DEVELOPMENT OF THE PLACE PRINCIPLE

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Two experiments using embryonic and hatchling chickens examined how the representation of frequency along the basilar membrane changed during hearing development. In experiment 1, chicks were exposed to high intensity pure tones (500, 1,500, or 3,000 Hz) at one of three different ages. Analysis of hair cell degeneration indicated a discrete region of damage which systematically changed as a function of exposure frequency and age. With maturation, each frequency produced damage at progressively more apical locations. In experiment 2, the representation of frequency in the brain stem auditory nuclei was compared in embryonic, hatchling, and adult chickens. Microelectrode recordings indicated a systematic shift in the frequency representation. Neurons, which are activated by high frequencies in the adult, initially respond to only low frequencies. These experiments indicate how the mature pattern of frequency representation along the basilar membrane gradually emerges during the stages of hearing development.

INTRODUCTION

The brilliant work of von Békésy demonstrated that the cochlea is organized in such a way that high frequencies maximally stimulate only the basal region and progressively lower frequencies maximally activate progressively more apical locations along the cochlea.[1] This allows the cochlea to receive a complex acoustic signal and instantaneously transform the spectral characteristics into a spatial array of eighth nerve activity. This spatial array is then maintained in register by the orderly projection of ganglion cells onto the cochlear nuclei, and remains in register at each successive level of the auditory pathways. The result is that at each level the neurons are tonotopically organized.

Literature on the development of hearing has been quite consistent. In each species that has been investigated, the development of hearing begins with low or low to midrange frequencies. These results are summarized in Fig 1 in which the adult frequency ranges and the approximate frequency range at the time of earliest hearing function of several species are indicated. In some species, like the human, hearing seems to begin with low to midrange frequencies. In others it begins with low frequencies for the species, and in some, such as the bat or mouse, the first responses are to frequencies actually below their adult range. Note that none of the animals first respond to high frequencies or even to tones in the upper half of their adult frequency range. These results parallel many other measures of hearing development.[2,3] In general, responsiveness to high frequencies lags behind low and mid-frequency responses.

This pattern of hearing development clearly predicts the pattern of receptor development. The apical turn or upper part of the middle turn should mature first; the delay in high frequency responsiveness indicates that the base should mature last.

Paradoxically, the opposite result has been found repeatedly. As exquisitely demonstrated by Retzius[4] and repeated many times since, the basal or midbasal region matures first. The middle coil is less mature, and the apex is very immature at the time hearing function begins. Thus, there is a generalized gradient from the base or the midbasal region to the apex. This general pattern has been found in virtually every animal investigated.[3] During this same period in the chick and hamster there is a spatial gradient of development of the brain stem auditory

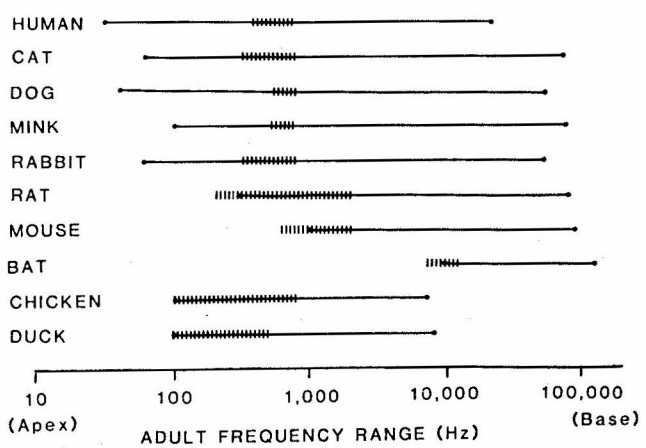

Fig 1. Frequency range at onset of hearing. Adult frequency range of hearing for each species (at approximately 70 dB SPL) is shown as solid line. Vertical lines mark frequency range that animals are believed to be sensitive to at youngest age that responses have been evoked. Behavioral and physiological data have been combined from the literature to make these estimates.[3]

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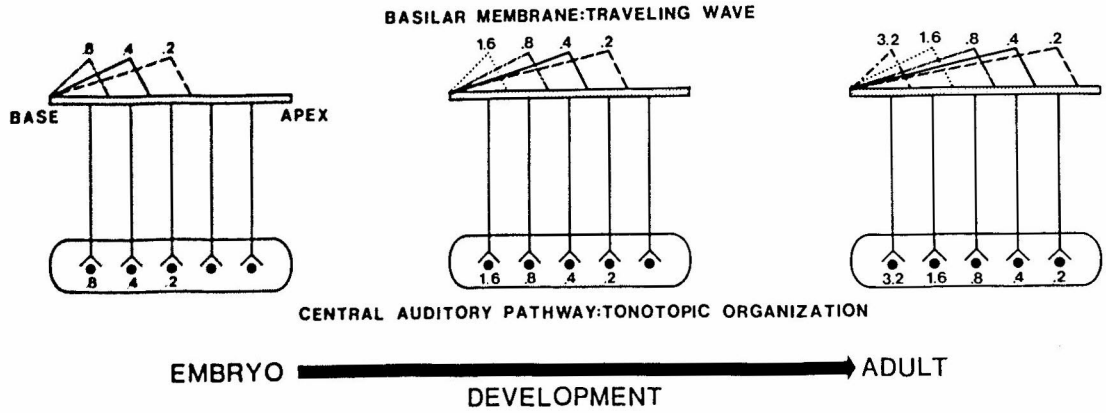

Fig 2. Hypothesized sequence of inner ear development (top) along with changes predicted in best frequency of neurons in CNS (bottom). At beginning of auditory function (left), basal half of cochlea is responsive to relatively low frequencies and CNS areas receiving projections from base respond to low frequencies. With maturation (middle and right), apex of cochlea begins responding to low frequencies and base becomes more and more sensitive to high frequencies. Resulting shift in neuronal best frequencies is indicated at bottom of each diagram. (Reprinted with permission from Rubel EW, Ontogeny of auditory system function, *Ann Rev Physiol* 1984; 46:218-29.)

nuclei that corresponds to that found in the cochlea.[5-7] One way to resolve this paradox would be if the frequency code is not stable. Specifically, we have proposed that the basal region is the first to mature, but it responds only to low frequencies early in development. Then, as we mature, more apical regions respond to low frequencies and the base becomes maximally responsive to higher and higher frequencies.[3-5] This hypothesis and two predictions are indicated in Fig 2.

The first prediction relies on the assumption that high intensity pure tones produce restricted damage to the place where the maximum traveling wave is generated.[8,9] If a developmental shift in the place code occurs, the place of damage produced by a pure tone should actually shift along the cochlea during development. Any given frequency should produce damage at progressively more apical locations with advancing age.

The second prediction is that the tonotopic organization will also shift during development. That is, if we record from a neuron within any brain stem auditory nucleus it will have some characteristic frequency (CF, the frequency to which it is most sensitive). The model presented in Fig 2 indicates that if we record from the same neuron or group of neurons throughout development their best frequency should start low and become most sensitive to progressively higher frequencies as they get older.

Both of these predictions were tested in the chicken embryo and hatchling. This animal model was used for the following reasons. 1) It has a history of hearing development like that of the human; it hears in ovo and is born with good but not quite fully developed hearing.[3,10] 2) There is a wealth of developmental information on the cochlea and brain stem auditory pathways in the chick.[6,11-14] 3) Because the cochlea is short and uncoiled, sectioning and quantification of the number of hair cells are easily accomplished.[15] 4) Most importantly, both the frequency code along the cochlea and the tonotopic organization centrally have been quantitatively described.[9,16] Thus, developmental changes could be assessed.

EXPERIMENT 1

The purpose of this experiment was to test the first prediction noted above. We examined changes in the position of structural damage to the basilar membrane when animals were exposed to intense pure tones at different ages during the final stages of hearing maturation.

Method. Domestic chickens (Hubbard x Hubbard) of three ages were used: embryonic day 20 (E20, 1 day prior to hatching), postnatal day 10 (P10), and postnatal day 30 (P30). Chicks begin hearing around embryonic day 12 and evoked potential thresholds are nearly adultlike by hatching. Behavioral thresholds to low frequencies are mature by the day of hatching while high frequency thresholds are elevated at this time. By ten days after hatching, both evoked potential and behavioral thresholds appear fully mature. Thus, these ages span the final stages of hearing development. Chicks were incubated, hatched, and maintained in our laboratory colony until the time of sound exposure.

At each of the above ages the animals were divided into four groups (4 to 8 chicks per group). One group served as normal control animals and were not exposed to intense acoustic stimulation. Subjects in the other three groups were exposed for 12 hours to a continuous intense (125 dB sound pressure level) pure tone of either 500, 1,500, or 3,000 Hz. Animals were exposed in pairs in a wire-mesh chamber placed under a power horn. Stimuli were calibrated before and after exposure at the level of the animals' ears using a General Radio electret microphone and a General Radio model 1900 A wave analyzer. All harmonics and other sounds were at least 40 dB below the signal level. In some animals, one ear canal was plugged using a

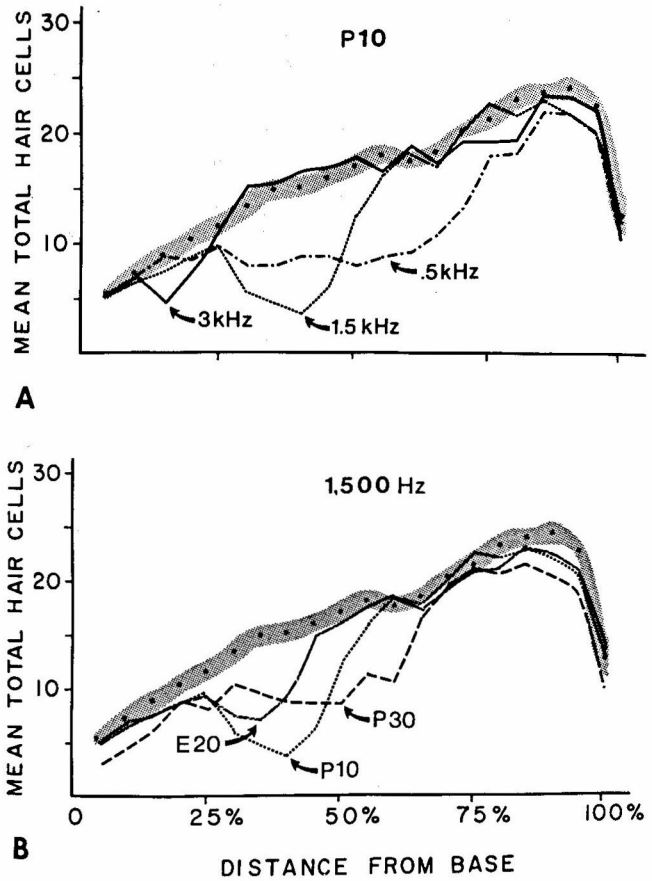

Fig 3. Total number of hair cells as function of position along basilar papilla from base to apex. Mean ± standard error for normal animals is shown by shaded area and points. Standard errors for experimental groups are omitted for clarity (they are comparable to those for normal animals). A) Mean number of hair cells along basilar papilla of P10 chicks exposed to 500, 1,500, or 3,000 Hz. B) Mean number of hair cells at each position from E20, P10, and P30 chicks exposed to 1,500 Hz. Arrows indicate positions of maximum damage. (Reprinted with permission from Rubel EW, Ryals BM, Development of the place principle: acoustic trauma, *Science* 1983; 219:512-4.)

commercial silicone earmold compound which provides approximately a 40-dB conductive loss,[17] thereby protecting that ear from damage. This attempt to provide a within-subject control proved unnecessary (see below). In order to achieve the same level of intense sound exposure in the embryos (E20), the shell overlying the large end of the egg was removed, exposing the chick's head which had already entered the air space. The ear canals were then gently aspirated to remove any remaining fluid. Care was taken to assure that normal temperature was maintained during the sound exposure. Control animals were subjected to the same operative and handling conditions.

Following sound exposure, the animals were returned to their brooder and were allowed to survive for ten days under normal laboratory conditions. The cochleas were then fixed by intralabyrinth perfusion, dissected free, osmicated, and embedded in Epon. Each cochlea was sectioned orthogonal (transverse) to the long axis. At 100-μm intervals along the entire length of the cochlea, beginning at the most basal (proximal) end, several 3-μm sections were collected and stained with toluidine blue. The number of hair cells across the basilar membrane was counted at each 100-μm interval. Counts were made under an oil immersion objective (N.A. = 1.0) at a total magnification of 500x. Three 3-μm sections were analyzed at each 100-μm interval and the average number of hair cells per section was recorded. Counts were expressed as a function of distance from the base, and then normalized across animals by converting to percent of the total length in 5% intervals.[18] More detailed descriptions of the exposure conditions and methods of hair cell analysis, as well as photomicrographs of normal and exposed cochleas can be found in previous reports.[9,15]

Results. There were no differences in hair cell counts between normal animals, sham-exposure animals, or the ears which had been protected by earplugs. Therefore, data from these normal ears were combined. The mean counts (± 1 SEM) from the normal ears are shown as the shaded line and dots in Fig 3. While in mammals there are four hair cells (one inner and three outer) in a transverse section, in birds and reptiles there is a sheet of five to 25 hair cells stretching about two thirds of the way across the basilar membrane.

The other functions in Fig 3 show the pattern of results found when chicks were exposed to pure tones of different frequencies (Fig 3A) or to the same frequency at different ages (Fig 3B). These data are plotted as a function of the position along

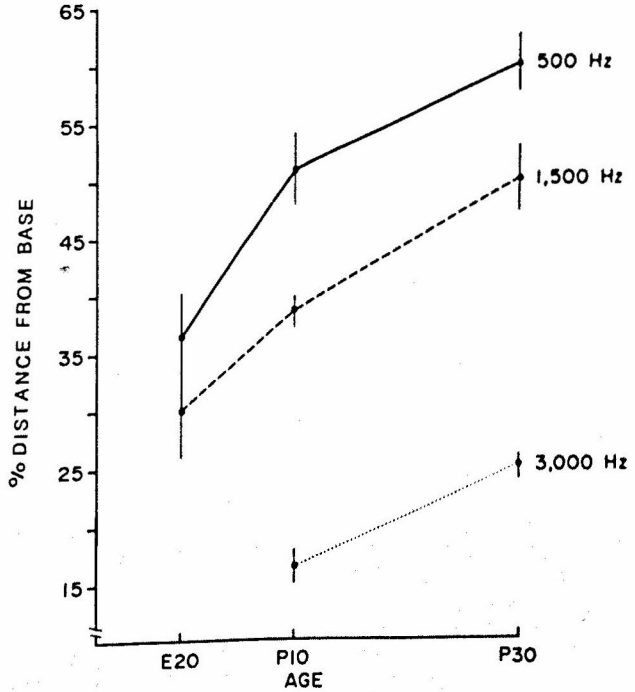

Fig 4. Position of maximum hair cell loss as a function of age. Ordinate represents percentile position, from base to apex, at which maximum hair cell loss occurred. Mean position is shown for each group of chicks exposed to each frequency (± SEM). (Reprinted with permission from Rubel EW, Ryals BM, Development of the place principle: acoustic trauma, *Science* 1983; 219:512-4.)

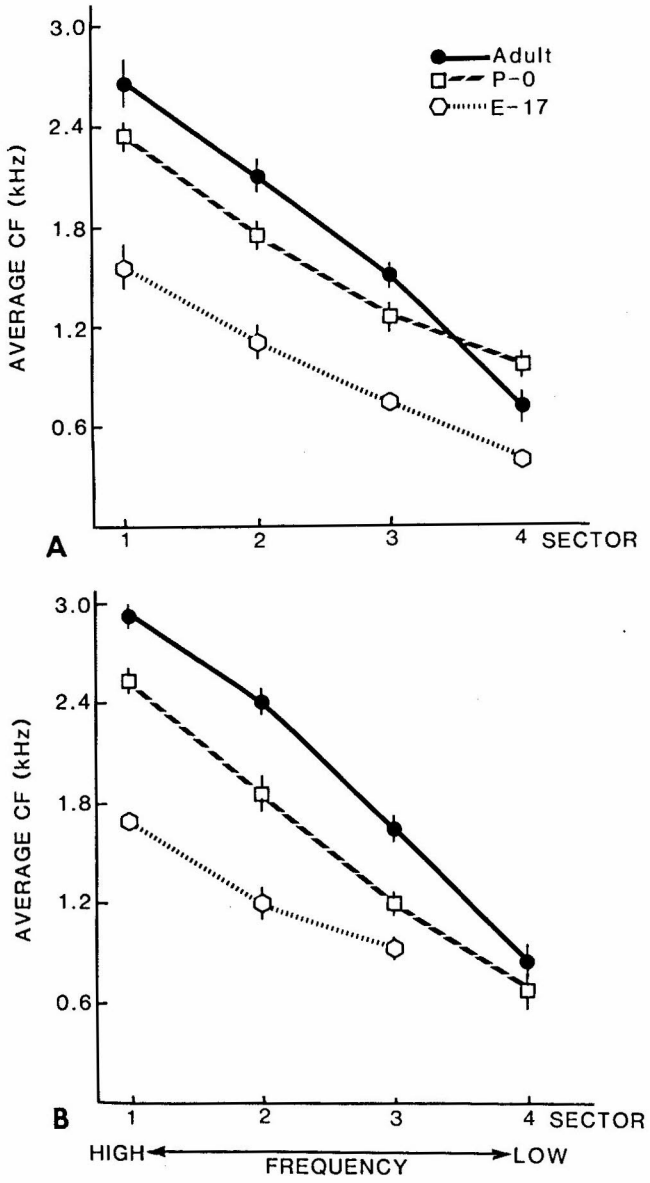

Fig 5. Average characteristic frequency of units and small groups of units in each nucleus for chicks at three ages studied: embryonic day 17 (E17), day of hatching (P0), and adults. A) Nucleus magnocellularis. B) Nucleus laminaris (NL). Each nucleus was divided into four equal sectors orthogonal to tonotopic axis. Average best excitatory frequency (CF) was then calculated for recordings made in each sector. These averages (± 1 SEM) are indicated. Symbols without error bars indicate that error bars would be smaller than symbol size. Data base for lowest frequency sector in NL at E17 was not sufficient to have a reliable estimate of average CF; therefore, this point is omitted.

the cochlea, from base to apex. The standard errors have been eliminated for clarity, but they were similar in size to those found in the control groups.

Figure 3A indicates that there is a restricted area of hair cell loss that differs systematically for the different exposure frequencies.[9] It is located progressively apically and becomes broader as the frequency is lowered from 3 kHz to 500 Hz. Figure 3B shows the results obtained if animals are exposed to a 1,500-Hz tone at different ages. The data from the P10 animals is repeated. The other two functions show average hair cell loss for animals exposed at E20 and P30. The position of damage in the youngest animals is centered most toward the base. At P10 the position of damage occurs more apically, and at P30 it is centered even more toward the apex. The arrows point to the position of maximum hair cell loss for each group.

This same trend was found for each of the frequencies examined (Fig 4). At the youngest age the animals were relatively insensitive to 3 kHz, thus no damage occurred. With each of the three frequencies the position of maximum hair cell loss consistently shifted toward the apex. This shift was statistically reliable ($p \leq 0.01$) for each frequency.

Conclusion. Therefore, the first prediction was confirmed; there was a shift in the position of sound damage that is consistent with the hypothesized shift in the place code. As the animals matured, the position of damage produced by intense stimulation with each frequency shifted toward the apex.

It is worth reiterating that with the exception of responses of the youngest ages to the highest frequency, normal or near normal thresholds are found at all the ages tested. Thus, changes in the position of damage are independent of the sensitivity of the auditory system for each frequency and these "place changes" seem to continue past the time when adult sensitivity is established.

EXPERIMENT 2

The second prediction was that the best frequency, or threshold frequency, of neurons in the CNS would change. We tested this by microelectrode recording from brain stem auditory nuclei in the chick at three ages. Quantitative microelectrode mapping was done to determine the threshold frequency (characteristic frequency) of the same neuronal subpopulations at different ages.

Method. Chicks of three ages were used: 16- to 17-day-old embryos (E17), immediate posthatch animals (P0), and adults. The adult data was combined from adult animals examined along with our younger subjects in this study and previous data from 20- to 30-day-old chickens studied in our laboratory.[16]

Standard microelectrode recording procedures were used.[16] Briefly, anesthetized chicks were secured in specially designed headholders (depending on age) within a double-walled booth (Industrial Acoustics Co). Body temperature was maintained at 37.5° C. The embryos remained within the egg. Their heads were exposed, attached to the headholder, and the external auditory canal was aspirated to expose the tympanic membrane. Calibrated sound pulses (50- or 100-ms duration, 5-ms rise-fall times) were delivered at a rate of one per second or one per 2 seconds (for embryos) through a closed system sealed to the external auditory meatus.

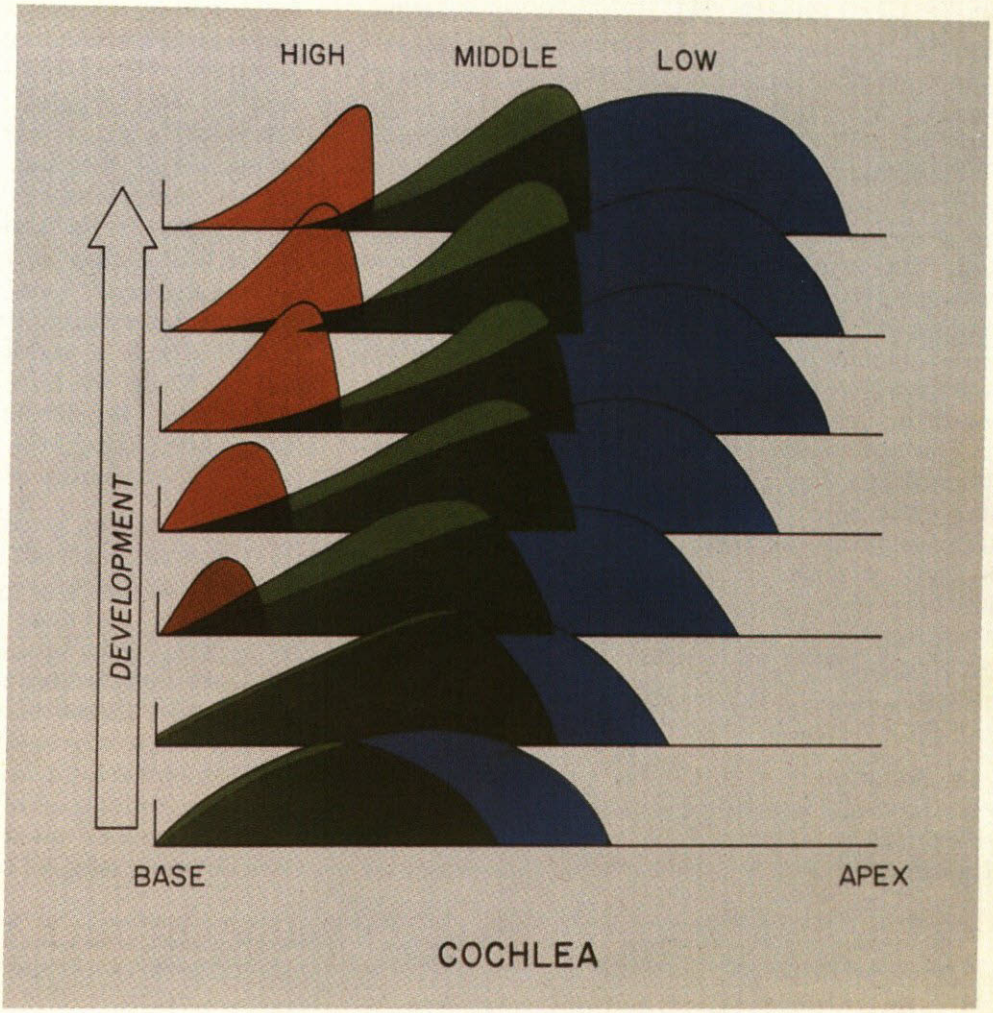

Fig 6. Schematic representation of shift in traveling wave during development for high, middle, and low frequency sounds.

Glass-insulated tungsten microelectrodes were used to explore two brain stem auditory nuclei. Nucleus magnocellularis (NM) contains second order neurons receiving excitatory input from the eighth nerve and considered homologous with the mammalian anteroventral cochlear nucleus; nucleus laminaris (NL) neurons are third order neurons receiving binaural innervation via NM on the two sides of the brain. The CF of NM and NL neurons was determined at several locations as the electrode was lowered through the brain stem. Several electrode penetrations were made in each animal. Marking lesions were made to facilitate reconstruction of the recording locations. At the end of each experiment the brain was fixed, embedded in paraffin, and sectioned in the coronal plane. The precise location of the electrode tip at each recording position was then determined relative to the length (posterior to anterior) and width (lateral to medial) of NM or NL. The recording positions were related to the best frequency (CF) of the neurons, providing quantitative description of the relationships between recording position within each nucleus and the preferred frequency (CF) of the neurons.[16]

Results. Each neuron or group of neurons in the 17-day embryos was optimally responsive to a lower frequency than neurons at the same place in the nucleus of the adult chick. The CFs in the embryos ranged from 33% to 69% (average 52%) of their predicted values from adult recording positions. Neurons from P0 animals had CFs intermediate between the E17 and adult animals. These relationships are shown in Fig 5. For this analysis, we divided each nucleus into four frequency sectors by plotting a line along the tonotopic organization and then dividing the nucleus into four equal sectors orthogonal to this line. We then computed the average frequency of neurons found in each of the regions at each age. The changes in average CF along each line indicate the tonotopic organization in NM and NL at each age. Difference in the overall positions of the lines indicate changes in the frequency tuning across age.

As indicated in Fig 5, there is a consistent and statistically significant increase in the best frequencies of neurons in each sector of NM between 17-day-old embryos and adults. In the lowest frequency region there is no reliable difference between the oldest two age groups. In NL the same result was confirmed; in each sector neurons in the embryo respond to tones much lower in frequency, by about an entire octave, than those same neurons in the adult.

Conclusion. The second prediction of the hypoth-

esis is also confirmed. The tonotopic organization shifts during development; neurons in a given region respond to progressively higher frequencies.

DISCUSSION

These two converging operations strongly support the proposition of a general theory regarding development of the place principle (Fig 6). It appears that early in development the basal region responds to sound first, but it is only responsive to middle and low frequencies. As an organism matures, maximum responses to the low frequencies and middle frequencies shift down the cochlea toward the apex, and the base becomes increasingly specialized to respond to progressively higher tones. There is now considerable evidence that this process is general across terrestrial vertebrates, including humans. First, as noted in the introduction, the indirect evidence is apparent in each species that has been investigated. Second, similar changes to those reported here have now been reported for the gerbil cochlea[19] and the cochlear nucleus.[20] Finally, earlier studies of the auditory cortex in neonatal cats show this same relationship.[21]

Recognition of a shift in the values of the place code has some interesting ramifications. First, it resolves the developmental paradox discussed in the Introduction. Second, it means that neurons in the brain will be optimally stimulated by different frequencies at different ages. At some age each will be maximally stimulated by low frequencies. Finally, and of most general interest, it means that the values of the place code are not fixed for any species or individual; the place code is mutable. We believe there is compelling evidence that it shifts during development. There is also some circumstantial evidence that it may also shift during aging and following trauma.[22] Lambert and Rubel have recently presented some preliminary observations suggesting a shift due to a middle ear effusion (unpublished data). While we do not yet know the structural basis underlying developmental or other changes in frequency coding, the most likely possibilities involve the mechanics of the cochlear partition.

It is intriguing to consider the possibility that if the mechanics are altered during development or other conditions to produce these changes, it might be possible to change the system intentionally, ie, surgically. In the future, could we intervene, modify the basilar membrane, and intentionally shift the place code, thereby allowing undamaged parts of the cochlea to maximally respond to the frequencies most important for language recognition? Experimental examinations of this question will encourage new approaches toward the development of effective inner ear surgical treatments.

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The American Otological Society is offering research grants for the study of otosclerosis including research in diagnosis, management, pathogenesis as well as underlying processes. Grant requests must be submitted by January 31, 1985. Grants received after January 31 will be considered for the following year. Further information and application forms may be obtained from the Office of the Secretary-Treasurer of the Board of Trustees of the Research Fund of the American Otological Society, Maxwell Abramson, MD, 630 West 168th Street, New York, NY 10032.

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CLINICAL AND RESEARCH ISSUES IN SWALLOWING AND SWALLOWING DISORDERS

A conference on Clinical and Research Issues in Swallowing and Swallowing Disorders will be held June 6-7, 1985, at the National Institutes of Health in Bethesda, Maryland. For further information contact Barbara C. Sonies, PhD, Department of Rehabilitation Medicine, Clinical Center, National Institutes of Health, Bethesda, MD 20205; (301) 496-4733.