

- ×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.
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 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.
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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

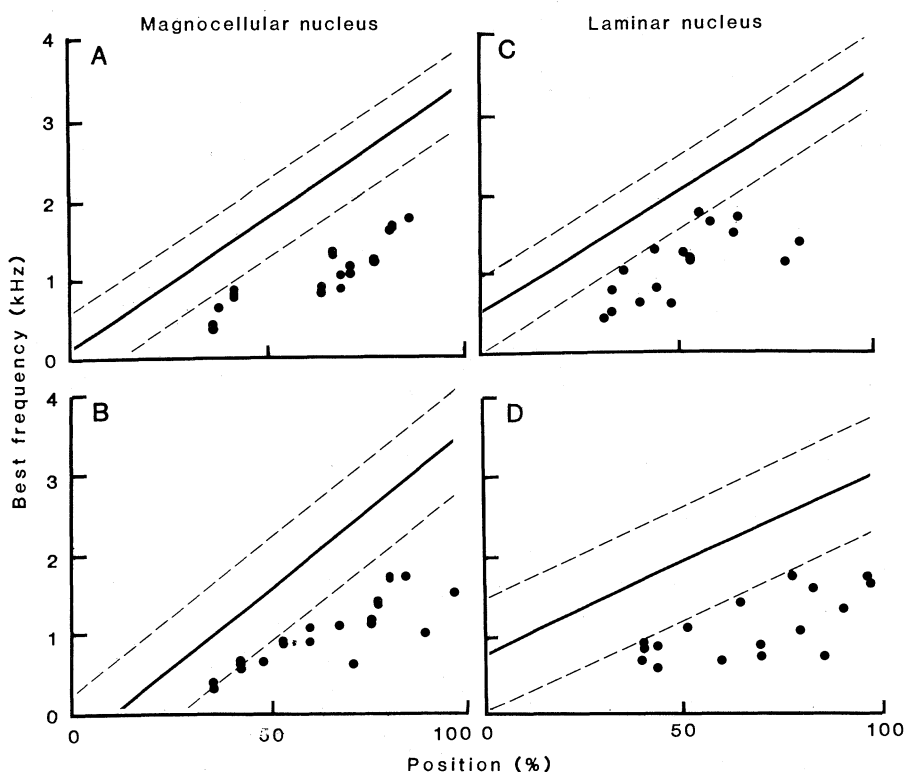


Fig. 1. Best frequency as a function of percentile position along the posterior to anterior (A and C) and lateral to medial (B and D) dimensions of the magnocellular and laminar nuclei. The linear regressions that predict the best frequency of neurons in hatchlings are shown by the solid lines; the dashed lines represent ± 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.

the recording tip for the embryos is shown by the scatter plots. Three findings are apparent. First, the embryos have a very limited range of high-frequency sensitivity. The highest best frequency recorded in the embryos (1829 Hz) is more than 1 octave lower than the highest best frequency recorded in the hatchlings (4100 Hz). This agrees with the low- to high-frequency gradient of functional development found in a variety of animals (8). Second, a well-defined tonotopic organization is present in the embryos and is qualitatively similar to that in the hatchlings; progressively higher best frequencies are found at successively anterior and medial positions in both the magnocellular and laminar nuclei. Finally, there is a striking quantitative difference between the tonotopic organization in hatchlings and embryos. At every position in the two nuclei the best frequencies in the embryos are markedly lower than the best frequencies in the hatchlings. The best frequencies in the embryos ranged from 33 to 69 percent (mean, 52 percent) of the predicted values (9).

In Figure 2 the tonotopic organization in embryos and hatchlings is compared by using two-dimensional reconstructions of the magnocellular and laminar nuclei (7). The nuclei have been divided into four sectors along the frequency axis. The range of best frequencies found in each sector is shown for the hatchlings and embryos. Clearly, the neurons are more sensitive to lower frequencies in the embryos than in the hatchlings for each region of the two nuclei.

These results, which indicate a systematic shift in tonotopic organization during late embryonic development, are consistent with the hypothesis that there is a change in the spatial encoding of frequency along the basilar papilla. The possibility that this shift in tonotopic organization results from a change in the relative location of neurons in the magnocellular and laminar nuclei during late embryonic development is unlikely. The morphogenetic events that would result in such a positional shift are complete by embryonic day 17 (10). It is also unlikely that the best frequencies of neurons are the same in embryos and hatchlings and that a relative attenuation of high-frequency sound transmission occurs in the immature middle ear. If this were occurring it might make some point on the low-frequency slope of the tuning curve of an embryonic neuron appear to be the best frequency. However, one would then expect an apparent shift to lower best frequencies to occur only for high-frequency units (those located in the

anteromedial parts of the magnocellular and laminar nuclei), whereas in the present experiment an equivalent shift toward lower best frequencies appeared to occur throughout the nuclei (11). Furthermore, developmental studies of middle ear admittance and evoked potential thresholds (12) and the complementary results of Rubel and Ryals (1) provide no evidence for a selective attenuation of high-frequency sound conduction. Finally, the change in tonotopic organization is probably not due to deterioration of the preparation (13) or to a shift in the central or peripheral connections of the eighth cranial nerve. While our results do not exclude the latter possibility, it would not account for the findings reported by Rubel and Ryals (1).

The change in tonotopic organization described here and the developmental shift in the locus of hair cell damage reported by Rubel and Ryals indicate that the spatial encoding of frequency along the cochlea is not fixed, but

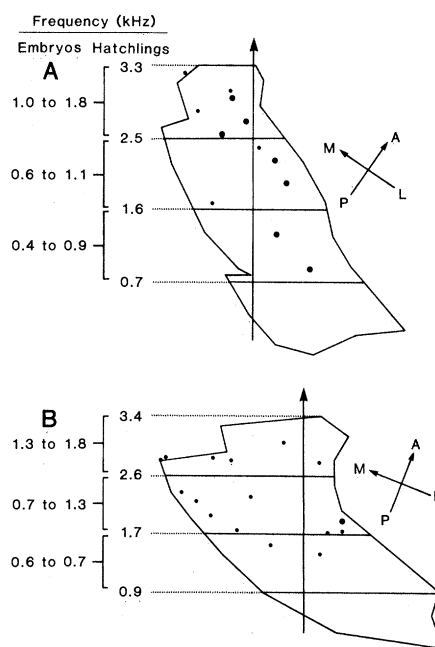


Fig. 2. Two-dimensional reconstructions of the magnocellular (A) and laminar (B) nuclei comparing the tonotopic organization in hatchlings and embryos. The nuclei are divided into four equal sectors along the frequency axis (long vertical arrows). The best-frequency ranges observed in the different sectors in hatchlings and embryos are indicated. Orientation of the frequency axes from posterolateral (low frequencies) to anteromedial (high frequencies) is predicted by multiple linear regression equations that quantitatively describe the tonotopic organization in hatchling chicks (2). Filled circles indicate where the 38-unit clusters were recorded in the embryos. Large filled circles show where more than one cluster was recorded. The orientation of the reconstructions with respect to the anteroposterior and mediolateral axes of the brain is indicated by the arrows to the right.

changes in an orderly manner during development. If this change is general across avian and mammalian species (14), it would account for the paradoxical relation between the structural and functional development of the vertebrate auditory system (1). A change in peripheral encoding would also imply that the frequencies which maximally activate any point in the central auditory pathway change during development. In this case, the relatively low frequency sounds that dominate the normal fetal acoustic environment (15) would, as development proceeds, maximally activate each neuronal subpopulation in the auditory pathway and not only the subregions that, in the adult, are tuned to low-frequency sounds. This may be of major importance in attempts to understand how the acoustic environment influences auditory system development. Finally, the observation that the numerical values assigned to the place code are not necessarily fixed may have broad implications for understanding audition. The possibility that this function shifts along the basilar membrane under conditions other than development was previously suggested (16) and should be examined further.

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5. The embryo's head was pulled through a hole in the shell, the head region was infiltrated with a local anesthetic, Flaxedil (2 mg) was injected into the neck muscle to eliminate movements, and the tympanic membrane was exposed. Electrophysiological and behavioral responses to sound are first recorded at 12 and 14 days of incubation, respectively [J. C. Saunders, R. B. Coles, G. R. Gates, *Brain Res.* **63**, 59 (1975); H. Jackson and E. W. Rubel, *J. Comp. Physiol. Psychol.* **92**, 682 (1978)].
6. Best frequency is defined as the sound frequency at which a neuron has its lowest threshold for activation. It was determined by presenting shaped tone bursts (duration, 100 msec; rise-fall time, 5 msec) at 2-second intervals and monitoring the evoked unit activity on an oscilloscope and over an audio monitor. Poststimulus time histograms were used to confirm the best frequencies in approximately 30 percent of the responses.
7. Each recording point was projected onto a horizontal planar projection of the magnocellular and laminar nuclei, and its posterior to anterior and lateral to medial percentile positions were calculated by setting the total anterior to posterior and medial to lateral dimensions of each nucleus equal to 100 percent.
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13. In one preparation we recorded from the same location for over 8 hours with no change in the tuning. In addition, there was a negative correlation between time into the experiment and the percentile difference in frequency between embryos and hatchlings ($r = -.27$, $P > .10$; $N = 25$).
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24 May 1982; revised 5 October 1982

Development of Hindlimb Locomotor Activity in the Bullfrog (*Rana catesbeiana*) Studied in vitro

Abstract. *The isolated central nervous system of the bullfrog larva (tadpole) is a valuable model system for studying the development of central motor control because the neural activity for locomotion is expressed in vitro. Patterned synaptic activation of immature hindlimb motoneurons is present before the bones and muscles of the hindlimb differentiate, and it develops against the background of the tadpole's functionally mature motor program for tail oscillations. This activation of hindlimb motoneurons later produces patterned bursting that underlies coordinated stepping and frog kicks.*

The frog occupies the evolutionary niche between aquatic and terrestrial vertebrates, a phylogenetic position that is reflected in the frog's locomotor development. The larval frog (tadpole) swims by means of tail oscillations, whereas after metamorphosis, the frog walks, hops, and swims using the hindlimbs either alternately (stepping) or synchronously (frog kicks). These two patterns of hindlimb coordination are the basis of the locomotor gaits used by all terrestrial quadrupeds (1). In this report we describe the developmental changes in central nervous system (CNS) motor activity that are associated with the frog's transition from aquatic to terrestrial locomotion at metamorphosis.

We have previously shown, using this in vitro CNS preparation, that tail beating in the tadpole is controlled by an endogenous locomotor program distributed throughout the spinal cord and expressed only by the axons of motoneurons that innervate the axial swimming musculature (2). This program produces alternate contractions of the axial musculature on the left and right sides of the body and tail and coordinates intersegmental activity of the muscles (2). Regulation of tail-beat rate and modification

of intersegmental coordination result from sensory interactions with the central locomotor program (3).

Motoneurons innervating the axial

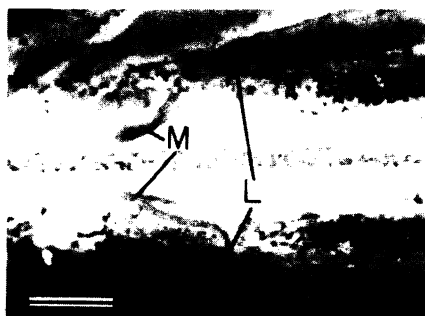


Fig. 1. Ventral spinal cord showing the medial and lateral rootlets that compose the ventral roots of the lumbar enlargement of the tadpole. The medial rootlets (M) contain only axons of primary motoneurons and innervate axial muscles that produce tail beating in the tadpole. The lateral rootlets (L) are composed of axons of lateral motor column motoneurons that innervate muscles of the hindlimbs. These two rootlets are normally enclosed in a common meningeal sheath, but can be dissected free of each other to be studied independently in electrophysiological experiments. The medial rootlets have been stained with toluidine blue for clarity. Calibration bar, 500 μ m.

swimming musculature are found at all levels of the spinal cord and are anatomically distinct from the lumbar motoneurons that innervate the hindlimbs (4, 5). The former (primary motoneurons) are born during embryonic stages, whereas hindlimb motoneurons that form the lateral motor column (LMC) proliferate and differentiate during larval life (5). In the lumbar enlargement, axons of these two populations of motoneurons exit the spinal cord in discrete rootlets that combine to form the ventral roots (2, 4). The medial rootlets that contain axons of primary motoneurons can be dissected free of the lateral rootlets that contain axons of hindlimb motoneurons (Fig. 1), and the electrophysiological activity of motoneurons subserving tail beating or hindlimb locomotor behavior can be studied independently (2).

We examined the development of the pattern generator for interlimb coordination of hindlimb locomotor activity and its relationship to the existing motor program for tail beating. We simultaneously recorded spontaneous activity of the medial and lateral rootlets of the ninth ventral roots in 101 isolated CNS preparations maintained in oxygenated Ringer solution (2, 3). Axons of primary motoneurons in the medial rootlets exhibited episodes of patterned bursting (2) until metamorphosis, consistent with the continued use of the tail for locomotion (Fig. 2, A to C).

In contrast to the unchanging pattern of bursting in primary motoneurons, we found that the activity of hindlimb motoneurons changes dramatically during larval development. Prior to stage VIII (6), activity in the lateral rootlets increased with the onset of medial rootlet bursting, but showed little evidence of patterned bursting. Slow potentials recorded from the axons of hindlimb motoneurons, however, revealed unmistakable rhythmicity by stage III (Fig. 2A), indicating that at least some of the hindlimb motoneurons are receiving patterned synaptic inputs at that time. Between stages VIII and XIV, spike activity gradually increased near the peaks of the slow potentials so that by stage XIV a clear pattern of alternating burst activity was evident (Fig. 2B). We determined phase relationships between bursts in different nerves by dividing the latency to burst onset by the total cycle period, using the onset of medial rootlet bursts as reference points. Both the alternating bursts and the rhythmic depolarizations that preceded were phase-shifted 180° from the homolateral medial rootlet bursts [mean phase \pm standard error = $0.50 \pm .01$, $N = 93$] and always occurred at the same fre-