

Repeated Atmospheric Pressure Alteration Effect on the Cochlea in Rats: Experimental Animal Study

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OBJECTIVE: This study aimed to evaluate the effects of repeated pressure alterations on cochlear structures in rats in an attempt to understand indirectly the inner ear status of flight crew who are repeatedly exposed to pressure alterations.

METHODS: There were 12 adult Wistar albino rats equally divided into 2 groups: Group 1 (controls) and Group 2 (study group). The animals in Group 2 were exposed to repeated pressure changes in a pressure cabin which is regulated by manometers. The animals in Group 1 were placed in the cabin without being exposed to pressure changes. Auditory brainstem response (ABR) testing was performed in all animals at the beginning and at the end of the study. After 12 wk the animals were sacrificed and their cochleas were investigated using scanning electron microscopy (SEM).

RESULTS: In the study group, hearing decreases at 2 kHz, 4 kHz, 6 dB at 8 kHz, and 32 kHz were encountered at the end of 3 mo. On SEM evaluation of the control group, the outer hair cells (OHC) and stereocilia were normal throughout the cochlea. In the study group, there were irregularities in lateral surface connections and separations, collapse, and adhesions in the basal segment of the cochlea and partial loss of stereocilia throughout the cochlea.

CONCLUSION: Repeated alterations in the atmospheric pressure can lead to damage in the inner ear with subtle or evident hearing loss. Frequent flyers like air workers may be at risk of inner ear damage, which may be considered an occupational health problem.

KEYWORDS: inner ear, barotrauma, flight, altitude.

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Inner ear barotrauma occurring during air travel is caused by a pressure difference between the air in the middle ear and the external atmosphere. It develops after ascent or more usually descent. The pressure difference occurs because of failure of the Eustachian tube to equilibrate middle ear and atmospheric pressures.⁶ At a pressure differential of > 90 mmHg (12 kPa), the Eustachian tube will be closed.¹²

The first cases of inner ear barotrauma were reported in the 18th century by Pilate de Rozier, Charles, and Robert, who described ‘aerotitis’ following descent from a balloon. Since then, inner ear barotrauma, which can cause permanent or transient hearing loss, has been a well-recognized entity, especially in aviation. Sudden or repeated pressure changes can endanger the ear in the form of labyrinthine window ruptures, hair cell damage, and synaptopathy despite the fact that the inner ear is a barometric sensor that detects atmospheric pressure changes.^{11,13}

Airplanes spend at least 20 min while ascending to their highest altitudes [almost 10,000 m (32,808. ft)] or while

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descending, which can cause a pressure change of 237 mmHg relative to the sea level. Although that pressure alteration is equalized within the cabin of the plane, this may not be optimal and pressure change may exert an effect to some extent on the inner ear, especially in the frequent flyers. A hidden hearing loss is possible as well.⁸ In this study, we aimed to evaluate the effects of repeated pressure alterations on inner ear structures in rats in an attempt to understand indirectly the inner ear status of flight crew who are repeatedly exposed to pressure alterations.

METHODS

Animals

In total, 12 healthy adult Wistar albino rats which were 4 mo old and weighing between 250 and 350 g were included in the study. All experimental protocols and surgical procedures were carried out in the experimental animals research and application center of the tertiary referral hospital. This study was approved by the institutional review board of the ethical committee on animal experiments of the same facility (IRB 2017-3-20) in accordance with the rules on animal care of the international Helsinki declaration. The experimental animals were housed and fed with pellet feed at 50% humidity at 16 to 21°C temperature conditions.

The animals were equally divided into two groups as follows: Group 1 ($N = 6$) served as controls and Group 2 ($N = 6$) was the study group. The animals in Group 2 were exposed to pressure changes in a pressure cabin which is regulated by manometers. The animals in Group 1 were placed in the cabin without being exposed to pressure changes.

Materials

Auditory brainstem response (ABR) testing was performed in all animals at the beginning and at the end of the study in order to exclude hearing loss and evaluate hearing change, respectively. After 12 wk the animals were sacrificed, their temporal bones were removed, and their inner ears were investigated using scanning electron microscopy (SEM).

Procedures

The animals were anesthetized intramuscularly with 65 mg · kg⁻¹ ketamine hydrochloride and 5 mg · kg⁻¹ xylazine hydrochloride. Otomicroscopy was done to inspect the ear canal for any cerumen, crusting or infection. ABR (Intelligent Hearing Systems, Miami, FL, USA) testing was made in a quiet environment. The active ABR electrode was placed on the vertex, the reference electrode was placed under the mastoid skin, and the ground electrode was placed under the skin. A neonatal ear tip suitable for the outer ear canal of the rats was used. Recordings were made ipsilaterally at 2 kHz, 4 kHz, 8 kHz, 16 kHz, and 32 kHz using tone burst stimuli averaging 1024 sweeps, and a 100–1500 Hz filter was used. The stimulus rate was set to 21.1 pps. An ER 3 insert earphone was used for the 2 kHz, 4 kHz, and 8 kHz tone burst stimulus and a high-frequency transducer was used for the 16 kHz and 32 kHz tone burst stimulus. The

stimuli were given at 80 dB SPL by decreasing in 20 dB steps until 20 dB. Threshold scanning was performed to the lowest level where the wave was seen. ABR threshold was defined as the minimum intensity level at which the second wave was observed. A 20 dB SPL or lower intensity at each test frequency was considered normal hearing whereas intensities higher than 20 dB SPL were considered hearing loss.

A custom-made pressure cabin was produced by one of the authors who has been in the practice of hyperbaric oxygen treatment. The cabin could maintain its own barometric pressure independently of the atmospheric pressure changes outside. Barometric pressure could be adjusted at a variety of rates and ranges. The cabin had a vacuum pump and manometers. The vacuum pump was 20 kg in weight, 430 mm in length, 210 mm in height, 250 mm in width, 3000 d/d in engine speed, 0.75 kW in engine power, 0–2 mbar (759 mmHg) in vacuum efficiency, and 21 m³/h in pump capacity. The manometer was analog and adaptable to the cabin.

The animals in Group 2 were taken into the pressure cabin as the pressure was set to 0 mmHg. Then the pressure was decreased to -237 mmHg (i.e., 316 hectaPascal, 0.31 atm, 0.32 Bar, or 322 cm H₂O) for 20 min. For pressure change balance, the pressure was set to 0 mmHg again and then increased to +237 mmHg for 20 min. Airplanes spend around 20 min while ascending to their highest altitudes or while descending, which can cause a pressure change of 237 mmHg relative to sea level. The rest of the time during flying, pressure is balanced. Because of this, we calculated the experiment's time with 20 min ascending and 20 min descending. The same procedure was performed 3 d/wk for 12 wk. The same procedure was applied in Group 1—increasing cabin pressure as for the other group, but without pressure change through opening the cabin's door and leaving it open during the experiment. It eliminated specific noise exposure hearing loss to one group.

At least 3 mo after the interventions and 1 wk after the second ABR testing, the animals were sacrificed by injecting 80 mg · kg⁻¹ ketamine hydrochloride and 20 mg · kg⁻¹ xylazine hydrochloride intramuscularly. Temporal bone removal was started with a vertical skin incision in the occipital area. The temporalis muscle and periosteum on both sides were elevated over the parietal bone and squamous portion of the temporal bone. The auricle was laterally retracted and the occipital and parietal bones were cut vertically. The intracranial structures were removed. Mastoid and tympanic parts of the temporal bone (bulla) were exposed by continuing the elevation of muscle tissues in the subperiosteal plane. The mastoid portion was dissected from the occipital bone. The squamous portion was dissected from the parietal, frontal, palatine, and ethmoid bones. The bulla was dissected from the occipital and sphenoid bones. The petrous portion was dissected from the sphenoid bone. The temporal bone was dissected from the surrounding muscle tissues and separated from the skull.

The tympanic cavity was instilled with 2.5% glutaraldehyde for fixation. The tympanic bulla was dissected and the cochlear structure was reached. It was kept in phosphate buffer solution (pH = 7.3) for 12 h and decalcified in 0.1 M Na-EDTA

(Sigma-Aldrich, Munich, Germany) solution for 2 wk at room temperature. After decalcification, the otic capsule was dissected asymmetrically from base to apex to view the cochlear

structures under the microscope (Olympus, 1 × 71 S8-F3, Japan) and routine SEM follow-up was done for 3 d at +4°C PBS. The tissues were dried with carbon monoxide at the critical drying point (CPD 010, Balzer Union, Liechtenstein) and fixed by carbon holders on brass blocks. The surface of the organ of Corti was covered with gold (SC502, Bio-Rad, Hercules, CA, USA) under argon gas. Routine follow-up procedures and SEM (JEOL 6335F SEM) imaging were performed.¹⁵ The outer hair cell (OHC) morphology was evaluated from the cochlear base to apex in the modiolar axis and the parameters were scored semiquantitatively (Table I).

Table I. Evaluation Parameters of the Inner Ear on Scanning Electron Microscopy.

PARAMETER	DEGENERATION GRADE	SCORE
General Cell Morphology (Surface, side connections)		
Normal cell morphology	Normal	0
Collapse, regular separation in side connections	Mild degeneration	1
Collapse, irregular separation in side connections	Moderate degeneration	2
Necrosis	Severe degeneration	3
Outer hair cells		
Normal stereocilia morphology	Normal	0
Irregularities in stereocilia	Mild degeneration	1
Adhesions and partial loss of stereocilia	Moderate degeneration	2
Total stereocilia loss	Severe degeneration	3
Location of degeneration		
No degeneration	Normal	0
Hair cell degeneration in 1/3rd in cochlea	Mild degeneration	1
Hair cell degeneration in 2/3rd in cochlea	Moderate degeneration	2
Hair cell degeneration in 3/3rd in cochlea	Severe degeneration	3

Statistical Analysis

Statistical analysis was performed using the Number Cruncher Statistical System (NCSS) 2007 Statistical Software (Kaysville, UT, USA). The Mann-Whitney *U*-test was performed to produce overall results for all groups by using median values (Table II, Table III, Table IV, and Table V).

RESULTS

On initial ABR testing, all animals had a hearing threshold level better than 20 dB SPL. There was no significant difference between the groups in the test frequencies ($P > 0.05$). In the control group, the pre- and post-intervention hearing thresholds were similar ($P > 0.05$). In the study group, hearing decreases at

Table II. *P*-Values for the Two Groups Before the Experiment (MWU Test, $N = 12$).

ABR	2 KHz		4 KHz		8 KHz		16 KHz		32 KHz	
	STUDY	CONT	STUDY	CONT	STUDY	CONT	STUDY	CONT	STUDY	CONT
Mean	16.67	16.67	13.33	15.83	16.67	17.50	18.33	19.17	15.00	16.67
<i>P</i> -Values	1.0000		0.2421		0.6870		0.5797		0.4372	

ABR: auditory brainstem response; MWU: Mann-Whitney *U* test; CONT: Controls.

Table III. *P*-Values for the Two Groups After the Experiment (MWU Test, $N = 12$).

ABR	2 KHz		4 KHz		8 KHz		16 KHz		32 KHz	
	STUDY	CONT	STUDY	CONT	STUDY	CONT	STUDY	CONT	STUDY	CONT
Mean	20.00	20.83	19.17	20.00	18.33	20.00	20.00	20.83	20.00	20.00
<i>P</i> -Values	0.3593		0.3593		0.1658		0.3593		1.0000	

ABR: auditory brainstem response; MWU: Mann-Whitney *U* test; CONT: Controls.

Table IV. *P*-Values for the Study Group Before and After the Experiment (MWU Test, $N = 12$).

ABR	2 KHz		4 KHz		8 KHz		16 KHz		32 KHz	
	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER
Mean	16.67	20.00	13.33	19.17	16.67	18.33	18.33	20.00	15.00	20.00
<i>P</i> -Values	0.0357		0.0043		0.3764		0.1658		0.0063	

ABR: auditory brainstem response; MWU: Mann-Whitney *U* test.

Table V. *P*-Values for the Control Group Before and After the Experiment (MWU Test, $N = 12$).

ABR	2 KHz		4 KHz		8 KHz		16 KHz		32 KHz	
	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER
Mean	16.67	20.83	15.83	20.00	17.50	20.00	19.17	20.83	16.67	20.00
<i>P</i> -Values	0.0247		0.0156		0.0780		0.1854		0.0357	

ABR: auditory brainstem response; MWU: Mann-Whitney *U* test.

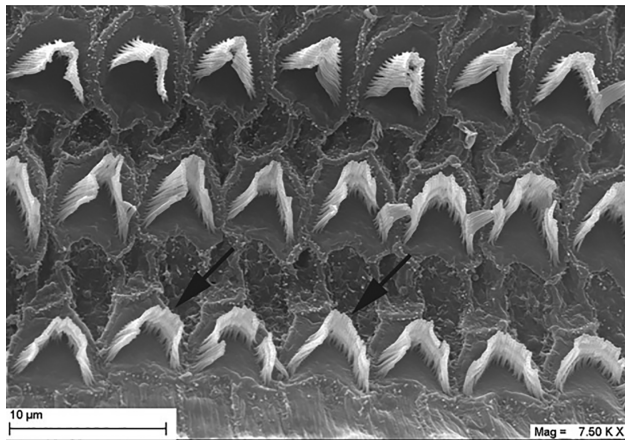


Fig. 1. Scanning electron microscopy of the control group at 7.50 K X. Note the normal surface topography of the organ of Corti and the normal arrangement of outer hair cells and their stereocilia (arrows).

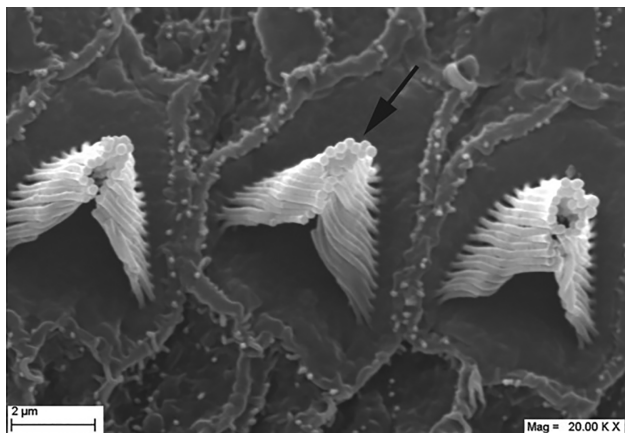


Fig. 2. Scanning electron microscopy of the control group at 20.00 K X. Note the normal surface topography of the organ of Corti and the normal arrangement of outer hair cells and their stereocilia (arrow).

2 kHz, 4 kHz, 6 dB at 8 kHz, and 32 kHz were encountered at the end of 3 mo ($P < 0.05$).

On SEM evaluation of the control group (Group 1), 12 cochleas of 6 rats were evaluated. In 10 cochleas, the OHC stereocilia were normal throughout the cochlea, which scored 0 points. In two cochleas, there were irregularities in the OHC stereocilia, which scored 1 point (average score of 0.16) (**Fig. 1** and **Fig. 2**).

On SEM evaluation of the study group (Group 2), 12 cochleas of 6 rats were evaluated. In seven cochleas, there were irregularities in the lateral surface connections and separations, collapse and adhesions in the basal segment of the cochlea, and partial loss of stereocilia of OHCs throughout the cochlea from base to apex. These cochleas scored 5 points. In five cochleas, there were irregularities, adhesions, and complete loss of OHC stereocilia (**Fig. 3** and **Fig. 4**). These cochleas scored 4 points. The average score of the study groups was 4.58. There was a statistically significant difference

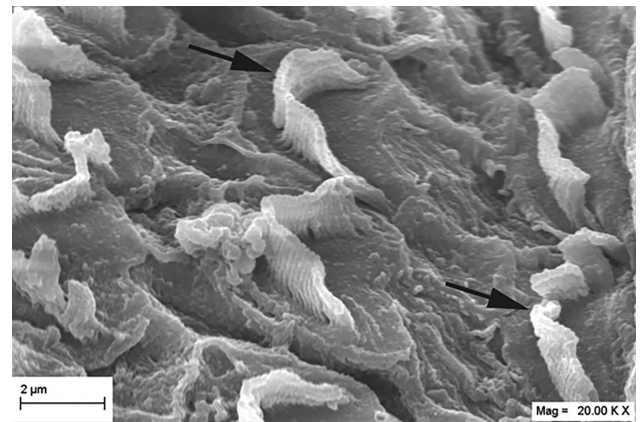


Fig. 3. Scanning electron microscopy in the study group at 20.00 K X. Note the adhesions and ruptures of the cuticular plates in the stereocilia of the outer hair cells (arrows).

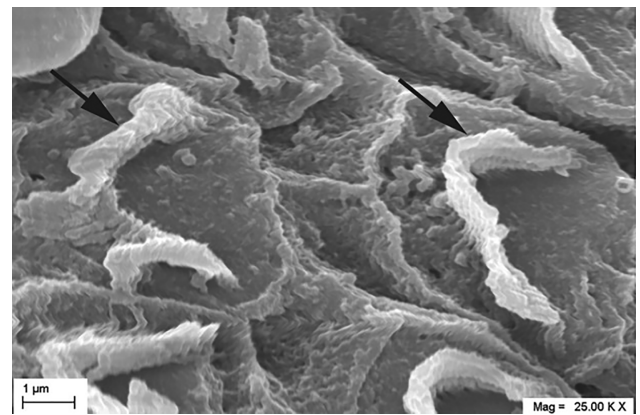


Fig. 4. Scanning electron microscopy in the study group at 25.00 K X. Note the adhesions and ruptures of the cuticular plates in the stereocilia of the outer hair cells (arrows).

Table VI. Inner Ear Evaluation Scores on Scanning Electron Microscopy.

PARAMETER	SEM SCORING OF MORPHOLOGY (N = COCHLEAS)		P-VALUE
	GROUP 1	GROUP 2	
General cell morphology	1 (N = 2)	5 (N = 7)	0.001
Outer hair cells	0 (N = 10)	4 (N = 3)	
Location of degeneration	0 (N = 0)	4 (N = 2)	
Score	0.16 (2/12)	4.58 (55/12)	

SEM: scanning electron microscopy.

between the SEM scores of the control and study groups ($P < 0.01$) (**Table VI**).

DISCUSSION

In barotrauma, equal dislocation may occur at every site in the basement membrane because the differences between the middle ear pressure and the inner ear or intracranial pressure

may cause equal pressure changes between the endolymph and perilymph at all cochlear basement membrane sites. Therefore, this nonspecific injury would occur equally throughout the entire basement membrane. Alternatively, the pressure difference between the middle ear and inner ear may cause the induction of air bubbles in the cochlea.³ An increase in middle ear pressure is commonly observed when diving or landing as an occupant of a pressurized aircraft.⁹

Barotrauma to the inner ear can happen by explosive or implosive means. The tympanic membrane will be pressed medially as the external pressure increases by descending, which will in turn push the stapes into the vestibule, causing increased pressure in the cochlea. An inward or outward movement of the stapes footplate can exceed the limits of normal extension. At a sound pressure of 1 Pa or 94 dB SPL, the vibratory displacement amplitude of the stapes is only 30 nm in the frequency range 100–1000 Hz.⁴ The scala vestibuli pressure in humans saturates somewhere between 1 and 2 kPa, depending on frequency.¹⁰ In addition to that, a sudden pressure increase in the external ear canal above 20 hPa is transmitted to the perilymph.⁷ Therefore, in our study the pressure alterations created in the pressure chamber were sufficient to exert effects on the cochlea.

High level acoustic energy can be more easily transferred from the ear canal to the human cochlea than to the rat cochlea. That is, human hearing might be damaged at much lower pressure than in rats.¹⁰ Although rats were used in our study, based on the difference between rats and humans, it would be plausible to say that the cochlear damage would be more severe in flight crew than what we detected in the rats.

There are also few manuscripts showing inner ear barotrauma in recreational divers. Although the cochlear problems that occur after the pressure experienced by sea divers are caused by more acute events, they show us the effects of pressure change on the cochlea. Vestibular symptoms and sensorineural hearing loss were observed in studies with divers.^{1,14}

Compression, decompression, or pressure alterations can cause transient and permanent threshold shifts. Hidden hearing losses can happen despite the normal auditory thresholds. Low pressure inner ear barotrauma can induce functional changes of the cochlea and cause temporal threshold shifts which are attributed to hair cells as detected by electrophysiology and scanning electron microscopy.¹⁷ Stereocilia injury of the hair cells following exposure to a high pressure environment is also possible.^{2,5} Repeated compression and decompression effects on the cochlea lead to ABR abnormalities, loss of hair cells, and fusion of stereocilia throughout the cochlea, which can be important for air workers.^{16,18}

In conclusion, repeated alterations in the atmospheric pressure can lead to damage in the cochlea with evident hearing loss. Frequent flyers like air workers may be under the risk of cochlear damage, which may be considered an occupational health problem. Further animal studies are recommended to understand the change in the vestibular part of the inner ear, duration of exposure effects in the cochlea, and a

broad-based pilot study on humans among aircrew or those exposed to constant changes in atmospheric pressure because this study was limited to rats and their cochleas.

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