Evoked otoacoustic emission test: a standartization in guinea pigs

Artigo Original

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RESUMO

A descriçãco da execução do exame e da audição normal das cobaias não foram adequadamente estabelecidas até o momento. Objetivo: Como uma das características fundamentais da pesquisa científica é poder ser repetida e validada em seus resultados, este estudo experimental em cobaias tem como proposta avaliar a técnica de Emissões otoacústicas produto de distorção, assi como definir os padrões de normalidade para um número significativo de cobaias. Material e Método: 71 cobais de 2 a 3 meses de idade, pesando de 250 a 450g, num total de 140 orelhas foram estudadas. Todos os procedimentos foram realizados sob anestesia geral. Os animais foram submetidos A teste de emissão otoacustica produto de distorção e as respostas positivas e diferença sinal-ruído foram analisados estatisticamente. Resultados: A média da diferença sinal-ruído para cada freqüência foi 38,11 em 8KHz, 23,04 em 6KHz, 18,81 em 4KHz, 20,20 em 3KHz, 15,88, em 2KHz, 12,67 em 1,5 KHz, 8,70 em 1KHz and 5,44 em 750Hz. Conclusão: O presente estudo foi capaz de determiner dentro de intervalos de confiança aceitáveis a distribuição normal para produtos de distorção em cobaias.

Descritores: emissões otoacousticas, padronização, normal, cobaias

ABSTRACT

The description of the execution of the exam and the actual normal hearing in guinea pigs were not properly described until now. Objective: As one of the landmarks of the scientific research is the ability of any method to be repeated and be validated, this experimental study in guinea pigs has the purpose of evaluate the use of the technique of OAEDP, as well as to define its normality patterns for a representative sample of guinea pigs. Material and Method: 71 male guinea pigs, from 2 to 3 months of life, weighing from 250 to 450 g, in a total of 140 ears were studied. All procedures were accomplished under general anesthesia. The animals were submitted to otoacoustic emission distortion products test and the positive answer and the signal/noise differences were statistically analyzed with the purpose of normalization. Results: The average signal to noise difference for every frequency was 38,11 in 8KHz, 23,04 in 6KHz, 18,81 in 4KHz, 20,20 in 3KHz, 15,88, in 2KHz, 12,67in 1,5 KHz, 8,70 in 1KHz and 5,44 in 750Hz. Conclusion: The present study was able to determine within acceptable confidence intervals the normal distribution curve for the distortion products and for the signal/noise difference in guinea pigs.

Keywords: otoacoustic emissions, standardization, normal, guinea pig

INTRODUCTION

A good animal model should assist the specific characteristics of the study that it intends to accomplish, as well as should keep the similarity in relation to the human's anatomy and physiology. Otolaryngology has adopted the guinea pig as one of its favorite experimental animal for the study of the ear, since it presents a hearing system similar to the human being.

The studies of the hearing function of the guinea pigs began in 1982, with the definitions of the characteristics of the normal acoustic reflexes. Due to the easy access and comfortable manipulation of the structures of the ear, with fair exposition of the internal and middle ears, the guinea pigs are often used as experimentation animals, especially in physiology and physiopathology research 1,2,3,4,5,6,7. There are several sophisticated exams to evaluate the hearing, in a quantitative and qualitative way. One of the tests used is the otoacoustic emissions test (OAE).

The measuring of OAE has the advantage of been fast, easy, objective and sensitive to any cochlear hearing loss. This test is of very recent clinical application, waking up an interest every time larger to the clinician. Kemp et al (1986) 8 were the first ones to enumerate the practical applications of OAE: a) as a cochlear screen test in neonates and small children; b) to establish otoacoustic basal parameters in patients with cochlear diseases, detecting early alterations; c) to contribute for the differential diagnosis of certain diseases that affect the hearing.

The OAE can be classified in: spontaneous, evoked transient and evoked distortion products (OAEDP).

OAEDP is used to examine specific frequencies. It measures the function of outer hair cells of the cochlea and informs not only about the amount of the hearing lost but about the quality of the loss. The majority of studies of the guinea pigs hearing through OAE was accomplished to evaluate the effects of drugs over the hearing 9,10,11. However, the description of the execution of the exam and the actual normal hearing in guinea pigs were not properly described until now, jeopardizing one of the landmarks of the scientific research that is the ability of any method to be repeated and validated.

This experimental study in guinea pigs has the purpose of evaluate the use of the technique of OAEDP, as well as to standardize it in a representative sample of guinea pigs.

MATERIAL AND METHOD

The animals studied were white male guinea pigs, from 2 to 3 months of life, weighing from 250 to 450 g.

The study was approved by the Animal Research Ethical Committee of our Institution. All the animals were submitted to the procedure in agreement with the technique described, by the same researcher. The animals were appraised in several periods of the day. The exams were accomplished in both ears. There were 140 ears under study, 70 of each side.

The equipment used was Scout Otoacoustic Emissions System, Version 3.22.00 (1994-2001 Bio-Logic Systems Corp) coupled to a portable computer Compaq Presario 1200. The available manufacturer protocol was used automatically: OAEDP 750-8000 Hz Diagnostic Test, with the following collection parameters: frequency at the beginning/end: 8000/750 Hz; F2/F1 ratio: 1.2; points for octave: 2; intensity of F1 (dB SPL): 65; intensity of F2 (dB SPL): 55. The Stop criteria were: minimum width (dB SPL): -5; basal noise (dB SPL): -17; signal/noise ratio (dB SPL): 8; Limit of time (seconds): 20.

Procedure

The animals were submitted to general anesthesia, with intraperitoneal injection of Xilazine (10mg/kg) and Ketamine (40 mg/kg), staying under spontaneous ventilation. Once anesthetized, all the animals had their ears examined bilaterally. They were positioned in a surgical table and with help of an optical microscope the evaluation of the external acoustic meatum and of the tympanic membrane was accomplished. The external ears were clean and the wax removed with a curette before the exam. The ears presenting any anatomic alteration were excluded of the study (2 ears).

The exam was accomplished with the animal in ventral position, with the mentum leaning over the surgical table. The acoustic pavilion was pulled upward and backward, in order to expose the cartilaginous and osseous meatum.

After the exposition, exam and preparation of the external ear, a probe of silicon, suitable for the manufacturer for use in neonates, was cut until the free border coincided with the end of the probe, introduced and maintained in the external ear in a perpendicular position to the eardrum. During the test, the pavilion was kept in the lateral-posterior direction by the examiner. The correct positioning of the probe and pavilion was determined by the automatic calibration of the apparel, repeated until the obtaining of acceptable conditions.

The following parameters were analyzed: width of the distortion products and the signal/noise ratio.

Statistical analysis

The significance level accepted was 5%. To verify which frequencies showed a normal distribution, an Anderson-Darling test was applied. A descriptive analysis of the results was made.

For the distortion products the existent differences among ears were only significant in the frequency of 1500 Hz ($p=0.033$), for the other frequencies the differences were not significant ($P>0.05$). Also to the signal to noise difference, the existent differences among the ears were not significant ($P>0.05$).

RESULTS

The results obtained and their descriptive statistics are exposed in the Tables 1 and 2.

Table 1 – EOAPD distribution in frequencies of 750, 1000, 1500, 2000, 3000, 4000, 6000 and 8000 Hz.

Distortion Product	8.000 Hz	6.000 Hz	4.000 Hz	3.000 Hz	2.000 Hz	1.500 Hz	1.000 Hz	750 Hz
Average	27,65	12,42	8,72	5,99	4,91	6,64	9,74	8,13
Median	31,25	13,70	9,70	5,75	5,55	7,80	11,35	8,80
Standard deviation	7,14	13,20	10,14	9,22	8,73	8,74	5,97	6,67
1st Quartile	18,88	8,48	2,65	0,68	1,08	3,38	5,33	2,93
3rd Quartile	38,28	19,43	15,53	11,53	10,20	10,53	14,48	12,73
N	140	140	140	140	140	140	140	140
Kurtosis	0,62	1,73	0,07	0,64	2,44	1,13	0,09	0,95
CI	2,19	1,68	1,53	1,45	1,45	0,99	1,10	1,18

Legend: CI – confidence interval; N – Sample size

Table 2 - Signal to noise differences in frequencies of 750, 1000, 1500, 2000, 3000, 4000, 6000 and 8000 Hz.

Signal to Noise Difference	8.000 Hz	6.000 Hz	4.000 Hz	3.000 Hz	2.000 Hz	1.500 Hz	1.000 Hz	750 Hz
Average	38,11	23,04	18,81	20,20	15,88	12,67	8,70	5,44
Median	40,80	23,75	19,25	20,15	14,85	11,40	9,20	8,00
Standard deviation	13,28	9,81	8,04	9,43	8,45	5,48	6,27	6,47
1st Quartile	28,95	16,03	11,85	12,48	11,05	8,90	8,08	1,00
3rd Quartile	49,05	29,33	25,03	26,33	21,68	15,63	11,23	9,48
N	140	140	140	140	140	140	140	140
Kurtosis	-0,33	0,30	-0,53	0,03	1,97	0,35	1,97	0,24
CI	2,20	1,62	1,33	1,56	1,40	0,91	1,04	1,07

Legend: CI – confidence interval; N – Sample size

DISCUSSION

References on detailed procedures for obtaining the OAEDP and on the results of OAEDP in normal guinea pigs are scarce. Therefore, we described the results of OAEDP in guinea pigs starting from a certain method, in order to supply reliable reference for other studies.

In this study only male guinea pigs were used, thus some studies did not show significant differences among the sexes, such as the ones of Kakigi et al (1998)9 and Ranieri et al (2001)10. The comparative analysis of the right and left ears, accomplished by the test of Wilcoxon, did not show statistical difference, so the data of both ears was analyzed as a whole.

The usual concern with the appropriate sealing of the external ear, due to the fact that it increases the basal noise and, consequently, harm the quality of the exam, could be avoided pulling the pinna backward. According to Kemp (1978) 11 and Lopes Filho et al (1996) 12, the good positioning and size of the probe guarantee the stimulus is directly addressed to the eardrum, making the exam more quickly.

Many are the elected frequencies by the researchers in their experiments, Lopes Filho et al (1995)13 adopt the frequencies ranging from 500 to 8000 Hz, and Vallejo et al (1998)14 chose the range 1000 Hz - 6300 kHz. In our intention of offering larger reliability to the present study, it was adopted the frequencies more tested in other experiments, reason for the use of frequencies of 750 Hz, 1000 Hz, 1500 Hz, 2000 Hz, 3000 HZ, 4000 Hz, 6000 Hz and 8000 Hz.

Nishino et al (2001) 15 in experiments with humans, stated the primary levels are more effective when L1>L2. The intensity of stimulus in 65/55 dB SPL, for L1/L2, was described by Gorga et al (1996) 16 as being the most efficient to distinguish ears without alteration from those presenting dysfunction. In this study the F2/F1 ratio was 1.2. Abdala (1996) 17 accomplished study to define the best F2/F1 ratio and it ended with a 1.22 ratio. Nielsen et al (1993) 18 for their time considered the ideal F2/F1 ratio among 1.20 to 1.25. The same value was found in the studies accomplished with animals, according to Kakigi et al (1998)9, Emmerich et al (2000) 19, Sockalingam et al (2000) 20 and Hyppolito et al (2005) 21.

We observed the noise of the system was higher in low frequencies, but did not harm the exam in any way. It was possible to obtain answers in 99,28% of the ears studied in the frequency of 8000 Hz; 97,14% in 6000 Hz; 95,71% in 4000 Hz; 97,14% in 3000 Hz; 95% in 2000 Hz; 95,71% in 1500 Hz; 80,71% in 1000 Hz; and 57,14% in 750 Hz. The signal/noise difference varied in average from 5.44 dB SPL in 750 Hz to 38.11 dB SPL in 8000 Hz.

To verify which frequencies possessed a normal distribution we applied the test of Anderson-Darling. We observed that the frequencies 4000 Hz (p=0.59) and 6000 Hz (p=0.36) had normal distribution curves for the distortion products and signal/noise ratios, without deviations or asymmetries of the curve of distribution. In the other hand, the frequencies of 750, 1000, 1500, 2000, 3000 and 8000 Hz presented curves considered not normally distributed. However the confidence intervals for the average were small and similar in their values, showing trustworthy and solid curves in the general. The distortion products became larger in agreement with the increase of the studied frequency, arriving to 27.65 dB SPL, the largest value, in 8000 Hz.

Also through the test of Anderson-Darling, we concluded the curve of distribution of the data in the frequencies of 750 Hz (p=0.10), 3000 Hz (p=0.41), 4000 Hz (p=0.06) had a normal distribution curve for distortion products, without deviations or asymmetries. The frequencies 1000, 1500, 6000 and 8000 Hz presented curves considered not normal. However, analyzing the confidence intervals, we observed results very close to the average, which statistically reveal consistence and reliability of the obtained results.

The best answer collected was the one 6 dB above the basal noise and was found at the higher frequencies. Such fact suggests that the best frequencies to be tested in guinea pigs are the one of 6000 Hz and 8000 Hz, what is in agreement with the study of Ranieri et al (2001) 10 where was observed better answers in the frequencies of 4000 and 6000 Hz; and of Poyatos et al (2002) 5 that found better answers among frequencies 8 to 16 kHz.

As observed by Gorga et al (1996) 16 that recommend the signal/noise difference above 6 dB to be the one to be trusted, we could notice that the frequency of 750 Hz was the one that presented smaller frequency of answers (57.14%) in this range, and should be avoid in studies of hearing function in guinea pigs.

The results presented as expected average of the answers of the distortion products and differences signal/noise, with a significance level of 5%, and their respective standard deviation for the confidence interval of 95%:

Distortion products -1000 Hz: 9,74 dB SPL (1,10); 1500 Hz: 6,64 dB SPL (0,99); 2000 Hz: 4,91 dB SPL (1,45); 3000 Hz: 5,99 dB SPL (1,45); 4000 Hz: 8,72 dB SPL (1,53); 6000 Hz: 12,42 dB SPL (1,68) and 8000 Hz: 27,65 dB SPL (2,19).

Signal/noise difference - 1000 Hz: 8,70 dB SPL (1,04); 1500 Hz: 12,67 dB SPL (0,91); 2000 Hz: 15,88 dB SPL (1,40); 3000 Hz: 20,20 dB SPL (1,56); 4000 Hz: 18,81 dB SPL (1,33); 6000 Hz: 23,04 dB SPL (1,62) and 8000 Hz: 38,11 dB SPL (2,20).

CONCLUSION

The present study was able to determine within acceptable confidence intervals, the normal distribution curve for the distortion products and for the signal/noise difference in guinea pigs.

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