Downregulation of GJB2 and SLC26A4 Genes Induced by Noise Exposure is Associated with Cochlear Damage

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Abstract

Noise can change the pattern of gene expression inducing sensorineural hearing impairment. There is no investigation on effects of noise frequency on the expression of *GJB2* and *SLC26A4* genes involved in congenital hearing impairment in cochlear tissue. This study investigated impacts of white and purple noise on gene expression and pathologic changes of cochlear tissue. In this study, 32 adult male Westar rats were selected and divided into experimental groups WN (animals exposed to white noise with a frequency range of 100-20000 Hz), PN (animals exposed to purple noise with a frequency range of 4-20 kHz) and control groups. All experimental groups were exposed to a sound pressure level of 118-120 dB for 8 hours per day. Cochlear tissue sampling was performed for tissue pathology studies, also RNA was extracted at 1 hour & 1 week after cessation of noise exposure. The results showed that Both white and purple noises caused permanent damage to the cortical, estrosilica systems of hair cells and ganglion of the hearing nerve. *GJB2* and *SLC26A4* were downregulated in both groups exposed with white and purple noise. However, differences are notably more significant in 1 weak post-exposure than 1 hour. Our Findings suggest *GJB2* and *SLC26A4* can be considered as biomarkers of response to noise frequency which is associated with the pathological response of cochlear tissue, leading to sensorineural hearing impairment. It would be suggested the demand for a more conventional approach to assessment of noise-induced hearing loss and subsequently the practice of hearing protection programs.

Introduction

Noise-induced hearing loss (NIHL) is the most common form of acquired hearing impairment in developed countries and therefore it is considered a public health issue [1]. A wide range of workers is exposed to noise pollution in the workplace, which has been shown to have detrimental effects, such as hearing impairment [2]. Limited studies have been conducted on the effects of high-frequency noises on the expression of genes involved in hearing impairment. One type of noise with a high-frequency range is purple noise and the other which had a range of audible frequencies is white noise. White or octave band noises centered on low frequency and purple noise centered on high frequency [3]. Hearing impairment could be induced by these noises or after exposure to high-intensity noises [4]. studies were demonstrated that exposure to sound pressure level (90−120 dB) and frequency range of 1−7 kHz could induce upregulation of heat shock protein genes [5], Protein 45β (GADD45β), CDK-interacting protein 1 (p21Cip1) [6], TACII, MHCII, [7] HDAC protein and Connexin 26 [8] in the atrium of inner ear suggesting them as biomarkers of audible frequencies noise response. Moreover, exposure to sound pressure level of 120 dB and frequency range of 7 kHz resulted in upregulation of C1s and Cfi proteins of the Corte system [9]. Stimulation with ultrasonic noises (16 kHz) and SPL = 130 dB significantly increased the expression of CRH (Corticotropin-Releasing Hormone) genes and its CRH-R1 receptor in PVN, also the physical stress caused by these noises had severely affected microglial cells [10]. Moreover, acute exposure to high-frequency noise (10−20 kHz) and SPL = 110−120 dB resulted in upregulation of cochlear tissue different proteins such as β-ACTIN, PRESTIN, and β-SPECTRIN [11]. Indeed, abnormal noise at different frequencies and times exposure can induce mutation ad changes in the gene expression pattern in

ganglion and cochlear tissue. In this manner, noise can induce morphological and physiological changes leading to hair-cell death and disorders in different sections of the human auditory system [6, 12–13]. Inner and particularly outer hair cells (IHC and OHC, respectively) especially those located in the basal turn of the cochlea in the auditory system, are very sensitive to noise damage. Thus, the disruption of stereocilia leads to a severe alteration in HC structural integrity that has been correlated to permanent threshold shifts (PTS) in many species [14–19].

It was documented that several genes are involved in the physiological response of the cochlea to noise-induced damage. These genes are associated with various biological processes such as oxidative stress and transcriptional signaling [6, 20]. The gap junction beta-2 (*GJB2*) gene-encoded one of the most important proteins involved in the regulation of potassium ions in the cochlea, which is located within the spiral cells of the limbus and the spiral ligament fibroblasts [21–22]. The solute carrier 26a4 (*SLC26A4*) gene consists of the DFNB4 locus, a recessive chromosomal locus, that causes sensorineural hearing impairment in both non-syndromic and syndromic forms (pendred syndrome, PDS) [23]. Studies have shown that pendred syndrome is alike an anion converter whose mutation leads to hearing impairment. Pendred syndrome is most often diagnosed with nerve deafness associated with goiter. Sensorineural hearing impairment due to pendred syndrome is progressive in some cases and causes varying degrees of deafness [23]. Since no research has been performed directly on the effects of white and purple noises exposure on the expression of *SLC26A4* and *GJB2* genes involved in congenital hearing impairment, in the present study we investigate their expression pattern after exposure with white and purple noise at a different time in the rat. Given that cochlear tissue is the most important sensorineural receptor of sound when exposed to noises, the pathologic changes in this tissue were also investigated.

Materials And Methods

Study group selection

In this experimental study, 32 adult male Westar rats were selected and divided into experimental groups (N = 12) WN (animals exposed to white noise with a frequency range of 100-20000 Hz), (N = 12) PN (animals exposed to purple noise with a frequency range of 4–20 kHz) and the control group (N = 8) (animals exposed to the same environmental conditions but without noise exposure). For each experimental group, 4 rats were selected as the control group (Table 1). Both experimental groups were exposed to a sound pressure level of 118–120 dB for 8 hours per day. 6 rats from the WN exposure group were exposed to white noise for 3 days (WN-3D) and the other 6 rats were exposed to white noise for 6 days (WN-6D). Also, 6 rats from the PN exposure group were exposed to purple noise for 3 days (PN-3D) and the other 6 rats were exposed to purple noise for 6 days (PN-6D). Samples of exposure groups were anesthetized and after biopsy of rat's cochlea, afterward gene expression analysis was performed on them at two-time period 1 hour and 1 week after cessation of noise exposure designated as 1h and 1w for each group (Table 1). Accordingly, to determine the mean normal expression level of *GJB2* and *SLC26A4* genes, control group samples were used under the same environmental conditions but without exposure to the noise. For cochlear tissue pathology studies in experimental groups, 8 rats from the

exposure groups, 1 hour & 1 week after cessation of noise exposure (2 rats from the WN-3D group, 2 rats from the WN-6D group, 2 rats from the PN-3D group, and 2 rats from the PN-6D group) were considered. Also, for studies of cochlear tissue pathology in a state that was not under any stress or acoustic stimulation, 1 rat was selected from the control group. In this study, the sample size and type of exposures in the tested time-periods, according to the nature of this study, were obtained experimentally and by surveying the results of similar studies in this field of research [5, 8, 24]. The study was done under the principles of the Ethics Research Committee of the School of Public Health, Tehran University of Medical Sciences (Code: IR.TUMS.SPH.REC.1398.220).

Table 1
The summary of groups exposed with white and purple noise in different time

Groups	Sample size (N)	Noise exposure	Time of exposure (hour per day)	Time of RNA extraction after exposure
WN-3D-1h	3	White noise*	8 hour/ 3D	1 hour
WN-3D-1w	3	White noise	8 hour/ 3D	1 weak
WN-6D-1h	3	White noise	8 hour/ 6D	1 hour
WN-6D-1w	3	White noise	8 hour/ 6D	1 weak
PN-3D-1h	3	Purple noise**	8 hour/ 3D	1 hour
PN-3D-1w	3	Purple noise	8 hour/ 3D	1 weak
PN-6D-1h	3	Purple noise	8 hour/ 6D	1 hour
PN-6D-1w	3	Purple noise	8 hour/ 6D	1 weak
*Frequency range: 100-20000 HZ, SPL (sound pressure level) = 118–120 dB				
** Frequency range: 4–20 kHz, SPL (sound pressure level) = 118–120 dB				

Laboratory conditions

Rats used in this study with a specific weight and age (7–8 weeks, 190-200g) were placed in a high-performance four-cell audio chamber. One week before the start of exposure, they adapted to the conditions of the experimental environment (12 hours of darkness and 12 hours of light, temperature of $25 \pm 2 \degree C$ and humidity of 45–52%) and then were exposed to noises at a specific time from 10 am to 6 pm [5, 8] and during the period the necessary tests, water, and food were sufficiently provided to the rats.

Exposure to white and purple noise took place inside a four-cell high-performance audio chamber measuring 60×52×40 cm^3. The sound transmission loss from inside the chamber to the outside was 50 decibels. The walls of this chamber are composed of layers of iron sheet, cellulose, air layer, cellulose, iron sheet, and cellulose, respectively. Two holes on the walls of the chamber were used for ventilation of the chamber and to supply enough oxygen for animal samples inside this chamber, a blowing peripheral pump with a flow of 70 liters per minute was used.

Noise exposure

The noise pollution was generated using computer software (ADOBE AUDITION with version 13.0.7) and distributed inside the chamber by an amplifier (Crown D75A, Elkhart IN) and 2 speakers (RadioShack Corp, Ft Worth TX). Frequency and sound pressure level monitoring were used with SPECTRUM ANALYZER software (version 5.0.2.). Also, to check the uniformity of the sound pressure level in different parts of the chamber, the sound pressure level in different parts was measured by an analytical sound meter (CELL491 catalog model).

Histology tests

First, the rats were anesthetized by exposure to carbon dioxide in a cubic chamber, then the skull bone was dissected, and the brain of the specimens was removed. The cochlear tissue, which includes the inner ear and is located inside the pterygium, was removed from the tympanic and temporal sections of the auditory system by surgical instruments. The cochlear tissue inside the microtube for extract RNA, as well as histological tests, were performed (tissue fixation, alcohol dehydration, clearing with xylene, impregnation with Paraffin, molding, cutting from the samples with a microtome in thickness of 5 to 10 μm and painting of hematoxylin-eosin). In the end, morphological-pathological examination of cochlear tissue was performed using light microscope images at a 200-micron scale [12, 25].

RNA extraction

Total RNA was extracted from tissue using Tripure reagent (Roch, Mannheim, Germany) according to the manufacturer's instruction. Briefly, 50 mg of tissue was homogenized and lysed using Tripure reagent. The lysate was mixed with chloroform and vortexed vigorously and then centrifuged at 12,000 g for 15 min at 4°C. Afterward, the upper colorless phase was precipitated by adding isopropanol and washed with 75% ethanol. Finally, RNA was dried at room temperature and dissolved in RNase-free water. The quantity and quality of RNA were evaluated by nanodrop (ND-1000, Thermo Scientific Fisher, Waltham, Massachusetts, USA) and gel electrophoresis, respectively. RNA was treated with DNase to avoid genomic contamination, (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

cDNA synthesis

Complementary DNA (cDNA) was synthesized using 1 μg RNA, 2.5 μmol/μl oligo-dT (Roche, Mannheim, Germany) and 60 μmol/μl random hexamers (Roche, Mannheim, Germany) incubated 10 min at 65°C followed by addition of 10 mM of dNTP mix (Deoxy Nucleoside Triphosphate mix) (Roche, Mannheim, Germany) 40U/μl RNase inhibitor (Roche, Mannheim, Germany), 4μL of 5X reverse transcriptase (RT)

buffer (Roche, Mannheim, Germany) and 20 U/μL reverse transcriptase enzyme (Roche, Mannheim, Germany) and then incubated at 25°C for 10 min followed by incubation at 50°C for 60 min. The integrity of cDNA was checked with GAPDH (Glyceraldehyde 3-Phosphate Dehydrogenase) primers (Table 1) as a housekeeping gene. The PCR condition as follows: an initial denaturing step of 94°C for 5 min and 35 repetitions of denaturation at 94°C for 30 s, annealing at 49°C for 30s, and extension at 72°C for 45s with a final extension of 72°C for 5 min.

Real-time RT-PCR analysis

To investigate the differences in the RNA expression level of target genes among exposure and control group Real-time reverse transcriptase-PCR (RT-PCR) was used. Specific primers were designed by Primer 3 software version 0.4.0 (http://www.ncbi.nlm..nih.gov/tools/primer-blast) (Table 2). Two independent RNA preparations were used for each real-time RT PCR experiment. The experiments were performed in 20 μl reactions containing 1 μl cDNA target, 100 nM forward and reverse primers, and 1x SYBR® Premix Ex TaqTM II (Takara, Tokyo, Japan). RT-PCR was carried out in duplicate for target genes and GAPDH as a housekeeping gene using a Real-Time System (Applied Biosystems [ABI], Foster City, CA, USA). The PCR condition was as follows: activation at 95°C for 3 min, amplification at 95°C for 10 s, 60°C for 32 s for 40 cycles followed by a melting curve, temperature increments of 0.2°C every 30 s to determine amplification of the expected product [26].

Table 2
Primer sequences of target genes used in real-time RT-PCR

Oligo Name		seq 5 – 3	TM
GJB2	Forward	5´-TCACTGTCCTCTTCATCTTCCG-3´	59.51
	Reverse	5´-CTTCCGTTTCTTTTCGTGTCTC-3´	57.88
SLC26A4	Forward	5´-CATCATGCCTGGCTGGTTCT-3´	60.40
	Reverse	5´-TGGACACCAACATTCCGTCA-3´	59.53
GAPDH	Forward	5´-AAGTTCAACGGCACAGTCAAGG-3´	61.58
	Reverse	5´-CATACTCAGCACCAGCATCACC-3´	61.32

Analysis of gene expression

The expression level of target genes is reported as the gene fold in the noise-exposure samples normalized to the internal control gene (GAPDH) and relative to the control group using the $2^{-\Delta\Delta CT}$ method as previously described [27].

Statistical method

Statistical calculations were performed by prism Graph pad software with version 9.0.0.121. Relative gene expression value data are mean ± standard deviation and were normalized to the internal control. The normal distribution hypothesis for quantitative variables was evaluated by the Shapiro-Wilk test and

P-value < 0.05 was considered statistically significant. Differences among the exposure & control groups were evaluated by Tukey's Multiple Comparison Test, followed by post hoc pairwise comparisons to determine which differences account for the result of the more inclusive test.

Results

Chamber sound pressure level measurement reports

The measurement results showed that the arrangement of sound systems in the chamber was such that the sound pressure level was constant in all parts of the chamber and all samples were in contact with white and purple noise and with increasing distance from the floor surface of the chamber, the sound pressure level has also increased. Also, the SPECTRUM ANALYZER software results showed that the frequency range and produced sounds pressure level in the chamber were quite like the frequency range and target sounds pressure level for the present study.

Results of pathological studies

Microscopic examination of tissues and their imaging showed that 1 week after cessation of noise exposure, neurons in the samples of groups exposed to white noise (WN-3D and WN-6D) were damaged more seriously than 1 hour after exposure termination (Fig. 1). Also, the nerve cells of the samples exposed to purple noise (PN-3D and PN-6D) were damaged more significantly 1 week after cessation of noise exposure compared to 1 hour after challenge. In the pathologic section, the auditory nerve ganglion and Reisner membrane showed higher structural damage in samples exposed to purple noise (PN-3D and PN-6D) compared to white noise (WN-3D and WN-6D). Besides, 1 week after termination of noise exposure more seriously damaged was detected in samples exposed to purple noise (PN-3D and PN-6D) in comparison with white noise (Fig. 1).

Expression pattern of GJB2 gene

The results showed that 1 week after cessation of noise exposure, the transcript level of *GJB2* was significantly decreased in exposure groups WN-3D, WN-6D, PN-3D, and PN-6D by 0.34-, 0.02-, 0.51- and 0.12-fold, *p*-value < 0.05. Also, 1 hour after cessation of noise exposure, the transcript level of *GJB2* was significantly decreased in exposure groups WN-3D and WN-6D by 0.51- and 0.12-fold, *p*-value < 0.05, while it was increased in PN-3D (1.26-fold, *p*-value > 0.05) as compared to control. 1 hour after cessation of noise exposure, the PN-6D exposure group was slightly downregulated which was not significantly (0.72-fold, *p*-value > 0.05). Overall, 1 week after cessation of noise exposure, the average mRNA levels of *GJB2* were significantly lower when compared to 1 hour after cessation of noise exposure in all of the exposure groups (Fig. 2).

Expression pattern of SLC26A4 gene

The results showed that 1 week after termination of noise exposure, the transcript level of *SLC26A4* was significantly decreased in exposure groups WN-3D, WN-6D, PN-3D, and PN-6D by 0.31-, 0.009-, 0.43- and

0.1-fold, p-value < 0.05. Also, 1 hour after cessation of noise exposure, the transcript level of *SLC26A4* was significantly decreased in exposure groups WN-3D, WN-6D, and PN-6D by 0.42-, 0.09- and 0.28-fold, p-value < 0.05, while it was slightly increased in PN-3D (1.21-fold, p-value > 0.05) as compared to control. Additionally, 1 week after cessation of noise exposure, the average expressions of *SLC26A4* exhibited more downregulation as compared to 1 hour after cessation of noise exposure in all of the exposure groups (Fig. 3).

Discussion

The cochlear tissue and hair cells of the Corte system are the most important sensorineural receptors for sound in the human auditory system. Noise exposure could trigger point mutation and alteration in gene expression which resulted in many sensorineural hearing defects [13]. Therefore, it hypothesizes that the cochlear tissue sample can be used as an ideal tissue sample to study the changes of target genes and understanding the mechanisms that induced syndromic/non-syndromic sensorineural hearing impairment. Accordingly, in this study, the effects of two noise types, which had different frequency limits were investigated on the expression of *GJB2* and *SLC26A4* genes in rate cochlear tissue. One type of noise had a high-frequency range (purple noise) and the other had a range of audible frequencies (white noise). Most studies in the field of gene expression and its relationship with noise have examined only the effects of noises with a certain frequency on the expression of genes [9–10, 24, 28], while in this study, sound with a different frequency range in the human hearing range (20-20000 Hz) were analyzed. The results of this study showed that *GJB2* and *SLC26A4* genes exhibited more downregulation after 6 days exposure of to both white and purple noises in comparison with 3 days exposure, suggested that increasing the duration of time-exposure could induce more alteration in gene expression. Additionally, when compare two-time points of RNA extraction after the termination of noise exposure, it was revealed that the average expression of *GJB2* and *SLC26A4* genes exhibited more remarkable downregulation 1 week after the termination of noise exposure as compared to 1 hour in all the experiment groups. Inconsistent with the results of gene expression, in pathologic examination of cochlear tissues, it was demonstrated that neurons were damaged more seriously 1 week after cessation of white and purple noise exposure compared to 1 hour after experiments. These results propose that more stable differences in gene expression, as well as more pathologic damage, could be seen in the long-term period after termination of noise exposure.

There is a lack of enough knowledge regarding the expression of *GJB2* and *SLC26A4* genes after white and purple noise exposure. However, recently several studies have evaluated the expression of some cochlear tissue and hair cells proteins, such as β-ACTIN, PRESTIN, and β-SPECTRIN after contact with sound pressure level between 110–120 dB, and the results demonstrated that gene expression was significantly increased in exposure groups [5, 9, 11, 24]. Furthermore, it was documented that after exposure of rats with white noise (4kHz) for 2 days the expression of connexin 26 (CX26) protein in the dorsal wall of the cochlear tissue increased significantly and the amount of protein in ligament fibrocytes was increased [28].

In this context, a similar study showed that the expression of heat shock proteins (HSP 27, 70) increased after 1 hour of noise exposure (100 dB), and the level of the hearing threshold was raised following 4 hours of exposure with the noises, suggesting that changes in HSP gene expression could lead to rapid physiological changes at the level of the auditory threshold [29]. Inconsistent with the former studies, Gratton et al. [6] stated that a sound pressure level of 105 dB (for 1 hour per day) induces overexpression of HSP70, HSP40, Protein 45β (GADD45β), and CDK-interacting protein 1 (p21Cip1) genes in cochlear tissue of mouse. However, the discrepancy between the results of these studies and the present study may be due to the difference in time exposure, type of noise, and false-positive and negative results of the real-time reverse transcriptase-PCR technique. Also, 1 hour after cessation of exposure, the expression of both *GJB2* and *SLC26A4* genes in one group exposed to purple noise (PN-3D) up-regulated and the other groups exposed to white and purple noise down-regulated significantly. These results are inconsistent with a study by Alagramam et. al., [2] in which two groups of mice were exposed to noise with sound pressure levels of 110 and 116 dB for 1 hour per day. The results showed that exposure to 110 dB reduced the expression of 221 genes and increased the expression of 55 genes in the cochlea tissue, while after exposure to 116 dB, the expression of 61 Genes was decreased and 243 genes enhanced. Additionally, the expression of Fos Proto-Oncogene in the cochlea increased significantly after exposure to 116 dB, while the expression of calcium-related genes decreased significantly after exposure to 110 dB suggesting mitogen-activated protein kinase (MAPK) signaling was the major pathway in both levels of noise exposure.

It was demonstrated that acute and chronic noise exposure could change gene expression differently. In agreement with this notion, a study by Eraslan et al. [30] revealed that after acute noise exposure the expression of both CRH-R1 and CRH-R2 (corticotropin-releasing hormone receptor) genes were downregulated, while after chronic noise-exposure the expression of CRH-R1 and CRH-R2 genes increased and decreased, respectively. These results are inconsistent with the results of the effects of purple noise on the expression of *GJB2* and *SLC26A4* genes in the present study in which the gene expression is lower after 6-day exposure compared to 3-day exposure. The results of this study are also in harmony with the findings of a similar study [7] that have shown a significant relationship between changes in gene expression pattern and the incidence of sensorineural hearing impairment. Furthermore, it was documented that *GJB2*, SOD2, and CAT genes may be involved interactively or independently in the development of NIHL [31]. Moreover, exposure of mice to 110 dB for 8 hours a day for 5 consecutive days decreased the expression of connexin 26 (CX26) in cochlear tissue and lead to impairment of hair cells in the cortical system and trigger the risk of NIHL [8].

Another important result in this study is that exposure to both white and purple noises has adverse effects such as axonal changes of nerve neurons, destruction of cochlear tissue and hair cells on the samples of the auditory system. These injuries have intensified with the increasing of time-exposure, also purple noise due to the high energy has more destructive effects than white noise. Likewise, it was reported that exposure to loud noises can lead to increased production of nitric acid in the spiral ganglion neuron of pregnant women leading to alteration in the gene-expression pattern of ganglion cell and thereby cell death, and development of congenital hearing impairment [32–33]. It is well documented that

after damage and destruction of the auditory system, restoration, and reconstruction of nerve cells such as hair cells in mammals is not possible and subsequently can cause cell death in the Corte system, the development of sensorineural hearing impairment, and permanent hearing threshold (PTS) changes [13]. In terms of pathology, cochlear injury induced by noise exposure is a multifactorial degradation process that occurs due to inflammation and direct mechanical responses contributing to apoptosis and necrosis [20]. In this line, changes in the neuron cell body's golico kanghogahse after noise exposure have been reported in ear tissue and other tissues such as the heart [34]. Evidence has also shown that changes in the expression level of proteins related to the auditory system noises cause permanent damage to the auditory nerve ganglion and cochlear tissue neuron tissue to lead to non-syndromic hearing loss or sensorineural syndrome [8, 13, 25]. Given that both white and purple noises have a significant effect on the expression of genes involved in sensorineural hearing impairment (*GJB2* and *SLC26A4*), therefore, research on this issue should be concerned. The discovery of genes that are affected by noises and cause sensorineural hearing impairment can be useful in pathophysiological studies and the development of an appropriate solution for prevention [35].

Conclusion

Our findings suggest that *GJB2* and *SLC26A4* genes can be considered as biomarkers of response to noise. Also, the pathological results show sensorineural hearing impairment and structural and cellular changes in cochlear tissue following noise exposure. To prevent the effect of noise on genetic changes in the auditory system, appropriate training, and advice should be suggested at the community level, also using engineering or therapeutic control methods could decrease the risk of injury to the hearing system.

Declarations

Funding

Conflict of interest /Competing interests

The authors declare that they have no competing of interest.

Availability of data and materials

Data will be available on special request to corresponding author.

Authors' contributions

AAG, MRME and EK collected and analyzed data. AAG and EK wrote the manuscript. MK, MMH and SM reviewed and revised the manuscript. EK and MRME supervised the entire processes. All authors read and approved the final manuscript.

Ethical approval and consent to participate

The study was approved by the Ethics Research Committee of the School of Public Health, Tehran University of Medical Sciences (Code: IR.TUMS.SPH.REC.1398.220).

Consent for publication

All authors read and approved the final manuscript.

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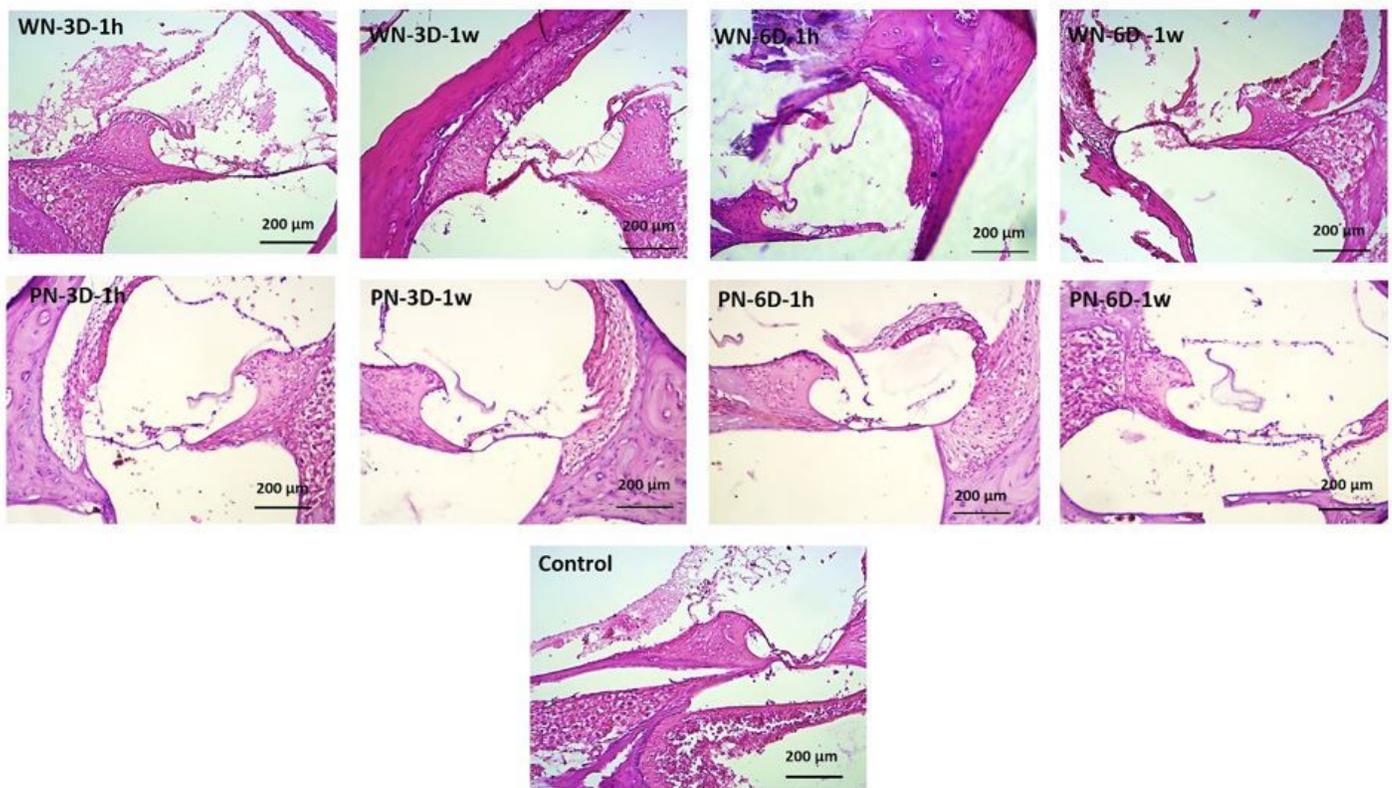
Figures

Figure 1

Tissue image of different exposure group samples, 1 hour and 1 week after cessation of noise exposure. WN-3D, group were exposed to white noise for 3 days; WN-6D, exposed to white noise for 6 days; PN-3D, group were exposed to purple noise for 3 days; PN-6D; exposed to purple noise for 6 days. Pathologic studies in experimental groups 1 hour (1h) & 1 week (1w) after cessation of noise exposure were conducted
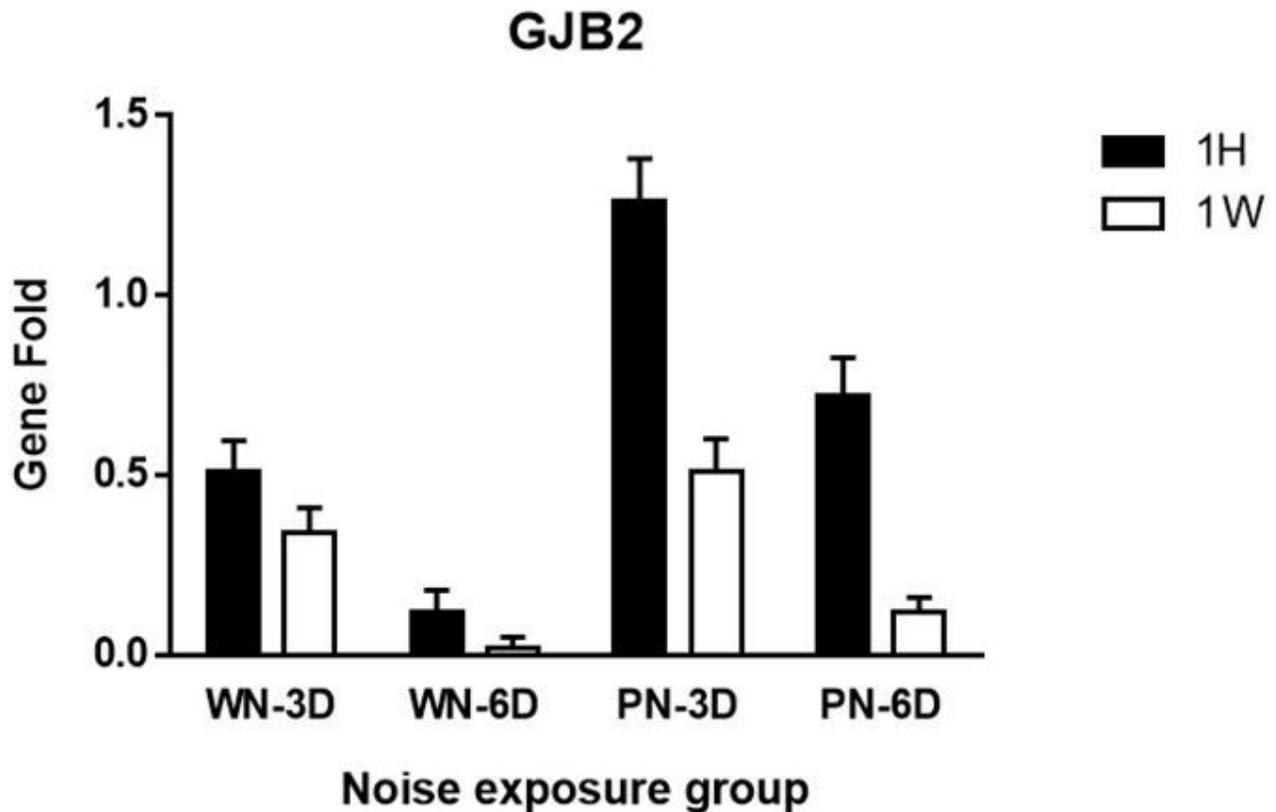

Figure 2

Effect of white & purple noise on Expression of GJB2 gene. Westar rats were exposed to white & purple noise (SPL=118-120 dB) for 8hour, and Cochlear RNA was subjected to qRT-PCR for exposure group WN-3D, WN-6D, PN-3D, and PN-6D conducted 1 hour (1h) and 1 weak (1w) after cessation of noise exposure. Gene fold change is the ratio of values for noise-exposed rats relative to unexposed controls
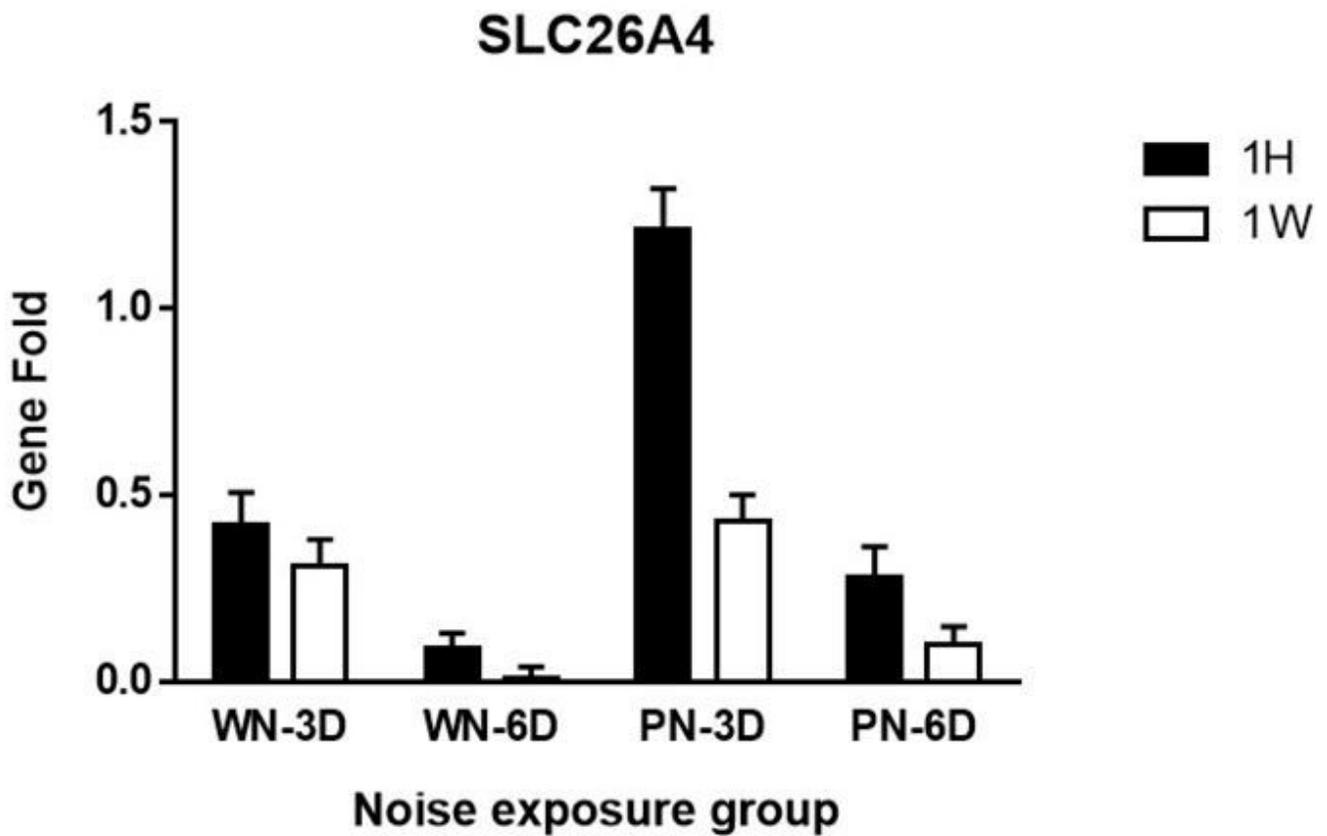
Figure 3

Effect of white & purple noise on Expression of SLC26A4 gene. Westar rats were exposed to white & purple noise (SPL=118-120 dB) for 8hour, and Cochlear RNA was subjected to qRT-PCR for exposure group WN-3D, WN-6D, PN-3D, and PN-6D conducted 1 hour (1h) and 1 weak (1w) after cessation of noise exposure. Gene fold change is the ratio of values for noise-exposed rats relative to unexposed controls