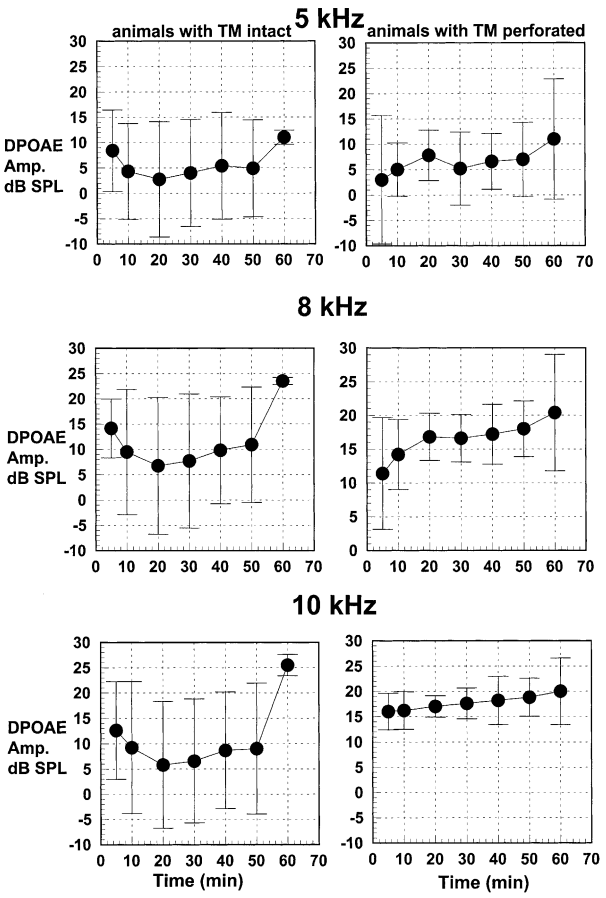

Fig. 5. Data summarizing the findings of Exp. 3, showing DPOAE responses (60–50 protocol) from animals with intact TMs (from the normal-dose group) and with perforated TMs at three f_2 frequencies. Each panel shows the mean DPOAE level (±1 S.E.M.) in the observation window from 5 to 60 min. The 'decay and recovery' pattern in the DPOAE responses of the normal-dose group, shown in the left-column panels, is not present in any of the right-column panels. The normal responses at $T = 60$ min show lower S.E.M. values than the other time points, because it was not possible to record data for 6 animals, who demonstrated signs of awakening.

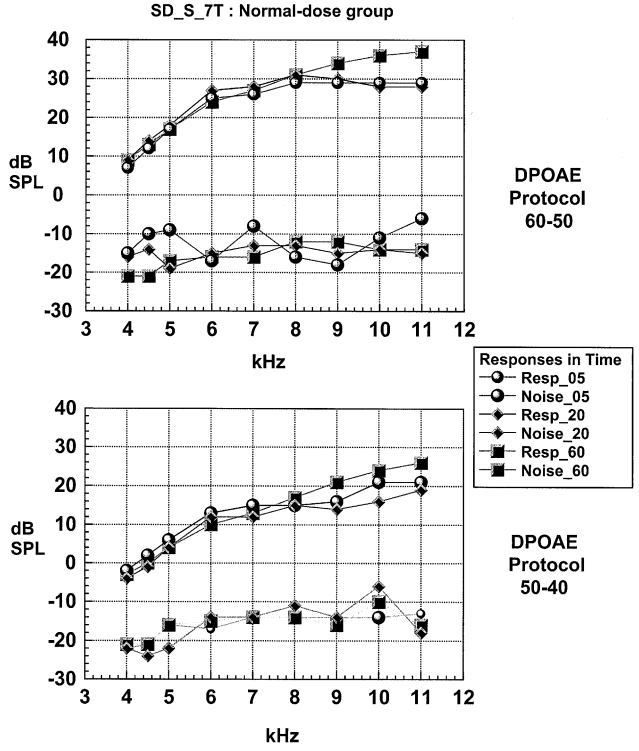

Fig. 4. Typical DPOAE responses from animal SG_S_7T. The top panel refers to responses from a 60–50 dB SPL protocol and the bottom panel to responses from a 50–40 dB SPL protocol. The y-axis shows frequencies referenced to f_2.

3.5. Mean arterial pressure

Guidelines for this experiment were based on previous unpublished telemetry data obtained in the laboratories of Fidia SpA, from awake Sprague–Dawley rats showing a MAP around 115 mm Hg.

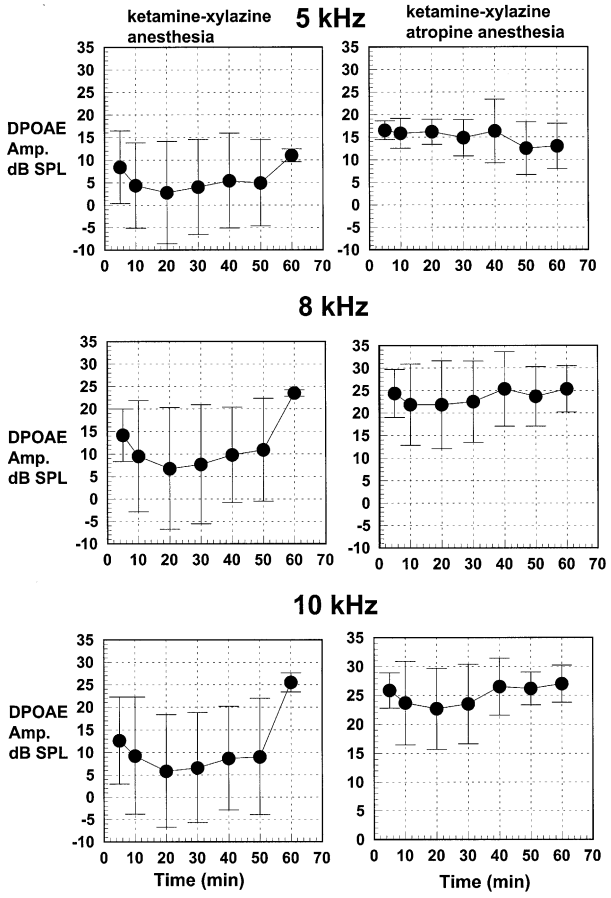

Fig. 6. Data summarizing the findings of Exp. 4, showing DPOAE responses (60–50 protocol) from the normal-dose group (left column) and from the atropine group (right column) in three f_2 frequencies. Each panel shows the mean DPOAE level (± 1 S.E.M.) in the observation window from 5 to 60 min. The responses from the atropine group do not present a strong 'decay and recovery' pattern, suggesting that this anesthetic mixture triggers less the middle-ear negative pressure mechanism. The normal responses at $T = 60$ min show lower S.E.M. values than the other time points, because it was not possible to record data for six animals, who demonstrated signs of awakening.

At time $T_0 = 15$ min the animals from the high-dose group presented a MAP of 100 ± 5 mm Hg. The pressure declined during the experiment with a rate of approximately 4 mm Hg/10 min. The average number of heart beats per min, during the 45 min observation (T_0 from 15 to 60 min) was estimated as 307 ± 14 beats.

The four animals from the normal-dose group showed an average MAP at time $T_0 = 15$ min, equal to 108 ± 6 mm Hg. The MAP declined during the experiment with a rate of 3 mm Hg/10 min. The average number of heart beats per min was estimated as 315 ± 20 beats.

No significant differences were found between the mean pressure values from the two anesthesia groups.

4. Discussion

Experiments in auditory physiology require that the tested animals should be immobilized. In most studies anesthetics are being used for that purpose. The principal objective of the study was the investigation of the possible effects of ketamine anesthesia on the TEOAE and DPOAE responses of the Sprague–Dawley rat. The results suggest that OAE responses from animals anesthetized with a normal-dose anesthesia (cumulative dose of 66.6 mg of ketamine and 13.2 mg of xylazine/kg) do not present any particular time trends. In contrary, the high-dose group animals which have received an additional subcutaneous anesthesia dose (cumulative dose of 50 mg of ketamine and 10 mg of xylazine/kg) present significant alterations of their TEOAE responses, approximately 20 min post the first intra-peritoneal anesthesia administration.

The data from the high-dose group (Exp. 1) suggest that increased doses of ketamine anesthesia affect the frequency structure of the TEOAE responses around 2.0 and 3.0 kHz. The hypothesis that anesthetic agents are altering the middle-ear dynamics, specially the eustachian tube function, has been verified in previous studies on pentobarbital (Zheng et al., 1997) and nitrous oxide (Chinn et al., 1997; Elam et al., 1998). These anesthetics induced a negative pressure in the middle ear. The relationship between negative middle-ear pressure and level of OAEs in human (Marshall et al., 1997; Konradsson et al., 1999; Avan et al., 2000) and animal models (Magnan et al., 1999) is now well established. For both human and animals the negative middle-ear pressure manifests as a frequency-specific attenuation of the OAE amplitude. The experimental data of the present study do not provide specific cues on the origin of the suppressive OAE mechanisms which are triggered by the administration of ketamine–xylazine anesthesia. Nevertheless, several factors point to a middle-ear mechanism: (i) the fact that lower TEOAE frequencies (2.0 and 3.0 kHz) were influenced more than higher frequencies; (ii) the fact that the animals from the TM group did not present any DPOAE-response profile characterized by a 'decay and recovery' pattern. An alternative hypothesis on the nature of the suppressing mechanism could be a process affecting the cochlea via an olivocochlear efferent activation. Data from a recent OAE study comparing efferent and middle-ear effects (Buki et al., 2000) have indicated that while the middle-ear manipulations present an OAE suppression at one or two peaks around the resonance frequency of the involved subsystem, the efferent effect exhibits a broadband-level OAE suppression. Within this context, an efferent mechanism affecting the cochlea should have attenuated the DPOAE responses

in a broader frequency range and not at two close-by frequencies (i.e. 2.0 and 3.0 kHz).

It was found that the normal-dose ketamine anesthesia does not induce any significant effects on the amplitude and spectral content of the DPOAEs and TEOAEs. The data in Fig. 5, showing data from animals with intact TMs, suggest that even at that anesthesia dose there is a negative middle-ear pressure build-up (see the delay and recovery profile of the DPOAE responses). Since no significant differences were found between the tested animals of the normal-dose group and the TM group, it must be deduced that the negative middle-ear pressure value was very small and the induced DPOAE alterations were not significant. These data indicate that when light ketamine–xylazine anesthesia is used, the prolongation of anesthesia by small quantities of anesthetic might increase the middle-ear negative pressure. In this context it is not suggested to conduct OAE experiments with anesthetized animals longer than 60 min.

The comparison of two anesthesia protocols indicated that the use of atropine generates significantly larger DPOAE responses. The data in Figs. 5 and 6 show that the DPOAE responses recorded from the atropine group are similar to the responses from the animals with perforated TMs. This observation suggests that the addition of atropine in the anesthetic protocol somehow inhibits the mechanism which gives raise to the negative middle-ear pressure. This point however needs further investigation.

The data from the high-dose group have indicated that the anesthesia effect on the TEOAE response and the TEOAE S/N ratio at 2.0 and 3.0 kHz reaches a peak approximately at 20 min post T_0. Similar observations have been reported in other studies from human subjects using inhalant anesthetics, such as nitrous oxide, propofol and isoflurane (Hauser et al., 1992; Ferber-Viart et al., 1998) and other animal models (Kettembeil et al., 1995). This observation suggests that despite the different means of anesthesia administration, the induced effects on the OAEs follow similar pathways. It is unclear, though, how the time constant (20 min) of the anesthesia effect is approximately the same in different auditory systems.

The effects of light anesthesia in a Sprague–Dawley animal model verify the data from the rabbit model reported by Lonsbury Martin et al. (1987) and the gerbil model reported by Zheng et al. (1997). In these studies, ketamine anesthesia did not produce any significant effects on the DPOAE responses. In contrast, Harel et al. (1997) found that light ketamine anesthesia (15 mg ketamine, 2.5 mg xylazine and 0.04 mg atropine/kg) increases the amplitudes of the OAEs in the chinchilla. In terms of TEOAE responses, Harel's study has reported (i) that the OAE recordings were acquired 15–20 min after the anesthesia administration and (ii) that significant (positive) changes of the TEOAE S/N ratio at 2.0 and 3.0 kHz were observed. According to the results of the present study these TEOAE variables were significantly reduced. We postulate that the reported differences between the chinchilla and the Sprague–Dawley rat model might have been the result of using different observation windows. In our study, we have recorded TEOAEs from a time $T_0 = 5$ min post-anesthesia and we have observed a 'decay and recovery' behavior (see data from Figs. 1 and 5) of the responses. Within this context, If our observation window would have shifted by 10–15 min we could have also described increasing TEOAE responses, as the TEOAE signals were recovering to an initial value. As a word of caution, the experimental data of the study provide information about the Sprague–Dawley rat strain and cannot be necessarily extrapolated to other species. It might be possible that anesthesia effects can be species specific and depend on how well the Eustachian tube remains patent during the anesthesia session.

The findings of this study can be summarized as follows:

1. Normal ketamine anesthesia (cumulative dose: 50 mg of ketamine and 10 mg of xylazine per kg) administered as 1 ml/kg i.p. and 0.5 ml/kg subcutaneously does not affect significantly the amplitude or the spectral structure of the TEOAE and DPOAE responses.
2. Increased subcutaneous doses of ketamine anesthesia attenuate significantly the TEOAE responses. The data suggest that the TEOAE frequencies of 2.0 and 3.0 kHz are more affected. The effect of the suppressing mechanism reaches a peak around 20 min and then slowly decreases (decay and recovery profile of the OAE response).
3. The use of atropine in the anesthetic protocol (ketamine–xylazine–atropine) results in DPOAE responses characterized by larger amplitudes in comparison to responses from ketamine–xylazine anesthetized animals, and lack of a 'decay and recovery' signal profile.

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References

Avan, P., Buki, B., Maat, B., Dordain, M., Wit, H.P., 2000. Middle ear influence on otoacoustic emissions. I: noninvasive investigation of the human transmission apparatus and comparison with model results. Hear. Res. 140, 189–201.

Brownell, W.E., 1990. Outer hair cell electromotility and otoacoustic emissions. Ear Hear. 11, 82–92.

Brownell, W.E., Bader, C.R., Bertrand, D., de Ribaupierve, Y., 1985. Evoked mechanical responses of isolated cochlear outer hair cells. Science 227, 194–196.

Buki, B., Wit, H.P., Avan, P., 2000. Olivocochlear efferent vs. middle-ear contributions to the alteration of otoacoustic emissions by contralateral noise. Brain Res. 852, 140–150.

Chinn, K., Brown, O.E., Manning, S.C., Crandell, C.C., 1997. Middle ear pressure variation: effect of nitrous oxide. Laryngoscope 107, 357–363.

Elam, M., Harell, M., Luntz, M., Fuchs, C., Sade, J., 1998. Middle ear pressure variations during 50% N2O anesthesia as a function of mastoid pneumatization. Am. J. Otol. 19, 709–711.

Ferber-Viart, C., Preckel, M.P., Dubreuil, C., Banssillon, V., Duclaux, R., 1998. Effect of anesthesia on transient evoked otoacoustic emissions in humans: a comparison between propofol and isoflurane. Hear. Res. 121, 53–61.

Harel, N., Kakigi, A., Hirakawa, H., Mount, R.J., Harrison, R.V., 1997. The effects of anesthesia on otoacoustic emissions. Hear. Res. 110, 25–33.

Hatzopoulos, S., Di Stefano, M., Albertin, A., Martini, A., 1999. Evaluation of Cisplatin ototoxicity in a rat animal model. In: Henderson, D., Salvi, R.J., Quaranta, A., McFadden, S.L., Burkard, R.F. (Eds.), Ototoxicity. Basic Science and Clinical Applications. Ann. N.Y. Acad. Sci. 884, 211–225.

Hatzopoulos, S., Di Stefano, M., Campbell, K.C.M., Falgione, D., Ricci, D., Rosignoli, M., Finesso, M., Albertin, A., Previati, M., Capitani, S., Martini, A., 2001. Cisplatin ototoxicity in the Sprague Dawley rat evaluated by Distortion Product Otoacoustic Emissions. Audiology 40, 253–264.

Hauser, R., Probst, R., 1991. The influence of systematic primary-tone level variation L_2-L_1 on the acoustic distortion product emission $2f_1-f_2$ in normal human ears. J. Acoust. Soc. Am. 89, 280–286.

Hauser, R., Probst, R., Harris, F.P., Frei, F., 1992. Influence of general anesthesia on transiently evoked otoacoustic emissions in humans. Ann. Otol. Rhinol. Laryngol. 101, 994–999.

Horner, K., Cazals, Y., 1989. Distortion products in early stage experimental hydrops in the guinea pig. Hear. Res. 43, 71–79.

Kemp, D.T., Ryan, S., Bray, P., 1990. A guide to the effective use of otoacoustic emissions. Ear Hear. 11, 93–105.

Kettembeil, S., Manley, G.A., Siegl, E., 1995. Distortion-product otoacoustic emissions and their anaesthesia sensitivity in the European starling and the chicken. Hear. Res. 86, 47–62.

Khvoles, R., Freeman, S., Sohmer, H., 1996. Transient evoked otoacoustic emissions can be recorded in the rat. Hear. Res. 97, 120–126.

Khvoles, R., Freeman, S., Sohmer, H., 1998. Development of Transient Evoked Otoacoustic Emissions in the neonatal rat. Audiol. Neuro-Otol. 3, 40–53.

Konradsson, K.S., Svensson, O., Carlborg, B., Grenner, J., 1999. Tympanic pressure gradients and otoacoustic emissions. Ear Hear. 20, 403–409.

Laird, N.M., Ware, J.H., 1982. Random-Effects Models for Longitudinal Data. Biometrics 38, 963–974.

Lonsbury Martin, B.L., Martin, G.K., Probst, P., Coats, A.C., 1987. Acoustic distortion products in rabbit ear canal. I. Basic features and physiological vulnerability. Hear. Res. 28, 173–189.

Magnan, P., Dancer, A., Probst, R., Smurzynski, J., Avan, P., 1999. Intracochlear acoustic pressure measurements: transfer functions of the middle ear and cochlear mechanics. Audiol. Neuro-Otol. 4, 123–128.

Marshall, L., Heller, L.M., Westhusin, L.J., 1997. Effect of negative middle-ear pressure on transient-evoked otoacoustic emissions. Ear Hear. 18, 218–226.

Mills, D.M., Norton, S.J., Rubel, E.W., 1993. Vulnerability and adaptation of distortion product otoacoustic emissions to endocochlear potential variation. J. Acoust. Soc. Am. 94, 2108–2122.

Shera, C.A., Guinan, J.J., 1999. Evoked otoacoustic emissions arise by two fundamentally different mechanisms: a taxonomy for mammalian OAEs. J. Acoust. Soc. Am. 105, 782–798.

Sochalingam, R., Freeman, S., Cherny, T.L., Sohmer, H., 2000. Effect of high-dose cisplatin on auditory brainstem responses and otoacoustic emissions in laboratory animals. Am. J. Otol. 21, 521–527.

Voss, S.E., Rosowski, J.J., Merchant, S.N., Peake, W.T., 2001. Middle-ear function with tympanic-membrane perforations. I. Measurements and mechanisms. J. Acoust. Soc. Am. 110, 1432–1444.

Whitehead, M.L., Lonsbury-Martin, B.L., Martin, G.K., 1992. Evidence for two discrete sources of $2f_1$-f_2 distortion product otoacoustic emissions in rabbit. II: Differential physiological vulnerability. J. Acoust. Soc. Am. 92, 2662–2682.

Whitehead, M.L., McCoy, M.J., Lonsbury-Martin, B.L., Martin, G.K., 1995a. Dependence of distortion-product otoacoustic emissions on primary levels in normal and impaired ears. I. Effects of decreasing L_2 below L_1. J. Acoust. Soc. Am. 97, 2346–2358.

Whitehead, M.L., Stagner, B.B., McCoy, M.J., Lonsbury-Martin, B.L., Martin, G.K., 1995b. Dependence of distortion-product otoacoustic emissions on primary levels in normal and impaired ears. II. Asymmetry in L_1, L_2 space. J. Acoust. Soc. Am. 97, 2359–2377.

Withnell, R.H., Yates, G., 1998. Enhancement of the transient-evoked otoacoustic emission produced by the addition of a pure tone in the guinea pig. J. Acoust. Soc. Am. 104, 344–349.

Withnell, R.H., Kirk, D., Yates, G., 1998. Otoacoustic emissions measured with a physically open recording system. J. Acoust. Soc. Am. 104, 350–355.

Zhao, F., Wada, H., Koike, T., Stephens, D., 2000. The influence of middle ear disorders on otoacoustic emissions. Clin. Otolaryngol. 25, 3–8.

Zheng, Y., Ohyama, K., Hozawa, K., Wada, H., Takasaka, T., 1997. Effect of anesthetic agents and middle ear pressure application on distortion product otoacoustic emissions in the gerbil. Hear. Res. 112, 167–174.

Zweig, G., Shera, C.A., 1995. The origin of periodicity in the spectrum of evoked otoacoustic emissions. J. Acoust. Soc. Am. 98, 2018–2047.