

PATTERNS OF HAIR CELL LOSS IN CHICK BASILAR PAPILLA  
AFTER INTENSE AUDITORY STIMULATION

*Exposure duration and survival time*<sup>1</sup>

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**Abstract.** Ten-day-old chicks were exposed to a pure tone (1.5 kHz) or white noise at 125 dB SPL avg. RMS for 4 to 24 hours, and were sacrificed either 10, 30 or 60 days after exposure. The basilar papillae were embedded in plastic, sectioned, and hair cells were counted at 100- $\mu$ m intervals throughout the length of the papilla. As sound exposure duration increased, both the maximum number of hair cells lost, and the extent of the damaged area along the basilar membrane increased. Short hair cells situated on the free area of the basilar papilla were more susceptible to damage than were tall hair cells. The location of hair cell loss varied as a function of frequency band of exposure; the pure tone produced a well localized basal lesion, while wide-band noise produced a more general lesion which extending toward the apex. Degeneration continued with increased survival time up to 30 days. It is concluded that avians respond to acoustic overstimulation in a manner very similar to mammals. The convenience of this preparation along with the relative simplicity of its cochlea may render it useful for future investigations of the mechanisms of acoustic trauma.

For many years it has been known that exposure to intense sound can cause permanent structural changes to the sensory epithelium of the mammalian cochlea (Haber-mann, 1890; Guild, 1921; Miller, Watson & Covell, 1963). While the use of other vertebrate classes has proven quite rewarding for studies of peripheral auditory function (Wever, 1974), central processing (Capranica, 1976), and auditory development (Rubel, 1978), experimental models to study the peripheral and central effects of acoustic overstimulation have generally been limited to mammals.

A great deal of literature is available describing both the development and structure of the avian middle ear (Smith, 1905; Borg, et al., 1979), inner ear (Takasaka & Smith, 1971; Tanaka & Smith, 1975; Cohen & Fermin, 1978; Hirokawa, 1978; Tanaka & Smith, 1978) and central auditory pathways (Rubel & Parks, 1975; Rubel, Smith & Miller, 1976; Parks & Rubel, 1978; Smith & Rubel, 1979). Moreover, the avian basilar papilla is convenient for histological preparation and analysis due to its short, uncoiled nature which allows serial section reconstructions (Guild, 1921) within a reasonable time frame. Finally, since avian auditory system development has been extensively documented, the developmental effects of perturbations of the auditory environment, such as noise exposure, can be related to other aspects of auditory system ontogeny.

Two previous reports concerning the response of the avian inner ear to acoustic trauma (Bohne & Dooling, 1974; Dooling & Saunders, 1974) used surface preparation techniques and suggested that there was essentially no hair cell degeneration after intense acoustic stimulation (106 dB SPL for 12 hours). Further investigation into the susceptibility of avian inner ear hair cells to

<sup>1</sup> A preliminary account of this study was presented at the Association for Research in Otolaryngology, Midwinter Research Meeting, 1979.

Table I. Summary of groups

Survival time	Duration of exposure (hours)	Stimulation condition		
		Pure tone (1.5 kHz)	White noise	Plugged or none
10 Days	4	4 Ears		
	8	4 Ears		
	12	4 Ears	4 Ears	8 Ears <sup>a</sup>
	24	4 Ears	4 Ears	
30 Days	24	2 Ears		2 Ears
60 Days	24	2 Ears		2 Ears

Total  $N=40$  ears.

<sup>a</sup> Data from ears that were plugged during sound exposure ( $n=4$ ) were combined with data from normal ears ( $n=4$ ) since there was no difference between these control groups.

acoustic trauma using serial sections and variations in acoustic intensity level, frequencies of stimulation, exposure durations and survival was felt to be warranted. In the next paper (Ryals & Rubel, 1981) we describe the location of hair cell damage as a function of the frequency spectra of acoustic overstimulation.

## METHODS

### 1. Subjects

Eight experimental and four control groups of 10-day-old Hubbard  $\times$  Hubbard chicks were used (see Table I). The 2–8 chicks in each group were obtained from random hatching groups. Normal control subjects not exposed to the acoustic trauma conditions or papillae from ears that had been protected by an ear plug during exposure served as controls. Since the intensity level at which hair cell destruction first occurred was unknown, a 125 dB SPL intensity level was chosen to maximize effects. One pure tone (1.5 kHz) chosen to be near the middle of the animals' frequency range and near their maximum sensitivity (Saunders et al., 1974; Kerr et al., 1979) was chosen. This allowed assessment of the specificity of damage. In

addition, a white noise stimulus was used to produce a widespread pattern of stimulation throughout the basilar membrane. Exposure durations extended from 4 to 24 hours of continuous stimulation. Chicks were sacrificed either 10, 30 or 60 days after noise exposure. A summary of all conditions is shown in Table I.

### 2. Sound exposure

**Signal generation.** Audio signals were generated by either a noise generator (GS 901B) or an audio oscillator (Wavetek Model 134), amplified (Southwest Technical Products 207/A) and led to a freefield speaker (IRS Power Horn 40-1238). Acoustic signals were calibrated using a  $\frac{1}{2}$ -inch electret microphone (GR 19-72) with preamplifier (15600P42) and a wave analyser (GR 1521-B). The acoustic environment of the chamber in which animals were confined during noise exposure was calibrated with a constant voltage input and was shown to be acoustically flat through 2000 Hz. Spectral analysis was performed with a microphone positioned near the animal's ear canal before, during and after auditory exposure and showed a flat frequency response for the white noise condition up to 4000 Hz. Sound pressure levels for the pure tone stimulation were measured at the level of the ear canal and were calibrated to  $125 \pm 2$  dB. Levels for the experimental tone and its harmonics were then measured at 10 other positions within the chamber. Both the 1.5 kHz tone and broad band noise were within 3 dB throughout the chamber. The first and second harmonics of the 1.5 kHz tone were  $>30$  dB below the fundamental throughout the chamber.

**Procedure.** Ten-day-old chicks were placed, in pairs, in a small wire mesh tubular chamber ( $5 \times 5 \frac{1}{2}$  inch) directly beneath the IRS power horn inside an acoustic chamber (IAC) and were continuously exposed to either the pure tone or white noise stimulus for the desired duration. Decibel levels near

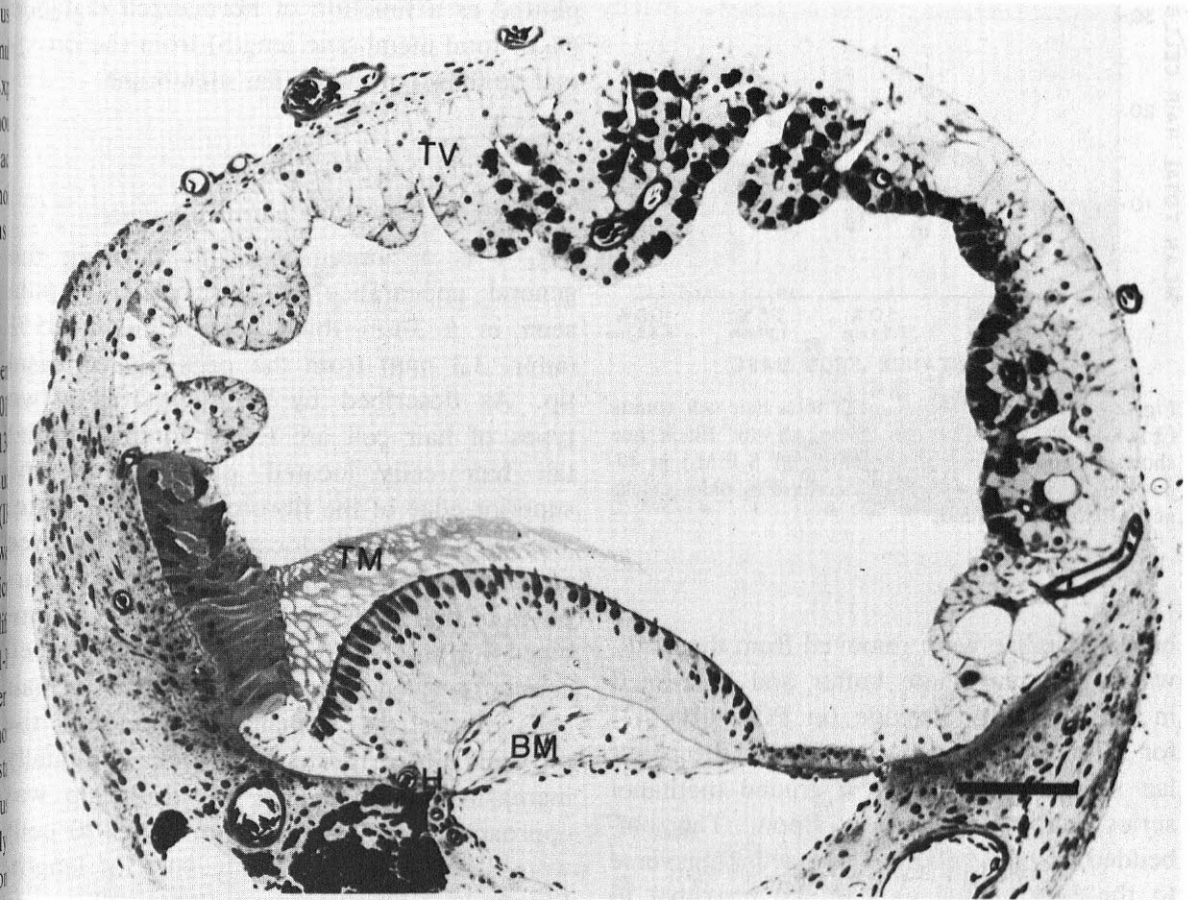


Fig. 1. Transverse section through basilar papilla (appr. 3.3 mm from proximal tip) in a normal 40-day-old chicken. At this point tall hair cells predominate the

width of the basilar membrane. TV = tegmentum vasculosum, BM = basilar membrane, H = habenula, TM = tectorial membrane. Bar indicates 100  $\mu$ m.

the ear canal were monitored continuously during exposure using a 1-inch condenser microphone (B&K 4145) attached to a sound level meter (B&K 2204) set either on a linear scale (for white noise stimuli) or the appropriate octave band during pure tone stimulation.

### 3. Ear plugging

Acoustic over-stimulation was provided monaurally in some animals so that the other ear could be used for within-animal comparisons. The ear plugging procedure used to protect one ear from over-stimulation has been described elsewhere (Kerr et al., 1979) and has been shown to provide at least 40 dB

attenuation from 125 to 4500 Hz. After acoustic over-stimulation earplugs were removed. It has previously been shown (Kerr et al., 1979) that the plugs can be left in place for as long as 10 days with no adverse effect on cochlear or eighth nerve function.

### 4. Fixation and tissue preparation

After 10, 30 or 60 days survival duration the chicks were deeply anesthetized by intravenous injection of Nembutal. A direct intralabyrinthine perfusion of 1% paraformaldehyde 0.75% glutaraldehyde mixture was performed bilaterally immediately after decapitation. The entire head was then immersed in cold fixative for 8–12 hours. The

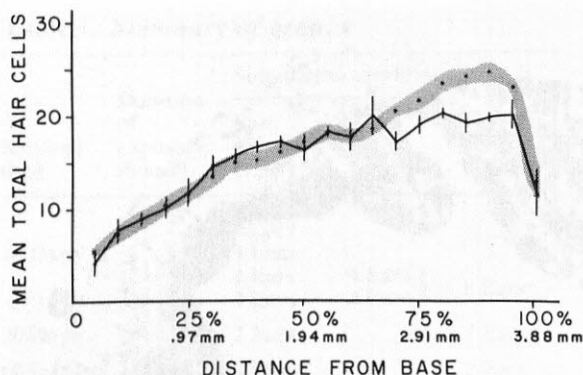


Fig. 2. Shaded area shows mean total hair cell counts ( $\pm 1$  S.E.M.) in 20-day-old chicks ( $N=8$ ). Black line shows mean total hair cell counts ( $\pm 1$  S.E.M.) in 40-day-old subjects ( $N=2$ ). Note decrease in older chicks at the distal (apical) end.

basilar papillae were removed from the skull, washed in phosphate buffer and post-fixed in 2% osmium tetroxide (in  $\text{PO}_4$ ,  $\text{pH}=7.3$ ) for 2 hours. Following osmication the papillae were dehydrated in a graded methanol series and embedded in Epon. The embedded papillae were sectioned transverse to the longitudinal axis in the proximal to distal (basal to apical) direction using an LKB Ultramicrotome. A group of three or four, 3- $\mu\text{m}$  thick sections were collected at each 100- $\mu\text{m}$  interval throughout the length of the papilla, mounted in serial order, and stained with toluidine blue.

### 5. Quantitative analysis of number of hair cells

Quantitative analysis of the number of hair cells at each level of the basilar papilla was performed by viewing each section under 40 $\times$  planapochromatic oil immersion objective ( $\text{NA}=1.0$ ) at a total magnification of  $\times 640$ . In order to maintain consistency and reliability between hair cell counts, counting criteria were established. A hair cell was counted when the following criteria were met: presence of cuticular plate, cilia, and cell body. The average counts from the three sections at each 100- $\mu\text{m}$  interval were then

plotted as a function of normalized distance (% of total membrane length) from the proximal tip (base) of the basilar membrane.

## RESULTS

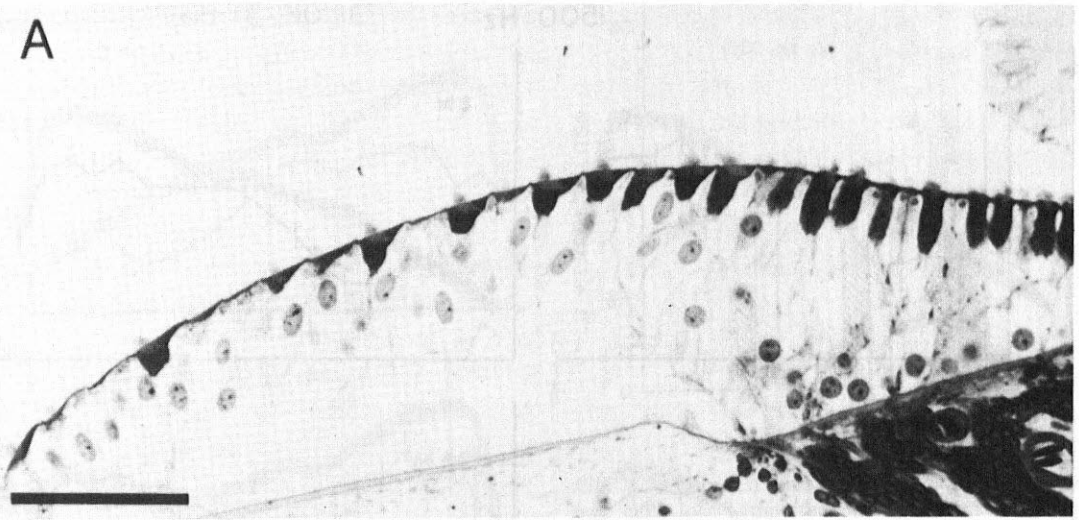
### 1. Normative baseline hair cell counts

Fig. 1 is a photomicrograph showing the general appearance of the basilar papilla seen in a 3- $\mu\text{m}$  thick section taken 85% (appr. 3.3 mm) from the proximal or basal tip. As described by Retzius (1884), two types of hair cell are found in the papilla: tall hair cells located primarily on the superior edge of the fibro-cartilaginous plate, and short hair cells located toward the free edge of the basilar membrane. For the purposes of this study both cell types were combined for total cell counts. In general, cell counts revealed a small number (5-6) of hair cells across the basilar membrane at the proximal tip of the basilar papilla, gradually increasing in number as the distal tip was approached until a maximum of 25-30 cells were seen at approximately 90% of length. Fig. 2 shows the mean total hair cell counts obtained throughout the length of the normal papilla. Since qualitative and quantitative analysis showed no difference in cell appearance or number ( $t_{\text{obs.}}=1.15$ ,  $\text{df}=158$ ) the plugged ear counts (4 ears) were combined with the counts from the non-exposed subjects (4 ears).

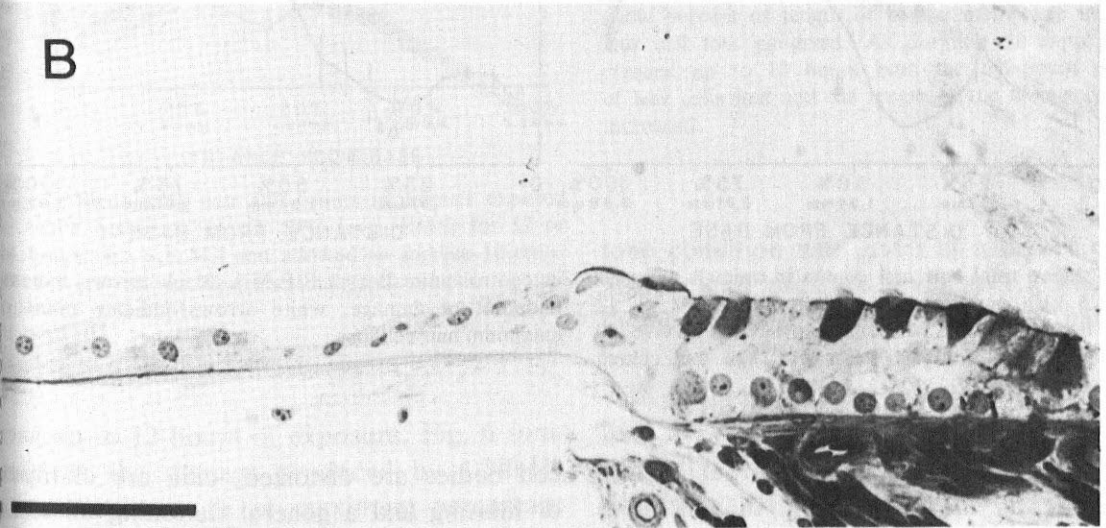
A comparison of the number and distribution of hair cells along the basilar papilla in 20-day-old chicks vis-à-vis the number found in 40-day-old chicks is also shown in Fig. 2. In older chicks the general appear-

Fig. 3. (a) Photomicrograph taken appr. 1.35 mm from the proximal tip of a normal basilar papilla. (b) Photomicrograph taken in same area (1.41 mm from proximal tip) of an animal exposed to 1.5 kHz for 4 hours. (c) Higher power photomicrograph of damaged hair cells seen in a section taken 1.5 mm from basal tip of the same animal shown in 3b (1.5 kHz, 125 dB, 4 hours). Pointers indicate cells which were considered abnormal and did not meet the criteria for counting. a & b, bars = 5  $\mu\text{m}$ ; c, bar = 20  $\mu\text{m}$ .

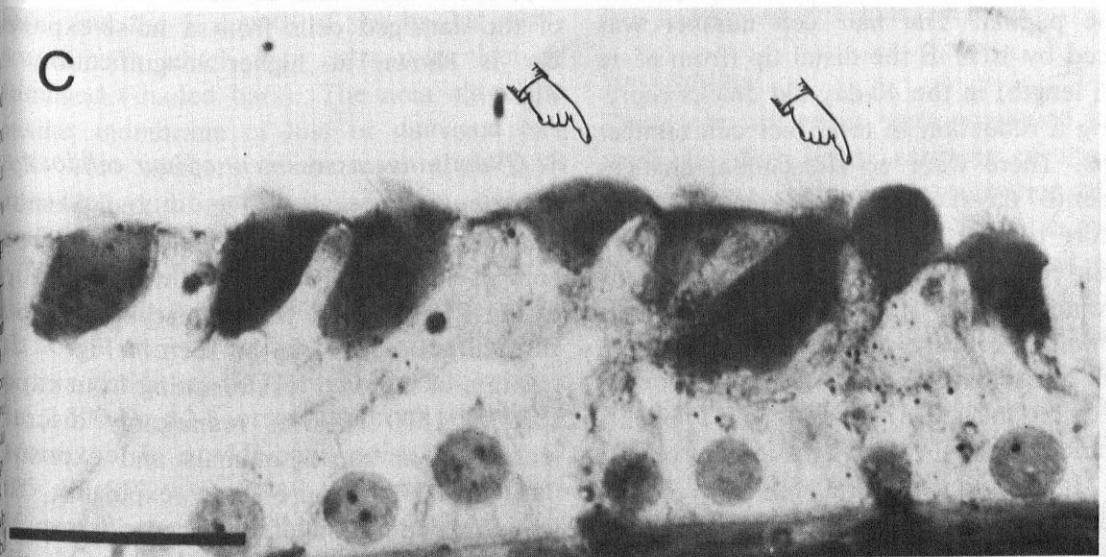
A



B



C



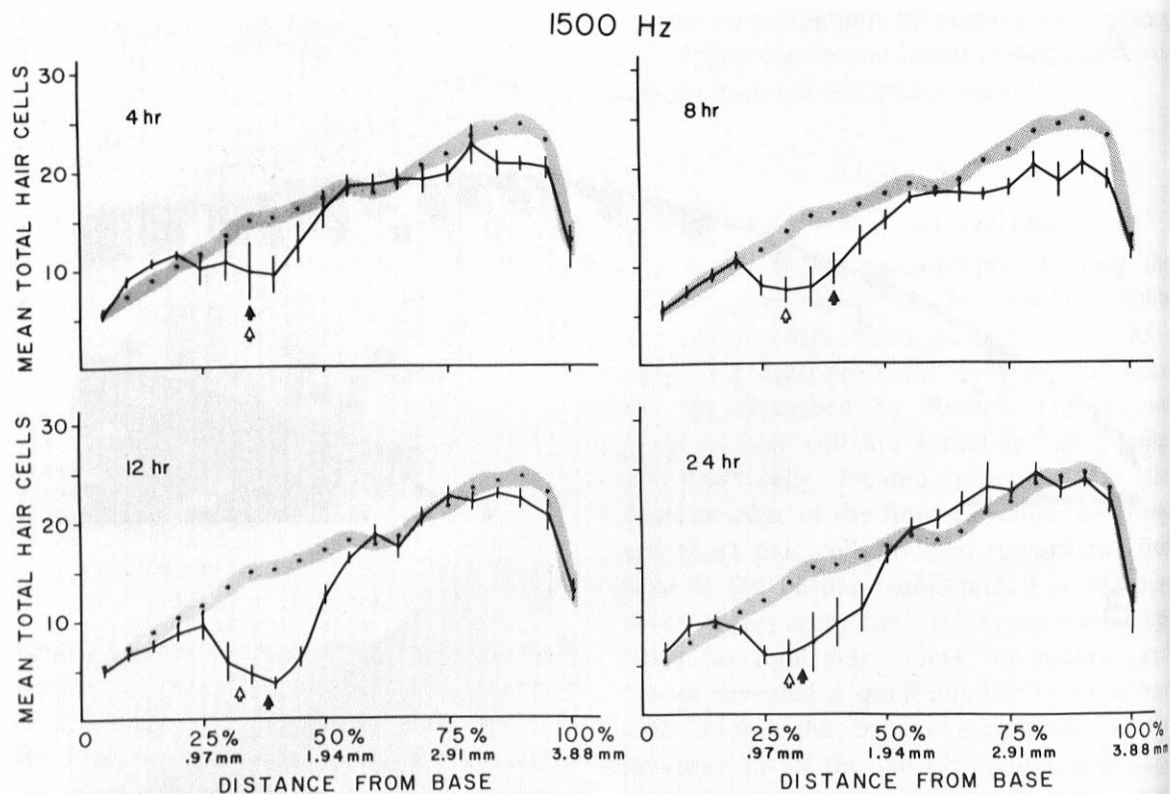


Fig. 4. Mean total hair cell counts in animals exposed to a 1.5 kHz pure tone at 125 dB SPL for 4, 8, 12 or 24 hours ( $\pm 1$  S.E.M.) and allowed to survive 10 days. Shaded area shows mean total hair cell counts

in control animals ( $\pm 1$  S.E.M.). Black arrows indicate midpoint of damage, white arrows indicate point of maximum hair cell loss.

ance of the hair cells along the papilla was the same as in 10-day-olds, though there were fewer hair cells in the distal portion of the papilla. The hair cell number was reduced by 16% at the distal tip (from 65 to 100% length) in the 40-day-old chicks representing a reduction in total hair cell number of 4%. There were no subsequent changes in older (67 days) chickens.

Before turning to the quantitative analysis of hair cell loss, qualitative observations regarding changes in hair cell morphology after noise exposure should be noted. Fig. 3 shows a comparison of a typical region of hair cell damage in a traumatized papilla (3b) and the same region in a normal papilla (3a). It is apparent that fewer cells are present and in particular, short hair cells seem lost. In addition, the remaining hair

cell bodies are distorted, cilia are clumped or missing and a general flattening of some supportive cells can be seen. An example of the damaged cells from a noise-exposed ear is shown at higher magnification in Fig. 3c.

### 3. Quantitative assessment of hair cell loss

*Duration of exposure.* Fig. 4 and 5 show the mean number of hair cell counts from animals surviving 10 days following exposure to the 1500-Hz and white noise at all exposure durations. As can be seen in Fig. 4 the position of hair cell loss resulting from exposure to 1500 Hz was remarkably discrete and constant across animals and exposure durations. With pure tone exposure, the longer the duration of the acoustic stimulation the larger the degree of total hair cell

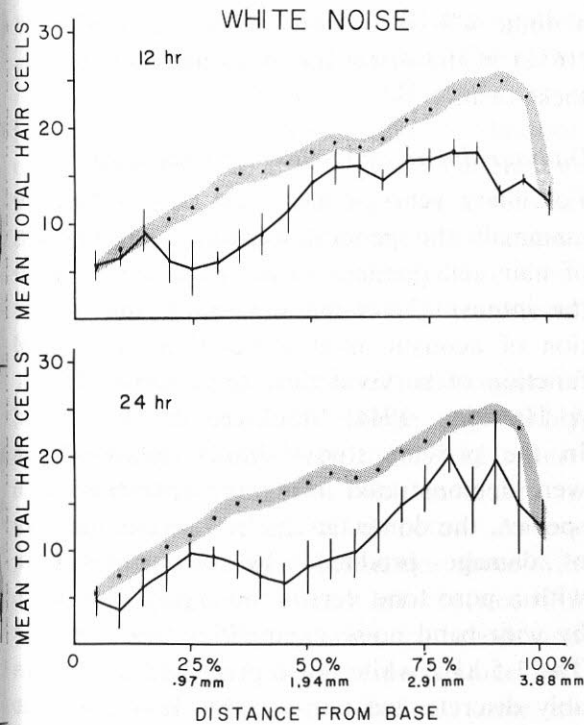


Fig. 5. Mean total hair cell counts in animals exposed to white noise at 125 dB SPL (avg. RMS) for 12 or 24 hours ( $\pm 1$  S.E.M.) and allowed to survive 10 days. Shaded area shows mean total hair cell counts in control animals ( $\pm 1$  S.E.M.).

loss up to 12 hours of exposure. Fig. 6 summarizes the damage produced by 1500-Hz exposure in terms of maximum percentage hair cell loss within the damaged area (open bars) and the % length of the basilar membrane over which hair cells were lost or damaged (shaded bars). The area along the basilar membrane of lost or damaged hair cells also appears to increase slightly as exposure duration increases from 4 to 8 hours and then shows little change.

The wide-band white noise exposure showed two regions of hair cell loss with 12 hours' exposure. The total amount of loss in this condition was similar to that seen in the 1500-Hz 12-hour condition. It should be noted that in a wide-band noise stimulus of 125 dB SPL avg. RMS the level per cycle of any frequency within the noise band is considerably less than 125 dB. The pure

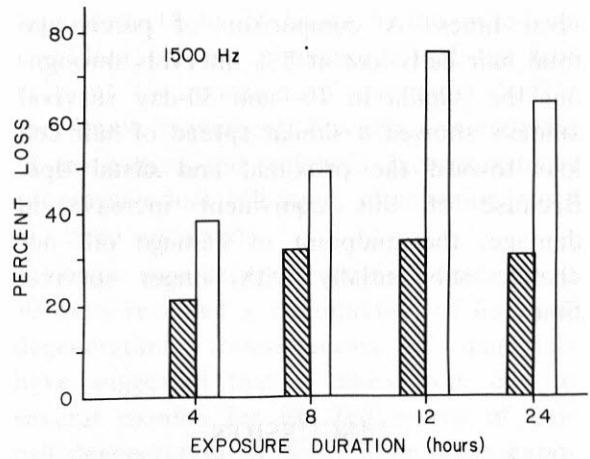


Fig. 6. Unshaded bars indicate maximum percent of hair cells lost within the damaged area of animals exposed to the 1.5 kHz pure tone condition. Shaded bars show percent of length of basilar membrane in which hair cell loss occurred. As duration of exposure increased up to 12 hours both the maximum percent of hair cells lost and the length of the damaged region increased.

tone condition was given at a higher equivalent sound pressure level at 1.5 kHz and therefore the subsequent degree of hair cell loss was predictably higher than that found with wide-band white noise. When the duration of wide-band noise exposure was increased from 12 hours to 24 hours the area of damage increased and a substantially greater degree of hair cell loss resulted (from 14% hair cell loss to 29% hair cell loss).

#### Survival time

Three survival times were employed in this study to allow some estimate of the time necessary for a final approximation of permanent hair cell loss after acoustic trauma. Hair cell counts in animals exposed to the pure tone for 24 hours and allowed to survive 10 days showed a 7% total hair cell loss, while those allowed to survive 30 days lost 20% of their hair cells. Further extension of the survival time to 60 days failed to reveal additional hair cell loss.

It has also been shown that the area of hair cell loss in mammals increases with longer sur-

vival times. A comparison of percentage total hair cells lost at 5% intervals throughout the papilla in 10- and 30-day survival animals showed a similar spread of hair cell loss toward the proximal and distal tips. Because of this equivalent increase in damage, the midpoint of damage did not change substantially with longer survival times.

## DISCUSSION

### *Normative hair cell counts*

The pattern of normal hair cell distributions along the basilar papilla found in the present study closely resemble hair cell counts taken along the length of the basilar papilla in adult chickens by other investigators (Hirokawa, 1978; Tanaka & Smith, 1978). One discrepancy was noted; the present study found a maximum number of 25-30 hair cells at the distal portion of the papilla, whereas previous studies found a maximum of 45-46 hair cells at the distal portion. The most likely explanation for this discrepancy is a general difference in criteria used in cell counting. No definition of criteria for cell counting was given in the aforementioned studies. Another possible explanation is that the previous authors used adult chickens (3-4 months old) while the present study used chicks ranging in age from 20 to 40 days. This explanation, however, does not agree with our finding of fewer hair cells in the distal tip of 40- and 70-day-old chickens than in 20-day-olds.

A decrease in number of hair cells with age has also been shown in mammals. Bredberg (1968) used fetal ears as the basis for normative cell counts, since he found a decrease in cell number between fetuses and young children. Coleman (1976) in a normative study of hair cell number in guinea pig cochlea has also shown decreases in hair cell number, especially at the apical tip, as early as 24 hours after birth. Our findings of

a total 4% reduction, with its maximum (16%) in the distal tip, is in agreement with these results.

### *Damage due to acoustic overstimulation*

For many years it has been known that in mammals the general location and amount of hair cell damage varies as a function of the intensity, frequency spectra, and duration of acoustic over-stimulation, and as a function of survival time (e.g. Lurie, Davis & Hawkins, 1944; Stockwell et al., 1969). In the present study similar relationships were demonstrated in a representative avian species, the domestic chick. The comparison of damage produced by over-stimulation with a pure tone versus the injury produced by wide-band noise exemplifies this parallel. The 1.5 kHz white noise produced a remarkably discrete and non-variable lesion with a maximum hair cell loss of over 80% at approximately  $\frac{1}{3}$  of the way from the base to apex. White noise, on the other hand, produced widespread damage resulting in a maximum loss of only  $\frac{1}{3}$  to  $\frac{1}{2}$  of the hair cells at any given location. These results are of course what would be expected on the basis of mammalian data and/or a consideration of the amplitude and shape of the travelling wave produced by these stimuli. Perhaps the most notable result of the present study was the lack of variability seen after 1.5 kHz pure-tone exposure. The extent to which this is due to differences in analytical methods, or true differences between birds and mammals is unclear.

The similarity in the pattern of morphological changes resulting from acoustic trauma in avians and mammals suggests a common mechanism is responsible for the position of hair cell loss at intense levels of stimulation (125 dB), even though the basilar papilla is very different from the mammalian cochlea in many morphological details. Two theoretical mechanisms for acoustic trauma, metabolic exhaustion and mechanical stress, are said to be competing when the level of the



stimulus is greater than 120 dB (Spoendlin, 1976). It has been postulated that these two mechanisms may interact differently as a function of the frequency spectra, duration or survival time of the subjects. Differences with respect to the frequency of overstimulation will be discussed in the next paper, while the possible effects of exposure duration and survival time are briefly considered here.

As exposure time increased, the region of hair cell loss did not change appreciably in either conditions. With the 1.5 kHz pure tone cell loss increased with increases in exposure duration up to 12 hours. As expected by the relative intensities there was a marked increase in cell loss between 12 and 24 hours of exposure to broad-band noise. Instead of analysing percentage hair cell loss with exposure duration, some investigators have considered length of damage along the basilar membrane as the dependent measure. Spoendlin & Brun (1973), using broad-band noise, have shown a consistent relationship between logarithmic increases in time of exposure and linear increases in the length of damage along the basilar membrane in the guinea pig. They saw an initial rather sharp increase in damage length when exposure time was increased with a gradual levelling off of damage length. Our results show a similar trend. With 1.5 kHz exposure there was an initial increase in the length of damage from 4 to 8 hours, with the length of damage at 8, 12 and 24 hours remaining relatively constant. Twenty-four hour exposure with wide-band noise produced reliable damage throughout the papilla which was not true in the 12 hour exposure condition. With respect to the proposed dichotomy between mechanical and metabolic factors, it seems reasonable that increases in exposure duration would have a progressive effect on metabolic factors, whereas an asymptotic effect on mechanical factors would soon be reached. If this is indeed the case it is not clear why increasing exposure dura-

tion from 12 to 24 hours in the pure-tone condition did not eliminate all of the hair cells at the position of maximum basilar membrane movement. Possibly the coupling of mechanical and metabolic factors reduces progressive hair cell injury after major injury has been sustained.

Longer post-exposure survival times up to 30 days revealed a continuation of hair cell degeneration. Investigations of mammals have suggested that it takes from one to several months for the full extent of hair cell degeneration to occur after noise exposure. In the chick it appears that hair cell loss can be detected by 10 days following sound exposure; it then continues, at a diminished rate, during the succeeding 20 days by which time it appears maximum. Longer survival times also caused hair cell degeneration to spread toward the base and apex. This general spread might be predicted as a function of continuing degeneration due to metabolic factors which were not revealed at short survival times.

## CONCLUSIONS

In the present report we have shown that the pattern of damage produced by acoustic overstimulation of the avian basilar papilla is very similar to that reported for mammals. Hair cell loss varies systematically with the acoustic spectra, exposure time and survival time in ways that are apparently identical with what has been found in mammals. In this respect it is clear that avian preparations, because of the accessibility of the organ and the relative ease of quantitative histological analysis, may provide an excellent model for future studies of acoustic trauma. In the following paper we will further develop this model by assessing the relationship between the frequency of acoustic over-stimulation and the position of hair cell loss. We will then further consider the implications for understanding acoustic trauma.

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## ZUSAMMENFASSUNG

Zehn Tage alte Hühnchen wurden entweder mit einem reinen Ton von 1,5 Hz oder mit weißem Rauschen beschallt. Den Untersuchungen wurden Reize mit den folgenden Eigenschaften zugrunde gelegt: 125 dB SPL durchschnittliche RMS mit einer Reizdauer von 4 bis 24 Stunden. Die Versuchstiere wurden 10, 30 oder 60 Tage nachher geopfert. Nachdem die Partes basilaris in Kunststoff eingebettet und durchgeschnitten wurden, konnte man die Haarzellen in histologischen Probe-schnitten jeweils nach Abteilungen von 100  $\mu\text{m}$  durch die Länge der Partis basilaris zählen. Indem man die Reizlänge erhöhte, vergrößerte sich die Maximalnummer der verschwundenen Haarzellen und es breitete sich der Umfang der Beschädigung entlang der Partis basilaris aus. Kurze Haarzellen, die sich in dem freien Teil der Partis basilaris befinden, schienen empfindlicher gegenüber größeren Haarzellen. Die Stellung des Haarzellenverlusts konnte auf die Breite des Reiztonbereichs zurückgeführt werden: Reine Töne veranlaßten klar umgrenzte Verletzungen, während breitere Rauschfrequenzbände eine verbreitete Verletzung, die sich zur Spitze dehnte, verursachten. Es wird vorgeschlagen, daß die Reaktion der Vögel auf akustische Überreizungen mit der von Säugetieren verglichen werden kann. Wegen der Bequemlichkeit und verhältnismäßig unkomplizierten Struktur der Cochlea eignet sich dieses Präparat für weitere Untersuchungen des akustischen Traumas.

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