

high levels reached at 36 hours after hepatectomy do not persist. In contrast, AFP mRNA remains high until at least 96 hours after the operation. These differences between AFP and *ras* transcripts might be a reflection of the half-lives of these messengers. In contrast to the pattern of change presented in Fig. 1b, albumin mRNA does not increase after partial hepatectomy (20).

Our results show that transcripts of a cellular oncogene increase concomitantly with the burst of DNA synthesis in regenerating rat liver and rapidly return to basal levels. While we have no data demonstrating that the expression of *c-ras* controls compensatory growth, this observation indicates that there is regulated transcription of the cellular *ras* oncogene during a physiological growth process.

MICHELE GOYETTE  
CHRISTOS J. PETROPOULOS  
PETER R. SHANK  
NELSON FAUSTO

Division of Biology and Medicine,  
Brown University,  
Providence, Rhode Island 02912

#### References and Notes

1. J. M. Bishop, *Cell* **23**, 5 (1981).
2. H. E. Varmus, *Science* **216**, 812 (1982).
3. E. Hunter, in *Viral Oncology*, G. Klein, Ed. (Raven, New York, 1980), p. 1.
4. G. M. Cooper, *Science* **217**, 801 (1982).
5. R. A. Weinberg, *Cell* **30**, 3 (1982).
6. D. DeFeo et al., *Proc. Natl. Acad. Sci. U.S.A.* **78**, 3328 (1981).
7. D. G. Blair, M. Oskarsson, T. G. Wood, W. L. McClements, P. J. Fischinger, G. G. Vande Woude, *Science* **212**, 941 (1981).
8. H. Langbeheim, T. Y. Shih, E. M. Scolnick, *Virology* **106**, 292 (1980).
9. E. Chang, M. E. Furth, E. M. Scolnick, D. R. Lowy, *Nature (London)* **297**, 497 (1982).
10. E. M. Scolnick, *Microbiol. Rev.* **45**, 1 (1981).
11. ———, M. O. Weeks, T. Y. Shih, S. K. Russett, T. M. Dexter, *Mol. Cell Biol.* **1**, 66 (1981).
12. R. Müller, D. J. Slamon, J. M. Tremblay, M. J. Cline, I. M. Verma, *Nature (London)* **299**, 640 (1982).
13. R. W. Ellis et al., *J. Virol.* **36**, 408 (1980).
14. N. L. R. Bucher and R. Malt, *Regeneration of Liver and Kidney* (Little, Brown, Boston, 1971).
15. P. Thomas, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5201 (1980).
16. M. Goyette, C. J. Petropoulos, P. R. Shank, N. Fausto, in preparation.
17. C. A. Scholla, M. V. Tedeschi, N. Fausto, *J. Biol. Chem.* **255**, 2855 (1980).
18. N. Fausto, G. Schultz-Ellison, V. Atryzek, M. Goyette, *J. Biol. Chem.* **257**, 2200 (1982).
19. C. Der, T. G. Krontiris, G. M. Cooper, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 3637 (1982).
20. C. J. Petropoulos, G. Andrews, T. Tamaoki, N. Fausto, *J. Biol. Chem.*, in press. The proportion of polysomal poly(A)<sup>+</sup> RNA which is AFP mRNA was determined with a <sup>3</sup>H-labeled complementary DNA probe by the titration method [B. D. Young, P. R. Harrison, R. S. Gilmour, G. D. Birnie, A. Hell, S. Humphries, J. Paul, *J. Mol. Biol.* **84**, 555 (1974)].
21. V. Atryzek and N. Fausto, *Biochemistry* **18**, 1281 (1979).
22. J. A. McGowan and N. Fausto, *Biochem. J.* **170**, 123 (1978).
23. R. D. Palmiter, *Biochemistry* **13**, 3606 (1974).
24. P. W. J. Rigby, M. Dieckman, C. Rhodes, P. Berg, *J. Mol. Biol.* **113**, 237 (1977).
25. The values designated "percentage of normal" in Fig. 1 were determined by dividing the absolute experimental values obtained with regenerating liver RNA's by that of normal adult liver RNA and multiplying by 100. The value for the hybridization of <sup>32</sup>P-labeled Ha-ras DNA to normal polysomal poly(A)<sup>+</sup> RNA averaged 150 count/min. We have determined that there are approximately 42 × 10<sup>12</sup> molecules of polysomal poly(A)<sup>+</sup> RNA per milligram of DNA in normal adult rat liver, 0.006 percent of which is AFP mRNA (20).
26. We thank R. W. Ellis for the generous gift of BS-9 DNA. Supported by National Cancer Institute grants CA 24829 (P.R.S.) and CA 23226 (N.F.).

20 September 1982; revised 17 December 1982

## Development of the Place Principle: Acoustic Trauma

**Abstract.** *Developmental changes in the site of receptor damage following pure-tone acoustic overstimulation were examined in the basilar papillae of embryonic and hatchling chickens. During development, a systematic shift in the position of damage toward the apex of the cochlea was produced by each of three frequencies, suggesting that the transduction properties of the sensory epithelium systematically shift with age. These results imply that neurons in the central nervous system may be maximally stimulated by different sounds during development.*

The basal region of the adult cochlea is maximally responsive to high frequencies, while apical positions are maximally responsive to lower frequencies. This spatial representation of frequency, known as the place principle in the cochlea, is preserved throughout the central auditory pathways. It is thought that this tonotopic organization helps to preserve spectral relations in the pattern of neural activity (1). As demonstrated in the classic experiments of von Békésy (2), place principle is determined by the position of the maximum amplitude of the traveling wave as a function of frequency. An indirect method used to con-

firm the "map" of frequency representation in the cochlea is to determine the position of hair cell damage produced by exposure to high-intensity pure-tone or narrow-band noise (3–5).

During ontogeny all birds and mammals initially respond to low or relatively low frequency sounds, and mature functional properties usually appear first for relatively low frequencies, whereas high-frequency responsivity is delayed (6–9). This sequence suggests that the apical (low-frequency) regions of the cochlea are the first to be responsive to sound and that only later are the basal (high-frequency) regions involved. Paradoxically,

it has been repeatedly demonstrated that differentiation of the organ of Corti in mammals and the basilar papilla in birds occurs in the opposite direction (9–10). In addition, differentiation of brainstem auditory regions in avian embryos occurs first in the basal projection area and only later in the apical, or low frequency, area (11, 12).

This paradoxical relation between the ontogeny of responses to sound and development of the auditory system cannot be accounted for by changes in middle ear transmission (13), suggesting that the part of the sensory epithelium that is maximally responsive to low or middle frequencies shifts during development. Specifically, it has been 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 (9, 12). The hypothesis predicts that the position of hair cell damage produced by exposure to an intense pure tone will shift apically during the late stages of hearing maturation and that the best frequency (14) of neurons at any given location in primary central auditory regions will shift toward higher frequencies during maturation. We tested these predictions by examining developmental changes in the functional organization of the cochlea (basilar papilla) and brainstem auditory nuclei of chicks (4, 15). In this report we discuss ontogenetic changes in the position of hair cell damage produced by acoustic overstimulation with pure tones; changes in frequency representation in the central nervous system are considered in the following report (16).

Domestic chickens (Hubbard × Hubbard) of three ages were used: embryonic day 20 (E20), postnatal day 10 (P10), and postnatal day 30 (P30). At each of these ages at least three animals were exposed to a continuous pure tone of 500, 1500, or 3000 Hz at 125 ± 3 dB (sound-pressure level) for 12 hours (4, 5, 17). 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.

After their exposure to sound, 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 cochleas (basilar papillae) were then fixed, dissected free, osmicated, embedded in

Epon, sectioned transversely, and stained. The hair cells extending across the basilar membrane were then counted at each 100- $\mu$ m interval from the basal to the apical end (18).

In each experimental animal (except E20 embryos exposed to 3000 Hz) there was a discrete region of the basilar membrane where many hair cells were missing and where the remaining hair cells appeared damaged. Outside this region the hair cells were normal in appearance and number. Figure 1A shows the mean number of hair cells as a function of distance along the basilar membrane for normal chicks and P10 animals. An area of hair cell loss that varied systematically in position and area was evident in each experimental group. As in mammals, progressively higher frequencies resulted in more restricted damage at progressively more basal locations.

Figure 1B shows the results of exposing animals at the three different ages to the same pure tone (1500 Hz). Both the extent and position of hair cell loss varied as a function of age (5, 19). More importantly, the overall region of hair cell loss and the position of maximum loss shifted toward the apex as the animals became older. These results are consistent with the hypothesis that the region of the basilar membrane that is maximally activated by a 1500-Hz tone has a relatively basal location initially and then, with increasing age, shifts toward the apex.

Examination of the developmental changes in the locus of damage produced by each frequency yielded similar results. In every experimental group except E20 animals exposed to 3000 Hz (no significant damage) there was a systematic apical shift in the position of hair cell loss as a function of age (Fig. 2) (20). Physiologically measured thresholds to 500 and 1500 Hz were equivalent and adult-like at all the ages studied; thresholds to 3000 Hz were elevated in the E20 animals but were equivalent and adult-like by postnatal day 10 or 30 (7, 8, 21).

These findings offer a resolution to the paradox between the structural and functional ontogeny of the auditory system. The data indicate that the site of maximum stimulation of the basilar membrane by an intense sound changes during development. This conclusion is based on the assumption that the spectral pattern of sound reaching the inner ear is similar across the ages in question. Available data on developmental changes in middle ear function, the lack of significant changes in threshold, and the fact that distortions and harmonics were well-attenuated in

this study all suggest that the spectral properties of the stimuli reaching the inner ear do not change markedly over the developmental period (22).

Two factors lead us to believe that these results parallel what would be found from direct measurements of the

spatial properties of hair cell activation by moderate or low-level sounds. First, while nonlinearities and distortion products of the cochlea are probably reflected in these functions, there is no a priori reason to believe that they differ systematically across the ages studied. Second, the results of mapping studies in the cochlear nucleus of late embryos with threshold-level stimuli provide complementary results (16).

Possible mechanisms for the ontogenetic shift in the spectral properties of the cochlea include (i) age-related changes in the dimensional, mass, or stiffness characteristics of the basilar membrane, resulting in mechanical changes; and (ii) changes in the properties of the stereocilia, resulting in different "filtering" characteristics.

EDWIN W. RUBEL\*

BRENDA M. RYALS†

Departments of Otolaryngology and Physiology, University of Virginia School of Medicine, Charlottesville 22908

#### References and Notes

1. J. E. Rose, R. Galambos, J. R. Hughes, *Bull. Johns Hopkins Hosp.* **104**, 211 (1959); M. Merzenich and M. D. Reid, *J. Neurophysiol.* **38**, 231 (1975).
2. G. von Békésy, *Experiments in Hearing* (McGraw-Hill, New York, 1960).
3. J. F. Schuknecht, *Acta Oto-Laryngol.* **58**, 377 (1953); W. W. Stockwell, H. W. Ades, H. Engstrom, *Ann. Otol. Rhinol. Laryngol.* **78**, 1144 (1969).
4. B. M. Ryals and E. W. Rubel, *Acta Oto-Laryngol.* **93**, 205 (1982).
5. E. W. Rubel and B. M. Ryals, *ibid.* p. 31.
6. G. Gottlieb, in *The Biopsychology of Development*, E. Tobach et al., Eds. (Academic Press, New York, 1971), pp. 67-128; D. R. Moore and D. R. F. Irvine, *Brain Res.* **163**, 49 (1979).
7. J. C. Saunders, R. B. Coles, B. R. Gates, *Brain Res.* **63**, 59 (1973).
8. G. Rebillard and E. W. Rubel, *ibid.* **229**, 15 (1981).
9. E. W. Rubel, in *Handbook of Sensory Physiology*, vol. 9, *Development of Sensory Systems*, M. Jacobson, Ed. (Springer-Verlag, New York, 1978), pp. 135-237.
10. G. Retzius, *Das Gehörorgan der Wirbeltiere* (Samson & Wallin, Stockholm, 1884); R. Pujol and R. Marty, *J. Comp. Neurol.* **139**, 115 (1970); N. Hirokawa, *J. Neurocytol.* **7**, 283 (1978).
11. H. Jackson, J. T. Hackett, E. W. Rubel, *J. Comp. Neurol.* **210**, 80 (1982).
12. E. W. Rubel, D. J. Smith, L. C. Miller, *ibid.* **166**, 469 (1976).
13. E. M. Relkin and J. C. Saunders, *Acta Oto-Laryngol.* **90**, 6 (1980); J. C. Saunders, personal communication.
14. The best frequency or characteristic frequency is the frequency to which the neuron has the lowest excitation threshold.
15. E. W. Rubel and T. N. Parks, *J. Comp. Neurol.* **164**, 411 (1975).
16. W. R. Lippe and E. W. Rubel, *Science* **219**, 514 (1983).
17. In embryos, the shell overlying the air space was removed, exposing the chick's head. The ear canals were gently aspirated to remove any remaining fluid. Sound exposure procedures, spectral analyses of the stimulation, histological procedures, and data collection methods are described in detail elsewhere (4, 5). Embryos were maintained at standard incubator temperature and humidity during sound exposure. Sound pressure levels were calibrated with a General Radio Electret microphone and wave analyzer. Measurements were made before and after exposure at the level of the ear canals.
18. The hair cells across the basilar membrane were counted under an oil immersion objective (numerical aperture, 1.0) at a total magnification of

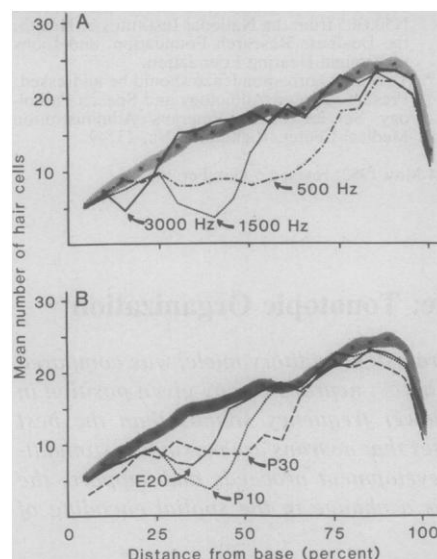


Fig. 1. (A and B) Total number of hair cells as a function of position along the basilar papilla from base to apex. The mean  $\pm$  standard error for normal animals is shown by the thick lines. Standard errors for the experimental groups are omitted for clarity (they are comparable to those for the normal animals). (A) Mean number of hair cells along the basilar papilla of P10 chicks exposed to 500, 1500, or 3000 Hz. (B) Mean number of hair cells at each position from E20, P10, and P30 chicks exposed to 1500 Hz. Arrows indicate positions of maximum damage (23).

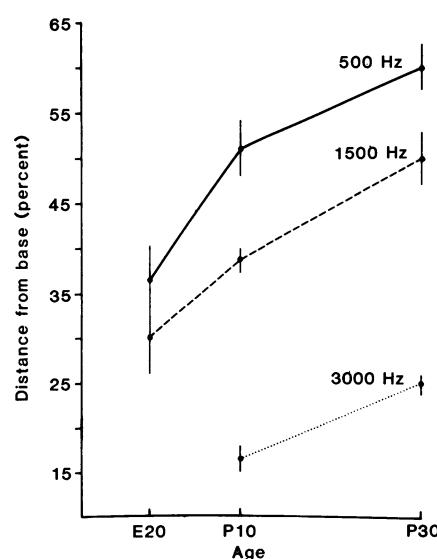


Fig. 2. Position of maximum hair cell loss as a function of age. The ordinate represents the percentile position, from the base to the apex, at which maximum hair cell loss occurred. Mean position is calculated for each group of chicks exposed to each frequency.

×500. 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 the percentage of the total length in 5 percent intervals.

19. The normal number of hair cells differed minimally between age groups; in older chicks there was a slight reduction in the number of hair cells in the apical (distal) 20 percent of the cochlea (5).
20. In all cases the resulting shifts were highly significant. For exposure at 500 Hz,  $F(2, 8) = 11.90$ ,  $P < .01$ ; at 1500 Hz,  $F(2, 8) = 8.18$ ,  $P < .01$ ; at 3000 Hz,  $F(1, 5) = 37.34$ ,  $P < .01$  (analysis of variance). Similar results were obtained by analyzing positions of the midpoint of damage as a function of age.
21. L. M. Kerr, E. M. Ostapoff, E. W. Rubel, *J. Exp. Psychol. Anim. Behav. Proc.* 5, 97 (1979).
22. In a separate experiment E20 and P10 chicks were exposed to 125-dB white noise. In the young animals damage was restricted to the

basal end of the cochlea, whereas in the hatchlings damage occurred throughout. This result indicates that the positional shifts reported cannot be accounted for solely by hypothesizing a low-frequency transmission bias in the young middle ear.

23. The position of maximum damage is considered the 5 percent region showing the greatest difference between exposed and normal cochleas.
24. We thank O. Steward, L. Gray, W. Lippe, S. Young, and J. Deitch for critical comments on the manuscript; P. Palmer, R. Kitch, and M. Wells for histological assistance; and S. Davis for typing and editorial work. This work was supported by grants NS 15478 and RCDA N500305 from the National Institutes of Health, the Deafness Research Foundation, and Lions of Virginia Hearing Foundation.
- \* To whom correspondence should be addressed.
- † Present address: Audiology and Speech Pathology Service (126), Veterans Administration Medical Center, Richmond, Va. 23249.

24 May 1982; revised 5 October 1982

## Development of the Place Principle: Tonotopic Organization

**Abstract.** *The tonotopic organization of brainstem auditory nuclei was compared in embryonic and hatchling chickens. In embryos, neurons at any given position in these nuclei were maximally sensitive to lower frequency sounds than the best frequency after hatching. This finding indicates that neurons are maximally stimulated by sounds of different frequencies as development proceeds and supports the hypothesis that during development there is a change in the spatial encoding of frequency along the cochlea.*

The hypothesis that the spatial encoding of frequency along the cochlear partition changes during development was outlined by Rubel and Ryals in the preceding report (1). In brief, we have proposed that in the immature cochlea only the early-developing basal region is responsive to sound and that, unlike this

region in the adult, it is maximally responsive to sounds of relatively low frequency. During development the maximum point of sensitivity to low and middle frequencies gradually shifts apically as the basal end becomes responsive to higher frequencies. This hypothesis predicts that neurons at any position

in a central auditory nucleus will be most sensitive to progressively higher frequencies as development proceeds. To test this prediction, we mapped the tonotopic organization (spatial representation of frequency) of the magnocellular and laminar nuclei in the chick embryo and compared it to the tonotopic organization in hatchling chickens (2).

The magnocellular and laminar nuclei are second- and third-order nuclei in the avian auditory system and have been considered to be homologous to the mammalian anteroventral cochlea nucleus and medial superior olivary nucleus, respectively (2, 3). The apical to basal dimension of the basilar papilla projects from posterolateral to anteromedial onto the magnocellular nucleus. The latter, in turn, sends a topographic, bilateral projection to the laminar nuclei (4).

Chick embryos (Hubbard × Hubbard;  $N = 40$ ) in day 16 to day 17 of incubation were prepared for electrophysiological recording and positioned in a temperature-controlled chamber (37.5°C) inside a sound-attenuated room (5). A calibrated, closed sound system permitted presentation of 200- to 500-Hz tones at sound pressure levels up to 115 dB. During recording, the best frequency of unit clusters was determined at several locations as a tungsten microelectrode was slowly advanced through the magnocellular and laminar nuclei (6). The location of each electrode penetration was marked by a microlesion. After several penetrations had been completed, the brain was fixed in situ, removed, and processed through standard histological procedures. The position of each unit cluster recorded in the two nuclei was then determined (2, 7).

Figure 1 compares the tonotopic organization of the magnocellular and laminar nuclei in the embryos and in hatchlings 2 to 3 weeks of age. The relation of best frequency of neurons to their posterior to anterior and lateral to medial positions in hatchlings is shown by the linear regression lines, which were replotted from a previous study (2). The relation of best frequency to position of

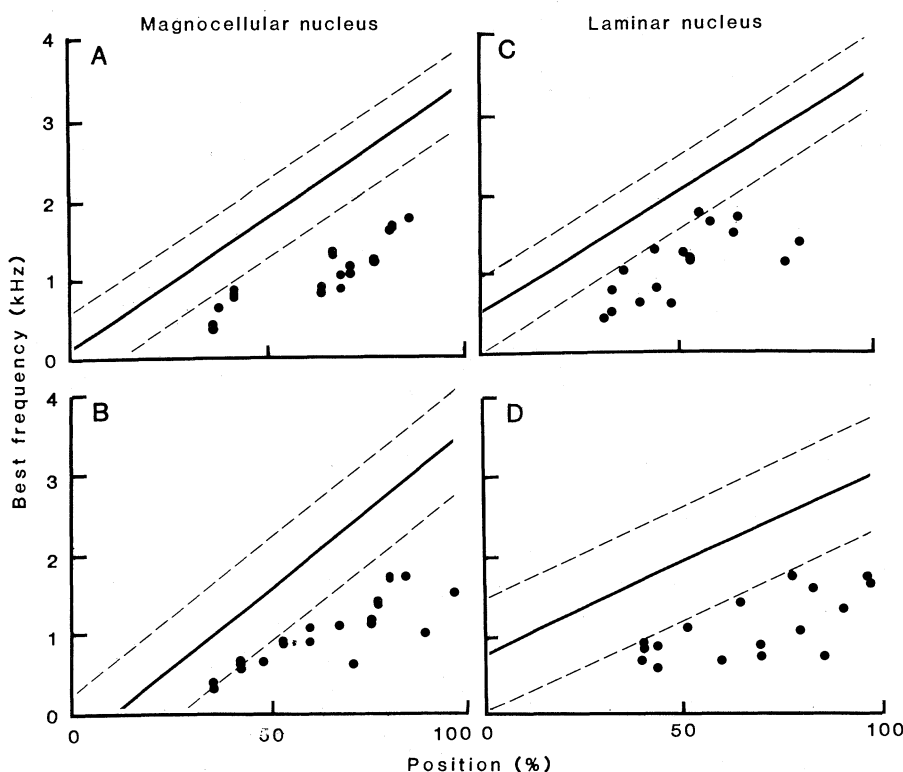


Fig. 1. Best frequency as a function of percentile position along the posterior to anterior (A and C) and lateral to medial (B and D) dimensions of the magnocellular and laminar nuclei. The linear regressions that predict the best frequency of neurons in hatchlings are shown by the solid lines; the dashed lines represent  $\pm 1$  standard error. The relation between position and best frequency actually observed in embryos is illustrated by the filled circles. Note that for each response in embryos the observed best frequency is markedly lower than that found in hatchlings.