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ORIGINAL ARTICLE

Assessment of the Effects of Different Sound Pressure Levels on Distortion Product Otoacoustic Emissions (DPOAEs) in Rats

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ABSTRACT

Excessive exposure to noise can lead to Noise-Induced Hearing Loss. Otoacoustic emissions affect the microscopic biomechanical activities of healthy outer hair cells. The present study aimed at assessing the influence of various sound pressure levels on Distortion Product Otoacoustic Emissions (DPOAEs) in rats. To this end, 27 adult male rats with an age range of 3 to 4 months and a weight of 200 ± 50 g were randomly divided into nine groups of three. Three groups were considered as the control groups and the rest (i.e. Six groups) as the case groups. Rats of the case groups were exposed to sound pressure levels of 85, 95, and 105 dBA. White noise was used as the noise to which the rats were exposed. The signal to noise ratio (SNR) of otoacoustic emissions of rats' ears was measured at different frequencies in an acoustic room using a DPOAE machine (4000 I/O manufactured by Homoth of Germany). The collected data were analyzed by the use of Statistical Package for Social Sciences (SPSS) version 18. The results of SNR measurement indicated that over 90% of the data had SNR values of 6dB or more. Furthermore, sound pressure level had a significant negative correlation with SNR, i.e. as the sound pressure level increased, the SNR declined ($p<0.001$). There was also a significant negative correlation between exposure time and SNR, meaning that increase in the exposure time led to decline in the SNR ($p=0.008$). It is thus concluded that higher sound pressure levels result in decrease in DPOAE levels.

KEYWORDS: *Noise, Hearing loss, Otoacoustic emissions, DPOAE, Rat*

INTRODUCTION

As one of the global profession-related health problems, exposure to excessive noise has measurable social and psychological impacts.

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One of these consequences is known as noise-induced hearing loss (NIHL) [1-5], which refers to a hearing impairment that is caused by excessive exposure to noise in the course of time. NIHL leads to bilateral and symmetrical impairment of hearing [6].

The ear has three major parts: the outer ear, the middle ear, and the inner ear [7], with the latter being mostly affected by exposure to high noise levels. NIHL causes mechanical and metabolic changes in the inner ear. These mechanical damages, which occur in delicate parts of the outer hair cells (OHCs) in the cochlea, are the result of high energy transfer, a phenomenon that causes anatomical and physiological consequences of overstimulation of the inner ear. Furthermore, high energy transfer results in metabolic stress within the endolymphatic fluids of the cochlea, which in turn leads to swelling and degeneration of the eighth nerve terminals attached to the inner hair cells [8-9]. Otoacoustic emissions (OAEs) are the consequence of microscopic biochemical activities of healthy outer hair cells (OHCs). These activities provide mechanical stimuli in the cochlea that move from the tympanum to the outer ear and are reflected in the auditory canal [10]. OAEs that are produced by DPOAE display the performance of the OHCs and are considered a valid test in evaluating changes in the cochlea [11-12]. As an objective and non-aggressive test, DPOAE utilizes the features of frequency sensitivity to assess otoacoustic damage [13]. In the current study, the otoacoustic performance of the cochlea was assessed by the use of DPOAE test. DPOAE is measured by recording the emissions made and reinforced in the cochlea by specific frequencies (f1 and f2) [14]. SNR is used to indicate the difference between the measured OAE and the background noise level. That is, positive SNR indicates a considerable response over the background noise [15-16]. Therefore, in the light of the influence of sound pressure level on OAEs, the present study was designed to:

1- Assessing the SNR of DPOAEs in rats' ears when they are exposed to various sound pressure levels
2- Comparing the changes in mean SNRs of various frequencies in different exposure times
3- Introducing the statistical model of the SNR of DPOAEs in rats

MATERIALS AND METHODS

Experimental animals: For the purpose of this study, twenty-seven adult male Sprague-Dawley rats were purchased from Pasteur Research Institute. Their age ranged from 3 to 4 months and they weighed 200 ± 50 g. They were kept in the animal unit of the School of Health, Tehran University of Medical Sciences until the study began. The rats were exposed to a photoperiodic cycle of 12 hours of light phase and 12 hours of dark phase, with the temperature being around 23±2°C. They had free access to water and food for animals. All the principles of the Declaration of Helsinki about conducting experiments on laboratory animals were observed. Before the study, the health of rats' auditory system was investigated by creating a specific sound around the rats' ear. If the rats were stimulated as a result of being exposed to the sound, it indicated that their ears were healthy. Conversely, if no stimulation was observed in rats, it was a sign for some defect in their auditory system. Rats with problematic auditory systems were excluded from the study through this screening process.

Instruments for noise exposure: Experiments on animals were conducted in a four-cell chamber with high efficiency. It was an echo chamber with the dimensions of 40×50×60 cm. In this echo chamber, sound energy was equally distributed in all directions, meaning that animals would receive an equal amount of sound no matter where they were in this chamber. During the exposure time, the room air must be replaced at a rate of 12 times per hour (based on recommended conditions for taking care of animals) [17]. Therefore, a ventilation with a flow of 24 liters per minute was installed in the chamber. This ventilation consisted of an environmental pump and a flow meter used to control the flow rate. During the experiment, the chamber's temperature was 25±2°C and the moisture content was 50%. It should be noted that, in each cell, there were three rats that were equally exposed to noise.

The software and source of noise generation: First of all, Signal was used to produce white noise. Cool Edit Pro (version 1-2, manufactured by Syntrillium Software Corporation in the United States in 1999-2000) was utilized to play the noise files. Two speakers (PROBIT, manufactured in Iran) were used to generate the noise. The speakers had input-output resistance of impedance: 4 (ohms) power: 5 (W) that was directly amplified through an amplifier (model ES-2000s, ES Audio Industrial Corporation) made in Taiwan. The speakers were positioned in the chamber ceiling in a way that they were symmetrical compared to the center of the ceiling.

Organization of experimental groups: In this study, twenty-seven rats were randomly assigned to nine groups, each one containing three rats [18]. The status of each group (control and case) has been explained in the following sections.

Control group: The control group consisted of three sub-groups whose features have been demonstrated in Table 1.

Table 1. Different sub-groups of rats in the control group

Sound pressure level (dB)	Exposure time (h)
	Before exposure
65	3
	8

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Case group: The rats in the case group were exposed to SPLs of 85, 95, and 105 dB. The sound to which they were exposed was white noise. The features of the six subgroups of the case group are illustrated in Table 2.

Table 2. Different sub-groups of rats in the case group

Sound pressure level (dB)	Exposure time (h)
85	3
	8
95	3
	8
105	3
	8

Noise *Measurement:* The sound pressure level in the four-cell chamber was measured by the use of a sound level meter (CEL-440, CASELLA, USA). This machine is equipped with octave parser and is thus able to show the SPL in octave band centers. Before using the machine, we calibrated it by the CEL-282 calibrator (CASELLA, USA). In each cell of the chamber, SPL was randomly measured in different spots.

Measuring DPOAEs: Rats should be unconscious in order to assess their DPOAEs. Two types of drugs (Ketamine and Xylazine) were used to anesthetize the rats, with a proportion of 60% to 40%. Three mL of this mixture was injected inside the peritoneum using insulin syringe. OAEs in animal phase were measured by the use of a DPOAEs machine (DPOAE 4000 I/O manufactured by Homoth of Germany) in the following frequencies: 6562.5 Hz, 5437.5 Hz, 3937.5 Hz, 2062.5 Hz, 750 Hz, 1125 Hz, 562 Hz, and 375 Hz. These measurements were conducted in the acoustic room of the physical factors laboratory of the School of Public Health, Tehran University of Medical Sciences. The ratio of sounds emitted to rats' ears was f1/f2=1/22, while their intensity were L1=65 dB and L2=55 dB. Distortion-product otoacoustic emission - noise floor (DP-NF) was also used to calculate SNRs for the three groups. The SNR of 6dB or greater was considered as the inclusion criterion. Before conducting DPOAE tests, the researchers made sure that the following prerequisites were met: (1) the external ear should not be obstructed; (2) the probe should be inserted property in the ear canal; and (3) the probe should be positioned properly inside the ear canal.

Statistical analysis: Statistical Package for Social Sciences (SPSS) version 18 was utilized for data analysis. After summarizing the data through descriptive indices, Shapiro-Wilk test was conducted to check the normality of distribution. Then, both within and between groups repeated measure analysis of variance (ANOVA) were employed to find if there were any significant differences among various groups. Finally, Tukey test was conducted to detect significant mean differences among various groups. The P-value was considered to be smaller than 0.05.

Ethical considerations: The ethical considerations of this research were approved by the Ethics Committee of Tehran University of Medical Sciences) ID: 1394.5 (. Moreover, all the principles of the Declaration of Helsinki about conducting experiments on laboratory animals were observed.

RESULTS

The results of SNR of DPOAEs in rats: Table 3 shows the SNR of control group rats' DPOAEs at the beginning of the experiment as well as 3 and 8 hours after exposure to the noise. The same table also displays the SNR after 3 and 8 hours of conducting the experiment among case group rats, which were exposed to 85, 95, and 105 dB white noise.

The results of comparing SNR mean changes in the light of various frequencies and various exposure times: Fig.1. shows SNR mean changes in various frequencies of the control group (65 dB) in three exposure times (before exposure, 3 hours after exposure, and 8 hours after exposure). It is indicated that as the exposure time increases, significant changes are not made to the SNR in various frequencies.

Figs. 2 through 4 show the mean changes in SNR in in various frequencies of the case groups (65 dB) in different exposure times (3 hours and 8 hours after exposure). It is observed that as exposure time goes up, SNR mean declines in various frequencies.

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Table 3. Mean and standard deviation of SNR among the three control groups and the six case groups

Sound pressure level (dB)	Exposure time (hour)		Frequency (Hz) 375	562	750	1125	2062.5	3937.5	5437.5	6562.5
65 (control group)	Before exposure	Mean	9.16	10.10	11.20	12.24	14.14	19.26	16.27	13.10
		SD*	0.30	0.21	0.05	0.15	0.12	0.17	0.14	0.10
	3	Mean	8.65	9.55	11.00	12.05	14.06	19.10	16.2	12.90
		SD	0.05	0.20	0.03	0.07	0.05	0.18	0.20	0.06
	8	Mean	8.12	9.14	11.20	11.91	13.95	19.00	16.07	12.45
		SD	0.11	0.06	0.07	0.13	0.032	0.10	0.06	0.07
85 (case group)	3	Mean	7.93	8.70	9.40	10.30	11.42	17.28	12.17	9.33
		SD	0.59	0.37	0.24	0.40	0.19	0.34	0.17	0.22
	8	Mean	6.91	7.76	9.13	9.50	10.48	16.53	11.27	8.66
		SD	0.25	0.29	0.05	0.20	0.26	0.17	0.50	0.43
95 (case group)	3	Mean	6.84	8.14	8.00	8.89	10.10	14.7	11.40	8.20
		SD	0.51	0.18	0.11	0.29	0.27	0.19	0.32	0.34
	8	Mean	6.88	7.20	7.20	7.91	9.39	13.73	10.49	7.15
		SD	0.42	0.15	0.15	0.44	0.21	0.35	0.59	0.14
105 (case group)	3	Mean	6.44	7.93	7.35	7.80	9.15	12.74	8.78	7.28
		SD	0.32	0.33	0.38	0.21	0.11	0.20	3.66	0.22
	8	Mean	7.82	6.44	7.05	7.36	8.41	11.80	9.38	7.08
		SD	0.62	0.38	0.13	0.32	0.38	0.40	0.39	0.65

**** SD: Standard deviation***

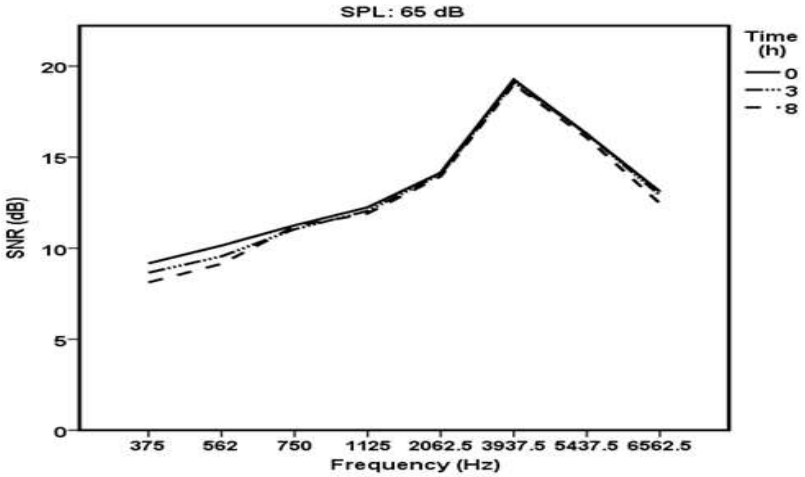

Fig.1. SNR mean changes in various frequencies of the control group (65 dB)

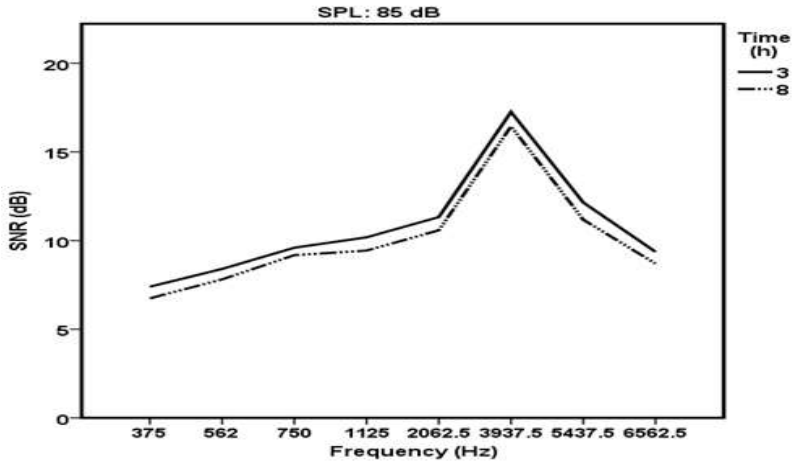

Fig.2. SNR mean changes in various frequencies of the case group (85 dB)

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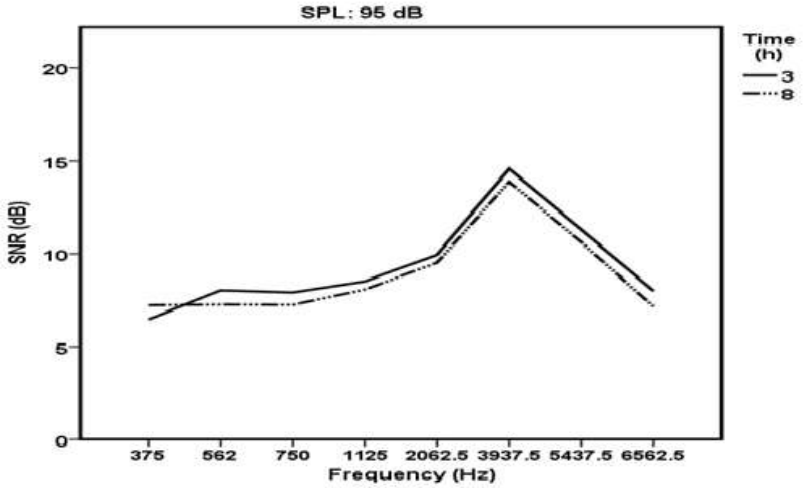

Fig.3. SNR mean changes in various frequencies of the case group (95 dB)

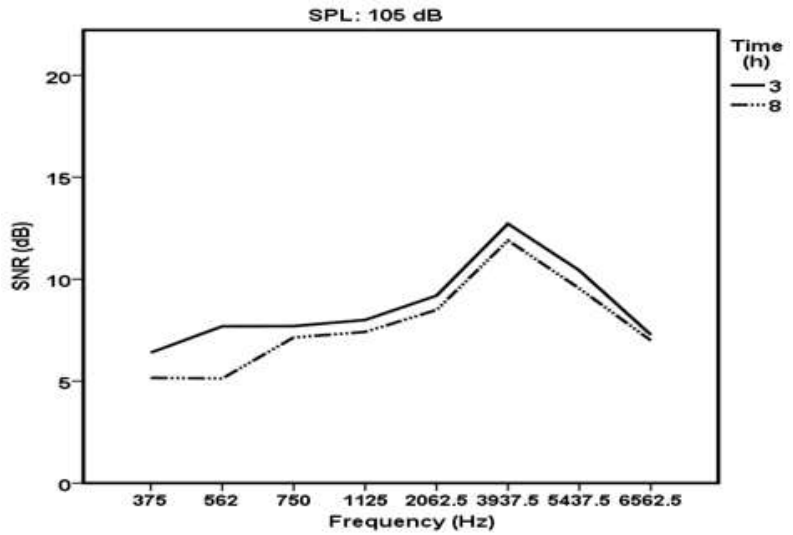

Fig.4. SNR mean changes in various frequencies of the case group (105 dB)

The results of investigating the significant mean differences in SNRs of various sound pressure levels: ANOVA was conducted to see if there was any significant difference in the SNR of various sound pressure levels. According to the results:

After 3 hours of exposure, significant differences were observed among the frequencies of 6562.2 Hz, 5437.5 Hz, 3937.5 Hz, 2062.5 Hz, 750 Hz, 1125 Hz, 562 Hz, and 375 Hz) in various sound pressure levels (65, 85, 95, and 105 dB).

After 8 hours of exposure, significant differences were observed among the frequencies of 6562.2 Hz, 5437.5 Hz, 3937.5 Hz, 2062.5 Hz, 750 Hz, 1125 Hz, 562 Hz, and 375 Hz) in various sound pressure levels (65, 85, 95, and 105 dB).

In order to detect significant differences between various means, Tukey was conducted as the post hoc test. Based on the results:

After 3 hours of exposure: significant SNR mean differences existed between frequencies of 6563.6 Hz, 5437.5 Hz, 2062.5 Hz, 750 Hz, and 1125 Hz in sound pressure levels of 85, 95, and 105 dB. In the frequency of 562 Hz, a significant difference was observed between sound pressure levels of 105 dB and 85 dB (P=0.04). However, no significant difference was seen between sound pressure levels of 105 dB and 95 dB (P=0.08). In the frequency of 375 Hz, a measurable difference was detected between sound pressure levels of 105 and 85 dB (P=0.15). In contrast, no significant difference was observed between sound pressure levels of 105 dB and 95 dB (P=0.95).

After 8 hours of exposure: in the frequency of 6562.5 Hz, a significant difference was observed between sound pressure levels of 105 dB and 85 dB (P=0.01). However, no considerable difference was detected between sound pressure

levels of 105 dB and 95 dB (P=0.77). In the frequency of 5437.5 Hz, a measurable difference was observed between sound pressure levels of 105 dB and 85 dB (P=0.009). On the contrary, no significant difference was detected between sound pressure levels of 95 dB and 85 dB (P=0.32). In the frequencies of 3937.5 Hz, 2062.5 Hz, and 1125 Hz, significant differences were observed between sound pressure levels of 85, 95, and 105 dB. In the frequency of 750 Hz, a measurable difference was observed between the sound pressure levels of 105 dB and 85 dB (P=0.01). In contrast, no significant difference was detected between the sound pressure levels of 105 dB and 95 dB (P=0.26). In the frequencies of 562 Hz and 370 Hz, significant differences were observed between the sound pressure levels of 105 dB, on the one hand, and 85 and 95 dB, on the other hand.

The statistical model of the SNR of DPOAEs in rats: The statistical model of SNR in the light of sound pressure level:

SNR (dB)= 20.87-0.123 SPL
SPL= sound pressure level (dB)
The statistical model of SNR in the light of exposure time:
SNR (dB)= 11.27-0.254Time
Time: Exposure time (hour)

DISCUSSION

In this study, 27 rats were randomly assigned to nine groups of three (three control groups and six case groups).

In order to record DPOAE (2f1-f2), two signals, i.e., f1 and f2 (with f2 > f1) were used. The f2/f1 ratio was kept at 1.22, and the levels of these signals were L1 = 65 dB and L2 = 55 dB. The SNR ≥ 6 also was considered as the inclusion criterion [19-20].

Attias et al. (2001) claimed that DPOAE test appropriately shows the changes in the case group (compared to the control group). Thus, it is a suitable test for assessing the performance of cochlea [21]. In addition, Vinck et al. (1999) concluded that noise exposure causes significant changes in DPOAE and TEOAE. Therefore, they stated that these tests can be used in order to evaluate the performance of cochlea [20]. The above mentioned studies clearly indicate the validity of DPOAE test. As a result, this test was used in the current study.

SNR measurement showed that, in more than 90% of the data, SNR was equal to or greater than 6, hence considering them as acceptable data. The rest of the data (10%), in which SNR was smaller than 6, were removed in the analysis phase. The results showed that there is a significant negative relationship between sound pressure level and SNR, meaning that as sound pressure level goes up, SNR declines (P<0.001). A significant negative correlation was also observed between exposure time and SNR; that is, the increase in exposure time resulted in decrease of SNR (P=0.008).

Lund et al. (2001) investigated the long-term effect of exposure time and low sound pressure level on the changes in threshold of hearing and DPOAE among rats. The results showed that DPOAE is a highly sensitive test in studying rats' threshold of hearing. Furthermore, the results of measuring DPOAE drop among anesthetized rats were acceptable [23].

Salehi et al. (2012) studied the performance of outer hair cells in rabbits that were exposed to noise. The results indicated that noise exposure reduces DPOAE threshold in frequencies of 4 to 10 KHz [17]. Similarly, the findings of the current study showed that, in sound pressure levels that are higher than 4 KHz, noise exposure leads to more significant drops in DPOAE threshold. Emerich et al. (2000) investigated the influence of industrial noise on DPOAE and destruction of cochlea hair cells in Hindi pigs. They concluded that noise exposure results in changes in the shape of hair cells and electrophysiological variations in the mid frequency ranges. They also found a significant relationship between DPOAE decline and decrease in sound pressure level [24]. Similarly, the results of this study showed that noise exposure causes decline in DPOAE thresholds.

CONCLUSION

Based on the above mentioned results, it is concluded that high sound pressure levels reduce DPOAE levels. Furthermore, compared to the control group, DPOAE levels dropped more sharply in the case group. It is also suggested that, since DPOAE test is sensitive to various frequencies, it can be used as a valid measure to evaluate the performance of cochlea (outer hair cells).

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