

Temporary and Permanent Level Shifts in Distortion Product Otoacoustic Emissions following Noise Exposure in an Animal Model

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Abstract

Background: Noise-induced hearing loss (NIHL) is one of the most common occupational illnesses. Most of the studies on NIHL were conducted at high noise levels that people are rarely exposed to but in industries. The function of the outer hair cells (OHCs) is impaired after exposure to industrial noise. Distortion product otoacoustic emissions (DPOAEs) are useful in examination of noise-induced level shifts.

Objectives: To assess the function of OHCs by DPOAE temporary and permanent level shifts (TLS_{dp} and PLS_{dp}) in rabbits exposed to white noise at realistic levels typically found in industrial settings over a broad range of frequencies.

Methods: 12 albino rabbits were divided into two groups: the experimental group rabbits which were exposed to 95 dB SPL white noise at 500–8000 Hz for 8 hrs/day for 5 consecutive days, and the control group rabbits with no exposure to noise. The function of OHCs was examined by DPOAE level (L_{dp}) in different occasions. The study groups were compared for DPOAE temporary and permanent level shifts (TLS_{dp} and PLS_{dp}) to assess the effect of noise on OHCs function.

Results: Noise-induced DPOAE levels (L_{dp}) were decreased up to 20.65 dB (on day 8) and 18.93 dB (on day 11) at 5888.50 Hz (p=0.081). TLS_{dp} and PLS_{dp} were significantly decreased up to 17.99 dB and 16.27 dB, respectively in the experimental group. The most and least L_{dp} were significantly different (p<0.05); they occurred at 5888.50 and 588.00 Hz, respectively. There were significant differences between temporary and permanent threshold shift at various frequencies (p<0.05). These differences were mainly related to 5888.50 Hz compared to other frequencies in each ear (p<0.05).

Conclusion: DPOAEs are an attractive tool for obtaining information about small temporary or permanent threshold shifts, even when the pure tone audiogram is normal.

Keywords: Hearing loss, noise-induced; Hair cell, auditory, outer; Otoacoustic emissions, spontaneous; Auditory threshold; Auditory fatigue

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Introduction

Noise is the most pervasive physical agent in many industries. Prolonged exposure to loud noise is one of the most common causes of sensory-neural hearing loss and hearing threshold level values (TLVs).¹ Sensory-neural hearing loss results from deterioration of the structures within the cochlea; usually owing to the loss of hair cells from the organ of Corti.¹ Noise-induced hearing loss (NIHL)—a type of sensory-neural hearing loss—is among the 10 most common occupational diseases.¹

Exposures to noise in life, environment and industries are mostly in the form of exposure to the white noise.² Exposure to excessive noise has many deleterious health effects including interference with communication, altered performance, annoyance and distraction; it may also cause interference with work or relaxation and physiological responses such as elevated blood pressure and sleep disturbances.³

Excessive noise can cause temporary or permanent damage to the auditory system.⁴ Repeated or prolonged exposure to intense noise gradually damages the cochlear hair cells of the inner ear, resulting in a permanent threshold shift (PTS) across multiple frequencies.⁵⁻⁷ Noise overstimulation can cause damage to the cochlea, hair cell membranes, and changes in the size and shape of the hair cells via different mechanisms.^{7,8}

Many studies reported the effects of noise on the function of the outer hair cells (OHCs), but most of them were conducted at high noise levels that people are rarely exposed to but in industries. Distortion product otoacoustic emissions (DPOAEs) are used for the assessment of the OHCs activity;⁸ therefore, it may be used for assessing the effects of exposure to excessive noise on the OHCs function as a proxy for hearing level.

DPOAEs are sounds measured in the ear canal that reflect mechanical activity of the OHCs.⁹ DPOAEs can be used to screen animal hearing by providing an objective means for confirming healthy cochlear function.¹⁰ DPOAEs are generated in response to simultaneous stimulation of the cochlea by two pure tones with frequencies f_1 and f_2 , where f_2 is slightly higher than f_1 .¹¹ The amplitude of the tone with the higher frequency is equal or lesser than the other one. DPOAEs are most likely generated from at least two locations on the basilar membrane—the overlapping region between f_1 and f_2 , nearer to the f_2 place; and the cubic distortion place ($2f_1-f_2$).¹² DPOAE information is commonly presented as distortion product-grams (DP-grams) that depict DPOAE levels (L_{dp}) as a function of f_2 for a selected combination of primary tone levels L_1 and L_2 .¹³

The objective of the present study was to assess the function of the OHCs by DPOAE temporary and permanent level shifts (TLS_{dp} and PLS_{dp}) in rabbits exposed to white noise at realistic levels typically found in industrial settings over a broad range of frequencies.

Materials and Methods

Twelve male albino rabbits with a mean±SD body weight of 2000±200 g were maintained in animal house at 20–22 °C, 30%–70% relative humidity, and 10 times per hour air displacement. Rabbits were had access to food and soft drink water *ad libitum*. Animals were treated according to the general principles of Helsinki law related to laboratory animals. The minimum sample size for each group was calculated to be six according to a pilot study.

The rabbits in the experimental group were exposed to 95 dBA SPL continuous noise at 500–8000 Hz for eight hours per

day for five consecutive days.

The experiment protocol included baseline audiometry on day 0, rest periods for three days (days 1 to 3), exposure periods (only for experimental group), second audiometry (an hour after the last exposure to noise on day 8), rest period for three days (days 9 to 11), and a third audiometry (72 hours after the last exposure on day 8). Control group was treated similarly except for exposure to noise. The rabbits were exposed to noise in a transparent polycarbonated Plexiglas chamber with dimensions of 50×50×50 cm; the box size was calculated based on clearances needed for six rabbits, ventilated air volume, and reverberation environment (that SPL was independent on distances).

Noise was generated by a signal generator software developed by Pardisan Technology and Science Park, and delivered using Cool Edit Pro ver. 2.1 (Syntrillium Software Corporation). The generated noise was amplified by an amplifier (model ES-2000s, ES Audio Industrial Corporation), and was delivered to animals by a pair of loudspeakers (Micro Lab, model Subwoofer M-563, Probit Co.) mounted on the roof of the box. The SPL was systematically monitored in the chamber by a sound level meter (SLM type Precision, model CEL-490, Cassella-CEL Company) attached to an analyzer located at animal hearing zone. The mean±SD background noise in the animal house and lab was below 20±2 dB.

The animals were anesthetized by a mixture of 60% ketamine (40 mg/kg) and 40% xylazine (10 mg/kg) and examined otologically to exclude any infection or ear channel blockage by wax. Middle ear health was examined by tympanometry. L_{dp} recording was done in the left ear using DPOAE analyzer (DPOAE Model 4000 I/O, Homoth Co.). L_{dp} audiograms were measured using two pure tone stim-

TAKE-HOME MESSAGE

- Noise-induced hearing loss is one of the most common occupational illnesses.
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uli: f_1-f_2 with f_2/f_1 ratio of 1.25. Intensity levels of the two tones, L_1 and L_2 , were set at almost 75 and 65 dB SPL, respectively. Before L_{dp} recordings, signal levels were calibrated in ear canal by using an emission probe microphone.

Both L_{dp} and signal to noise ratio (SNR) were measured at $2f_1-f_2$ and plotted against the geometric mean of f_1 and f_2 . Measurements with a SNR of 6 dB or more were used for in analyses. The function of the OHCs was assessed by measurements of DPOAE levels (L_{dp}) on days 0, 8, and 11 of the study. DPOAE temporary and permanent level shifts (TLS_{dp} and PLS_{dp}), and its magnitude (pre-exposure level minus post-exposure level) were compared to assess the effect of noise on the function of the OHCs. The rabbits' body temperature was kept constant during the test for avoiding interference with L_{dp} measurement.

Kolmogorov-Smirnov test was used to determine the normality of the distribution of the continuous data. Repeated-measures analysis of variance (ANOVA) was used to compare means of several measurements of L_{dp} and L_{nf} across time.

Table 1: DPOAE levels (L_{dp}) and the corresponding noise floor levels (L_{nf}) at different times in the control group

Frequency (Hz)	Mean±SD DPOAE levels (L_{dp}) (dB)			Mean±SD noise floor levels (L_{nf}) (dB)		
	Day 0	Day 8	Day 11	Day 0	Day 8	Day 11
588.00	4.98±0.09	5.27±0.07	5.13±0.09	-0.72±0.01	-0.16±0.06	-0.61±0.05
867.00	9.28±0.07	9.53±0.08	9.48±0.11	-1.41±0.04	1.03±0.04	-1.03±0.08
1133.00	13.43±0.09	13.21±0.10	13.71±0.14	-1.56±0.03	-1.96±0.06	-0.97±0.03
1677.00	18.74±0.12	18.48±0.14	18.51±0.11	-2.55±0.06	-1.02±0.10	-2.65±0.06
1967.00	23.23±0.11	22.98±0.08	22.99±0.15	-2.08±0.04	-2.77±0.8	-2.08±0.02
3098.50	27.31±0.07	27.57±0.11	27.07±0.10	-2.75±0.03	-3.05±0.04	-2.98±0.07
3956.00	31.22±0.13	31.48±0.09	31.06±0.13	-3.16±0.06	-2.01±0.06	-3.75±0.09
5888.50	36.55±0.18	36.31±0.12	36.82±0.09	-4.27±0.09	-3.89±0.10	-3.89±0.07
8166.50	34.78±0.16	34.53±0.06	34.96±0.12	-4.43±0.11	-3.12±0.07	-4.34±0.10
9855.00	33.18±0.13	33.43±0.09	33.34±0.08	-4.12±0.08	-5.25±0.11	-5.07±0.07

One-way ANOVA was used to compare means of L_{dp} and L_{nf} at different frequencies. Tukey's HSD was used as the *post hoc* test. Paired-sample *Student's t* test was used to compare L_{dp} and its L_{nf} between the right and left ears. A *p* value <0.05 was considered statistically significant.

Results

Analysis of the pre-exposure and post-exposure L_{dp} revealed that it was not significantly different across times in the control rabbits (*p*=0.072) (Table 1). L_{nf} over a vast range of frequencies was not different across occasions (*p*=0.093). L_{dp} was not significantly different between the two ears (*p*=0.075).

Pre-exposure DPOAE level (L_{dp}) in the experimental (noise-exposed) rabbits was not different from that recorded in the control rabbits (*p*=0.081) (Table 2). Noise-induced L_{dp} was decreased up to

18.93 dB on day 8, and 20.65 dB on day 11, at 5888.50 Hz. The least diminished L_{dp} was 0.66 dB on day 8, and 2.02 dB on day 11, at 588.00 Hz. Differences of L_{dp} over frequency range were statistically significant across time in the experimental (noise-exposed) rabbits (*p*=0.003). There were significant differences in L_{dp} recorded at any frequency among days 0, 8, and 11 (*p*=0.009). It seems that this difference was mainly attributed to 5888.50 Hz (*p*<0.002). L_{dp} was not significantly different between right and left ears in noise-exposed rabbits (*p*=0.082).

Both TLS_{dp} and PLS_{dp} were significantly different between the two studied groups (*p*=0.003, *p*=0.021, respectively) (Table 3). TLS_{dp} had a mild decreasing trend in noise-exposed rabbits compared to the control rabbits; the trend in PLS_{dp} was sharper than that for TLS_{dp} . Both TLS_{dp} and PLS_{dp} changed significantly (*p*=0.016) over frequency range in noise-exposed rabbits. Differences were mostly

Table 2: DPOAE levels (L_{dp}) and the corresponding noise floor levels (L_{nf}) at different times in the experimental (noise-exposed) group

Frequency (Hz)	Mean±SD DPOAE levels (L_{dp}) (dB)			Mean±SD noise floor levels (L_{nf}) (dB)		
	Day 0	Day 8	Day 11	Day 0	Day 8	Day 11
588.00	5.39±0.03	0.66±0.02	2.02±0.04	-6.02±0.02	-5.46±0.03	-6.43±0.02
867.00	9.07±0.06	3.03±0.05	3.57±0.03	-6.84±0.05	-6.97±0.05	-6.06±0.04
1133.00	13.02±0.02	5.48±0.04	6.97±0.04	-6.95±0.06	-7.57±0.02	-6.03±0.02
1677.00	18.97±0.03	11.01±0.07	12.05±0.06	-6.86±0.02	-8.25±0.03	-7.63±0.03
1967.00	23.93±0.05	12.97±0.03	15.11±0.02	-8.57±0.05	-7.83±0.05	-8.71±0.05
3098.50	27.38±0.08	15.15±0.06	16.09±0.05	-8.32±0.03	-9.38±0.06	-8.56±0.06
3956.00	31.42±0.04	18.08±0.03	18.96±0.08	-9.87±0.02	-10.61±0.05	-9.54±0.05
5888.50	36.92±0.07	18.93±0.05	20.65±0.05	-10.76±0.07	-9.03±0.03	-10.76±0.03
8166.50	34.19±0.09	16.75±0.07	18.85±0.07	-11.92±0.08	-10.11±0.07	-10.22±0.04
9855.00	33.61±0.10	17.23±0.09	18.88±0.04	-12.13±0.03	-11.32±0.06	-12.44±0.08

due to the frequency of 5888.50 Hz. The highest and lowest TLS_{dp} and PLS_{dp} values were recorded at 5888.50 Hz and 588 Hz, respectively.

Discussion

Exposure to noise caused a drop in DPOAE levels (L_{dp}) up to 20.65 dB on day 8 and 18.93 dB on day 11 at 5888.50 Hz. Consistently, most studies showed that prolonged and repeated exposure of awake animals to continuous noise led to a significant decrease in L_{dp} in a wide range of frequencies as decrease in the function of the cochlear OHCs depends on exposure duration, and frequency and intensity of the noise.⁷⁻¹³ We observed different results in different frequencies that might be attributed to the use of a broadband noise in our study.

TLS_{dp} and PLS_{dp} were significantly decreased up to 17.99 dB and 16.27 dB, respectively in animals exposed to noise.

Brief exposure to extremely high-intensity sounds may cause PLS_{dp} , but it is more commonly caused by prolonged repetitive or continuous exposure to lower levels of hazardous noise.^{7,8} Susceptibility to NIHL is highly variable; while some individuals are able to tolerate high noise levels for long periods, others who are subjected to the same environment rapidly lose hearing.^{7,8,11} Risk of PLS_{dp} is related to the duration and intensity of the exposure as well as to genetic susceptibility to noise trauma.^{9,11} Inner ear is believed to be partially protected from the effects of continuous noise by the acoustic reflex which is triggered when the ear is subjected to noise louder than 90 dB that causes the middle ear muscles (the stapedius and tensor tympani) to contract and thereby stiffen the conductive system, making it more resistant to sound entry.^{4,13} Because this protective reflex is neural, it is delayed for a period ranging from 25–150 ms, depending on the noise intensity.^{10,12}

Table 3: Comparison of TLS_{dp} and PLS_{dp} between the noise-exposed and control groups. Numbers are expressed as mean±SD.

Frequency (Hz)	Control group		Noise-exposed group	
	TLS_{dp}^* (dB)	PLS_{dp}^\dagger (dB)	TLS_{dp} (dB)	PLS_{dp} (dB)
588.00	0.29±0.02	0.15±0.01	4.73±0.03	3.37±0.05
867.00	0.25±0.04	0.20±0.03	6.04±0.02	5.50±0.03
1133.00	0.22±0.06	0.28±0.05	7.54±0.06	6.05±0.02
1677.00	0.26±0.02	0.23±0.02	7.96±0.03	6.92±0.06
1967.00	0.25±0.04	0.24±0.04	10.96±0.04	8.82±0.02
3098.50	0.26±0.01	0.24±0.05	12.23±0.07	11.29±0.04
3956.00	0.26±0.03	0.16±0.02	13.34±0.02	12.46±0.05
5888.50	0.24±0.04	0.27±0.01	17.99±0.06	16.27±0.02
8166.50	0.25±0.06	0.18±0.05	17.44±0.03	15.34±0.07
9855.00	0.25±0.03	0.16±0.03	16.38±0.06	14.73±0.08

*Temporary level shifts—TLS, †Permanent level shifts—PLS

Reasons for reduced L_{dp} include misalignment of hair bundles on adjacent hair cells, non-linearity in stiffness of stereocilia, and damage of the tectorial membrane.¹⁻⁵ Most studies found that the noise exposure causes permanent loss of hair cell stereocilia with apparent fracture of the rootlet structures and destruction of the sensory cells, which are replaced by non-functioning scar tissue.^{2,5} NIHL results from trauma to the sensory epithelium of the cochlea.^{3,7-9} In TLS, several potentially reversible effects such as regional decrease in stiffness of stereocilia secondary to contraction of rootlet structures, which are anchored to the cuticular plate of hair cells, intracellular changes within the hair cells including metabolic exhaustion and microvascular changes, edema of the auditory nerve endings, and degeneration of synapses within the cochlear nucleus, can be occurred.^{1,3-5} While in PLS, the changes become irreversible and include breaks in the rootlet structures, disruption of the cochlear duct and organ of Corti causing

mixing of endolymph and perilymph, loss of hair cells, and degeneration of cochlear nerve fibers.^{2,3,5}

An important cause of cochlear OHCs' dysfunction (presented as decreased L_{dp}) and damage to organ of Corti is based on oxidative stress mechanism.¹⁴ Metabolic damage or exhaustion is believed to occur when toxic waste products, the so-called free radicals including reactive oxygen species or reactive nitrogen species, are formed after cochlear cells are stressed by reductions in cochlear blood flow, excessive and toxic levels of neurotransmitters like glutamate, altered intracellular calcium balance, and other stress-related changes that are induced by noise.^{15,16} These free radicals injure a wide variety of critical structures in the cochlea, causing cell damage and cell death.^{17,18} Noise exposure affects several structural elements in hair cells, including the cell membrane and intracellular biochemical pathways.¹⁹ These changes may evoke the formation of free radicals, resulting in

sensorineural hearing loss.²⁰ Free radicals may increase dramatically within a few minutes or hours after exposure to an intense noise.^{14,18} Noise-induced cochlear free radicals endanger hair cells intrinsic antioxidant system—the glutathione system, which is a powerful natural antioxidant system in the cochlear hair cells.¹⁹ Depletion of glutathione contents in the organ of Corti due to exposure to noise can cause more susceptibility to hearing loss.^{17,20}

DPOAEs levels (L_{dp}) were not significantly different between right and left ears in animals exposed to noise. Creation of reverberation field in the exposure chamber seems to be the most important reason. Some studies reported similar results,^{9,10,12} but others did not.²¹⁻²³ Sato, *et al*, showed that an efferent influence may also help to explain the difference some researchers observed between the L_{dp} in the left and right ears.²¹ Another study indicated that tone-evoked L_{dp} are larger in the left ear.²² van den Brink reported pitch differences between left and right ears when they are exposed to the same frequency stimulus.²³

We studied albino rabbits; however, interspecies differences should be appreciated in interpreting results. There are clear interspecies differences in the dependence of L_{dp} on frequency and that L_{dp} tends to be largest in the regions of best hearing sensitivity in each species.²⁴ It has been shown that systematic variations in DPOAEs parameters such as L_1 and L_2 , f_1 and f_2 generally produce qualitatively similar changes in emission levels in humans, monkeys, cats, rabbits, and rodents.¹³ These similarities occur despite the quantitative differences, particularly in the f_2/f_1 ratio that elicits the largest DPOAEs, which is greater in rabbits and rodents (1.25) than in humans (1.22).¹³

Sex differences seem to play a key role in the measurement of L_{dp} —only male rab-

bits were used in the present study. Some reported L_{dp} are larger in female humans and rhesus monkey than in males.^{25,26} Larger L_{dp} may be correlated to a better hearing threshold for females of the same species.^{25,26} Some researchers believe that this difference is partly attributed to different hormonal exposure,^{25,26} while others believe that it can be attributed to a sex difference in the OHC electromotility and/or in the mechanism(s) responsible for stereociliary bundle motility.^{25,26} Both of these reasons can be the result of gender differences in membrane lipid profiles that would alter lipid-protein interactions.^{25,26} Another possibility is the shorter length of female cochleae,²¹ or gender differences in the size of the middle ear.²⁶ The observed L_{dp} would have been larger if the studied animals were female rabbits.²⁶

In conclusion, DPOAEs might be used as a screening and diagnostic test for early detection of NIHL in people with normal audiograms. OHCs were affected early in NIHL, and DPOAEs can detect subtle changes in OHCs function as temporary or permanent level shifts.

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