Hearing Research, 19 (1985) 135–142
Elsevier

HRR 00625

Ontogenetic changes in the position of hair cell loss after acoustic overstimulation in avian basilar papilla

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(Received 6 February 1985; accepted 26 June 1985)

High intensity sound was used to produce localized hair cell damage within the basilar papilla of chicks at three different ages: embryonic day 20, post-hatch day 10 and post-hatch day 30. At each age separate groups of animals were exposed to broadband white noise or pure tones at 500, 1500 or 3000 Hz for 12 h at 125 dB SPL. Chicks were killed 10 days later. Their basilar papillae were then fixed, dissected free, osmicated, embedded in Epon, sectioned serially and stained. Hair cells were counted at 100 μm intervals throughout the length of the papilla. There was a systematic developmental shift in the position of damage produced by each of the acoustic stimuli. Broadband white noise produced damage only in the basal one half of the cochlea in the embryonic animals while at later ages it produced damage throughout the length of the papilla. Exposure with each of the pure tones produced a discrete area of hair cell loss. However, with each frequency the region of damage shifted apically as a function of the age of the animal at the time of sound exposure. These results suggest that the frequency representation along the basilar papilla is not fixed, but changes during the development of hearing.

ontogeny, development, basilar papilla, frequency organization

Introduction

A paradoxical relationship exists between functional and structural properties of auditory development. Functionally, all birds and mammals respond first to low or relatively low frequency sounds; mature functional properties usually appear first for relatively low frequencies, whereas high frequency responsivity is delayed [7,14,25,29]. Structurally the maturation of the cochlea progresses from the base or mid-basal region and the apex is the last region to differentiate [5,15,20,21,25]. This suggests that initially it is the basal portion of the cochlea which is responding to low frequency sound stimulation. Enigmatically, this is just the opposite of what is known to occur in adults. It has been well established that in adults the basal portion of the cochlea preferentially responds to high frequency stimulation while the apical portion is most responsive to low frequency stimulation [1,4].

We have previously suggested that developmental changes within the cochlea may help to explain this apparent paradox [22,23,25,26]. Specifically, we have hypothesized that early in development low frequencies are transduced by the basal region of the cochlea; with maturation these same frequencies cause maximum responses at successively more apical positions while the basal regions respond maximally to progressively higher frequencies. This hypothesis predicts that frequency/place mapping of the cochlea using intense pure tone stimulation will show a systematic change in position of hair cell loss toward the apex during the late stages of hearing maturation. The present study uses this method by exposing chicks at various ages to intense acoustic stimulation and analyzing the position of hair cell loss along the basilar papilla.

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Methods

Domestic chickens (Hubbard × Hubbard) of three ages were used: embryonic day 20 (1 day prior to hatching, E20); postnatal day 10 (P10) and postnatal day 30 (P30). Chicks were incubated, hatched and brooded on site under standard conditions. At each of the above ages at least three animals were exposed to either wide-band white noise at 125dB SPL avg. RMS or a continuous pure tone of 500, 1500 or 3000 Hz at 125 ± 3 dB SPL for 12 h. Specific methods of pure tone exposure and sound field calibration have been described previously [24,27]. Briefly, chicks were placed in a wire mesh enclosure within a sound-treated booth directly beneath a power horn. The intensity and spectral purity of the sound field were measured at the entrance to the animal's ear canal and at 10 other positions within the wire enclosure. The sound field showed a relatively flat frequency response for the white noise condition from 200 to 4000 Hz (±5 dB). Pure tones were determined to be within ±3 dB of 125 dB at the exposure frequency; harmonics were all at least 30 dB below the exposure tone. In order to achieve these exposure conditions in embryos, the shell overlying the air space was removed, exposing the chick's head which had already entered the air space; the ear canals were gently aspirated when necessary. The chamber was maintained at 37.5°C for the embryos. Animals exposed to the same handling and operative conditions as the experimental chicks (sham exposure) as well as normal animals of the same age served as controls.

Following sound exposure the animals were returned to the brooder (the E20 animals invariably hatched during the exposure period) and were allowed to survive for 10 days under normal laboratory conditions. The basilar papillae were then fixed by intralabyrinthine perfusion of 1% paraformaldehyde/0.75% glutaraldehyde, dissected free, osmicated for 1 h with 2% osmium tetroxide, embedded in Epon, sectioned transversely, and stained. Specific details of this dissection and histological preparation are found in previous publications [24,27]. A group of three or four 3-μm thick sections were collected at each 100 μm interval throughout the length of the papillae and were mounted and arranged in serial order from the proximal tip (base) to the apex.

Quantitative analysis of the number of hair cells present at each sampling interval was also identical to that previously described [24,27]. Briefly, hair cells were viewed under 40 × planapochromatic oil immersion objective (NA = 1.0) at a total magnification of ×640. A hair cell was counted when cuticular plate, cilia and surrounding recognizable cytoplasm were present. The average counts from the three sections at each 100 μm interval were then plotted as a function of normalized distance (% of total membrane length) from the proximal tip (base) of the basilar membrane. These were then averaged across the animals within each group.

Results

Normative baseline hair cell counts

The total number of hair cells located across the width of the basilar membrane increases progressively from proximal to distal tip. A comparison of the total number of hair cells along the basilar papilla in 10-, 20- and 40-day-old normal control or sham condition chickens is shown in Fig. 1. The normal number of hair cells differed minimally between age groups; in older chicks there was a slight (4%) reduction in the number of hair cells in the apical (distal) portion of the basilar papilla (24).

Total hair cell loss after pure tone exposure

Fig. 2 shows a comparison between the mean

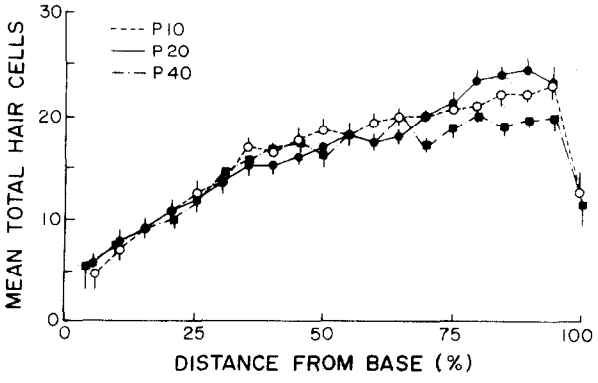

Fig. 1. Mean total hair cell counts (±1 S.E.) in normal control 10-day-old chicks ($n = 5$ ears), 20-day-old chicks ($n = 8$ ears) and 40-day-old chicks ($n = 2$ ears).

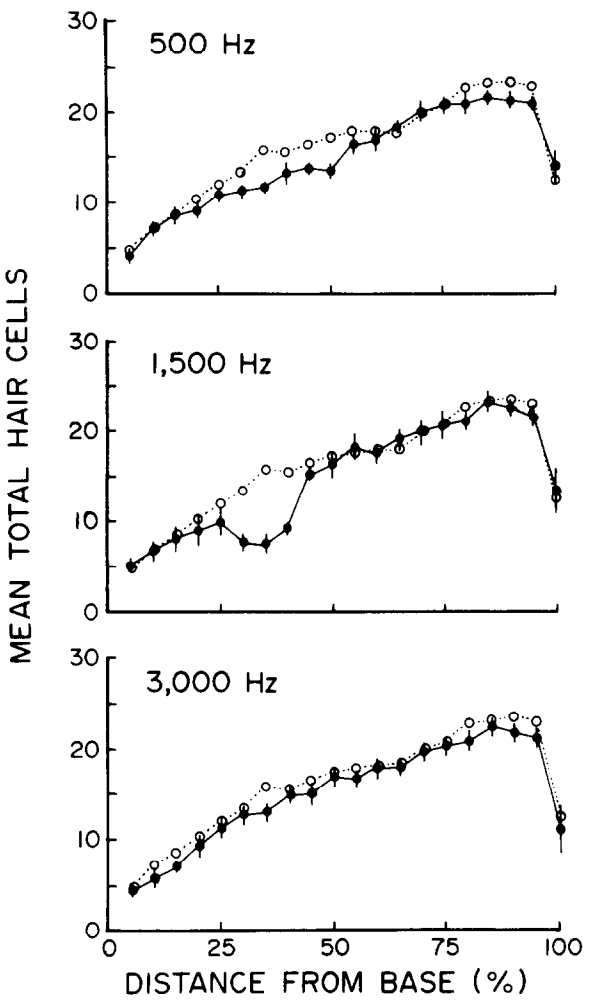

Fig. 2. Mean total hair cell counts (±1 S.E.) in chicks exposed at E20 to either 500 Hz (n = 5 ears), 1500 Hz (n = 4 ears) or 3000 Hz (n = 6 ears) pure tone stimulation at 125 dB SPL for 12 h. Open circles show mean total hair cell counts in control animals from the E20 group. All hair cell loss is located in the basal half of the papilla (0–50% of length).

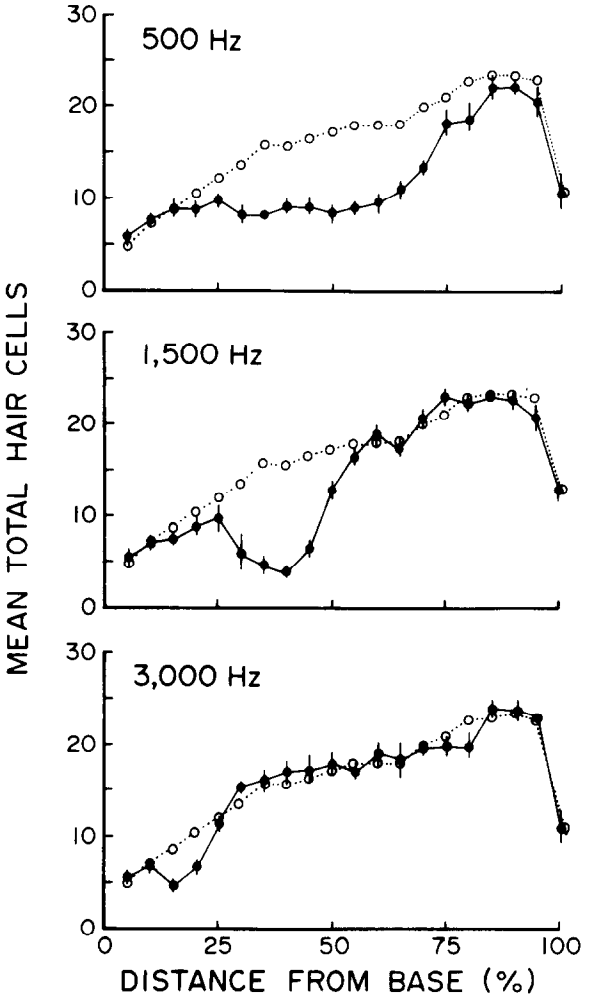

Fig. 3. Mean total hair cell counts (± S.E.) in chicks exposed at P10 to either 500 Hz (n = 3 ears), 1500 Hz (n = 4 ears) or 3000 Hz (n = 4 ears) pure tone stimulation at 125 dB SPL for 12 h. Open circles show mean total hair cell counts from the P10 normal control group. Position of maximum hair cell loss varies as a function of frequency (Hz) of exposure. Low frequency stimulation now causes hair cell loss in the apical half of the papilla.

number of hair cells in normal control animals and chicks exposed to either 500, 1500 or 3000 Hz pure tones at embryonic day 20 (E20). Only the 1500 Hz condition caused a discreet region of well defined cell loss. Lower frequency (500 Hz) stimulation caused a much smaller and more generalized amount of hair cell loss. No difference in cell number was seen after exposure to the 3000 Hz stimulus condition. In order to quantify the position of hair cell loss, the boundaries of hair cell loss were defined as the 5% region (5% sections of normalized length along the basilar papilla from the proximal tip) at which standard errors of hair cell counts did not overlap with those of normal. The position of maximum cell loss was considered that 5% region within the boundaries of hair loss showing the greatest difference between exposed and normal ears. The boundary of hair cell loss for both 500 and 1500 Hz were similar (25–50% of length), however, the position and degree of maximum cell loss were different. Position of maximum

hair cell loss for the 500 Hz condition was 36.4% of length (8.2% = 1 S.E.); position of maximum hair cell loss for the 1500 Hz condition was 30% of length (8.2% = 1 S.E.). Thus there was only a slight change in the position of hair cell loss as a function of frequency (Hz) of exposure. All hair cell loss occurred in the basal half of the basilar papilla.

Differential position of hair cell loss as a function of the frequency (Hz) of exposure was much more clearly defined in chicks exposed at P10. Boundaries of hair cell loss overlapped, however, the position of maximum hair cell loss for each frequency condition did not. Fig. 3 Shows mean total hair cell loss for each frequency condition in chicks exposed at P10. Average position of maximum loss changed as a function of the frequency (Hz) of exposure. The 3000 Hz exposure condition caused the most basal position of loss (16.3 ± 2.5(S.E.)% of length). The mid-frequency 1500 Hz condition caused maximum hair cell loss at approximately one third of length from the base (38.7 ± 5.8% of length) and the low frequency 500 Hz condition showed a wide region of loss with its

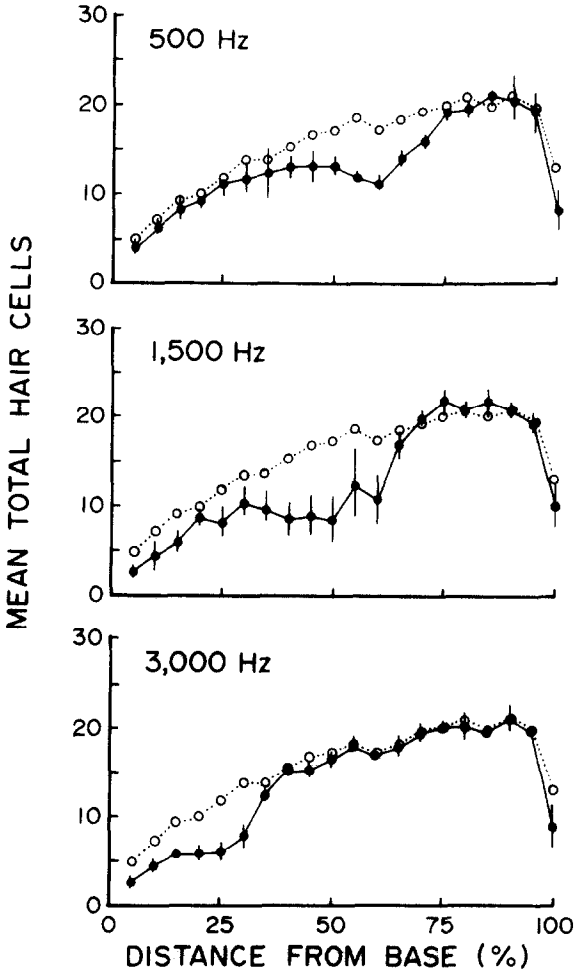

Fig. 4. Mean total hair cell counts (± S.E.) in chicks exposed at P10 to either 500 Hz (n = 3 ears), 1500 Hz (n = 3 ears) or 3000 Hz (n = 3 ears) pure tone stimulation at 125 dB SPL for 12 h. Open circles show mean total hair cell counts from P30 normal control group. Position of maximum hair cell loss continues to vary as a function of the frequency (Hz) of stimulation; all positions are shifted toward the apex as compared to younger animals.

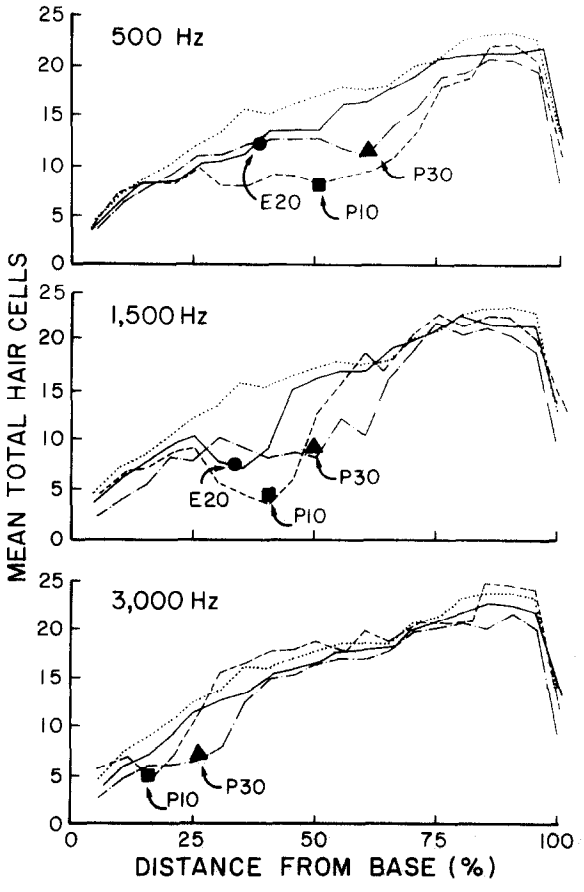

Fig. 5. Mean total hair cell counts for each exposure frequency as a function of age of stimulation. Dotted line shows mean total hair cell counts from control groups. Standard error bars are omitted for clarity. The position of maximum hair cell loss shifts toward the apex as a function of age; the most basal position of loss for all frequency exposure conditions is seen in the E20 or youngest group while the older P30 group shows more apical region of loss to the same frequency exposure.

maximum in the apical one-half of the basilar papilla (50.7 ± 6.0% of length).

Fig. 4 shows the same comparisons for chicks exposed at P30. Again the differential position of maximum hair cell loss is maintained as a function of frequency. All positions of loss have shifted slightly, however, toward the apex as compared to the position of loss in animals exposed at P10. Fig. 6 summarizes these mean hair cell counts in experimental and normal chicks for all three ages and frequency conditions.

When the position of maximum hair cell loss for individual frequency exposure conditions are compared at each age we see a progressive apical shift in position as age of exposure increases (see Figs. 5 and 6). Using the 1500 Hz condition as an example, the position of hair cell loss is most basally located at the youngest age of exposure, E20 (30% of length). At post-hatch day 10 the position of hair cell loss to the same frequency condition has moved toward the apex (38.7% of length) and at P30 it is shifted even more (48.7% of length). This same trend was found for each of the experimental frequency conditions. In all cases the shift in position of maximum hair cell loss as a function of age of exposure was highly significant.

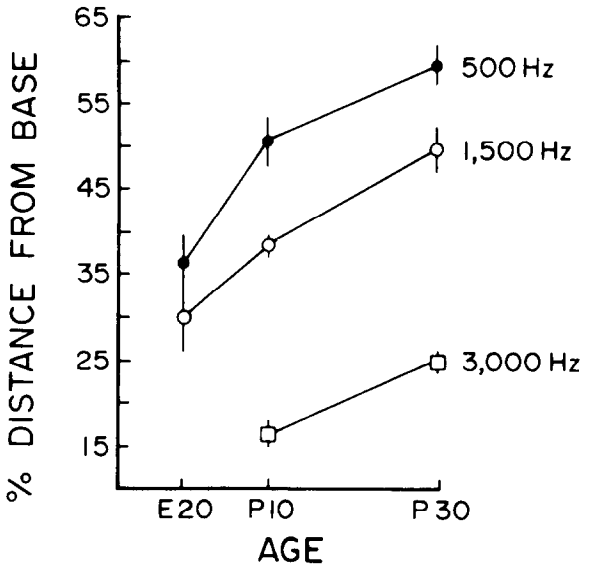

Fig. 6. Position of maximum hair cell loss as a function of age. The ordinate represents the percentile position from base to apex, at which maximum hair cell loss occurred. Mean position (± 1 S.E.) is calculated for each group of chicks exposed to each frequency.

For exposure at 500 Hz, $F(2,8) = 11.90$, $P < 0.01$; at 1500 Hz, $F(2,8) = 8.18$, $P < 0.01$; at 3000 Hz, $F(1,5) = 37.34$, $P < 0.01$ (analysis of variance). When the midpoint of the region of hair cell loss was used instead of the position of maximum loss similar results were obtained.

The more basal position of loss in animals exposed at E20 can also be seen when the basilar papilla is divided into basal and apical halves and the proportion of hair cell loss in each half is analyzed. For example, with 500 Hz exposure stimulation the ratio of hair cell loss in the basal and apical halves of the basilar papilla is 75:100 in animals exposed at P10, while in animals exposed at E20 the ratio of basal to apical loss is 100:69. This type of analysis reveals that the basal portion of the basilar papilla was consistently damaged in animals exposed to acoustic overstimulation at E20, while animals exposed at older ages showed a shift from basal location which was frequency and age dependent.

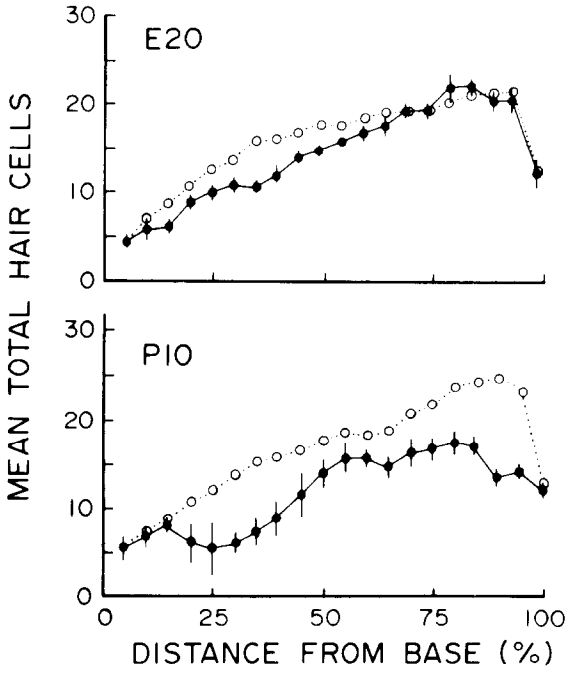

Fig. 7. Mean total hair cell counts in chicks exposed to white noise at 125 dB SPL avg. RMS for 12 h at embryonic day 20 ($n = 5$ ears) or post-hatch day 10 ($n = 4$ ears). Open circles show mean total hair cell counts in control animals. Hair cell loss is located only in the basal one half of the papilla for the youngest group while hair cell loss is seen in basal and apical halves for the older group.

Total hair cell loss after wide-band white noise exposure

Fig. 7 shows the mean total number of hair cells present in chicks exposed at either E20 or P10 to a wide-band white noise stimulus of 125 dB SPL avg. RMS. This experimental condition created generalized hair cell loss throughout the basilar papilla in the older chicks. The greatest loss is apparent at both basal and apical ends. For chicks exposed at E20, hair cell loss occurred only in the basal one-half of the papilla. It should be noted here, that in a wide-band noise stimulus of 125 dB SPL avg. RMS the level per cycle of any single frequency within the noise band is considerably less than 125 dB. The pure tone conditions were, therefore, given at higher equivalent sound pressure levels and thus the subsequent maximal amount of hair cell loss (within any one specific 5% interval of length) was predictably higher than that found with wide-band white noise. Nevertheless, again if one divides the papilla into basal and apical halves it becomes apparent from Fig. 7 that younger chicks showed hair cell loss only in the basal half of the papilla, while older chicks showed loss in both basal and apical ends of the papilla.

Discussion

The results of the present study show a consistent and systematic shift in the position of maximum hair cell loss following intense pure tone stimulation as a function of age. The basal (proximal) portion of the basilar papilla is maximally damaged in the youngest animals following wide-band white noise and mid and low frequency stimulation. As the age of stimulation increases the position of maximum damage moves toward the apical (distal) end of the basilar papilla. That is, the site of maximum stimulation of the basilar membrane by an intense sound changes in a systematic way during development.

It is important to note that this experiment was carried out during the very late stages of hearing development in the chick. Hearing thresholds are at adult levels for 500 and 1500 Hz by hatching. At E20, auditory thresholds are elevated for 3000 Hz but are adult-like by post-hatch day 10 [6,17,29]. Thus changes in the position of damage are independent of the sensitivity of the auditory system for each frequency. The lack of sensitivity to 3000 Hz at the youngest age probably accounts for the lack of hair cell loss in this group.

By E20, the middle ear system (typmanum and columella) is in the last stages of morphological maturity and the middle ear and ear canal are fluid free [11,30]. It has been suggested that middle transduction bias for low frequencies might help explain the initial developmental increased responsivity for low frequency stimulation [19]. The wide-band noise experiment in the present study, however, shows the opposite anatomical effect of what would be predicted by a middle ear low frequency bias in the young animals. If the low frequency portion of the white noise spectrum had the greatest energy transmission, it is likely that hair cell loss would be seen throughout the basilar papilla with perhaps more damage seen in the apical or presumably low frequency sensitive portion of the papilla. To the contrary, hair cell loss in the young chicks was restricted to the basal half of the basilar papilla. These factors, combined with the fact that auditory thresholds are similar across ages studied, suggest that middle ear transduction properties are not totally responsible for the changing position of hair cell loss seen as a function of age.

Morphologically, the basilar papilla is fully differentiated and appears adult-like for all ages studied. Hair cell types are fully differentiated by E20, the tectorial membrane is situated in a position similar to adults and synaptogenesis is complete. At E20 the efferent terminals are not as fully developed as in adults, however, they have formed synaptic endings on hair cells [2,3,9,10,31,32]. Recently, it has been shown that the basilar papilla continues to increase in overall size from E20 to P30 [28]. New sensory cells are not being added during this time so this growth reflects the addition of new supporting cells and/or the growth of existing structures. The position of hair cell loss would not be affected by this growth if the papilla grows equally along its length. However, if growth occurs primarily at the basal end, then the position of damage would shift systematically during development even if the place code remains fixed. In this case, exposure to a given tone will damage the same population of hair cells (i.e., the same absolute position along the papilla) at different ages.

However, the relative location of damage will shift progressively towards the apex in birds exposed at later times during development. Two observations make it unlikely that this accounts for the systematic change in the location of damage which occurred. First, the location of maximum damage does not differ significantly between birds which are exposed to the same frequency sound but allowed to survive for different periods of time [24]. This is inconsistent with the idea that the papilla grows primarily at its basal end. Second, we have calculated the change in location of damage that would occur under the assumption that the basilar papilla grows only at its basal end [13]. We found that even under this unlikely condition, cochlear growth cannot account fully for the ontogenetic shift in position of hair cell loss that occurred.

Although the chick basilar papilla is morphologically and functionally very similar during the ages studied, subtle changes in cochlear transduction properties may account for the changing frequency map shown during late stages of hearing development. These may include changes in the dimensional, mass or stiffness characteristics of the basilar membrane and/or changes in the properties of the stereocilia. Alternatively, changes in the innervation, particularly of the short hair cells [18], may be involved.

It appears that early in development the basal region of the basilar papilla responds to sound first, but it is only responsive to middle and low frequencies. As the chick matures, maximum responses to low and mid frequencies shift toward the apex of the basilar papilla and the base becomes increasingly specialized to respond to progressively higher tones. Other recent experiments lend substantial support to this hypothesis. Lippe and Rubel [12,13] describe corresponding changes in the frequency map in the chick brainstem auditory nuclei, and Harris and Dallos [8] have shown a systematic change in the cochlear microphonic cut-off frequency in the basal turn of the gerbil cochlea.

These results are of interest for several reasons. First, they offer a resolution to the paradox between the structural and functional ontogeny of the auditory system mentioned in our introductory remarks [25]. Secondly, they suggest that the frequency representation along the cochlear partition is not fixed for the life of an organism. If it changes during the development of hearing, one wonders if it may also change during other times in the life span, for example, during periods of stress or during aging.

Acknowledgements

This research was supported by NIH Grant NS15478, funds from the Lions of Virginia Hearing Foundation, the University of Virginia Pratt Foundation and the Veterans Administration Rehabilitation Research and Development Service.

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