RESEARCH PAPER

Acta Neurobiol Exp 2019, 79: 155–168
DOI: 10.21307/ane-2019-014

Short-term exposure to high-intensity sound induces hearing loss and apoptosis in guinea pigs

Yanqing Liu[1,2†], Hui Wang[3†], Ziquan Liu[1], Yanjun Gu[2], Limin Xin[1], Jinyang Liu[1] and Haojun Fan[1*]

[1] *Institute of Disaster Medicine, Tianjin University, Tianjin, China,*
[2] *Department of Medical Service, Logistics University of Chinese People's Armed Police Forces, Tianjin, China,*
[3] *Department of Experimental Pathology, Institute of Radiation Medicine, Academy of Military Sciences, Beijing, China,*
[†] *Both authors contributed equally to this work,*
** Email: fanhaojun999@126.com*

The aim of this study was to investigate the effect and underlying mechanisms of short-term high-intensity sound exposure on guinea pigs to mimic the effects of non-lethal anti-riot weapons. A total of 92 male adult guinea pigs were exposed to high-intensity sound at 0 dB, 110 dB and 130 dB for 5 min. Basic clinical observation, repellent behaviour detection, peripheral blood routine examination, serum cortisol detection and hearing ability assessment were performed to analyse the functional changes after high intensity sound exposure. Meanwhile, routine haematoxylin and eosin staining, scanning electron microscopy and transmission electron microscopy were used to observe the structure of the cochlear tissue. To investigate the mechanisms underlying the tissue changes, the levels of apoptosis and caspase 3, 8 and 9 were detected using TUNEL staining and immunohistochemistry. After short-term exposure to high-intensity sound, the guinea pigs exhibited fear and agitation, increased repulsive behaviour, high serum cortisol and an increase in auditory threshold. The inner hair cells and outer hair cells exhibited degeneration. In addition, apoptosis was observed in the cochlear tissue. After short-term exposure to high-intensity sound, the guinea pigs exhibited not only stress reactions but also impaired hearing and signs of hair cell degeneration. Apoptosis in the cochlear tissue may play an important role in the functional and structural injuries caused by high-intensity sound.

Key words: high-intensity sound, hearing, apoptosis, caspase, guinea pigs

INTRODUCTION

It is well-known that noisy environments are inevitable because of the rapid development of industrialization, and noise-induced hearing loss (NIHL) has become a severe disease worldwide (Ide and Morimitsu, 1990; Akdogan et al., 2009; Jacob et al., 2013). However, high-intensity sound exposure, which may not occur commonly in the environment, has been transformed as a novel non-lethal weapon that can be used by authorities to disperse crowds.

Hearing detection is the most direct way to evaluate damage caused by high-intensity sound (Pawlaczyk-Luszczynska et al., 2011). Specifically, the evaluation of auditory thresholds is one of the most important and common methods used in hearing detection (Saedi et al., 2013; Chen et al., 2014). Cochlear injuries were found to be the most significant change following noise or exposure to intensive sound (Maison et al. 2013). Varying degrees of structural and functional changes and recovery can be observed in the hours to weeks following the exposure (Fernandez et al., 2015). Cochlear changes have become a pri-

Received 10 May 2018, accepted 2 April 2019

mary focus in the study of sound-induced hearing problems (Liberman et al., 2015). Changes of inner hair cells (IHCs) and outer hair cells (OHCs) are the most significant effect in sound-induced hearing loss due to various causes, such as aging and noise (Han et al., 2013; Sergeyenko et al., 2013; Heeringa and van Dijk, 2014). Studies have shown that after noise exposure, necrosis and apoptosis occurred simultaneously in the cochlea, and apoptosis was the main cause of the early death of hair cells (Zhang et al., 2014). In a study in which guinea pigs were subjected to a narrow band noise centred at 4 kHz with 110 dB, 115 dB or 120 dB sound pressure levels (SPL), it was suggested that the apoptotic process may be involved in intense noise-induced hair cell death (Hu et al., 2000). Adler et al. (1992) and Shoji et al. (2000) exposed one-day-old chicks and guinea pigs to 120, 125 and 115 dB noise for 5 h or 48 h to observe the injuries and investigate possible treatments. These and other studies primarily employed long exposure times, while there are few studies on the effects and mechanisms of short-term noise exposure. Therefore, in the present study, to clarify the effects of high-intensity sound exposure and the underlying mechanisms, guinea pigs were subjected to experiments in which we investigated their hearing ability, recovery situation, cochlear structure and occurrence of apoptosis.

METHODS

Animals and groups

To eliminate differences due to body weight, 152 adult male guinea pigs, 250±20 g, were randomly divided into the following three groups: the sham group (n=44), the 110 dB sonic exposure group (n=54) and the 130 dB sonic exposure group (n=54). All experiments were performed between 08:00 and 15:00. Ethical approval was obtained from the affiliated Hospital of Logistic College of Chinese People's Armed Police Forces. To eliminate psychophysiological effects, the guinea pigs in the sham group were also handled and processed in parallel to those in the sonic exposure group but without sonic radiation.

High-intensity sound exposure

A directional high intensity sound exposure system that is currently employed by police, model DSAD-2E (Third Institute of Electronic Corporation of China, Beijing, China), was used in this study. The frequency ranged from 600 Hz to 6 kHz with a maximum sound pressure of 152 dB. In this study, the sound pressure was set to 110 dB and 130 dB for 5 min. A Nor140 sound level meter (Norsonic Corporation, Norway) was used to calibrate and detect the exposure dose (Fig. 1).

Repellent behaviour detection

A custom-made repulsive behaviour device was used to evaluate the reaction of the animals to the high-intensity sound exposure. A schematic diagram of this repulsive device is shown in Fig. 2. The device consisted of three parts, the inner cage, the outer cage and the cuboid cage. The diameter of the inner round cage and the outer round cage was 30 cm and 70 cm, respectively. The length of the cuboid cage was 60 cm. All cages were made of stainless steel. Moreover, the inner round cage contained six small doors through which the animals could move to the outer

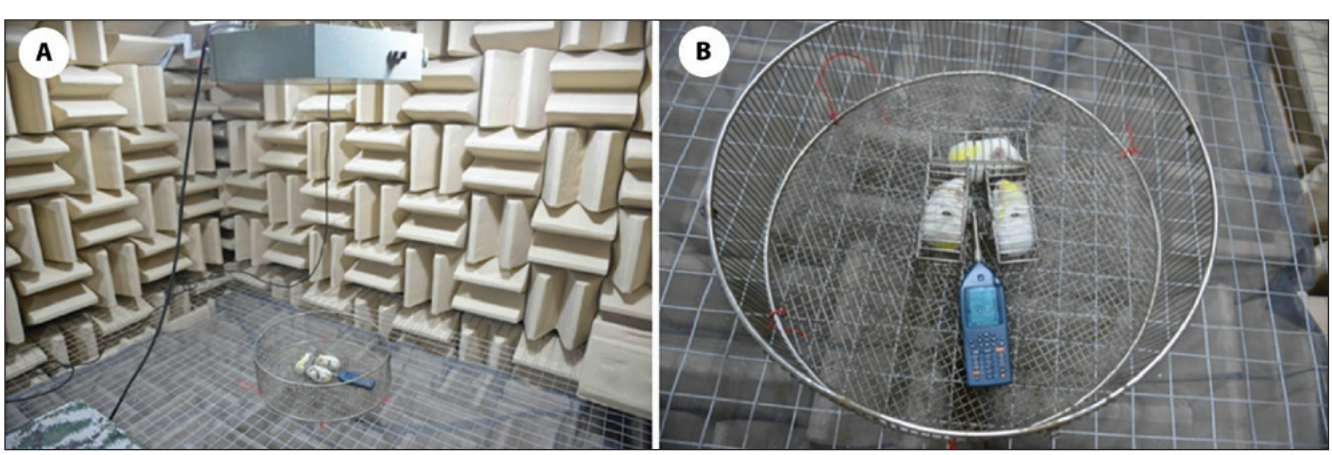

Fig. 1. Strong sonic exposure system and sound pressure device. (A) the strong sonic exposure system; (B) the sound level detecting meter.

round cage. There was one door in the outer round cage, which allowed the animals to enter the cuboid cage. The high-intensity sound generator was placed at the centre of the inner round cage during the experimental periods, and the animals were placed in the inner cage. Therefore, when the animals experienced intolerable sound, they were able to leave through any of the six inner doors to the outer round cage or even to the end of the cuboid cage. In this test, 90 guinea pigs (n=10 per group and 3 repeated experiments) were used. After the sound exposure, the number of animals that escaped was recorded for the subsequent statistical analysis.

Routine examination of peripheral blood and serum cortisol detection

Guinea pigs were anesthetized by intraperitoneal injection with 1% pentobarbital sodium (1 mL/100 g) immediately, 3 d, 7 d and 14 d after the strong acoustic exposure. A total of 100 μL of whole blood was added to a plastic centrifuge tube containing 5 μL of 7.5% EDTAK2, mixed, and subjected to haematological analysis using an MEK722 haematology analyser (Japan Optoelectronic Industry Co., Ltd, Japan). The red blood cell count (RBC), haemoglobin (HGB) levels, white blood cell count (WBC), platelet (PLT) levels and other cytological indicators were detected. In addition, at the indicated observation times, 1 mL of blood was also collected to prepare serum for analysis of cortisol (COR) concentration by radioimmunoassay.

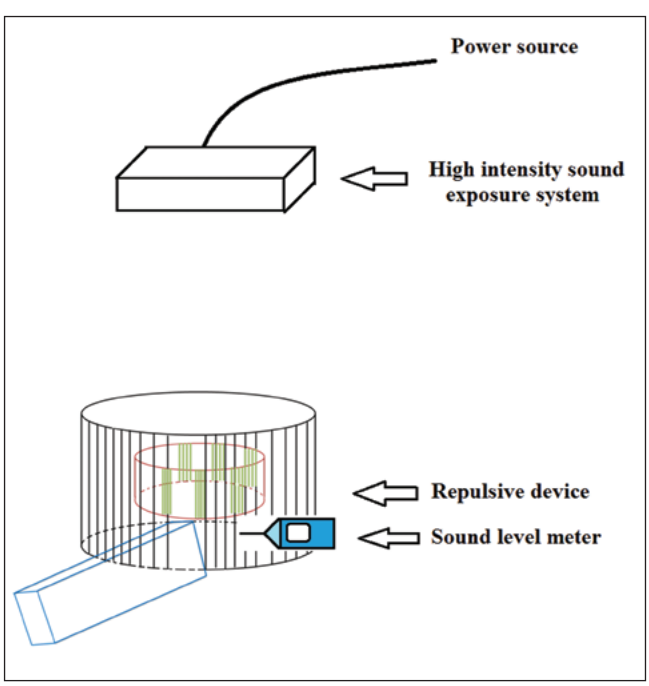

Fig. 2. Schematic diagram of the custom-made repulsive device. The inner round cage is outlined in red and, the six doors are shown in green. The outer round cage, which is marked in black, contains only one door leading to the cuboid cage, which is shown in blue. The high intensity sound exposure system was above the repulsive device.

Hearing ability assessment

The EP2.22 hearing system (Intelligent Hearing Systems, USA) was used in a sound-proof room to detect the auditory brainstem response (ABR). In this system, a 2-mm (inner diameter) plastic tube connected to a high-frequency converter passed the stimulation tone to the guinea pig's external auditory canal. The recording electrode was connected with the cranium, the reference electrode was connected to the test ear, and the ground electrode was placed on the nose tip. Short test tones of 1 kHz, 2 kHz, 4 kHz and 8 kHz were administered as stimulus tones. These tone bursts were performed according to the Blackman package mode with a 1-ms rise/fall time, 4 ms for the short pure tone stimulus time, a stimulus frequency of 19.9 times/s, a bandpass filter of 0.1 to 3 kHz, 1024 superimposition times and 10 ms for the scan time. Moreover, the stimulus intensity began at 100 dB SPL and decreased 10 dB per time until a repeatable ABR waveform could not be drawn. Then, the stimulus intensity increased 5 dB per time until the ABR waveform was distinguished, which was set as the threshold value. This entire process was repeated three times to determine the mean value and standard deviation.

Cochlear structure observation

The guinea pigs were sacrificed immediately, 3 d, 7 d and 14 d after the strong acoustic exposure. The temporal bone was taken from the dorsal side. Under the dissecting microscope, the auditory vesicle was opened, the bony cochlea was exposed and the osseous bones were drilled. Then, the orange bones were removed, and the round window membrane was punctured. Neutral formalin fixation fluid was extracted with a glass dropper and perfused at the snail tip holes and round windows for 3 min. Then, the cochlear tissues were fixed in 10% neutral formalin fixation fluid for 24 h and decalcified with 10% EDTA for 10 to 14 days, after which routine haematoxylin and eosin (H&E) staining was performed. A light microscope was used to observe the basic structure and images were obtained with a DMI3000 camera (Leica Company, Germany).

Immediately and 7 d after the strong acoustic exposure, the cochlear tissues were removed using the abovementioned methods, perfused with 3% glutaraldehyde phosphate buffer (0.1 M PBS, pH 7.4) for 3 min and fixed with 4% glutaraldehyde phosphate buffer for 24 h. The volute was removed under a dissecting microscope and perfused with a phosphate buffer solution and fixed in 1% osmium acid for 1.5 h to prepare the samples for scanning electron microscopy (SEM) observation. The S-4800 SEM (Hitachi, Japan) was used and pictures were taken.

The cochleae were isolated, washed with phosphate buffer and fixed with 1% osmium acid for 1 h. Then, the samples were dehydrated by gradient alcohol and embedded in epoxy resin to prepare the area for transmission electron microscopy (TEM) observation by the H7650 TEM (Hitachi, Japan).

Detection of cell apoptosis in the cochlea

The cochlear tissues were selected immediately, 3 d, 7 d and 14 d after the strong acoustic exposure. The paraffin sections were stained with TUNEL and all steps were performed according to the TUNEL Apoptosis Assay Kit (Roche, Switzerland). Violet blue granules in the nucleus indicated apoptotic cells. The number of positive cells was recorded under the light microscope at 200× magnification. Ten visual fields were randomly selected from each group, and the percentage of positive cells was calculated for statistical analysis.

To further clarify the location and intensity of caspase 3, 8 and 9 expression, immunohistochemical staining was performed in the exposure groups and the sham group. Anti- caspase 3, 8 and 9 antibodies (1:100; Bioworld Technology, USA), which were produced in rabbits, were the primary antibodies, and a polink-2 plus polymer horseradish peroxidase (HRP) detection system was used in this assay. Positive staining was visualized with a diaminobenzidine (DAB) kit (BOSTER, China), and sections were counter-stained with haematoxylin. Positive cells exhibited brown cytoplasm and light blue nuclei. The number of positive cells was recorded under the light microscope at 200× magnification. Ten visual fields were randomly selected from each group, and the percentage of positive cells was calculated for statistical analysis.

Statistical analysis

The data were presented as the mean (± standard deviation). Two-way analysis of variance (ANOVA) with repeated measures was performed using the software SPSS 11.0. Multiple groups were compared using SNK analysis. Differences at $p<0.05$ were considered to be significant. Group effect: compared to the sham group, *$p<0.05$, **$p<0.01$; compared to the 110-dB group, $^{\triangle}p<0.05$, $^{\triangle\triangle}p<0.01$.

RESULTS

Changes of repulsive behaviours

In these experiments, when a guinea pig escaped from the inner round cage, the animal was classified as exhibiting repulsion behaviour. After three repeated experiments, compared to the sham group, the repulsion behaviour of guinea pigs in the 110-dB group and the 130-dB group significantly increased ($p<0.01$) (Table I). The results indicate that strong acoustic exposure at 110 dB and 130 dB caused guinea pigs to exhibit signs of fright and evasion.

Blood changes in routine examination and serum cortisol

Compared to the sham group, there were no significant changes in WBC, RBC, HGB and PLT levels in the guinea pigs from the two high-intensity sound exposure groups (Fig. 3A-D). However, the serum level of cortisol increased significantly immediately following the sonic exposure in the 110-dB and 130-dB groups when compared with the sham group ($p<0.05$ or $p<0.01$). In addition, compared with the sham group, the corti-

Table I. Effect of strong acoustic exposure on the repulsion behaviour in guinea pigs.

Groups	Numbers of escaped animals (n=10 per group)
The sham group	0.333±0.577
The 110-dB group	4.333±0.577**
The 130-dB group	7.667±0.577**

sol concentration was also significantly increased in the 130-dB group at 3 d and 7 d ($p<0.05$) (Fig. 3E).

Hearing changes

Immediately after the 110-dB and 130-dB high-intensity sound exposure, the hearing threshold of the guinea pigs increased significantly ($p<0.05$) compared with the pre-exposure level (Fig. 4B and Fig. 5B). At 3 d after the sound exposure, the threshold in the 110-dB and 130-dB groups remained at a significantly elevated level compared to the pre-exposure level (Fig. 4C and Fig. 5C). At 7 d, the hearing level recovered to normal in the 110-dB group, while the hearing ability did not return to normal until 14 d in the 130-dB group (Fig. 4D and Fig. 5D) (Table II).

Morphological changes of the cochlea

In the sham group, the structures of the cochleae in the guinea pigs were normal, exhibiting three rows of OHCs, round nuclei, homogeneous chromatin and red cytoplasm. In addition, the IHCs exhibited mild oedema, and the structures of the supporting cells were also normal (Fig. 6A, D and G). Immediately after the high-intensity sound exposure, the nuclei of the outer hair cells moved slightly to the side of the cell, and the cytoplasm of the OHCs and IHCs exhibited mild oedema in the 110- and 130-dB groups (Fig. 6B and C). At 7 d after the sonic exposure, the shift, deformation and deeply dyed state of the nuclei of the outer hair cells, and the cytoplasmic oedema of the OHCs and IHCs, were also found in the two exposure groups, especially in the 130-dB group (Fig. 6E and F). At 14 d after the sonic exposure, the structures of the cochleae were restored to the normal state.

The ultrastructure of the cochlea was observed by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). As shown in Fig. 7, the cochlea initially exhibited a neatly arranged single layer of IHCs and three layers of OHCs with complete chenille (Fig. 7A and D). Immediately after the 110- and 130-dB high-intensity sound exposure, the first row of OHCs was arranged irregularly and the stereocilia of the second and third row of OHCs were scattered and were no longer properly situated with the IHCs, especially for the 130-dB group (Fig. 7B

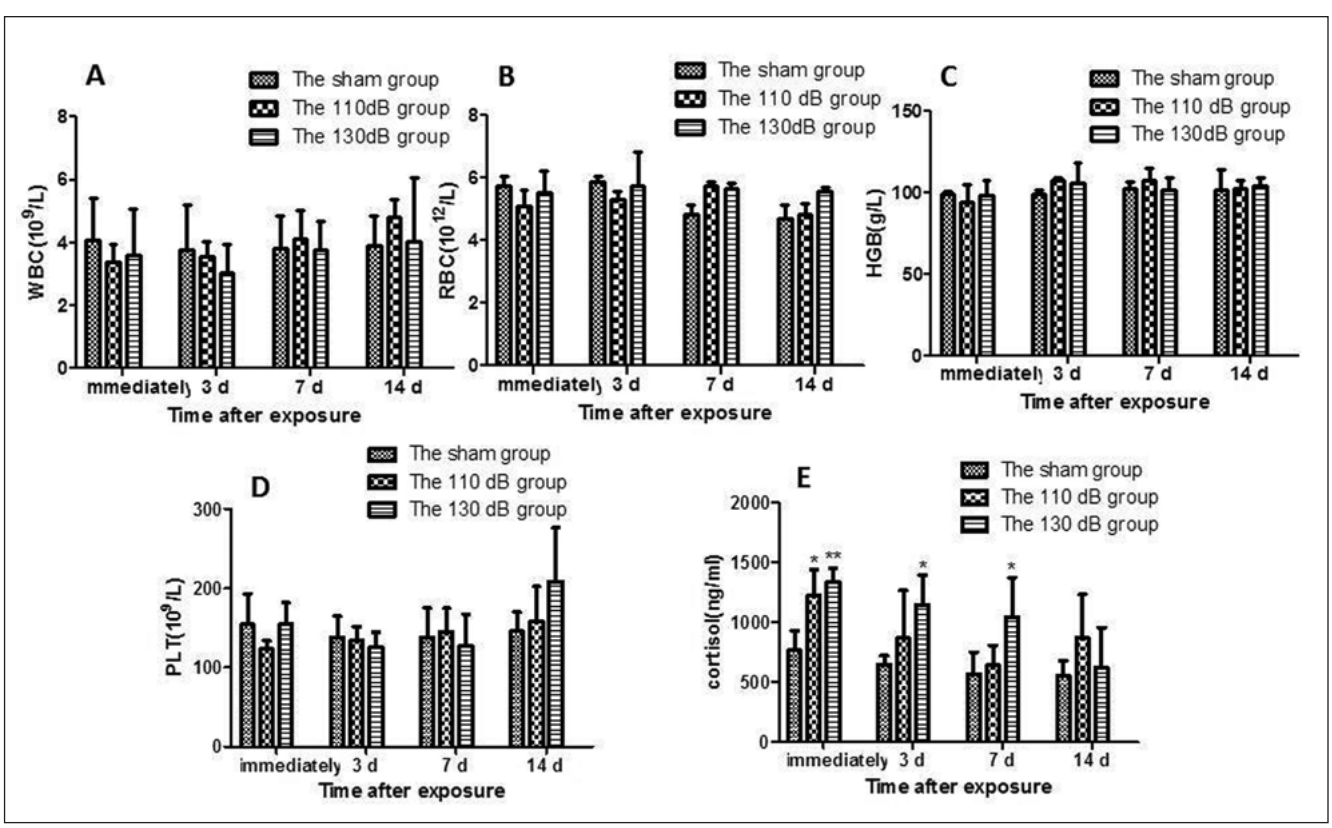

Fig. 3. Levels of WBC, RBC, HGB, PLT and serum cortisol in the sham group and the two sonic exposure groups. (A) WBC; (B) RBC; (C) HGB; (D) PLT; (E) serum cortisol. *$p<0.05$, **$p<0.01$.

Table II. Effect of strong acoustic exposure on the auditory threshold in guinea pigs (dB).

Groups	Pre-exposure	Time after strong acoustic exposure			
		immediately	3 d	7 d	14 d
The 110-dB group	29.17±2.04	42.50±9.35**	34.17±3.76*	30.00±4.47	–
The 130-dB group	30.00±4.47	67.50±6.89**	51.67±4.08**	37.50±2.74*	33.33±2.58

and C). At 7 d after the sonic exposure, the 110-dB group exhibited a partial reversal of the changes, with a slightly neat arrangement of OHCs and lightly scattered and lodged IHCs (Fig. 7E). However, in the 130-dB group at 7 d, the absence of stereocilia in the first row of OHCs and the irregularly arranged stereocilium in the second and third rows of OHCs were observed (Fig. 7 F).

In the sham group, the outer hair cells of the cochlear organ of Corti were normal, exhibiting a clear boundary, round nucleus, complete and clear nuclear membrane, abundant mitochondria, Golgi and an endoplasmic reticulum in the cytoplasm without obvious swelling (Fig. 8A). Small vacuoles were occasionally observed in the outer hair cells, while corresponding support cells with normal structure could be observed around the hair cells (Fig. 8D). Swelling of the outer hair cells, cavitation of organelles and lipid droplets could be seen immediately after the 110- and 130-dB sonic exposure, especially after

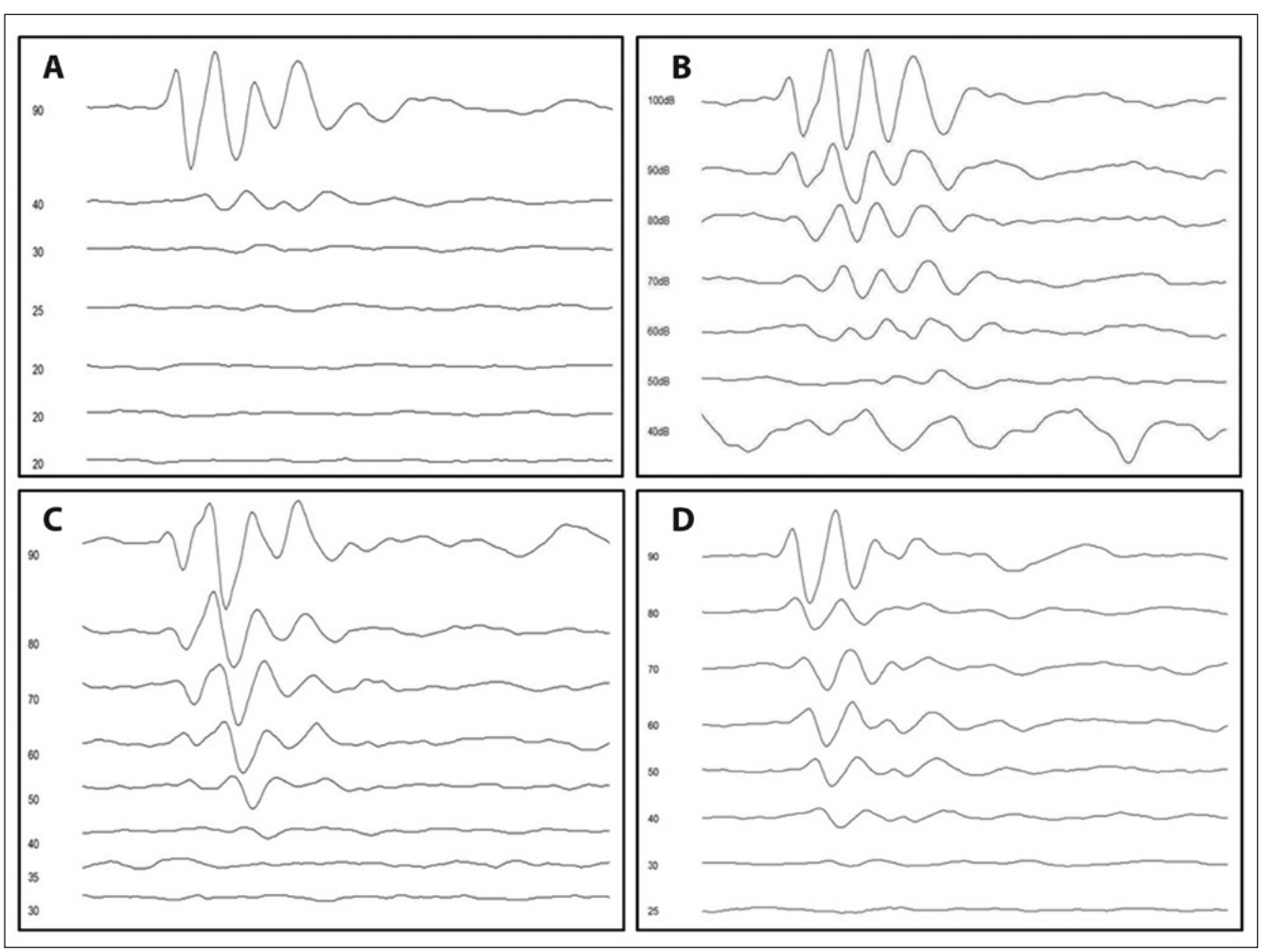

Fig. 4. Audiogram of guinea pigs after strong auditory exposure at 110 dB. (A) the audiogram before exposure; (B) immediately after the sound exposure; (C) at 3 d after the sound exposure; (D) at 7 d after the sound exposure.

the 130-dB exposure (Fig. 8B and C). At 7 d after the high-intensity sound exposure, the abovementioned injuries were restored to normal in the 110-dB group (Fig. 8E), while the lesions persisted at 7 d in the 130-dB group (Fig. 8F).

Apoptotic changes in the Cochlear cells

There were no obvious positive IHCs or OHCs in the sham group after the TUNEL staining (Fig. 9A and D). In the 110-dB group, no obvious positive cells were observed immediately after the sonic exposure (Fig. 9B), but at 7 d after the sonic exposure, a significant increase in apoptotic cells was observed ($p<0.01$) (Fig. 9E) (Table III). However, in the 130-dB group, significant apoptosis of the IHCs and OHCs was observed immediately and at 7 d after the sonic exposure ($p<0.01$) (Fig. 9C-D) (Table III).

Changes in the expression of caspase 3, 8 and 9 in the cochlea

Compared with the sham group, the expression of caspase 3 in the outer hair cells of the cochlea increased significantly at 7 d in the 110-dB group and immediately and at 7 d after the sonic exposure in the 130-dB group ($p<0.01$) (Fig. 10) (Table IV). Changes in the expression of caspase 8 and 9 were similar; significant positive changes were only detected in the 110- and 130-dB exposure groups at 7 d after the sonic exposure ($p<0.01$) (Fig. 11 and 12) (Table V-VI).

DISCUSSION

Loud sounds are a common cause of hearing loss. Intense sounds may result in permanent hearing loss, but lower levels typically cause a transient decrease in

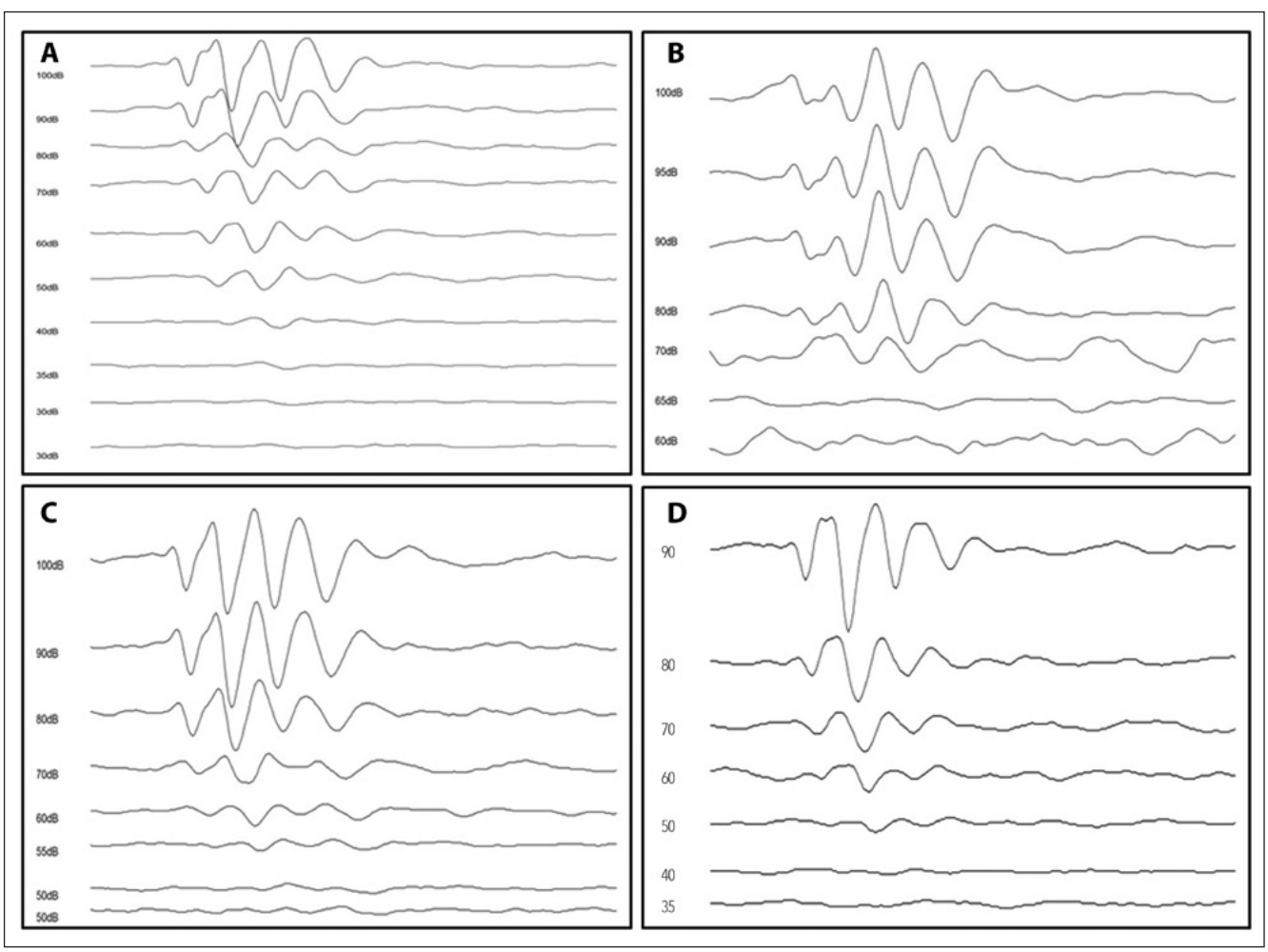

Fig. 5. Audiogram of the guinea pigs after strong auditory exposure at 130 dB. (A) the audiogram before exposure; (B) immediately after the sound exposure; (C) at 3 d after the sound exposure; and (D) at 7 d after the sound exposure.

auditory sensitivity. The most common consequence of excessive exposure to noise is an elevation in hearing thresholds. Such elevations in thresholds may be permanent or temporary (Chen et al., 2014). Noise exposure at low levels or low doses can damage hair cell afferent ribbon synapses without causing permanent threshold shifts (Saedi et al., 2013; Heeringa and van Dijk, 2014). Studies have reported that initial damage to ribbon synapses in the cochleae of guinea pigs is largely repairable, and the repair process in ribbon synapses has been reported in guinea pigs after noise exposure (Shi et al., 2013). In our study, we evaluated high-intensity sound exposure in order to determine whether it is suitable for use by the police as a non-lethal weapon to disperse crowds. Repulsive behaviours were observed immediately after the 110- and 130-dB sound exposures in the guinea pigs, and the auditory thresholds recovered to normal at 7 d and 14 d in the 110-dB and 130-dB group, respectively, which indicated that the sound exposure applied in our experiment did not induce permanent injuries. Meanwhile, the basic haematological index also reflected essential physiological changes. In our study, we did not detect changes in WBC, RBC, HGB and PLT levels in the guinea pigs, which indicated the safety of the high intensity sound exposure.

Cortisol, which is the major glucocorticoid produced in the adrenal cortex, has been used as a measure of hypothalamic-pituitary-adrenal axis activity in stress research (Carrasco and Van de Kar, 2003; Miller et al., 2007). Previous studies have revealed that industrial noise at levels > 80 dB has signifi-

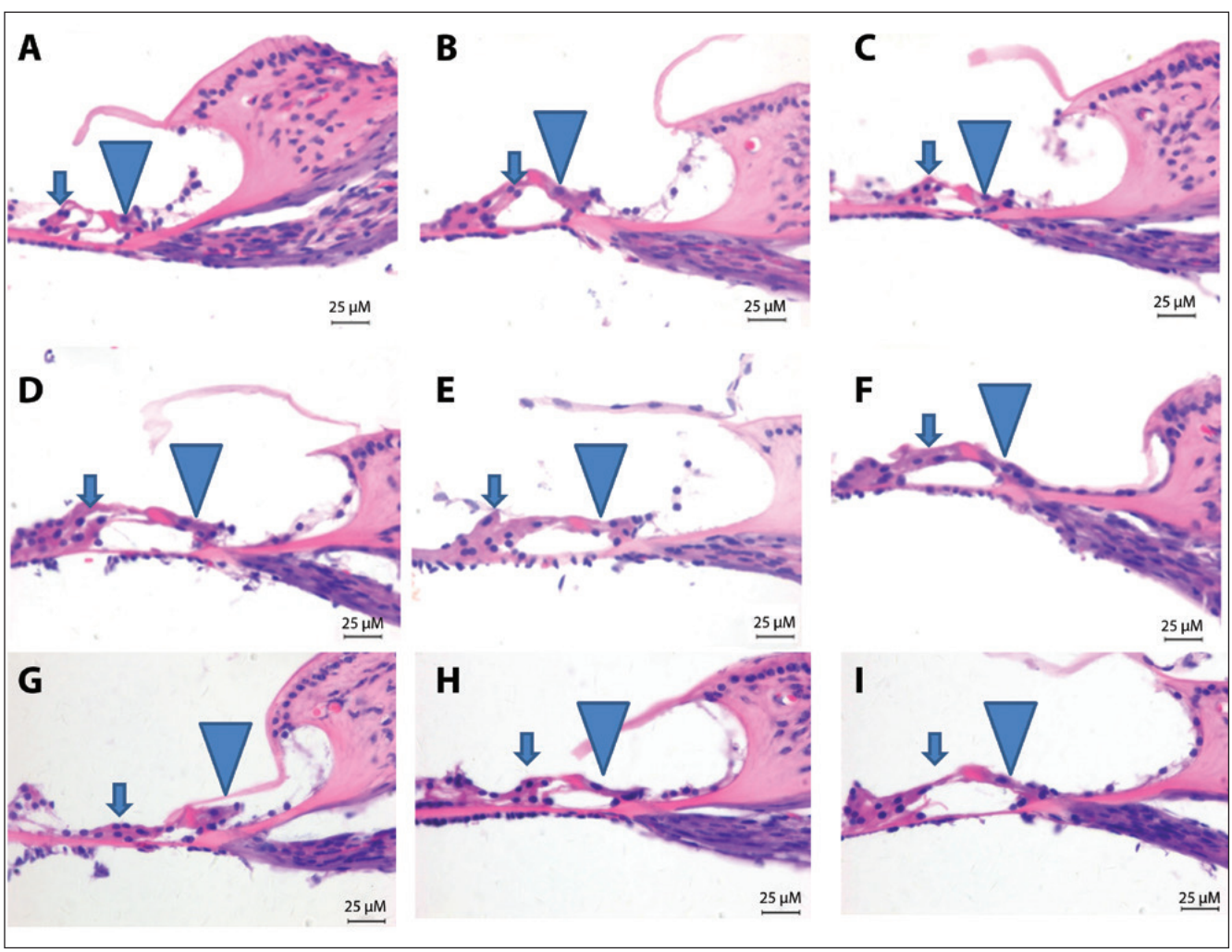

Fig. 6. Effects of strong sonic exposure on the morphological changes in the cochlea. A-C: immediately after sonic exposure for the sham group, the 110-dB group and the 130-dB group, respectively; D-F: at 7 d after the sonic exposure for the sham group, the 110-dB group and the 130-dB group, respectively. G-H: at 14 d after the sonic exposure for the sham group, the 110-dB group and the 130-dB group, respectively. Scale bars=25 μm. ⬇ indicates the OHCs, ▼ indicates the IHCs.

cant effects on salivary cortisol levels in blue collar industrial workers (Fouladi et al., 2012). Muchnik et al. (1998) also found that at the time of the complete arousal state in guinea pigs, norepinephrine levels and pulse rate increased significantly during noise exposure. Reaction to stress is a very important component in evaluating the basic physiological state. In our study, the serum cortisol levels in the guinea pigs exhibited a transient increase after the high-intensity sound exposure. The increase in serum cortisol in the 130-dB group persisted greater than 7 d after the sound exposure, but was no longer detectable at 14 d, indicating that the stress increase induced by the high-intensity sound was recoverable.

Observation of the basic structure and ultrastructure of the cochlea was important for evaluating the effect of sound exposure on hearing because structural changes are the basis for functional abnormality. Jensen et al. (2015) reported that moderate acoustic overexposure in adult rodents caused acute injuries in IHCs and delayed degeneration of the auditory nerve; moreover, various sound pressure levels (SPLs) caused a reversible or permanent threshold shift in mice. The structural and functional injuries were aggravated with an increase in sound pressure level (SPL). Both the IHCs and OHCs may degenerate after noise exposure (Heeringa and van Dijk, 2014; Wang F et al., 2013). In our research, at 7 d and 14 d after the high-intensity sound exposure, the IHCs and OHCs exhibited varying degrees of recovery, according to the H&E staining, which indicated that the injuries caused by the sound exposure in our experiments were not permanent. Combining the results of the auditory threshold tests and H&E staining, we concluded that the injuries recovered to normal by 7 d and 14 d in the 110- and 130-dB group, respectively. To further clarify the effects of sound exposure on the cochlea, we explored the underlying mechanisms and determined the type of damage that occurred in the IHCs and OHCs.

Necrosis and apoptosis are two main processes of cell death. Necrotic cell death, which is a passive form of cell death, is associated with cell swelling. Necrosis eventually leads to damage of the surrounding tissue and the initiation of an inflammatory response, which can induce severe functional changes (Majno and Joris, 1995). Apoptotic cell death is a programmed and highly regulated form of cell death. Apoptosis may occur as a means for the body to neatly eliminate unwanted or damaged/dying cells

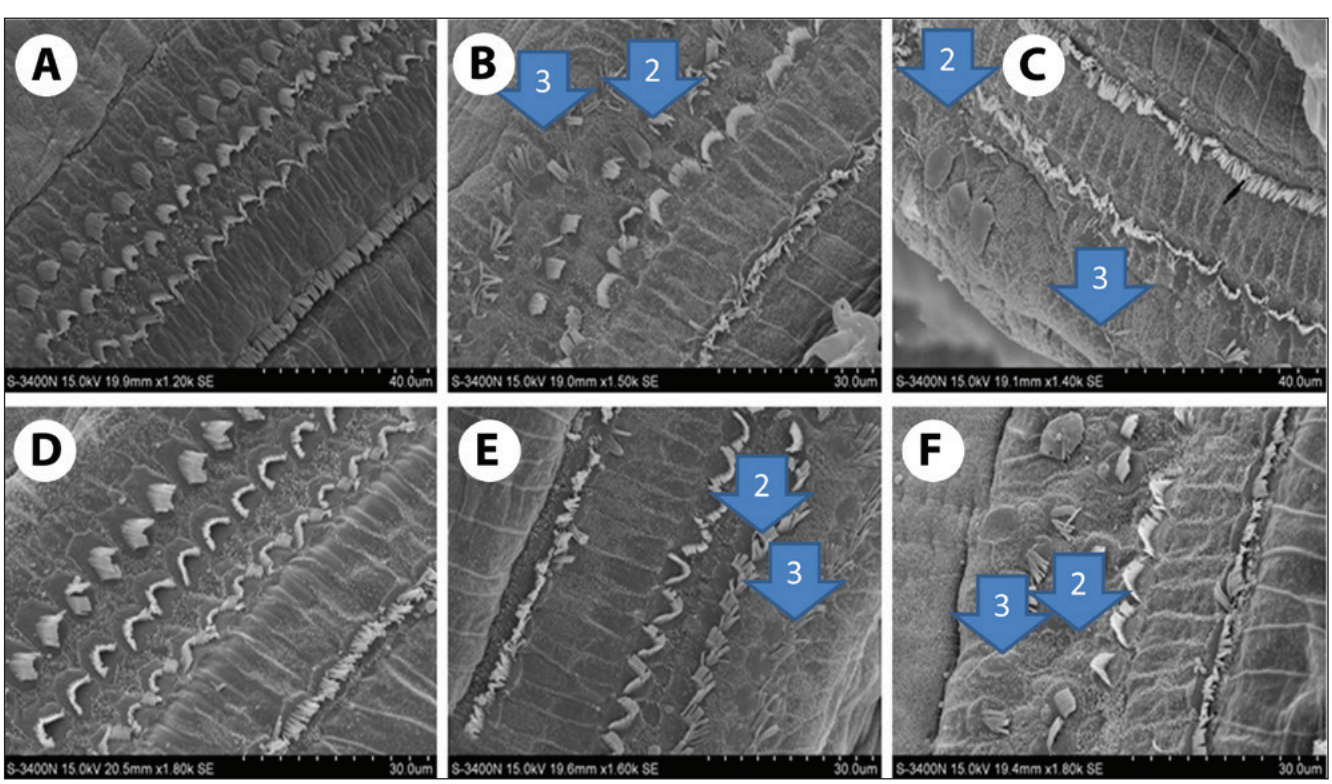

Fig. 7. Effects of two doses of strong sonic exposure on the Corti, as observed by SEM. A-C: the sham, 110-dB and 130-dB group, respectively, immediately after the sonic exposure. D-F: the sham, 110-dB and 130-dB group, respectively, at 7 d after the sonic exposure. ⬇ and ⬇ indicate the second and third rows of the outer hair cells; the cells were scattered, floating and missing.

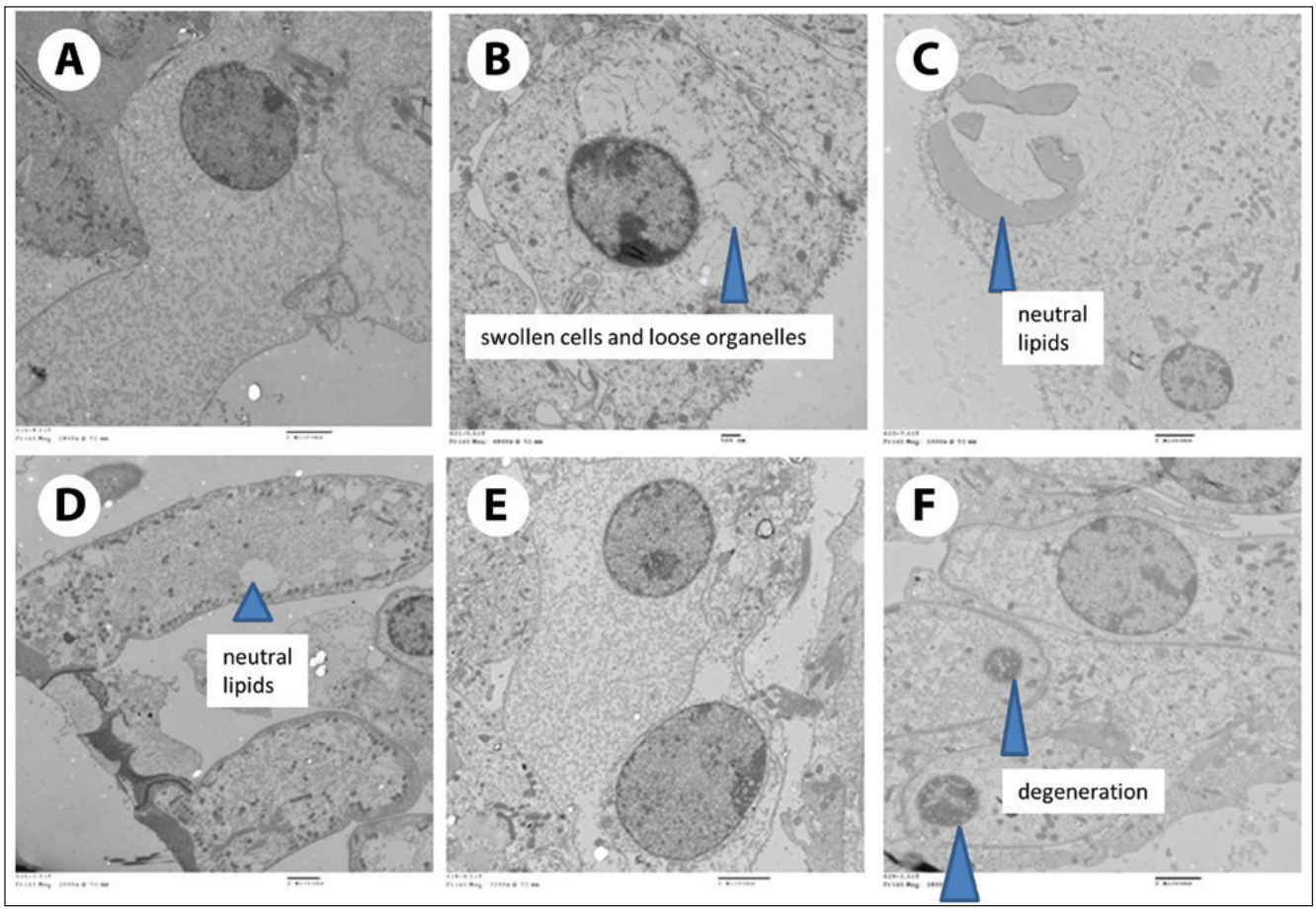

Fig. 8. Effect of various doses of strong sonic exposure on the organ of Corti, as observed with STM. A-C: the sham, 110-dB and 130-dB group, respectively, immediately after the sonic exposure. D-F: the sham, 110-dB and 130-dB group, respectively, at 7 d after the sonic exposure.

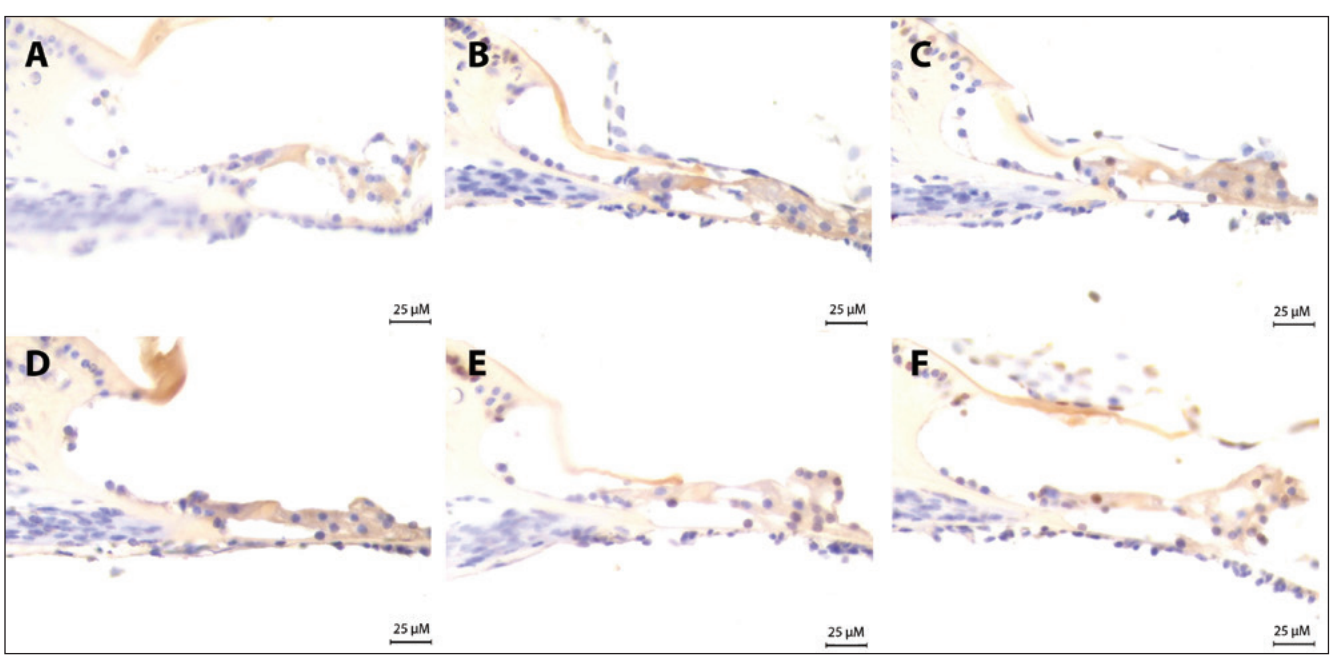

Fig. 9. Effect of high-intensity sound exposure on apoptosis of the cochlear cells, as measured by TUNEL staining. A-C: the sham, 110-dB and 130-dB group, respectively, immediately after the sonic exposure. D-F: the sham, 110-dB and 130-dB group, respectively, at 7 d after the sonic exposure.

Table III. Percentages of apoptotic cells in cochlear cells after strong acoustic exposure (%).

Group	Time after strong acoustic exposure	
	immediately	7d
Sham group	1.50±0.25	4.49±1.12
110-dB sonic exposure group	1.76±0.36	22.48±2.43**
130-dB sonic exposure group	5.69±0.54**	30.53±2.82**

that could potentially damage neighbouring healthy cells (Hu et al., 2002; Han et al., 2013). Recent studies have reported the occurrence of apoptosis in the noise-damaged cochlea (Hu et al., 2000; Hu, 2007). Our results confirmed the occurrence of apoptosis in the noise-exposed cochlea by TUNEL staining, and indicated that apoptosis is the main process involved in the generation of the OHC and IHC lesion. Based upon the results of H&E staining and the auditory threshold tests, we concluded that apoptosis would not induce permanent injuries. In addition, the caspase family are known to play important roles in apoptosis (Han et al., 2007). Moreover, caspases are central components of the machinery responsible for apoptosis (Shalini, 2015). Caspases involved in apoptosis are generally divided into two categories, the initiator caspases, caspase-2,-8,-9 and -10, and the effector caspases, which include caspase-3, -6, and -7 (Shi, 2002). The expression of caspase 3, 8 and 9 also exhibited the same pattern as the occurrence of

Table IV. Percentage of caspase 3-positive cochlear cells after strong acoustic exposure (%).

Group	Time after strong acoustic exposure	
	immediately	7d
Sham group	1.50±0.25	4.49±1.12
110-dB sonic exposure group	1.76±0.36	22.48±2.43**
130-dB sonic exposure group	5.69±0.54**	30.53±2.82**

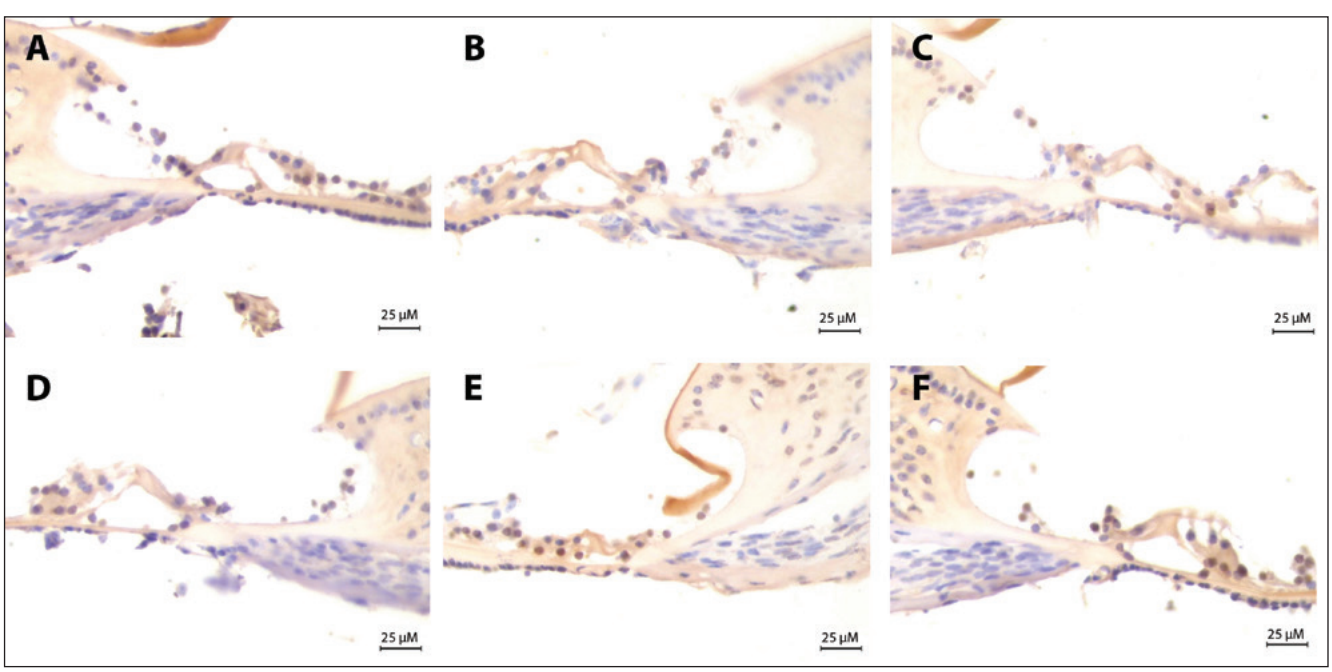

Fig. 10. Effect of high-intensity sound exposure on the expression of caspase 3 in the cochlea. A-C: the sham, 110-dB and 130-dB group, respectively, immediately after the sonic exposure. D-F: the sham, 110-dB and 130-dB group, respectively, at 7 d after the sonic exposure.

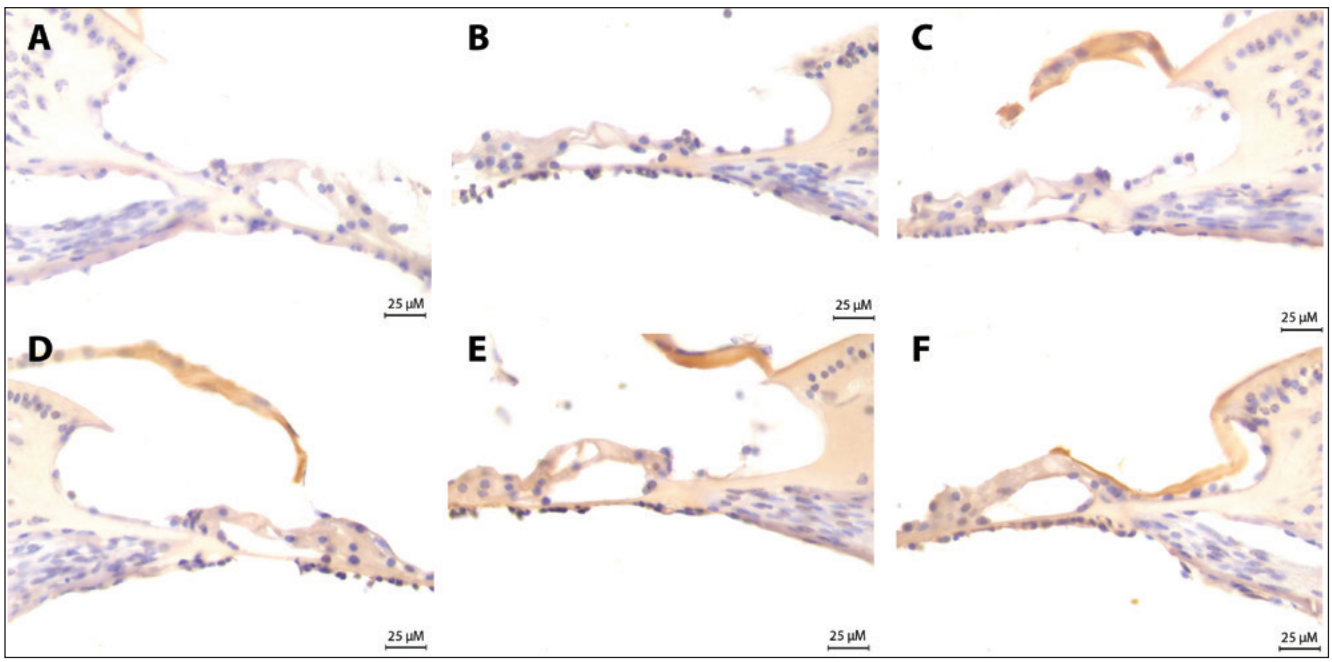

Fig. 11. Effect of high-intensity sound exposure on the expression of caspase 8 in the cochlea. A-C: the sham, 110-dB and 130-dB group, respectively, immediately after the sonic exposure. D-F: the sham, 110-dB and 130-dB group, respectively, at 7 d after the sonic exposure.

apoptosis (Kominami, 2012). The occurrence of apoptosis and the increased expression of caspase 3, 8 and 9 may be the basis for the effects of high-intensity sound exposure.

Effects of sound exposure on aural ability have been studied for years, including Hes1 expression changes, deficits in repaired synapses, increased oxidative stress and decreased ATPase activity, reduced organ of Corti stiffness, alterations in cyclooxygenase 1 (COX-1) and 5-lipoxygenase (5-LO) expression and free radical formation (Matsunobu et al., 2009; Heinrich et al., 2010; Jacob et al., 2013; Lo et al., 2013; Shi et al., 2013; Wang B et al., 2013). However, most of these studies involved normal noise, and the exposure time was at least several hours; the effect of exposure to high-intensity sound for a short time is still unclear. Therefore, additional research on high-intensity sound exposure is warranted.

We found that in guinea pigs the injuries caused by exposure to 110 dB and 130 dB for 5 min were recoverable. The 130-dB high intensity sound induced more serious injuries compared with the 110-dB sound, and the injuries in the 130-dB group occurred earlier and lasted longer than those in the 110-dB group, indicating a dose-effect relationship. Moreover, apoptosis of IHCs and OHCs was the primary cause of cell death.

ACKNOWLEDGEMENTS

This work was supported by grants from the Project of Logistic University of CAPF (JY1401 and WHB201414 and FYQ201561 and FYM201539) and the Project of Science and Technology in Tianjin (15ZXLCSY00040).

Table V. Percentage of caspase 8-positive cochlear cells after strong acoustic exposure (%).

Group	Time after strong acoustic exposure	
	immediately	7d
Sham group	7.68±1.79	8.57±1.08
110-dB sonic exposure group	8.43±1.58	23.28±1.78**
130-dB sonic exposure group	9.61±1.55	32.16±1.55**

Table VI. Percentage of caspase 9-positive cochlear cells after strong acoustic exposure (%).

Group	Time after strong acoustic exposure	
	immediately	7d
Sham group	3.30±0.58	4.53±0.92
110-dB sonic exposure group	3.51±0.53	12.47±1.32**
130-dB sonic exposure group	3.68±1.33	16.74±3.39**

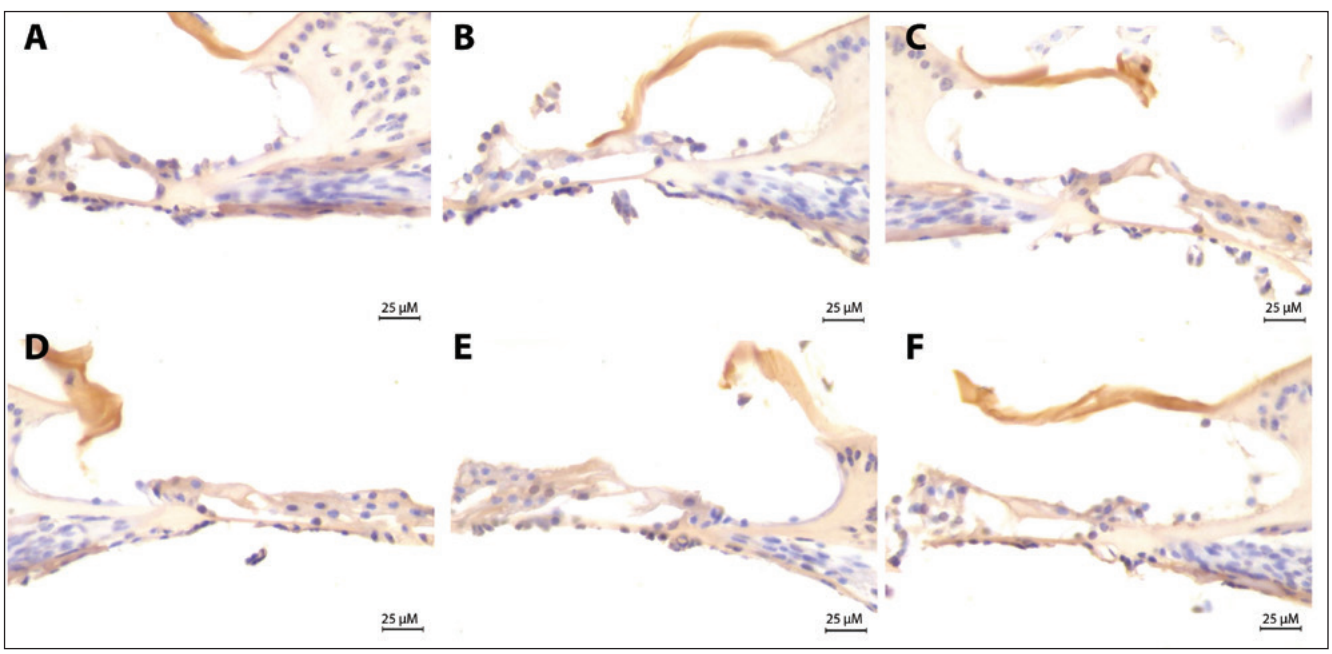

Fig. 12. Effect of high-intensity sound exposure on the expression of caspase 9 in the cochlea. A-C: the sham, 110-dB and 130-dB group, respectively, immediately after the sonic exposure. D-F: the sham, 110-dB and 130-dB group, respectively, at 7 d after the sonic exposure.

REFERENCES

Adler HJ, Kenealy JF, Dedio RM, Saunders JC (1992) Threshold shift, hair cell loss, and hair bundle stiffness following exposure to 120 and 125 dB pure tones in the neonatal chick. Acta Otolaryngol 112: 444–454.

Akdogan O, Selcuk A, Take G, Erdogan D, Dere H (2009) Continuous or intermittent noise exposure, does it cause vestibular damage? An experimental study. Auris Nasus Larynx 361: 2–6.

Carrasco GA, Van de Kar LD (2003) Neuroendocrine pharmacology of stress. Eur J Pharmacol 463: 235–272.

Chen GD, Decker B, Krishnan Muthaiah VP, Sheppard A, Salvi R (2014) Prolonged noise exposure-induced auditory threshold shifts in rats. Hear Res 317: 1–8.

Fernandez KA, Jeffers PW, Lall K, Liberman MC, Kujawa SG (2015) Aging after noise exposure: acceleration of cochlear synaptopathy in "recovered" ears. J Neurosci 35: 7509–7520.

Fouladi DB, Nassiri P, Monazzam EM, Farahani S, Hassanzadeh G, Hoseini M (2012) Industrial noise exposure and salivary cortisol in blue collar industrial workers. Noise Health 14: 184–189.

Han W J, Shi XR, Nuttall A (2007) Caspase 3 activation and apoptosis inducing factor translocation in noise exposure induced out hair cells apoptosis (in Chinese). Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 42: 515–519.

Han WJ, Shi XR, Nuttall A (2013) Noise-induced nitrotyrosine increase and outer hair cell death in guinea pig cochlea. Chin Med J 126: 2923–2927.

Heeringa AN , van Dijk P (2014) The dissimilar time course of temporary threshold shifts and reduction of inhibition in the inferior colliculus following intense sound exposure. Hear Res 312: 38–47.

Heinrich UR, Selivanova O, Schmidtmann I, Feltens R, Brieger J, Mann WJ (2010) Noise exposure alters cyclooxygenase 1 (COX-1) and 5-lipoxygenase (5-LO) expression in the guinea pig cochlea. Acta Otolaryngol 130: 358–365.

Hu BH, Guo W, Wang PY, Henderson D, Jiang SC (2000) Intense noise-induced apoptosis in hair cells of guinea pig cochleae. Acta Otolaryngol 120: 19–24.

Hu BH, Henderson D, Nicotera TM (2002) Involvement of apoptosis in progression of cochlear lesion following exposure to intense noise. Hear Res 166: 62–71.

Hu BH (2007) Delayed mitochondrial dysfunction in apoptotic hair cells in chinchilla cochleae following exposure to impulse noise. Apoptosis 12: 1025–1036.

Ide M, Morimitsu T (1990) Long term effects of intense sound on endocochlear DC potential. Auris Nasus Larynx 17: 1–10.

Jacob S, Johansson C, Fridberger A (2013) Noise-induced alterations in cochlear mechanics, electromotility, and cochlear amplification. Pflugers Arch 465: 907–917.

Jensen JB, Lysaght AC, Liberman MC, Qvortrup K, Stankovic KM (2015) Immediate and delayed cochlear neuropathy after noise exposure in pubescent mice. PLoS One 10: e0125160.

Kominami K, Nakabayashi J, Nagai T, Tsujimura Y, Chiba K, Kimura H, Miyawaki A, Sawasaki T, Yokota H, Manabe N, Sakamaki K (2012) The molecular mechanism of apoptosis upon caspase-8 activation: quantitative experimental validation of a mathematical model. Biochim Biophys Acta 1823: 1825–1840.

Liberman LD, Suzuki J, Liberman MC (2015) Dynamics of cochlear synaptopathy after acoustic overexposure. J Assoc Res Otolaryngol 16: 205–219.

Lo WC, Liao LJ, Wang, CT, Young, YH, Chang YL, Cheng PW (2013) Dose-dependent effects of D-methionine for rescuing noise-induced permanent threshold shift in guinea-pigs. Neuroscience 254: 222–229.

Maison SF, Usubuchi H, Liberman MC (2013) Efferent feedback minimizes cochlear neuropathy from moderate noise exposure. J Neurosci 33: 5542–5552.

Majno G, Joris I (1995) Apoptosis, oncosis, and necrosis. An overview of cell death. Am J Pathol 146: 3–15.

Matsunobu T, Satoh Y, Ogawa, K, Shiotani A (2009) Heme oxygenase-1 expression in the guinea pig cochlea induced by intense noise stimulation. Acta Otolaryngol Suppl 562: 18–23.

Miller GE, Chen E, Zhou ES (2007) If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. Psychol Bull 133: 25–45.

Muchnik C, Rosenthal T, Peleg E, Hildesheimer M (1998) Stress reaction to intense sound exposure under different arousal levels in guinea pigs. Acta Otolaryngol 118: 646–650.

Pawlaczyk-Luszczynska M, Dudarewicz A, Zamojska M, Sliwinska-Kowalska M (2011) Evaluation of sound exposure and risk of hearing impairment in orchestral musicians. Int J Occup Saf Ergon 17: 255–269.

Saedi B, Ghasemi M, Motiee M, Mojtahed M, Safavi A (2013) Transient threshold shift after gunshot noise exposure. B-ENT 9: 133–139.

Sergeyenko Y, Lall K, Liberman MC, Kujawa SG (2013) Age-related cochlear synaptopathy: an early-onset contributor to auditory functional decline. J Neurosci 33: 13686–13694.

Shalini S, Dorstyn L, Dawar S, Kumar S (2015) Old, new and emerging functions of caspases. Cell Death Differ 22: 526–539.

Shi L, Liu L, He T, Guo X, Yu Z, Yin S, Wang J (2013) Ribbon synapse plasticity in the cochleae of Guinea pigs after noise-induced silent damage. PLoS One 8: e81566.

Shi Y (2002) Mechanisms of caspase activation and inhibition during apoptosis. Mol Cell 9: 459–470.

Shoji F, Yamasoba T, Magal E, Dolan DF, Altschuler RA, Miller JM (2000) Glial cell line-derived neurotrophic factor has a dose dependent influence on noise-induced hearing loss in the guinea pig cochlea. Hear Res 142: 41–55.

Wang B, Liu Y, Chi F, Zhang Y, Yang M, Zhu X (2013) Dexamethasone suppresses cochlear Hes1 expression after noise exposure. Acta Otolaryngol 133: 233–238.

Wang F, Zuo L, Hong B, Han D, Range EM, Zhao L, Sui Y, Guo W, Liu L (2013) Tonotopic reorganization and spontaneous firing in inferior colliculus during both short and lo ng recovery periods after noise overexposure. J Biomed Sci 20: 91.

Zhang B, Zhou, HS, Cheng Q, Lei L, Hu B (2014) Low-frequency ultrasound induces apoptosis of rat aortic smooth muscle cells (A7r5) via the intrinsic apoptotic pathway. Genet Mol Res 13: 3143–3153.