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SEM Observation for the Damage of Inner Hair Cell Stereocilia of Guinea Pig Cochlea after Loud Tone Exposure

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Abstract

The inner hair cell stereocilia of the guinea pig cochlea was examined under a scanning electron microscope (SEM) after loud tone exposure onto the ear drum of the animal. Before and after guinea pigs were exposed to intensive and continuous tone such as 106 dB SPL in intensity, the functioning of the cochlea was monitored by N1-N2 audiograms. The structural damage of the stereocilia of inner hair cells (IHCs) and outer hair cells (OHCs) was examined using the SEM in x1500 magnification. The comparison between the functional change of the cochlea and the structural damage of the IHC stereocilia is done by means of photographic observation. It can be shown that the functional change might be related to the structural damage of the IHC stereocilia after intensive acoustic trauma.

Keywords: *SEM, Loud Tone Exposure, Guinea Pig, Cochlea, Inner Hair Cell, Noise Induced Hearing Loss.*

I. Introduction

Acoustic physiology about hearing loss problems caused by loud tone exposure, drug injection and surgical operation was studied for long years. Derbyshire and Davis[1] showed the action potential of the auditory nerve could be weakened by external noise. Dallos and Cheatham[2] analyzed the relationship between compound action potential or gross neural action potential and individual neural action potentials. Brown et. al.[3] showed that surgical trauma more influenced on the guinea pig compound action potential than anesthesia in high frequency sound. In 1978, Kemp[4] measured stimulated acoustic emissions from the human auditory system, and changed the traditional view point about cochlear functioning. In 1980s the cochlear bio-mechanics were investigated by cochlear functioning deterioration caused with the intentional acoustic trauma[5]. In 1990s the cochlear functioning was studied with numerical modeling[6,7]. Kim and Hong applied different anesthesia for various CAP observation[8].

The main function of the cochlea is like an active transducer because it actively transforms mechanical vibration into auditory neural pulses. The cochlea actively amplifies incoming mechanical energy if the energy is too small to transform. Outer hair cells (OHCs) are known to have the essential role for the active amplification[9]. If OHCs are damaged by any reason, then the phenomena of the active amplification would not be observed. How is the OHC triggered for the active amplification? One of the most positive answers may be the stereocilia rooted on the cuticular plate[10]. The energy amplification triggered by OHC stereocilia may be physically transmitted adjacent cell tissues such as Deiter cells. However another point of view has strong reasoning. That is, the active amplification may be triggered by efferent nerve fibers connected on the lower part of the OHC[11]. The energy amplification triggered by OHC efferent nerve fibers may be transmitted to tectorial membrane through OHC stereocilia. Both answers have a common idea of OHC stereocilia as an important cochlear functioning.

Very different results are reported about the structural deformation of the stereocilia at the type and time of the loud

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tone exposure experiments. Nilsson et al.[12] observed that IHC stereocilia were preserved while OHC2 and OHC3 stereocilia were severely damaged by wideband loud tone exposure. And Robertson[13] found that OHC1 and IHC stereocilia were apparently damaged by pure loud tone exposure while OHC2 and OHC3 stereocilia were more damaged by impulse noise (wideband) exposure. Other paper reported that when the exposure time was increased for the same frequency and intensity, the morphological damage was increased in the order of OHC1, IHC, OHC2 and OHC3 stereocilia respectively[14]. Liberman et al.[15] found no stereocilia damage to 40 dB hearing loss caused by acute acoustic trauma. Hunter-Duvar[16] reported that the collapse of the stereocilia caused by short time loud tone exposure was temporary phenomena and was restored after recovery. These reports show that the structural damage of the cochlear stereocilia caused by loud tone exposure are affected by both the type and time of the acoustic trauma for the same animal. The more morphological research carried out with acoustic trauma in parallel is needed to find out the cochlear bio-mechanism.

This paper observed the guinea pig cochlear stereocilia damage after loud pure tone exposure with N1-N2 audiogram monitoring [17]. The experimental procedure was similar to Robertson[13] but 106 dB SPL pure tone was exposed only 10 minutes. The present results show that the short time loud tone exposure causes apparent damages on IHC stereocilia unlike the results of Liberman et al.[15] or Hunter-Duvar[16].

II. Experimental Method

Guinea pigs were exposed with loud pure tone and their cochlear functioning was monitored with N1-N2 audiogram measurement before and after acoustic trauma. After acoustic trauma the cochlear specimen was prepared for SEM (Scanning Electronic Microscope) observation.

2.1. Guinea Pig Anesthesia, Surgical Operation and Acoustic Coupler Fitting

Guinea pigs were anaesthetized with 25 % Urethane (5.5 ml/kg), injected intra-peritoneally. A tracheotomy was then performed for better breathing. Surgical operation on external auditory meatus was done for proper contact between the tympanic membrane and an acoustic coupler[18]. The temporal bone of the bulla which was 5mm away from the external auditory meatus in a lateral direction was drilled for a small hole. The hole allowed a copper wire electrode to be placed on the edge of the round window. A Bruel & Kjaer ½" condenser microphone (type 4134) was used as a sound generator, and its sound pressure was transmitted to the ear drum by the acoustic coupler (Fig. 1). The animal was kept at a constant temperature of 38 ℃ by a thermistor probe.

2.2. Frequency Calibration for Sound Pressure

It was important to apply the accurate sound pressure onto the ear drum. A ½" calibration condenser microphone (type 4134) was used with 5cm long and thin stainless probe for sound pressure calibration.

2.3. Input Stimulating Sound Generation and Output Reaction Potential Recording

The input continuous pure tone signal was gated for 0.6msec

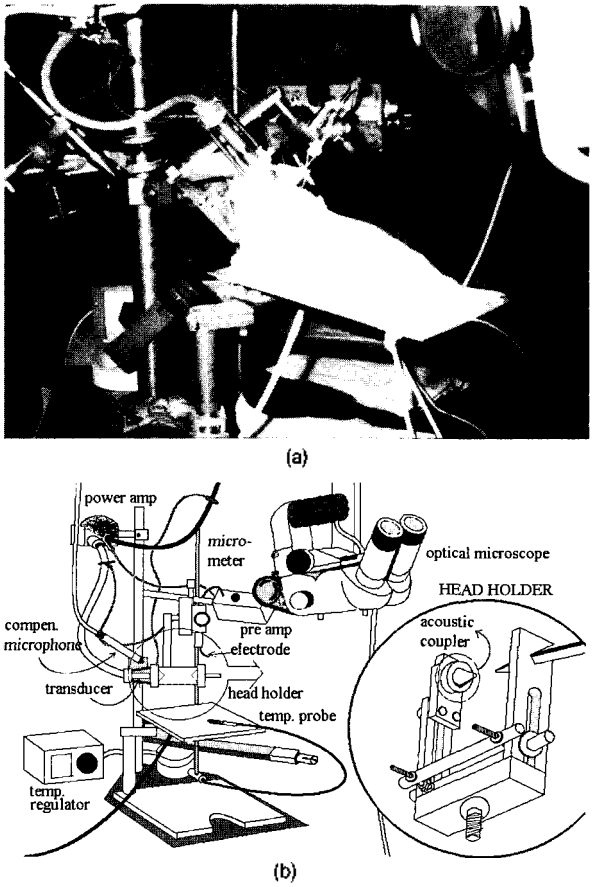


Fig. 1. Acoustic Coupler : An animal holder, shaped like '⊂', is suspended to a thick iron bar (in the middle). A coupler in the animal holder is inserted into the left external auditory meatus to form a closed acoustic system. A transducer is interfaced to the ear drum through the acoustic coupler.

with a ramp time of 0.2msec and its amplitude was controlled by a programmable attenuator and was amplified by a power amplifier. The output gross neural action potential (N1-N2 potentials) was measured from the round window through the wire electrode. Input stimuli and output records were done in manual as well as in automat.

2.4. N1-N2 Audiograms

The time from the start of the input stimulus (0 msec) to the positive peak P1 (charging of the ionic potential) was defined as the latency of the output. The potential amplitude between N1 (1st discharging of the ionic potential) and P1 was set as a criterion, and the sound amplitude of each input frequency was controlled to produce the same N1-N2 criterion. The N1-N2 audiogram shows the criterion of the sound intensity as a function of frequency[18].

2.5. Loud Tone Exposure Control

Guinea pigs were exposed with a 106 dB SPL continuous tone at 10 kHz for 10 minutes. The N1-N2 audiograms were recorded before and after (4-5 hours) the acoustic trauma[17].

2.6. Fixative Injection

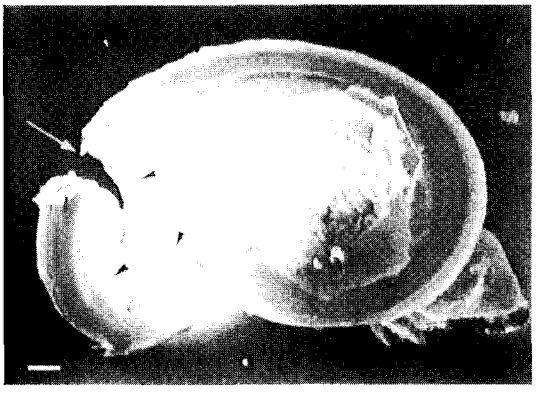
A mark was made on the cochlear duct with a curved metal needle (0.5 mm dia.) put through the round window. This mark served as a reference to measure distance along the cochlea to allow further examination of different types of damage. The tissue around the bulla was cut and removed in a ventral direction and the jaw bones were broken in order to see the apex of the cochlea. A small hole (1 mm dia.) was made in the apex of the cochlea using the point of a needle. Cold (4 ℃) fixative was gently perfused into the cochlea through the round window for fixation by a micrometer driver syringe (0.05 ml/min). The fixative leaked out of the hole cut at the apex. For the present experiment, the fixative consisted of 1 %-2.5 % glutaraldehyde in 0.05M phosphate buffer at pH 7.4.

2.7. Cochlear Specimen Preparation

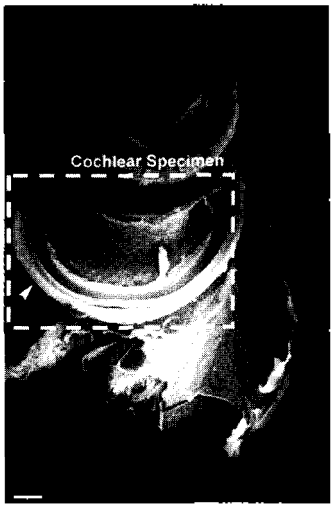
The animal was killed by cutting the brainstem. Both cochleas with some parts of the temporal bones were left in cold fixative for at least 24 hours. The stub of cochlea including the modiolus and organ of corti were dissected and removed from the surrounding bone. The stubs were dehydrated in acetone and then dried by the critical point technique with liquid CO2. The dried cochlear specimens were fixed on copper stubs and sputter-coated with platinum to a nominal depth of 18-25 nm and examined by a JEOL 120CX. The acceleration voltage was 40 kV.

III. Results

The noise-induced threshold shift of the N1-N2 audiogram measured electrophysiologically as a function of frequency can be compared with the ultrastructural change of the hair cell stereocilia assessed morphologically as a function of distance [15,19]. The functional loss in the cochlea after acoustic trauma is related not only to the threshold shift of the N1-N2 audiogram at a specific frequency range but also to the damage of the hair cell stereocilia structure at a specific cochlear location. 5 guinea

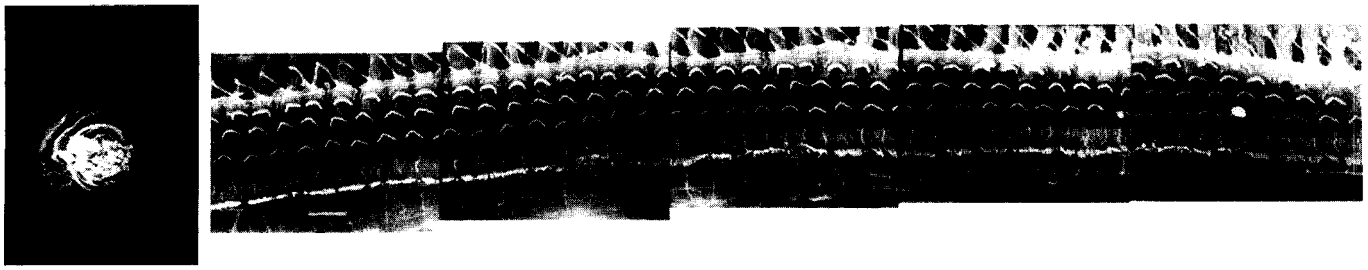


(a)



(b)

Fig. 2. (a) A scanning electron micrograph of the guinea pig cochlear duct with a reference mark (arrow) and broken parts (arrowheads). Scale Bar: 0.217 mm.
(b) A scanning electron micrograph of the lateral view of the right guinea pig cochlea. Arrowhead : Cochlear partition. Scale Bar : 0.22 mm.



(a) (b)

Fig. 3. (a) The unexposed cochlear duct: The arrowhead indicates the position where the IHCs stereocilia suddenly appear damaged. Scale Bar: 0.833 mm.
(b) The inner and outer cell stereocilia displayed in progression from the arrowhead of (a) towards the apex. Arrow: Reference Mark. Scale Bar: 11.0E3 nm

pigs had passed both in electrophysiological experiments and in morphological specimen preparation. After exposure to sound 106 dB SPL at 10 kHz for 10 minutes, the threshold loss curves were greatest 0.26–0.4 octave above 10 kHz, that is, at 12–13.5 kHz [16]. Fig. 2 shows the first 1½ turns of the cochlea from the base. The other turns towards the apex were removed, so that the specimen could be properly placed in the electron microscope. The reference causes mark damage (arrow) and breakage of basal parts (arrow heads). The noise damage appeared mostly in the stereocilia of the IHCs at approximately ¼–½ a turn (approx. 1.5~1.6 mm) of the cochlea from the reference mark.

Fig. 3 (a) shows another cochlear specimen with a reference mark (arrow). The cochlea was removed without loud tone exposure and prepared for examination. The stereocilia of the IHCs and OHCs were apparently normal under low magnification (x1500) around the reference mark. As the specimen was examined from the reference mark towards the cochlear apex, stereocilia remained normal on both IHCs and OHCs. At about 1.5 mm from the reference mark, the stereocilia of IHCs suddenly appeared damaged while those of OHCs were normal. Fig. 3(b) shows the stereocilia of IHCs and OHCs in progression from a position (arrowhead) which is about 1.5 mm from the reference mark. Further towards the apex, the stereocilia of IHCs and OHCs were again apparently normal.

After acoustic trauma, the stereocilia of most OHCs looked normal under low magnification and only occasionally was separation or bending of stereocilia found. However, the stereocilia damage of IHCs clearly appeared at approximately similar places for different cochleas with the same noise exposure. Stereocilia of IHCs were examined under low magnification (x1000 or x1500) in order to relatively and comparatively assess the percentage of stereocilia damage after the loud tone exposure[20]. The upright and straight line of the 'I' typed IHC stereociliary turfs without disconnection was judged as a normal array (0%). Other damaged IHC stereocilia were relatively examined according to the structural state. The criterion of the judgment was divided into 5 groups, and the degree of damage was summed and normalized for mixed criterion.

1. Missing -- 10 %
2. Collapse -- 15 %
3. Flexion -- 20 %
4. Detached -- 25 %
5. Fused -- 30 %

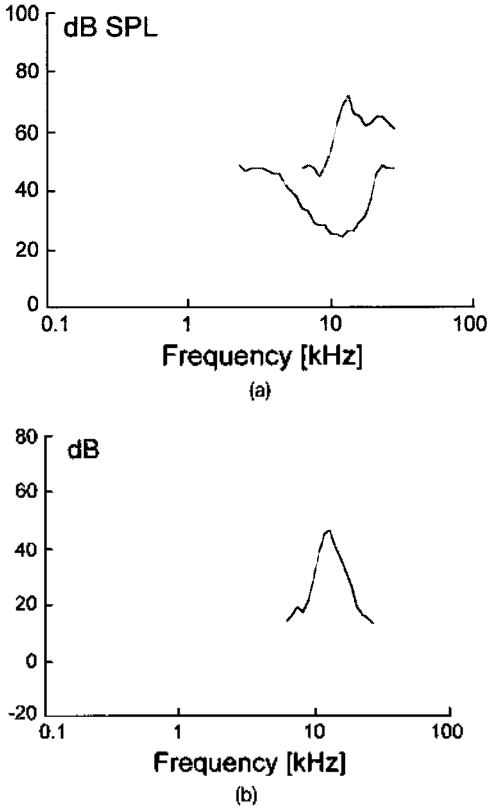


Fig. 4. (a) The pre-(lower line) and post-(upper line) exposure N1-N2 audiograms.
(b) The threshold loss curve of the N1-N2 audiograms.

The assessed percentage of IHC stereocilia damage was plotted as a function of distance along the organ of Corti (IHC Stereocilia Damage Curve) (Fig. 4).

Fig. 4 (a) shows the pre-exposure (lower line) and post-exposure (upper line) audiograms respectively. And fig. 4(b) shows the threshold loss of the N1-N2 audiogram after the loud tone exposure. At approximately 1.58 mm from the reference mark, the IHCs showed the start of the stereocilia damage along the cochlear duct. The noise damage appeared mostly (all of 5 guinea pigs) in the stereocilia of the IHCs at approximately ¼-½ a turn (approx. 1.5~1.6 mm) of the cochlea from the reference mark. This may indicate that the threshold loss of the audiogram caused by loud tone exposure could be related to localized structural damage to the IHC stereocilia (morphological change) when the loud tone intensity is moderate. The discontinuity of the IHC stereocilia damage curves corresponds to preparation damage on the outer edges of the cochlear specimen. The IHC stereocilia damage curve in Fig. 4 is narrower than the threshold loss curves of the corresponding cochleas.

Fig. 5 shows the IHC stereocilia damage curve after the loud tone exposure with scanning electron micrographs (x1000) corresponding to the marked position on the curves (arrowheads). In Fig. 5, it was observed that the IHC stereocilia flop towards the modiolus between the normal and severely damaged area, so that the collapse of the IHC stereocilia indicated the start and end of the stereocilia damage along the cochlear duct. The stereocilia of the OHCs were found to have sideway-links and tip-links at the regions where the stereocilia of IHCs had collapsed. The IHC stereocilia damage curve of Fig. 5 shows that the 10 minutes' acoustic trauma with 106 dB SPL causes localized IHC stereocilia damage.

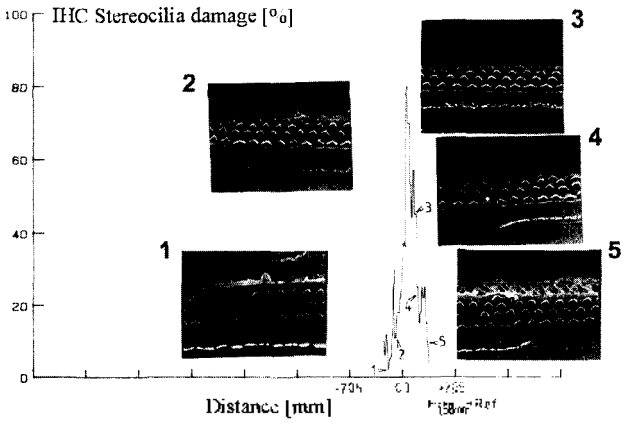


Fig. 5. The IHC stereocilia damage curve together with scanning electron micrographs of particular position indicated by arrowheads on the curve. Ref indicates the reference mark on the cochlear duct around the round window.

IV. Conclusion

For an accurate correlation of the threshold loss curves with structural changes in the organ of Corti, an adequate place-frequency map of the cochlear duct is important to relate a particular cochlear location to a corresponding frequency. Single neuron recordings from the spiral ganglion of the guinea pig cochlea have been studied to derive a place-frequency map for the basal turn[21]. From the present experiments, it is concluded that when the cochlea is exposed to a moderately loud continuous tone (106 dB SPL) for a short period of time (10 minutes). The threshold loss of the N1-N2 audiogram caused by loud tone exposure could be related to localized structural damage to the IHC stereocilia. Therefore the stereocilia of the OHCs were found to have sideway-links and tip-links at the regions where the stereocilia of IHCs had collapsed.

The present results show that the short time loud tone exposure causes apparent damages on IHC stereocilia unlike the results of Liberman et al.[15] or Hunter-Duvar[17]. This difference shows that the structural damage of the cochlear stereocilia caused by loud tone exposure are affected by both the type and time of the acoustic trauma for the same animal. The more morphological research carried out with acoustic trauma in parallel is needed to find out the cochlear mechanism.

If IHC stereocilia are locally damaged while adjacent OHC stereocilia are normal, the N1-N2 audiogram may show threshold loss at corresponding frequencies because of the weakened functioning of the IHC stereocilia. However the actively amplifying mechanism of OHC stereocilia would be alive. Kemp reported that the cochlea with the acoustic trauma produced more tinnitus which was believed to come from the abnormality of the OHC functioning[22]. In the present result the OHC stereocilia were normal after the acoustic trauma. One of reasons of tinnitus after acoustic trauma may be the efferent nerve fibers connected on the lower part of the normal OHC. The weakened IHC afferent nerves may stimulate the normal OHC efferent nerves to trigger some active amplification for poor hearing ability. Then over amplified OHC reaction may produce tinnitus in backward feedback.

The experiments of the cochlear study was carried out at the Dept. of Physiology, Birmingham University, U.K. and the evaluation of the experimental apparatus and the method was confirmed by the faculty of the medical school, Birmingham University[23]

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