Hearing Research, 8 (1982) 77–81
Elsevier Biomedical Press

Short Communication

Relationship between hair cell loss on the chick basilar papilla and threshold shift after acoustic overstimulation

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(Received 25 January 1982; accepted 5 May 1982)

Three groups of chickens were continuously exposed to 125-dB SPL pure tones at either 500, 1500 or 3000 Hz for 12 h. Ten days after the exposure to noise, a good correlation was found between the percentage of hair cell loss and the threshold shifts of the eighth nerve action potential. The data suggest possible roles for the two types of hair cells present on the basilar papilla. In addition it was discovered that the chicken is a good model in which to study acoustic trauma.

Key words: acoustic trauma; chick; basilar papilla.

Structural and functional changes that occur in mammalian cochlea as a result of acoustic overstimulation have been well documented [1,3,4,7,8]. However, although non-mammalian vertebrate auditory systems have often been used in studies of auditory function, the development and central processing of sounds which occur in these systems have rarely been used to study the effects of acoustic trauma. Both similarities and differences between the avian auditory system and the mammalian auditory system have been pointed out in the literature [10,11,15]. The avian basilar papilla (BP) is not coiled and lacks a true organ of Corti, but it does house two types of sensory cells: tall hair cells (THC) and short hair cells (SHC), the innervation patterns of which are similar to those of inner and outer hair cells in the mammalian auditory system [6,14,15]. We felt that because of its structural organization the avian BP is a different and perhaps more primitive organ than the mammalian cochlea. An analysis of the response of the avian BP to acoustic trauma might increase our understanding of the processes involved in acoustic trauma in mammals.

It has recently been shown that the pattern of sensory cell damage produced by

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0378-5955/82/0000–0000/$02.75

acoustic overstimulation in the avian BP is very similar to that reported for mammals [11,12]. However, we still lack electrophysiological and behavioral evidence that would allow us to correlate these structural changes with alterations in function. The purpose of the present work was to correlate patterns of anatomical damage with patterns of hearing loss secondary to overstimulation in the chick BP. The threshold of the VIII nerve compound action potential (AP) response, recorded from the round window, was used to assess auditory activity. After serial reconstruction of the sensory cells of the BP, histological damage to this structure was assessed by light microscopy. The auditory nerve AP responses and histological findings were then correlated.

Four groups of 10-day-old chickens (Hubbard × Hubbard) ($\mathbf{n} = 44$) were used in the present study. Chicks were placed in pairs in a small tubular wire-mesh chamber beneath an IRS power horn inside a small acoustical chamber, and were constantly exposed to a 125 dB SPL pure tone at either 500 Hz ($\mathbf{n} = 8$), 1500 Hz ($\mathbf{n} = 8$), or 3000 Hz ($\mathbf{n} = 8$) for 12 h. The methods for signal generation and calibration were identical to those previously reported [11,12]. 10 to 15 days after acoustical overstimulation, half of the chicks from each experimental group were anesthetized with 80 mg/kg of Ketalar (i.m.) and 1.5 ml/kg of Chloropent (i.p.). The middle ear was exposed by a surgical incision posterior to the external auditory meatus, leaving the tympanic membrane and the columella intact, and a silver electrode was secured at the round window. Compound VIII nerve action potentials were recorded at ten frequencies ranging from 250 Hz to 4000 Hz using a 30-ms duration tone-burst at a rate of 2 bursts/s with 5 ms rise and fall times. After amplification, the potentials were averaged on-line by a PDP 11/04 computer. For each frequency the tones were attenuated until the response disappeared. The lowest intensity at which a clearly observable response was evoked was taken as the threshold [9].

The other half of the animals from each experimental group were killed, and their BP were perfused with a 1% paraformaldehyde–0.75% glutaraldehyde solution. The BP were then postfixed in 2% osmium tetroxide, dehydrated, and embedded in Epon. Transverse 3-μm sections, sampled at 100-μm intervals were made from the base to the apex of the BP. The sections were stained with toluidine blue and the hair cells present were then counted in each section. In order to be counted, a hair cell had to exhibit a cell body, cuticular plate and stereocilia.

Both histological and electrophysiological data were compared with data from two control groups of chickens raised in a normal acoustic environment ($2 \times \mathbf{n} = 10$).

Analysis of AP thresholds in chicks exposed to noise and the control chicks revealed that the chicks in each group exposed to noise suffered significant threshold shifts. Fig. 1 shows the mean shift from normal threshold seen after exposure to each noise condition. Overstimulation with 500 Hz noise caused a broad threshold shift, from 250 to 3500 Hz, with a maximum (20 dB) at 500 Hz. The threshold shift following overstimulation with 1500 Hz noise was greater in magnitude (35 dB) but was sharper in frequency, with a maximum shift at 2000 Hz. The magnitude of the threshold shift after exposure to 3000 Hz noise was small (18 dB), with a maximum at 3000 Hz.

Total hair cells present in chicks exposed to noise were compared with the total

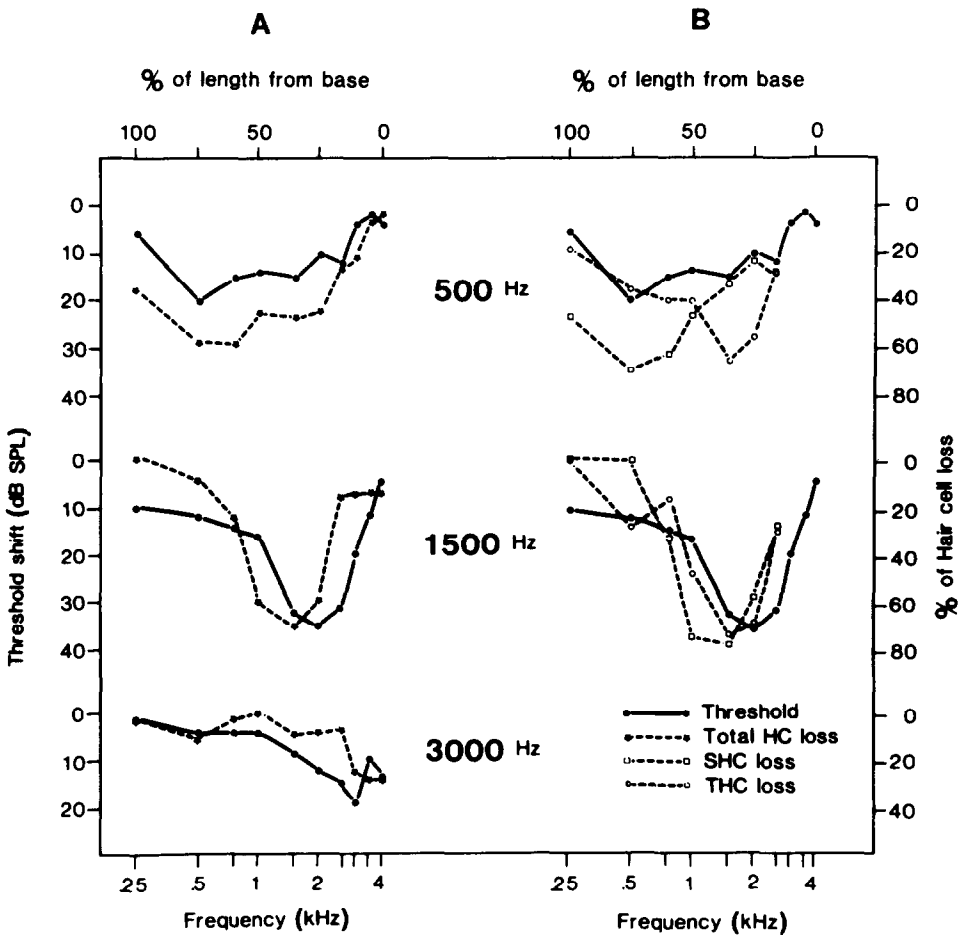

Fig. 1. Comparison between threshold shift and hair cell (HC) loss in the chicken ear after exposure to traumatic levels of three tones (500, 1500 and 3000 Hz). (A) The threshold shifts (———) are plotted as a function of frequency. On the same graph, total HC loss (- - - - - -) is plotted as a function of position along the basilar papilla (BP). (B) As in A, the solid lines represent the threshold shift. The two interrupted lines show the tall hair cell (THC) loss (○) and the short hair cell (SHC) loss (□). Note that for 3000 Hz the total HC loss corresponds to SHC loss since THC are not represented in this part of the BP.

number of hair cells present in control chicks along the entire length of the BP. Hair cell counts were taken at 100-μm intervals. Fig. 1A shows the percent of total hair cell loss as a function of position along the BP and as a function of frequency according to the regression curve determined by Ryals and Rubel [12]. Hair cells remaining were then analysed according to cell type. Cells were classified as THC and SHC in the manner of Tanaka and Smith [15]. Fig. 1B shows the percentage of THC and SHC loss along the BP for chickens in each experimental group. At 3000 Hz, the hair cells were damaged in a part of the BP containing only SHC. In this

case the percentage of total hair cell loss is equal to the percentage of SHC loss.

A non-parametric test of correlation (Sperman's coefficient of rank correlation, ρ) was performed to determine the relationship between histological and physiological data. A strong correlation was seen for the percentage of total hair cell loss along the BP and the threshold shift throughout the frequency range in each experimental group: for 500 Hz stimulation, $\rho = 0.92$; for 1500 Hz stimulation, $\rho = 0.73$; and for 3000 Hz stimulation, $\rho = 0.63$. When a correlation between types of hair cells lost and threshold shift was sought, it was found that there was better agreement between SHC loss and the threshold shift due to 500 Hz stimulation ($\rho = 0.80$) than there was for THC loss due to 500 Hz stimulation ($\rho = 0.60$). At 1500 Hz stimulation the correlation between THC loss and threshold shift ($\rho = 0.79$), and SHC loss and threshold shift ($\rho = 0.74$) were very similar.

The correlation between threshold shift and total hair cell loss was at least as good as the correlation between threshold shift and the loss of cells of each individual type. This suggests that both SHC and THC are necessary for auditory acuity in the chicken. This hypothesis is supported by the similarity between the relative quantities of hair cells lost and amplitude of threshold shift.

The location of maximum threshold shift correlates well with the frequency of stimulation for each experimental group. However, if we review the location of SHC loss or THC loss for each experimental group, a discrepancy can be seen. With 3000 Hz stimulation, damage is located in the area approximately 1/8 of the length of the BP from the base of the BP, where only SHC are found; in these animals, SHC loss was the same as total hair cell loss. With 1500 Hz stimulation maximum THC loss and maximum SHC loss occurred in essentially the same location along the BP (approximately 1/3 of the distance from the base), and for both types of cell the location of the SHC loss corresponds well with the peak of the threshold shift. However, the location of the maximum THC loss and SHC loss noted after stimulation with 500 Hz noise is very different. A maximum THC loss is again seen 1/3 of the length from the base of the BP, as was seen after stimulation with 1500 Hz noise, but maximum SHC loss is seen approximately 3/4 of the length from the base of the BP. The position of SHC loss corresponds to the location of loss of SHC seen by Ryals and Rubel [12] after stimulation with 500 Hz noise. Thus, for two of the three experimental frequency conditions the location of THC loss is constant (1/3 of the way from the base of the BP), while in all cases the location of the SHC loss varies as a function of the frequency of stimulation. The frequency-related location of SHC loss is in good agreement with data for OHC loss in mammalian studies reported in the literature [5,13]. SHC seem to be more sensitive to the maximum amplitude of the traveling wave than are THC. An intriguing finding is that when THC are damaged, it is always at the same place, regardless of the frequency of overstimulation. Since this place is located just below the oval window, one explanation may be that THC are more susceptible than SHC to the displacement of the columella which occurs at high sound pressure levels. Another explanation for this discrepancy might be that THC are not frequency-specific while the SHC are; this could have implications for frequency coding in the chick.

Our results have shown a very close and reliable correspondence between histo-

logical and physiological findings in the chicken ear. The chick BP may be a useful model to study in order to further our understanding of the relationships between anatomy and function in the ear, and thereby to uncover the mechanism of acoustic trauma.

Acknowledgements

This work was supported by NIH grant NS 15395 and funds from the Deafness Research Foundation. We gratefully acknowledge the assistance of Dr. Remy Pujol who read this manuscript carefully and Brigitte Etchecopar who typed the manuscript.

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