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PATTERNS OF HAIR CELL LOSS IN CHICK BASILAR PAPILLA AFTER INTENSE AUDITORY STIMULATION[1]

Frequency Organization

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Abstract. Ten-day-old chicks were continuously exposed to pure tones (500, 1 500, or 3 000 Hz) at 125 dB SPL for 12 hours and sacrificed 10 days after exposure. The basilar papillae were embedded in plastic, sectioned and hair cells were counted at 100-μm intervals throughout the length of the papilla. The position of hair cell loss along the basilar membrane varied systematically as a log-linear function with the frequency of stimulation. This systematic relationship was used to predict the frequency organization of the chick basilar papilla. It is concluded that although the avian basilar papilla differs greatly in morphological detail from the mammalian cochlea, its response to intense acoustic stimulation is quite similar.

The frequency organization of mammalian cochlea has been studied by analysing the location of hair cell loss along the basilar membrane following acoustic trauma (Schuhkneckt, 1953; Stockwell et al. 1969). A close correlation has been found between the location of hair cell loss and the site of maximum displacement of basilar membrane. Although the site of maximum basilar membrane displacement has been studied in avians (Békésy, 1944), the location of hair cell loss as a function of the frequency of acoustic overstimulation has not previously been investigated.

In the preceeding paper (Rubel & Ryals, 1981) we showed that the avian basilar papilla is affected by acoustic overstimulation in a manner very similar to mammals when exposure duration and survival times are varied. In the present study we describe the position of hair cell loss in avian basilar papilla as a function of the frequency spectra of stimulation. In this way we provide a 'frequency map' of the avian cochlea and examine the correlation between maximum basilar membrane displacement described by Békésy (1944) and the location of hair cell loss.

METHODS

The methods for sound exposure, fixation and tissue preparation were identical to those previously described (Rubel & Ryals, 1981). Three frequencies were used in order to examine the relationship of this variable to the location of hair cell damage. Pure tones of 500, 1 500 and 3 000 Hz were chosen as representative of low, middle and high frequency regions; avian hearing has been shown to extend from approximately 40 to 8 000 Hz (Saunders et al., 1974; Kerr et al., 1979).

Sound pressure levels for pure tone stimulation were measured at the level of the ear canal and were calibrated to 125 dB ± 2 dB. Levels for the experimental tone and its harmonics were then measured at 10 other

[1] Preliminary account of this study was presented at the Association for Research in Otolaryngology, Midwinter Research Meeting, 1979.

Table I. *Mean, standard deviation and range of sound pressure levels measured at 10 locations within the animal's sound field*

Frequency	Hz	Sound pressure level: Mean for 10 positions (dB)	S.D.	Range over 10 positions (dB)
Fundamental	500	124	1.3	120–126
First harmonic	1 000	94	1.3	91– 95
Second harmonic	1 500	78	2.0	75– 82
Fundamental	1 500	125	1.0	123–126
First harmonic	3 000	90	2.3	87– 93
Second harmonic	4 500	61	4.9	52– 68
Fundamental	3 000	124	2.5	121–129
First harmonic	6 000	98	3.3	92–102
Second harmonic	9 000	73	4.6	62– 79

positions within the chamber; means, ranges and standard deviations are shown in Table I.

Ten-day-old chicks were placed, in pairs, in a small wire mesh tubular chamber ($5\times5^1/_2$ inch) directly beneath a power horn inside an acoustic chamber and were continuously exposed to one of the three pure-tone stimuli for 12 hours. After pure tone exposure, the chicks were returned to standard brooders for 10 days.

After 10 days survival, the chicks were sacrificed and their basilar papillae were prepared for serial sectioning (see Rubel & Ryals, 1981, for details of fixation and embedding procedure). The Epon-embedded papillae were sectioned transverse to the longitudinal axis in the proximal to distal direction. A group of three or four 3-μm-thick sections were collected at each 100-μm interval throughout the length of the papilla. The average number of sections analysed per papilla was 94, or 3 sections at approximately 32 sampling intervals over the length of the basilar papilla. Quantitative analysis of the number of hair cells at each level of the basilar papilla was identical with that described by Rubel & Ryals (1981).

The criterion for counting a hair cell was an observable cell cytoplasm, cuticular plate and stereocilia. The average counts from the three sections at each 100-μm interval were plotted as a function of normalized distance (% of total membrane length) from the proximal tip of the basilar membrane.

RESULTS

A qualitative description of the hair cell damage produced by acoustic overstimulation was presented in the preceding paper (Rubel & Ryals, 1981) and will therefore not be repeated here. It is worth noting, however, that areas near the edges of the missing hair cells looked normal in all respects.

Fig. 1 shows the pattern of hair cell damage as a function of the frequency of sound exposure. The three insets at the top show the mean hair cell counts (±1 S.E.M.) as a function of distance along the papilla for each exposure condition, as compared with counts from normal animals. At the bottom the mean hair cell counts for each frequency exposure condition are compared with each other and with mean normal hair cell counts. The 500 Hz exposure conditions created a rather widespread loss in hair cell number from approximately 25% to 75% of the length of the basilar papilla. The 1 500-Hz exposure condition created a more discretely localized region of hair cell loss from ap-

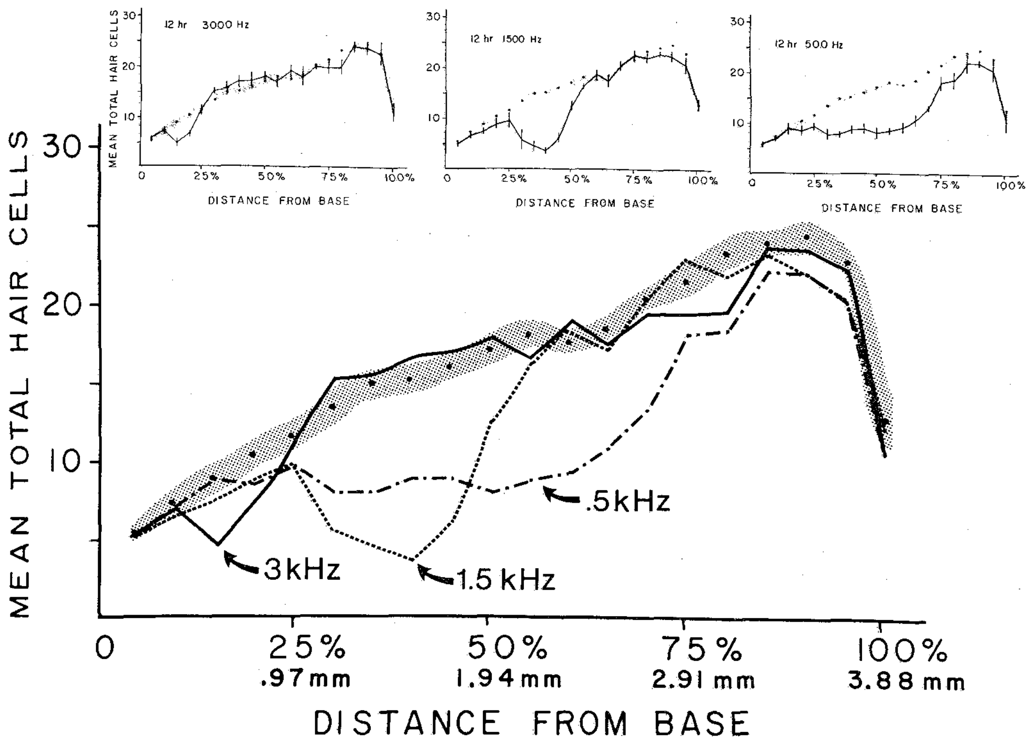

Fig. 1. Mean total hair cell counts (±1 S.E.M.) in chicks exposed to 500 Hz (N=3), 1500 Hz (N=4) or 3000 Hz (N=4) at 125 dB SPL for 12 hours. Shaded area in each figure shows mean hair cell counts in control chicks (±1 S.E.M., N=8). Discrete areas of hair cell degeneration are seen as a function of the frequency (Hz) of exposure; arrows indicate approximate midpoints of lesion for each frequency.

proximately 25% to 55% of length of the basilar papilla. The 3000-Hz exposure condition again created a well localized, discrete region of hair cell loss from approximately 10% to 25% of length of the basilar papilla. A progression of regions of hair cell loss from proximal (base) to distal (apex) can be seen as the frequencies of sound exposure decreased from 3000 to 500 Hz. It is of interest that short hair cells were lost to a greater degree than tall hair cells at the location of maximal hair cell loss.

Two methods were used to quantitatively assess the location of the damage. First, the midpoint of the area of damage was calculated by using the borders of damage as defined by the 5% (0.13 mm) steps at which the standard errors of the hair cell counts did not overlap those of normals. The average midpoint of the region of hair cell loss in the basilar papilla of chicks exposed to 500 Hz was 51% (S.E.M.=2.4) of length from base to apex, to 1500 Hz was 38% (S.E.M.=1.8) of length and to 3000 Hz was 18% (S.E.M.= 0.6) of length (see arrows in Fig. 1). Using these midpoints of damage from each subject, for each frequency condition, an exponential regression equation provides the line of best fit with a coefficient of determination of r^2=.89. The predicted frequency range of the basilar papilla, using this equation, is 55 to 7855 Hz, quite similar to the evoked potential threshold curve for audibility seen in chicks (Saunders et al., 1974; Kerr et al., 1979). Other equations (linear, log, power) using these midpoints also produce high correlation coefficients; however, the predicted frequency range of the

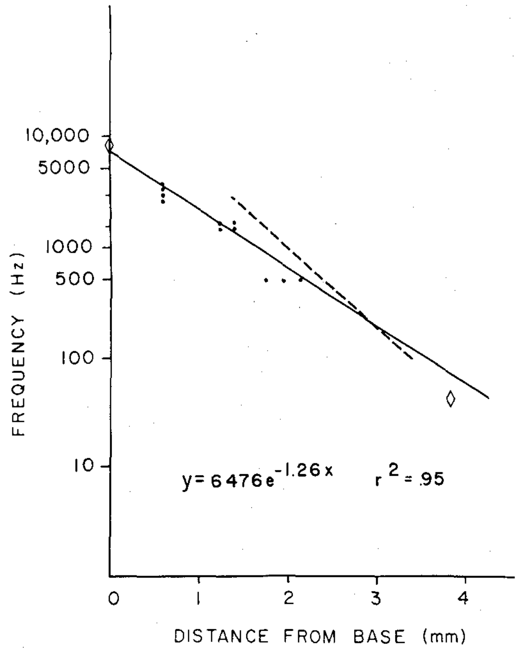

Fig. 2. Exponential regression equation derived from points of maximum hair cell loss along the basilar papilla for each frequency (Hz) exposure condition. ◇, Boundaries of chick auditory frequency range taken from evoked potential audiograms; these were also included to formulate the regression equation. —, Predicted points of maximum hair cell damage as a function of the frequency stimulation. ---, Function of maximum basilar membrane displacement in adult chickens, taken from Békésy (1944) and normalized for 3.88 mm length.

papilla is quite dissimilar to the threshold audibility curves known for chicks (example: a linear equation predicts a frequency range from −305 Hz to 429 Hz, a power function predicts a frequency range from 258 Hz to 231,552 Hz).

The second method for assessing the location of damage used the position of maximal percentage hair cell loss. The position of maximum percentage hair cell loss was defined as the maximum percentage loss within two consecutive 5% positions of hair cell counts (10% or 0.388 mm) along the basilar papilla. The means position of maximum hair cell loss along the basilar papillae of chicks exposed to 500 Hz was 52% (S.E.M.=4.5) of length from base to apex, to 1 500 Hz was 32.5% (S.E.M.=1.4) of length and to 3 000 Hz was 15% (S.E.M.=2.0) of length. This method, like the midpoint of the lesion, again provides discrete, non-overlapping locations of loss for each frequency condition. The points were located slightly closer to the proximal tip using this method. Again, an exponential regression equation provides a very high correlation (r^2=.95) and an appropriate frequency range (40 to 6476 Hz). This regression is shown graphically in Fig. 2, along with the data points from the individual subjects. Also shown (diamonds) are best estimates of the upper and lower limits of hearing in the domestic fowl at 120 dB SPL (estimated from Rubel & Parks, 1975; Kerr, et al., 1979). Békésy (1944) demonstrated a log-linear relationship of frequency to position of maximum displacement along the basilar membrane for adult chickens. This maximum displacement curve is also shown in Fig. 2 and reveals a good agreement for position of maximum displacement with location of hair cell loss. While there are differences in the slopes of the lines, the general prediction of a log-linear function is still maintained.

DISCUSSION

The goal of the present study was to determine the relationship between the location of hair cell loss and the acoustic spectra of stimulation. As shown in Fig. 1, a different pattern of hair cell loss was found along the length of the basilar papilla after pure-tone stimulation at 500, 1 500 and 3 000 Hz. Stimulation with the 3 000-Hz tone produced a narrow, well-defined region of hair cell loss approximately 0.7 mm from the proximal tip; the 1 500-Hz tone also produced a narrow, well circumscribed region of hair cell loss approximately 1.3 mm from the proximal tip; the 500 Hz tone produced a wider but still localized region of hair cell loss approximately 2.3 mm from the proximal tip. These differential positions of maximum hair cell loss created as a function of the frequency of stimulation were used to predict the frequency organization of the basilar papilla in 10-day-old chicks. An exponential regression equation showing an inverse log-linear relationship of frequency to position revealed a high correlation and the most reasonable prediction of auditory frequency range. The results of the present study show a close correspondence of position of maximum total hair cell loss to position of maximum basilar membrane displacement along the basilar papilla as shown by Békésy (1944).

For many years it has been known that, in mammals, the general position of hair cell damage varies as a function of the frequency of the stimulating tone or noise (Lurie, Davis & Hawkins, 1944; Stockwell et al., 1969). A consistant relationship between the position of hair cell loss and position of maximum displacement is presumed to correspond with the peak or leading edge of the travelling wave and thus patterns of hair cell loss have been correlated with the deflection pattern along the basilar membrane created by the travelling wave. The results of the present study suggest that the deflection pattern along the basilar membrane due to travelling wave motion can be studied by using intense pure-tone frequency stimulation in the chick. The similar pattern of morphologic change resulting from acoustic overstimulation in avians and mammals suggests that a common mechanism is responsible for the location 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.

In the foregoing discussions (see Rubel & Ryals, 1981) we have stressed the similarities between our results with avians and those using mammalian preparations and have provided interpretations based on current assumptions regarding the mammalian cochlea. On the other hand, it is important to note some of the differences between the avian and mammalian inner ear; these morphological differences, coupled with similar response patterns, may provide the more important clues as to the actual mechanisms of acoustic trauma and suggest avenues for future investigations. Among the structural differences between the avian and mammalian cochlea, the most obvious are: 1) the relatively short length and reduced frequency range of the avian basilar papilla; 2) the lack of a true organ of Corti or Reisner's membrane in avians; 3) the presumed dichotomy of 'long' and 'short' avian hair cells which may homologize with mammalian inner and outer types; 4) and the existence of a continuous series of hair cells extending across about two-thirds of the basilar membrane instead of 3–4 discrete rows. The results presented here and in the previous paper essentially obviate these factors as critically relevant to mechanisms of acoustic trauma.

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ZUSAMMENFASSUNG

Zehn Tage alte Hühnchen wurden fortlaufend mit reinen Tönen (500, 1 500 oder 3 000 Hz) beschallt. Den Untersuchungen wurden Reize mit den folgenden Eigenschaften zugrunde gelegt: 125 dB SPL mit einer Reizdauer von 12 Stunden. Zehn Tage nachher wurden sie geopfert. Die Partes basilares wurden in Kunststoff eingebettet, durchgeschnitten und die Haarzellen in histologischen Probeschnitten jeweils nach Abteilungen von 100 μm durch die Länge der Partis basilaris gezählt. Die Stellung des Haarzellenverlusts veränderte sich systematisch als Funktion der Reizfrequenz. Man verwendete dieses systematische Verhältnis, um die Frequenzeinordnung der Partis basilaris vorherzusagen.

Es wurde festgestellt, daß sich die Partes basilares der Vögel, obwoohl morphologisch bei Säugetieren gänzlich andersartig, bei intensiven akustischen Anregungen ähnlich verhalten.

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