

Evidence for an Alteration of the Tonotopic Map in the Gerbil Cochlea During Development

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ABSTRACT

We have investigated developmental alterations in the tonotopic projection of the gerbil lateral superior olive. Single neurons were characterized in the frequency domain and the recording site marked with fast green. Transverse tissue sections from the auditory brainstem of each animal were visualized with a video-equipped microscope, and the image was digitized for subsequent alignment. The three-dimensional display indicated little variation in the rostrocaudal axis, allowing us to collapse the data into a two-dimensional tonotopic map. The tonotopic map was found to change with age such that the characteristic frequency of neurons in a given anatomical location became successively higher during development. These results are consistent with the hypothesis that the place code gradually shifts in the developing cochlea.

Key words: tonotopy, ontogeny, lateral superior olive, auditory, image analysis

In all sensory systems that have been carefully examined, the brainstem nuclei exhibit an orderly and continuous functional representation of the respective peripheral receptor epithelia (Apter, '45; Rose et al., '63; Gacek, '69; Darian-Smith, '73; Heiligenberg, '84). Within the auditory system the representation of the cochlea proceeds from low to progressively higher frequency sounds (Rose et al., '59; Rose et al., '63; Tsuchitani and Boudreau, '66; Goldberg and Brown, '68; Aitkin et al., '70). During vertebrate development, the cochlea and its central representations are at first electrically responsive only to relatively low and middle frequency stimuli (Rubel, '78; Ehret, '83; Romand, '83; Rubel et al., '84). As maturation proceeds, the entire frequency range is capable of eliciting neural and behavioral responses. Paradoxically, the section of the cochlea that will respond to high frequencies in adults appears quite mature in morphology at the time when the organism is unresponsive to those very frequencies (Retzius, 1884; Wada, '23; Anggard, '65; Kikuchi and Hilding, '65; Mikaelian and Ruben, '65; Bredberg, '68; Pujol and Marty, '70; Chandler, '84; Fermin and Cohen, '84).

One resolution to this problem would be that a given position along the length of the cochlea responds to successively higher frequencies during maturation (Rubel et al., '76; Rubel, '78). There are now experimental results supporting such a view in three species (Lippe and Rubel, '83, '85; Rubel and Ryals, '83; Harris and Dallos, '84; Ryals and

Rubel, '85; Yancy and Dallos, '85; Hyson and Rudy, '87; Arjmand et al., '88). Two converging lines of evidence for a systematic shift in the place code come from studies of the chicken auditory system. Rubel and Ryals ('83) were able to show that the positions of maximal damage from high intensity pure tonal stimuli were found at progressively more apical locations (i.e., toward the section encoding lower frequencies) in older animals. Lippe and Rubel ('83), recording neural responses from first- and second-order auditory nuclei, found that a given anatomical position encoded successively higher frequencies as maturation progressed. A similar shift in frequency coding has been shown for the higher frequency range of both the gerbil (Harris and Dallos, '84; Yancey and Dallos, '85) and the rat (Hyson and Rudy, '87).

Among the questions that must be addressed are whether or not a shift in frequency coding is apparent in the low frequency range, and whether the change has a variable time course in contradistinct frequency ranges. The present

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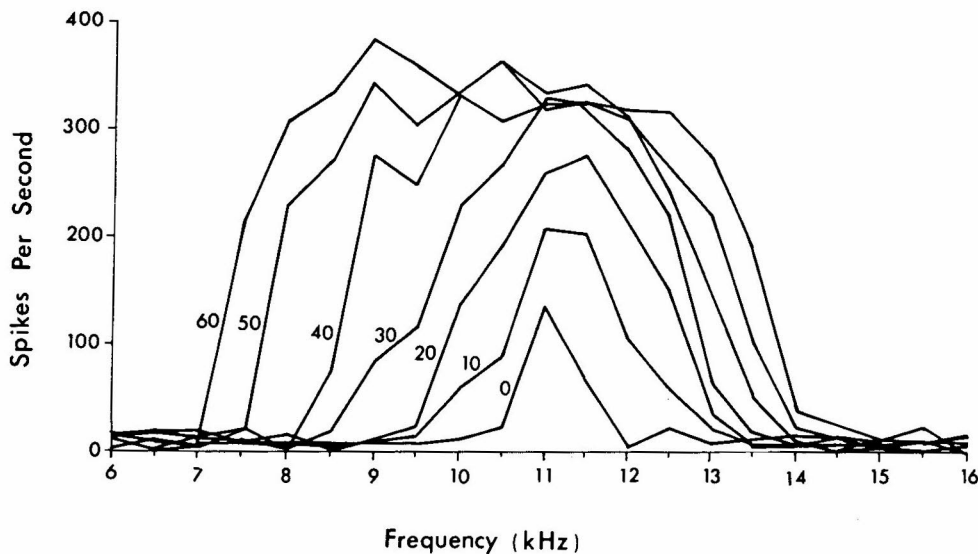


Fig. 1. A response area from an LSO neuron with a characteristic frequency of approximately 11 kHz. The iso-intensity curves show the discharge rates evoked by tonal stimuli presented to the ipsilateral ear begin-

ning at 0 dB SPL with 10 dB increments. This response area was obtained at the dye-marked site illustrated in Figure 2.

study is conceptually similar to that conducted by Lippe and Rubel ('83, '85) in chicks. The characteristic frequency of single neurons was ascertained at different postnatal ages in the lateral superior olivary nucleus (LSO). Two major obstacles in such a study are the three-dimensional reconstruction of the central nucleus, and the merging of data from individual animals. A computer-aided, three-dimensional reconstruction system allowed us to align serial sections through the brainstem, display physiological information in three dimensions, and quantitatively compare nuclei from animals of the same age and of different ages. Our results indicate that a shift in frequency coding does occur for both high and midrange frequencies.

METHODS

Electrophysiology. Gerbils (*Meriones unguiculatus*) of 12–16 weeks, 15–16 days postnatal, and 13–14 days postnatal were tranquilized with Ketamine hydrochloride (15–40 mg/kg; i.p.), anesthetized with sodium pentobarbital (40–55 mg/kg; i.p.) or chloral hydrate (350–400 mg/kg; i.p.), and given a single injection of atropine sulfate (0.08 mg/kg; i.m.) to prevent aspiration. In addition, animals of 12–16 weeks were tracheotomized. All animals were maintained at 37–38°C in a double-walled acoustic isolation room (IAC).

The preparatory surgery consisted of exposing the foramen magnum and removing the external auditory meatus. Glass microelectrodes filled with 2 M NaCl and saturated with fast green were used to record from single neurons in the LSO. Electrode impedance varied between 5–15 M. The physiological signal was amplified (Grass P15), bandpass filtered at 300 Hz and 5000 Hz (Krohn-Hite 3550R), displayed on an oscilloscope (Tektronix 5103N), and fed into a window discriminatory (WPI 121) that supplied TTL pulses for on-line digital processing.

To mark the location of each electrode site, negative current was passed at each recording site (10–30 μ A; 5–20 μ m) to iontophorese the fast green. There were 1–3 fast green marks per animal, and all recording sites were determined directly from these marks. In cases where there were more than one spot per animal, the position of the electrode was noted prior to each penetration so that the relative position

of the dye marks could subsequently be assigned to each neuron.

Tonal pulses were generated (Krohn-Hite 5910B), shaped (4 ms rise/fall; Wisconsin Switch), timed (50 ms on; 2–3.3 Hz), attenuated, and delivered through a closed acoustical system with dynamic ear speakers (Beyer DT48). A calibration of the peak sound level across the system's frequency range (40–20,000 Hz) was performed prior to each experiment with a probe tube.

Once a single neuron had been isolated, we presented tone pulses of ascending frequency at a preset sound level and, thereafter, increased the level in 5 dB steps. An excitatory frequency response area was thus generated. The characteristic frequency (CF) was defined as the frequency eliciting the greatest response at the first sound level to elicit any response at all. Only neurons that were ipsilaterally excited and contralaterally inhibited (EI units) were characterized and marked in this study. A representative response area is shown in Figure 1.

Anatomy. At the termination of each experiment the animal was injected with a lethal dose of sodium pentobarbital and perfused intracardially with 10% buffered formalin. The brain was immediately removed and stored in fixative. Brains were frozen and sectioned at 60 μ m on a sledge microtome. Serial sections were mounted onto gelatin-subbed slides. The deposits of fast green were localized before further processing in the event that they should be washed out by the staining solutions. After drying, the slides were rapidly stained in 0.25% thionin and coverslipped.

Image analysis and 3-D reconstruction. An image processing and graphics display system was utilized for generating the three-dimensional reconstructions. The system was based on a Masscomp MC-5500 computer system equipped with Multibus image acquisition, display, and frame buffer boards (Imaging Technology, Inc.). The reconstruction process consisted of several steps, which are outlined below.

A Leitz Orthoplan microscope with attached Dage-MTI 68 video camera was utilized for visualization of the sections and acquisition of the video image. The video signal

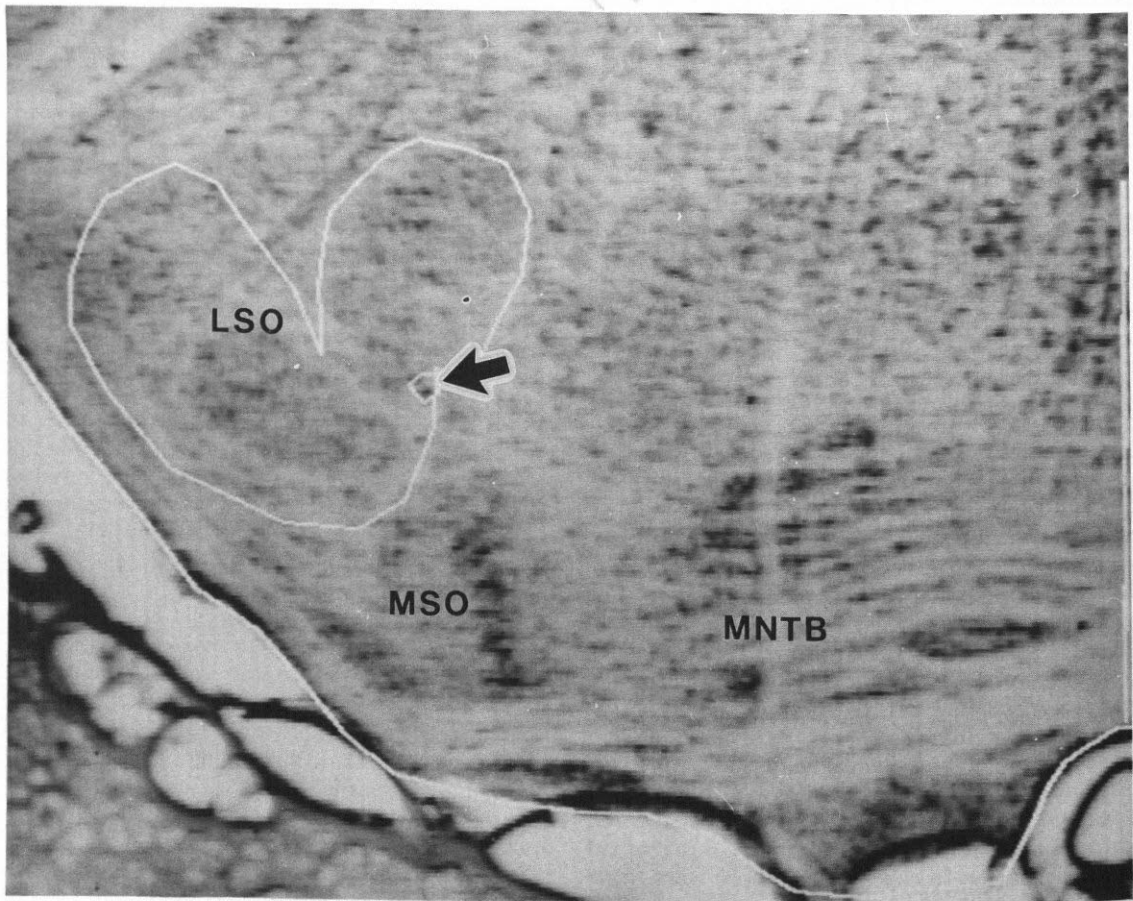
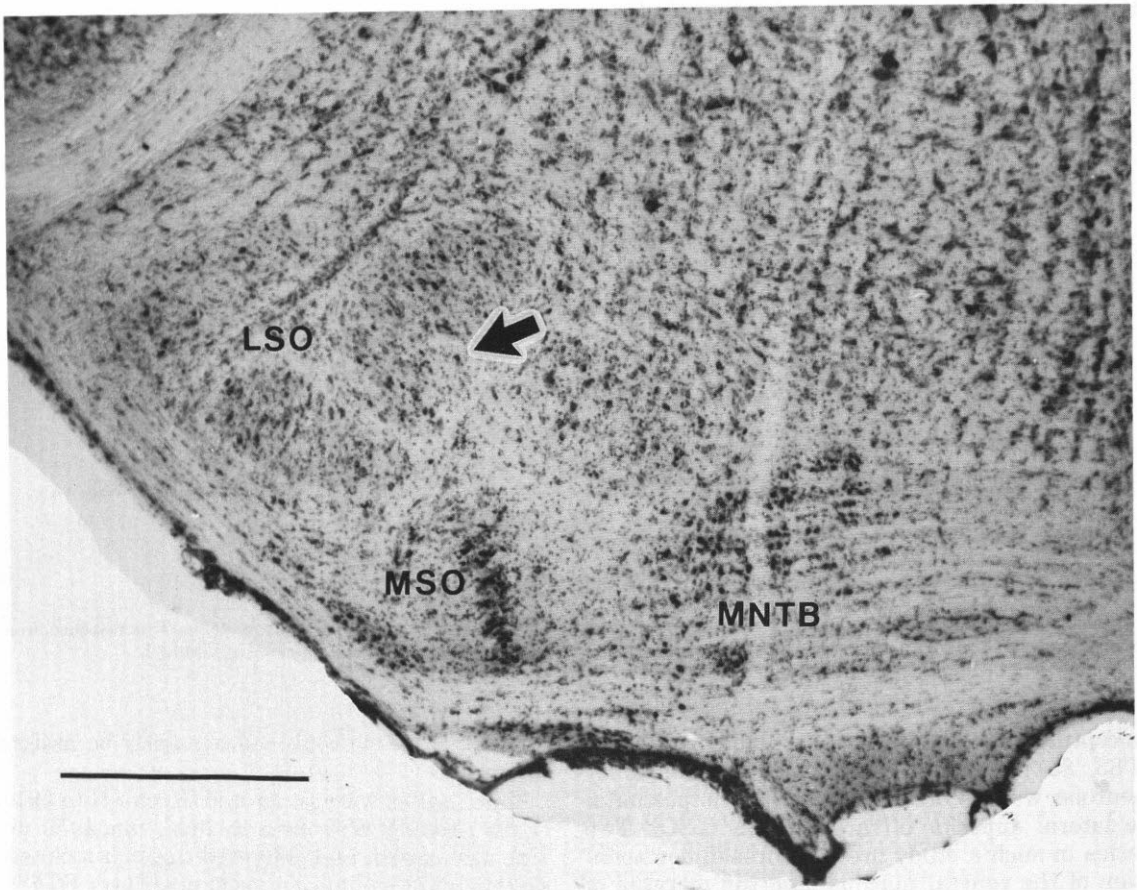


Fig. 2. The ventral brainstem region containing the superior olivary complex. **Top:** Photographic image. **Bottom:** Video image with contours of ventral surface, midline, the LSO, and the dye-marked recording site (ar-

row) drawn in. The recording site corresponds to the response area in Figure 1. LSO, lateral superior olive; MSO, medial superior olive; MNTB, medial nucleus of the trapezoid body. Bar = 500 μ m.

from the Dage camera was then digitized by using Imaging Technology acquisition boards. A cursor controlled by a "mouse" was then utilized to trace the contours of desired structures that consisted of the LSO nucleus, brainstem midline (used for later alignment), electrode recording sites, and the ventral brainstem. The contours of neighboring sections were then registered or aligned to one another using a software based procedure (regis) developed by one

of the authors (Merickel and McCarthy, '85). The three-dimensional database of contours were then displayed and edited with the biostruct editor (bsed) package of the CARTOS software system (Kropf et al., '85). Volume and surface area of the LSO nucleus were calculated using appropriate programs in the CARTOS system. A wire frame model was then created, using CARTOS, which was shaded and rotated, using the MOVIE.BYU three-dimensional display package (Christiansen and Stephenson, '84).

Figure 2 shows an example of the first three steps of the above procedure when applied to a single section. The circumscribed electrode recording site corresponds to the representative response area illustrated in Figure 1.

RESULTS

Three-dimensional perspectives of an individual reconstructed adult LSO are shown in Figure 3, where the rostrocaudal axis has been expanded threefold for viewing clarity. The aligned sections have been rotated about the Y-axis at 20° intervals. The adult structure of LSO is U-shaped in the center and tends to lose the dorsomedial portion at rostral and caudal extremes. A solid model of the nucleus in Figure 3 is shown in Figure 4.

The uniformity of shape across animals is illustrated in Figure 5, where three reconstructed adult nuclei are overprinted using different line types. The similarity of LSO shape between animals was quantified in three ways (Table 1) for nuclei within each age group. Measures of nuclear volume, surface area, and radius differed by less than 14%

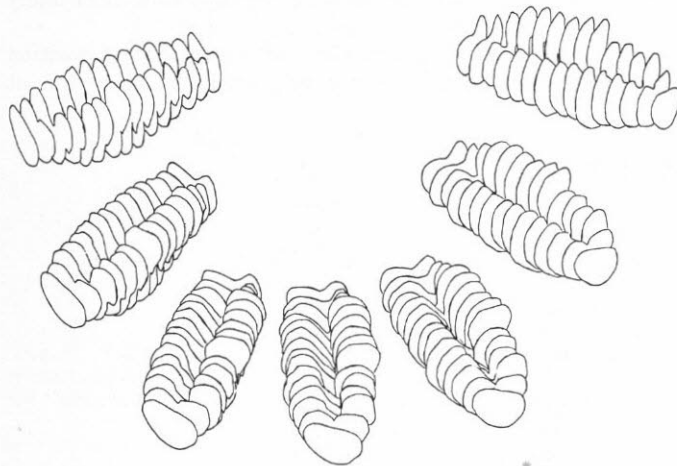


Fig. 3. The aligned contours of an adult LSO nucleus rotated about the dorsoventral axis. The view of the centralmost composite is from caudal to rostral, and medial is to the right. The sections were taken at 60- μ m intervals.

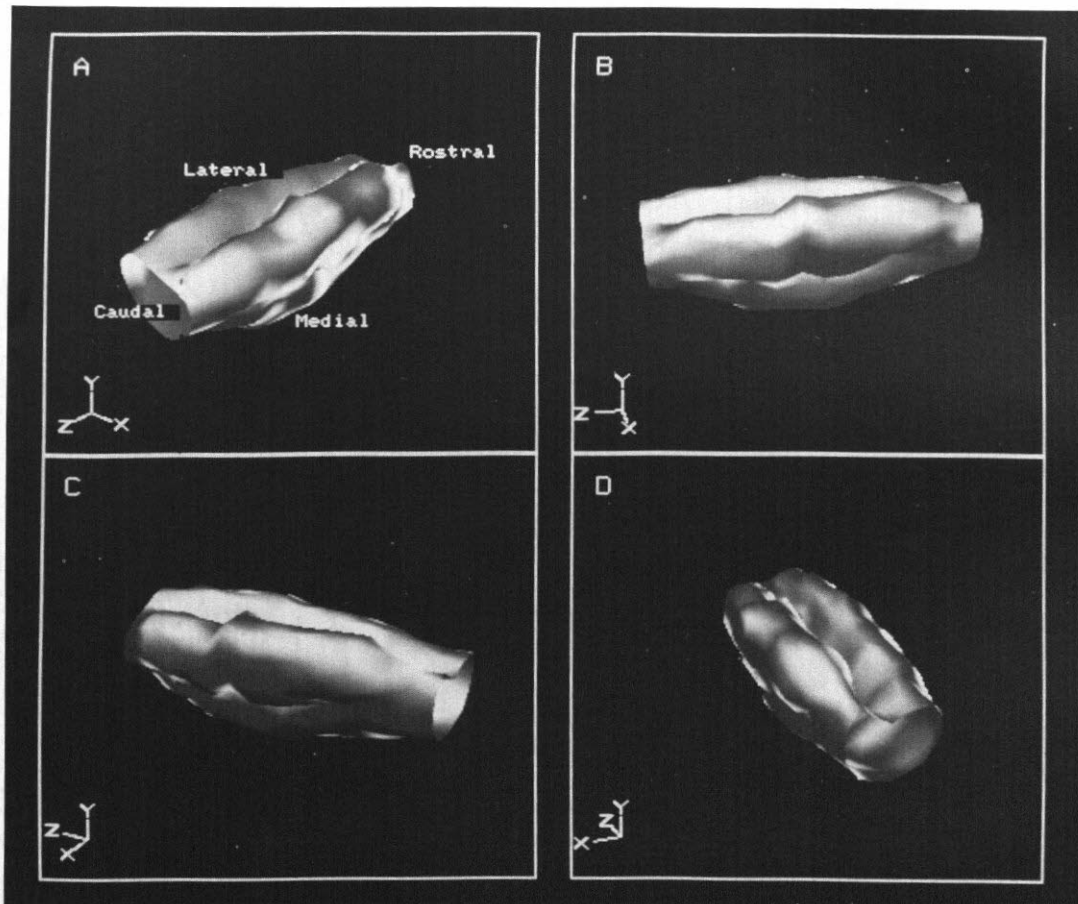


Fig. 4. A solid model of an adult LSO nucleus rotated about the dorsoventral axis (same nucleus shown in Fig. 3). The surfaces were interposed

between the "wire" model outlines, and shaded by positioning several "light" sources using MOVIE.BYU.

of the mean within an age group. The increase in LSO volume with age is roughly twofold from 13–14 days post-natal to 12–16 weeks. The average rostrocaudal length of LSO was 900 μm in the adult, 720 μm in 15–16-day animals, and 660 μm in 13–14-day animals.

The morphological uniformity among nuclei from animals of the same age suggested that we could merge dye-marked recording sites onto a single reconstruction. Furthermore, an examination of recording site location in three dimensions (Fig. 6) indicated that the tonotopic map was evidently not compressed rostrally and caudally, where the physical size is smaller. Rather, the high frequency region is physically and functionally absent at the extreme ends.

These observations allowed us to project data points onto a two-dimensional representation in the transverse plane. This transformation then facilitated the comparison of tonotopic maps between age groups.

When data points from adult animals were merged onto a transverse two-dimensional representation, the tonotopic axis was unmistakable (Fig. 7). High frequencies were represented dorsomedially and responses to successively lower frequencies were obtained ventrally and laterally. There were some slight discontinuities in the map that most likely derive from the merging process.

The same general relationship between CF and position within the LSO existed for young animals. However, at

TABLE 1. Development of LSO Size

Animal #	Volume (mm^3)	Avg. radius (μm) ¹	Surface area (mm^2)
<i>Adult</i>			
1082	0.543	506	3.81
1078	0.604	524	5.14
1083	0.630	531	4.41
<i>15/16 day</i>			
1092	0.376	448	3.01
1096	0.431	468	2.97
1019	0.356	439	2.98
<i>13/14 day</i>			
1080	0.322	425	2.81
1084	0.315	422	2.98
1085	0.283	407	2.56

¹Average radius is calculated on a section by section basis. First, the centroid is calculated for each section by averaging the X and Y coordinates for all the contour points in the section. The average radius is then calculated as the average of the Euclidian distance between the centroid and each contour point.

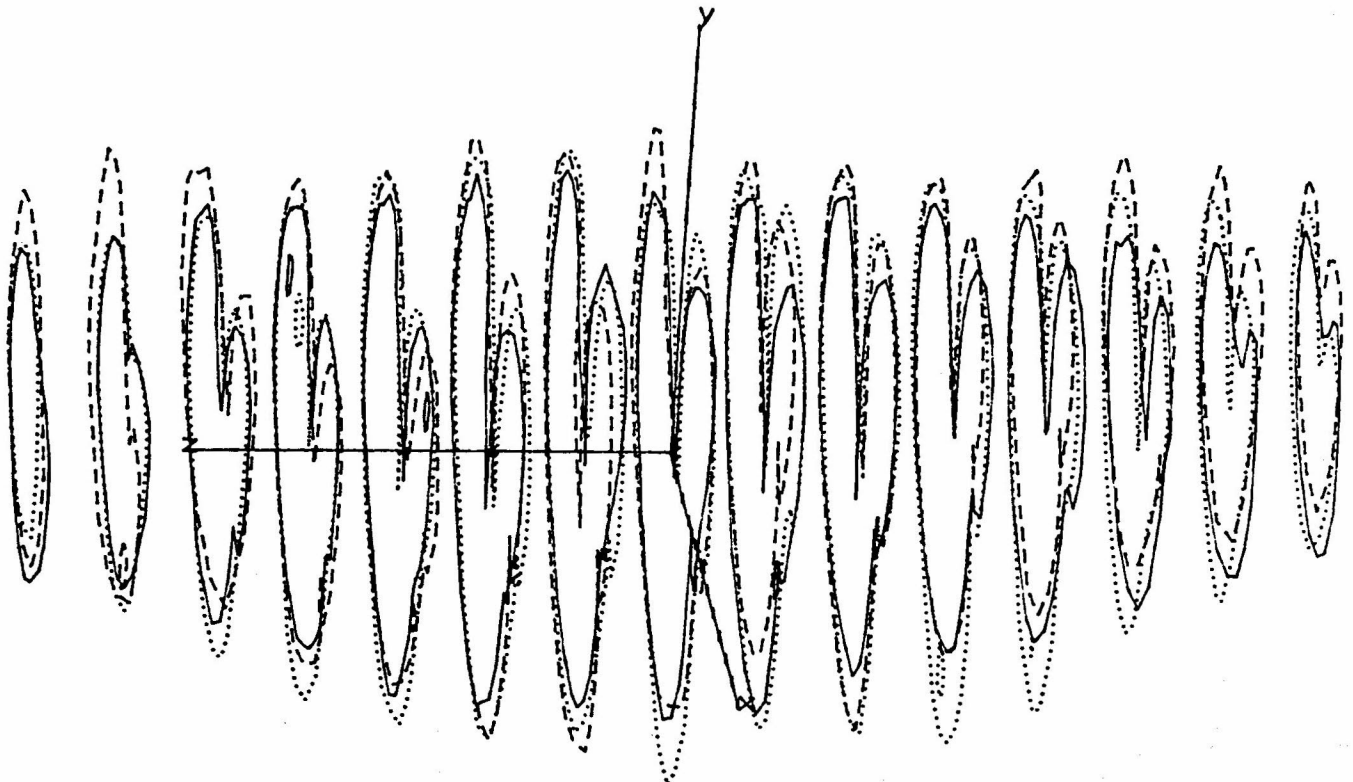


Fig. 5. The reconstructed and aligned LSO nuclei from three adult animals. The nuclei are viewed from a medial and slightly caudal location.

Note that there is very little difference between the size or shape of these nuclei.

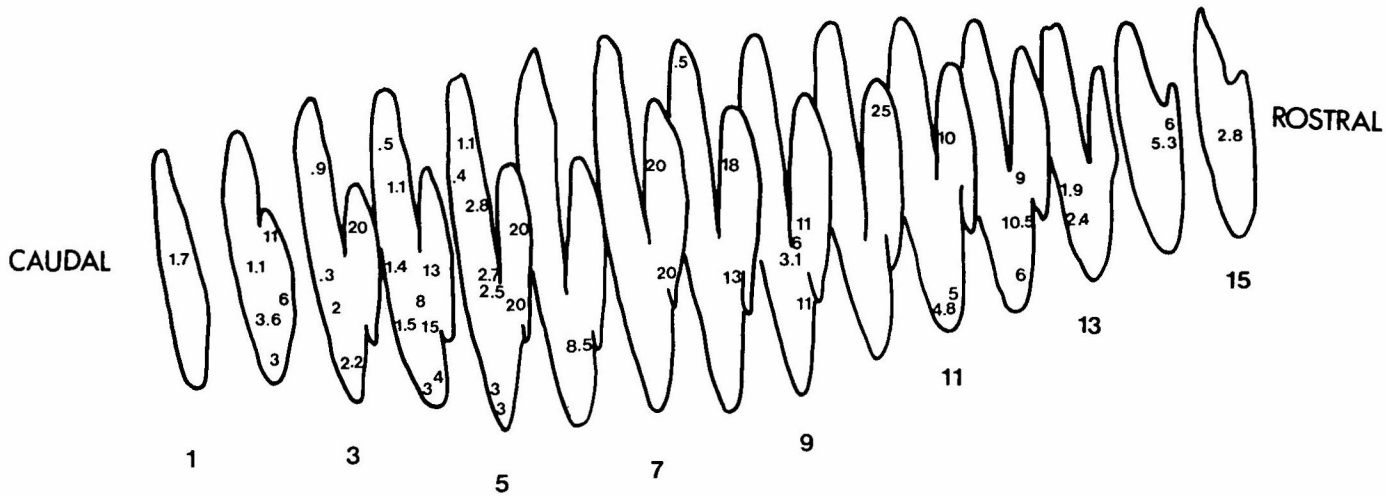
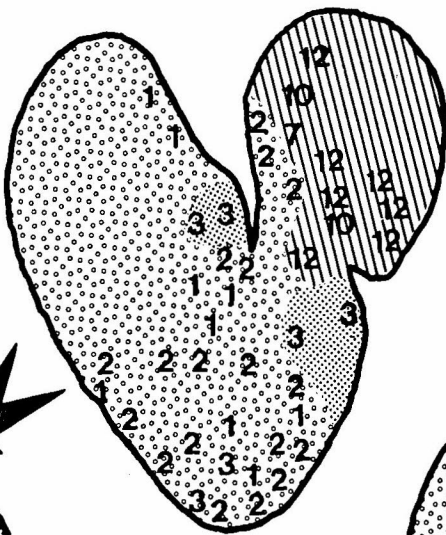


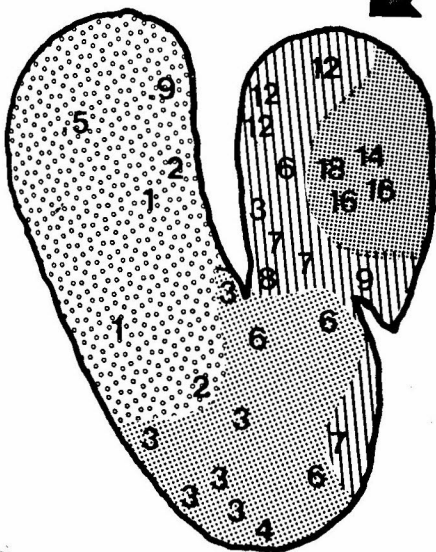
Fig. 6. The characteristic frequencies from all recording sites in adult animals merged onto a single nucleus. The nucleus is viewed from a medial and slightly caudal location. Note that the LSO becomes abbreviated at its

caudal and rostral extremes, apparently due to the loss of the high frequency projection region (dorsomedial).

13-14 days



15-16 days



ADULT

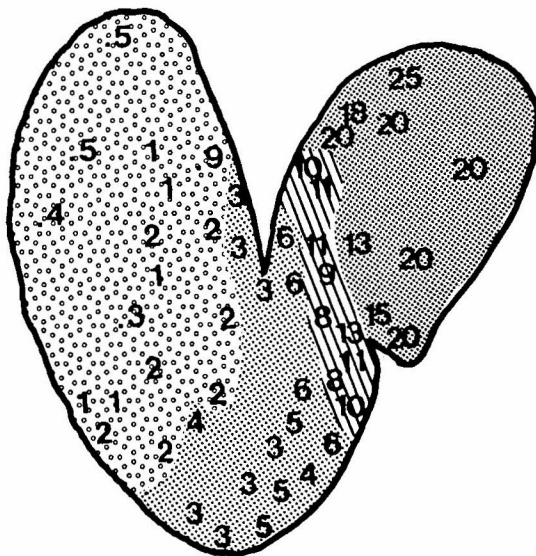


Fig. 7. The characteristic frequencies from all recording sites in 13/14, 15/16, and adult animals merged onto a two-dimensional transverse section. In each case, the transverse section represents the greatest cross-sectional boundaries. The shading delimits regions of similar characteristic fre-

quency. The highest frequency region (fine stipple) appears at 15/16 days, and its boundary increases in the adult. Characteristic frequencies below 1 kHz were not observed in 13/14-day animals.

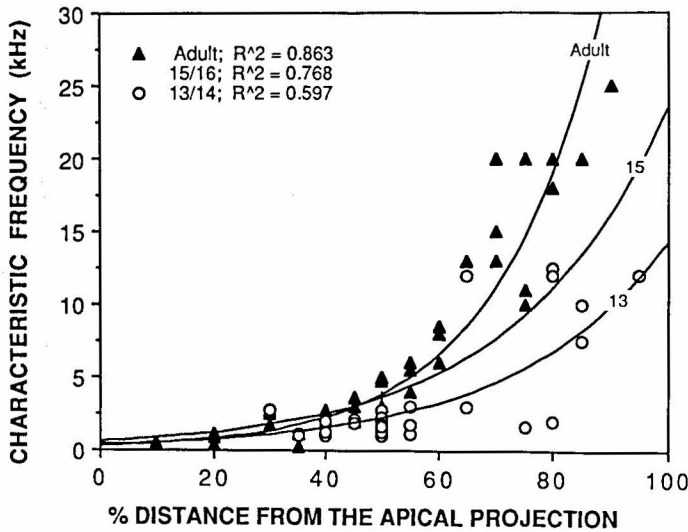


Fig. 8. Scatter plots and best-fit regression functions showing the relationship between CF of units in the LSO and the anatomical position of the unit along the adult tonotopic axis (see text). Data are pooled across animals within each age group, so scatter (and R^2) represent both within and between animal variability. Data points for both adult and 13/14-day groups are shown, along with exponential regression functions. Only the regression function for the 15/16-day group is shown; the R^2 value (0.768) indicates that the variability within this group is approximately midway between that of the younger and older groups.

these ages it was clear that neural responses at a given position in the transverse plane of LSO were to lower frequencies at 13–14 days than in adults. For example, if one compares the CFs of units localized to the most dorsomedial aspect of LSO, it is apparent that the range of values are 18–25 kHz in the adult, and 10–12 kHz in 13–14-day animals (Fig. 7). There appeared to be a similar frequency shift in the ventromedial aspect of LSO between 13–14 days and 15–16 days of age (Fig. 7).

The developmental shift in frequency coding was quantified by measuring the distance of each recording site from the apical projection region of the LSO (i.e., ventrolateral). A line was drawn up the center of each two-dimensional LSO map (Fig. 7), from ventrolateral to dorsomedial, and the position of each CF was computed as a percent distance, with the dorsomedial edge being 100%.

The data from each recording site (CF and percentile position of the electrode tip) were combined across animals of each age group. Regression analyses indicated that the prediction of CF could be best achieved by an exponential equation of the form $CF = a \cdot 10^{bx}$, where x is the percentile position of the electrode tip. This analysis provided an excellent fit to the data points, accounting for 86% of the variance in adult animals despite the fact that data were pooled from 22 individuals. The slopes and elevations of the regression functions were statistically compared using the method of Snedecor and Cochran ('67).

Figure 8 summarizes the relationship between position of the recording electrode and CF for all age groups. Individual data points for the 15/16-day-old gerbils are not plotted, but the R^2 value provides an indication of the relative variability around the regression function. Even in the youngest age group, approximately 60% of the CF variance was accounted for by the regression function, although the range of CFs was only 1–12 kHz.

Examination of Figure 8 reveals two trends that are born out by the inferential statistical analyses. First, the greatest differences between age groups are seen in the basal projection areas (high frequency representations). For example, at the 80th percentile the predicted CF for adult animals is 19 kHz, whereas it is only 7 and 12 kHz for the 13/14- and 15/16-day groups, respectively. Second, at more apical projection areas (e.g., below the 60th percentile), there appears to be a small but consistent difference between the 13/14-day and older animals, but no difference between the 15/16-day group and adults. Overall comparisons between the age groups yielded significant F ratios for both slope and elevation comparisons (slope, $F_{2,95} = 10.5$, $p < 0.001$; elevation, $F_{2,98} = 35.9$, $p < 0.001$). Individual comparisons revealed identical slopes of the regression functions (log CF on percentile) for 13/14- and 15/16-day groups, but an increase in the elevation for the 15/16-day group ($F_{1,56} = 55.8$, $p < 0.001$), suggesting a parallel shift in the entire tonotopic organization between 13 and 16 days postnatal. Due to the persistent change in the CF of neurons in the basal projection area after 16 days of age, significant differences are found between the slope of the adult function and the slopes for the other two age groups (13/14 vs adult, $F_{1,75} = 7.17$, $p < 0.01$; 15/16 vs adult, $F_{1,61} = 7.58$, $p < 0.01$).

DISCUSSION

We have demonstrated an ontogenetic modification of auditory function in the gerbil. Neurons that were located in a delimited region of LSO responded to successively higher frequencies as maturation progressed. This was evident both for high and midrange frequency regions, yet the latter appeared to reach an adultlike state by 16 days of age. Although the data was, ultimately, analyzed in the transverse plane, the three-dimensional, computer-assisted analyses provided us with the necessary confidence to employ such a transformation.

A superposition of three adult nuclei (Fig. 5) gave a subjective impression of the similarity of shape. Measures of nuclear volume, surface area, and radius (Table 1) lend quantitative support to this impression. The computer reconstructions also allowed us to examine the rostrocaudal distribution and location of recording sites (Fig. 6), ensuring that the data could be collapsed into the transverse plane for comparative purposes.

Developmental studies on the gerbil cochlea (Harris and Dallos, '84; Yancy and Dallos, '85; Arjmand et al., '88) provide a strong argument for mutability of the place code. When one differentially records the cochlear microphonic (CM) from within the scala tympani and at the round window, it is possible to ascertain the frequency above which the CM threshold is elevated. This procedure was repeated (e.g., with the intracochlear electrode located at the basal turn of the cochlea) for gerbils of various postnatal age, and it was found that the cut-off frequency increased with age (Harris and Dallos, '84). At the recording site examined by these investigators the cut-off frequency was approximately 6 kHz at 13–14 days, approximately 10 kHz at 15–16 days, and approximately 15 kHz above 30 days. These values are quite similar to those obtained from recording sites in the LSO at a position 75–90% from the lateral (i.e., low frequency) border. Our results are also consistent with the report of Arjmand et al. (1988) demonstrating that the place code is not altered at a second turn location responding to approximately 2.5 kHz. It has also been reported that low

frequency, tone-evoked, 2-deoxyglucose utilization shifts to progressively ventral loci (i.e., region encoding lower frequencies) in the cochlear nucleus of the gerbil (Ryan and Woolf, '88).

Two such independent lines of research for a place code shift have previously been obtained in the developing chick auditory system (Lippe and Rubel, '83, '85; Rubel and Ryals, '83; Ryals and Rubel, '85). In addition, it has recently been shown that 15-day rat pups, classically conditioned to respond to an 8 kHz tone, subsequently respond more readily to a 16 kHz tone (Hyson and Rudy, '87). Thus a behavioral analysis of frequency coding indicates that mid- to high frequency information is retained according to its original place of transduction along the cochlea, this changing by approximately one octave during development.

The precise mechanism by which the functional architecture of the cochlea changes is unclear. It is possible that local changes along the basilar membrane endow each place with specific frequency coding properties. At present, it is considered likely that an increase in stiffness and decrease in mass come about as a result of several cellular changes (Harris and Dallos, '84; Lippe and Rubel, '85). One must also consider the possibility that transduction along a seemingly mature length of cochlea may be affected by the immature morphological and physiological state of the neighboring apical region. Although the data for very low frequencies is scanty in the present study, it appears that there is a negligible response below 1 kHz in 13-14-day animals, whereas there is some response for this frequency range in the adults. Such an observation is consistent with the predictions inherent in the model of a shifting frequency code (Lippe and Rubel, '85).

It should be kept in mind that our sampling was biased heavily toward the basal two-thirds of the nucleus, since we only considered EI cells in the LSO that are primarily responsive to frequencies above 2-3 kHz. However, it is interesting to note that whereas the sampling was incomplete at the low frequency end of the nucleus, responsive units were sampled at the basal projection area even in our youngest age groups. This suggests that the lack of a response to high frequencies is not due to unresponsive neurons, but rather to neurons responding to lower frequency stimuli.

The implications of dynamic frequency coding in the developing cochlea has been amply discussed by several authors (Harris and Dallos, '84; Lippe and Rubel, '85; Ryals and Rubel, '85). It remains to be emphasized, however, that physiological studies aimed at contrasting adult and immature animals must not simply compare response properties based on frequency range. Rather, one must take into consideration that cochlear and neural elements at a given location may encode different frequency information during ontogeny. Therefore, a comparison of neural response properties based upon the anatomical position of neurons seems most equitable.

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