

Ontogenesis of tonotopy in inferior colliculus of a hipposiderid bat reveals postnatal shift in frequency-place code

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Summary. The postnatal development of midbrain tonotopy was investigated in the inferior colliculus (IC) of the south Indian CF-FM bat *Hipposideros speoris*. The developmental progress of the three-dimensional frequency representation was determined by systematic stereotaxic recordings of multiunit clusters from the 1st up to the 7th postnatal week. Additional developmental measures included the tuning characteristics of single units (Figs. 3f; 4f; 5f), the analysis of the vocalised pulse repertoire (Figs. 3e, 4e, 5e), and morphometric reconstructions of the brains of all experimental animals (Fig. 1).

The maturation of auditory processing could be divided into two distinct, possibly overlapping developmental periods: First, up to the 5th week, the orderly tonotopy in the IC developed, beginning with the low frequency representation and progressively adding the high frequency representation. With regard to the topology of isofrequency sheets within the IC, maturation progresses from dorsolateral to ventromedial (Figs. 3c, 4c). At the end of this phase the entire IC becomes specialised for narrowly tuned and sensitive frequency processing. This includes the establishment of the 'auditory fovea', i.e. the extensive spatial representation of a narrow band of behaviorally relevant frequencies in the ventromedial part of the IC. In the 5th postnatal week the auditory fovea is concerned with frequencies from 100–118 kHz

(Fig. 4c, d). During subsequent development, the frequency tuning of the auditory fovea increases by 20–25 kHz and finally attains the adult range of ca. 125–140 kHz. During this process, neither the bandwidth of the auditory fovea (15–20 kHz) nor the absolute sensitivity of its units (ca. 50 dB SPL) were changed. Further maturation occurred at the single unit level: the sharpness of frequency tuning increased from the 5th to the 7th postnatal weeks (Q₁₀-dB-values up to 30–60), and upper thresholds emerged (Figs. 4f, 5f).

Although in the adult the frequency of the auditory fovea matches that of the vocalised pulses, none of the juvenile bats tested from the 5th to the 7th weeks showed such a frequency match between vocalisation and audition (Figs. 4e, 5e).

The results show that postnatal maturation of audition in hipposiderid bats cannot be described by a model based on a single developmental parameter.

Introduction

In mammals and birds considerable changes occur within the auditory system during postnatal development (Rubel 1978; Romand 1983). Many rodents and bats are deaf at birth, and hearing only starts in the first 2 weeks of life (rat: Uziel et al. 1981; mouse: Shnerson and Pujol 1983; hamster: Schweitzer and Cant 1984; gerbil: Woolf and Ryan 1984; bat: Konstantinov 1973; Brown and Grinnell 1980; Rübsamen 1987). The frequency range heard is initially restricted to the low- and mid-frequencies and progressively expands towards higher frequencies during growth (Rubel 1978).

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Abbreviations: *BF* best frequency; *CF* constant frequency; *Cer* cerebellum; *CN* cochlear nucleus; *CO* auditory cortex; *CUF* cuneiform nucleus; *DAB* days after birth; *FAL* forearm length; *FM* frequency modulation; *IC* inferior colliculus; *NLL* nucleus of the lateral lemniscus; *PAG* periaqueductal gray; *SC* superior colliculus

An explanation for the maturation of audition was proposed by Rubel and coworkers (1984) and by Harris and Dallos (1984). They reported experimental evidence for shifts of the frequency map in the cochlea due to developmental changes of the hydromechanics, resulting in an alteration of the cochlear transfer function. Their model suggests that the basal end of the basilar membrane, which in the adult state is maximally displaced by high frequencies, is initially sensitive to low pitched sounds. In the course of postnatal development these basal parts of the basilar membrane become tuned to progressively higher frequencies.

This model is still disputed, since other experimenters have reported contradictory results from investigations in chickens (Cousillas and Rebillard 1985; Cotanche et al. 1987; Manley et al. 1987).

An alternative experimental approach which might help to resolve the controversy is to examine the tonotopy of the various nuclei of the ascending auditory pathway (Lippe 1987). This approach is based on the fact that the frequency representation throughout the central auditory pathway is cochleotopically organised and presumably is a faithful reproduction of the cochlear frequency map (review: Irvine 1986). In addition, the afferent and efferent innervation of the brainstem nuclei is already fully established at the onset of hearing (Tokimoto et al. 1977; Schweitzer and Cant 1984); thus changes in frequency maps due to rewiring may be ruled out. Studying the ontogeny of frequency maps by recording neuronal tonotopies avoids possible artifacts caused by experimentally applied lesions to the delicate micromechanical system of the cochlea.

Among echolocating bats, hipposiderid and rhinolophid species from the superfamily Rhinolophoidea are excellent models with which to test for the direction of developmental tonotopic shifts. For echolocation these bats use a narrow band of high frequencies which is represented in an expanded fashion on the basilar membrane (Bruns and Schmieszek 1980; Vater et al. 1985; Peters 1987). The large cochlear overrepresentation of the narrow echolocation frequency band is preserved in the tonotopy of all auditory nuclei from the cochlear nucleus to the auditory cortex (CN: Feng and Vater 1985; IC: Pollak and Schuller 1981; auditory cortex: Ostwald 1984). In an analogy to the visual fovea, this species-specific frequency overrepresentation has been named 'auditory fovea' (Schuller and Pollak 1979; Rübsamen et al. 1988). If Rubel's hypothesis is correct, that during ontogeny frequency representation progresses from lower to higher frequencies, we might expect that

the foveal overrepresentation of the high frequency range would not yet be present in the central auditory nuclei of young bats.

In horseshoe bats, the expanded foveal frequency representation provides a high resolution isofrequency sheet within which changes in the cochleotopic frequency map during development can be examined. These changes should be detectable with a frequency and spatial resolution unavailable in the conventional frequency maps of other mammalian species.

Developmental changes in the hearing of young horseshoe bats (*Rhinolophus rouxi*) have already been studied using evoked potential recordings from the inferior colliculus (Rübsamen 1987): auditory sensitivity began to appear as soon as 1 week after birth, and by the 3rd week the central auditory fovea representation emerged. During postnatal development, the high frequency limit of audition gradually shifted from 25–40 kHz towards higher frequencies, and the centre frequency of the auditory fovea progressed from 58–60 kHz in the 3rd postnatal week to 73–77 kHz in the adult bat.

In this paper we report the postnatal development of the tonotopic organisation in the inferior colliculus (IC) of *Hipposideros speoris*. This species emits brief (5–8 ms) pure tone echolocation signals which are terminated by a downward sweeping frequency modulated component (CF-FM pulse; Habersetzer et al. 1984). The frequency of the pure tone emitted varies among individuals within the species, ranging from 127 kHz to 138 kHz (2nd harmonic). In young *H. speoris*, the fundamental of vocalised frequencies shifted from 26–28 kHz in the 1st postnatal week to about 65 kHz in the 7th week after birth (Habersetzer and Marimuthu 1986). In the inferior colliculus of adult *H. speoris*, the frequency band of 120–145 kHz is enormously overrepresented in the medioventral 60% of the central nucleus and is the reflection of an acoustical fovea tuned to the echo frequencies (Rübsamen et al. 1988).

Materials and methods

The experiments were performed at the Kamaraj University at Madurai, south India. Twelve young specimens of the bat *Hipposideros speoris*, caught in a cave near the campus, were investigated. Within the first 7 postnatal weeks the collicular tonotopy was recorded in its weekly progress (Table 1): first week HS17, HS19; second week HS11, HS21; third week HS12, HS8; fourth week HS9, HS13; fifth week HS15, HS16; seventh week HS18, HS23. The analysis of postnatal growth of the brain was based on data from 9 of these bats. According to Habersetzer and Marimuthu (1986) the age of each individual was determined by measuring its forearm length (FAL). New-

Table 1. See text

Postnatal age [week]	Bat	FAL [mm]	Hearing range at 60 dB SPL [kHz]	Auditory fovea range [kHz]	Vocalisation [kHz]		
					1 Harmonic	2 Harmonic	3 Harmonic
1	HS19	19.1	18– 60 ^a	—	32.8 ± 1.6	66.4 ± 2.9	102.1 ± 3.9
	HS17	22.0	20– 94	—	35.5 ± 2.0	74.5 ± 3.6	111.6 ± 8.0
2	HS11	26.3	18– 98	—	41.2 ± 2.5	83.1 ± 5.1	127.8 ± 4.1
	HS21	26.7	14–102	—	43.2 ± 1.4	86.6 ± 2.7	131.1 ± 3.9
3	HS12	31.2			48.3 ± 3.6	96.9 ± 6.5	147.1 ± 3.1
	HS 8	32.8	16–107	—	45.0 ± 4.0	90.0 ± 8.0	132.0 ± 9.0
4	HS 9	35.4	19–105	—	54.3 ± 3.6	109.5 ± 7.3	—
	HS13	37.5	33–118	104–118	47.6 ± 5.3	96.3 ± 10.4	—
5	HS15	39.5	20–123	110–123	56.4 ± 6.4	112.3 ± 11.2	—
	HS16	40.5	19–125	110–125	45.0 ± 3.0	91.0 ± 7.0	—
7	HS23	47.1	16–127	110–127	—	133.0 ± 1.0	—
	HS18	50.5	20–132	110–128	—	137.0 ± 3.0	—

* Hearing range determined at 80 dB SPL

born *H. speoris* have forearm lengths of 16–17 mm, which is about 30% of the adult values, and after 7 weeks the FAL increases to 46–49 mm (about 92% of the adult values).

Comprehensive maps of the frequency representation in the inferior colliculus – based on recordings of multiunit clusters – were obtained from 7 specimens. This was accomplished by using a specific stereotaxic recording technique: In a single experimental animal all the electrode tracks penetrated the dorsal IC surface from a single point. First, a central electrode track was oriented perpendicularly to the surface of the IC, and the subsequent ones systematically tilted by 10° or 20° in the rostral, caudal, medial or lateral direction. Along each track, best frequency (BF) readings were obtained every 50 µm. This accurate and replicable stereotaxic recording technique enabled us to test, in each specimen, a fixed matrix of 200–360 recording sites. Each BF reading was regarded as representative for the volumes surrounding it. Consequently, each site in this recording matrix was assigned to a spatial section delimited from those spatial sections calculated for the 6 neighbouring recording sites, both in the same and in neighbouring penetrations. These volumes are taken to be frustums of pyramids with the recording site in its centre. The volumes were calculated by the formula:

$$V = (d * \sin \alpha)^2 * \delta T$$

(*d* = distance from electrode tip to brain surface; α = angle between neighbouring electrode tracks; *T* = distance between successive test sites in a single electrode track)

In each experimental animal the frequency/volume distribution was then calculated for the entire volume of the IC that we sampled. From these data we calculated the relative spatial volumes representing each frequency of the auditory range of the individual. For comparison with one another, the results from each individual were then plotted on a graph showing the cumulative frequency distribution.

The tuning characteristics of single units as a function of age were determined in 10 of the 12 bats. In all experimental animals, both the isolation calls and the echolocation calls were analysed with regard to frequency content and time structure. The recording sites within the IC were marked by electrolytic lesions and reconstructed by camera lucida drawings from serial frozen sections.

The experimental procedures including surgery, stereotaxic techniques for electrophysiological recording, histological analysis and sound analysis were identical to those described earlier (Rübsamen et al. 1988).

Results

Postnatal growth of the brain

In order to evaluate developmental changes in frequency representation, it is necessary to know the postnatal growth patterns of the inferior colliculus (IC) and of the total brain as well. We therefore made three-dimensional reconstructions of the brain volumes of each specimen computed from serial sections. To obtain the values used to denote the dimensions of each brain and IC, we took the maximal rostrocaudal and mediolateral extents of both structures and plotted these values as a function of forearm length (Fig. 1). As a reference the same measurements were obtained from adult bats. It is not possible to define unambiguously the ventral limit of the IC due to the massive fibre bundles of the lateral lemniscus entering the ventral IC; therefore, the dorsoventral extent of the IC was not included in the brain size/age correlation.

At birth, the size of the bat's head is disproportionately large compared with the body. Postnatal growth of the skull is mainly in the rostrocaudal dimension. However, this growth occurs predominantly in the region of the jaws and has very little effect on the size of the brain cavity or on the size of the brain itself. In newborn bats, the brain is more spherically shaped than in the adult. From the 1st postnatal week to the adult stage the total

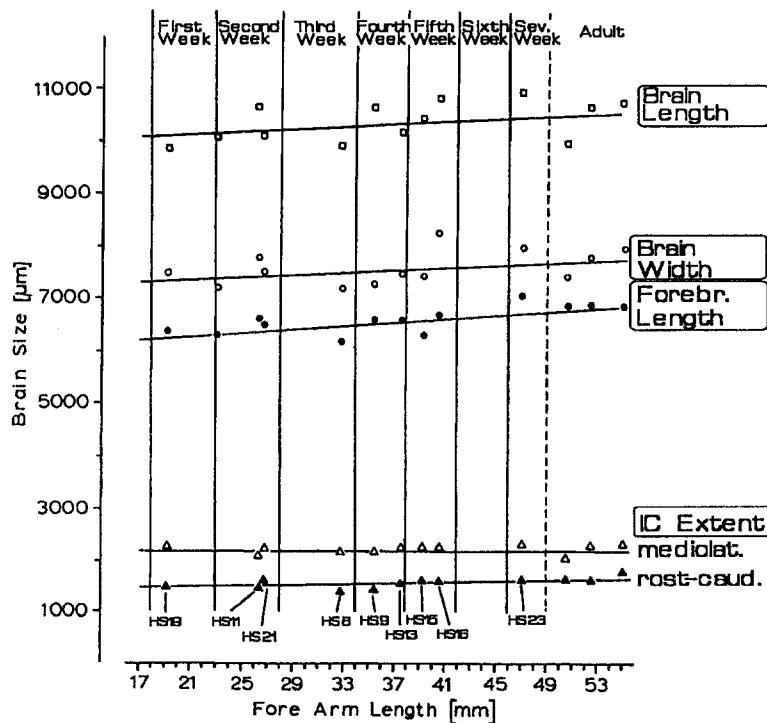


Fig. 1. Postnatal growth of the brain and the inferior colliculus (IC) during the period from the 1st to the 7th weeks of age. Measurements from 3 adult specimens are included for comparison, and the age scale should not be transferred to them. Regression lines and correlation coefficients [r] are calculated for all brain measures from estimation of linear correlations. (□) total brain length [$r=0.39$]; (○) brain width [$r=0.30$]; (●) forebrain length [$r=0.68$]; (Δ) mediolateral [$r=-0.05$] and (\blacktriangle) rostrocaudal [$r=0.60$] extent of the IC. According to Davis (1938) these coefficients are beyond the limits of confidence for the assumption of brain/body growth correlation

brain size did not increase by more than 10% mediolaterally and 20% rostrocaudally (Fig. 1). Such differences were within the margin of error of our stereotaxic recordings and assignment of three-dimensional frequency maps to the morphometrical brain reconstructions. Therefore, possible effects of postnatal growth on frequency map organisation were not taken into further consideration.

Postnatal development of inferior colliculus tonotopy

The youngest bat tested was 1-week-old (HS19; Table 1). This bat vocalised multiharmonic and pure tone 'isolation calls' (Habersetzer and Mariamuthu 1986) through the mouth or through the nostrils. The fundamental frequency was 32.8 ± 1.6 kHz, the second harmonic 66.4 ± 2.9 kHz, and the third harmonic 102.1 ± 3.9 kHz. Mean sound duration was 5.7 ± 1.1 ms ($n=10$).

In the IC of this bat, tonotopic organisation was restricted to a superficial dorsolateral sheet 300–500 μ m thick (Fig. 2). Within this sheet, frequencies from 18 to 60 kHz were represented in a dorsoventral sequence (Fig. 2c, d; the positions of the electrode tracks within the IC are indicated in Fig. 2a, b). Response thresholds of single units and multiunit clusters varied between 60 and 90 dB

SPL, with the lower thresholds for frequencies from 30 to 35 kHz.

In sharp contrast, units in the rest of the IC were uniformly broadly tuned in the range of 40–60 kHz and had no distinct BFs. There was no discernable tonotopic arrangement in this large, ventromedially located neuronal mass of the IC (Fig. 2c, d). Thresholds were high and ranged from 80 to 100 dB SPL. Within this diffusely tuned ventral part of the IC, small areas were interspersed which were unresponsive to auditory stimulation. In older bats, no such 'silent islands' were encountered. In adult bats these ventromedial and caudal parts of the IC contain the tonotopically arranged frequency representation of the auditory fovea, from 120 to 145 kHz.

In all 5 penetrations, angled in different directions, the transition from the tonotopically organized dorsal sheets to the broadly tuned and insensitive part of the IC occurred abruptly within a distance of 50 μ m, resulting in a physiologically well-defined borderline between the small tonotopically organised region and the large non-tonotopically arranged part of the IC.

At the end of the first postnatal week the auditory range broadened to include frequencies up to 90 kHz (HS17, Table 1).

Two bats were tested in their 2nd week (HS11, HS21; Table 1). These bats also vocalised multi-

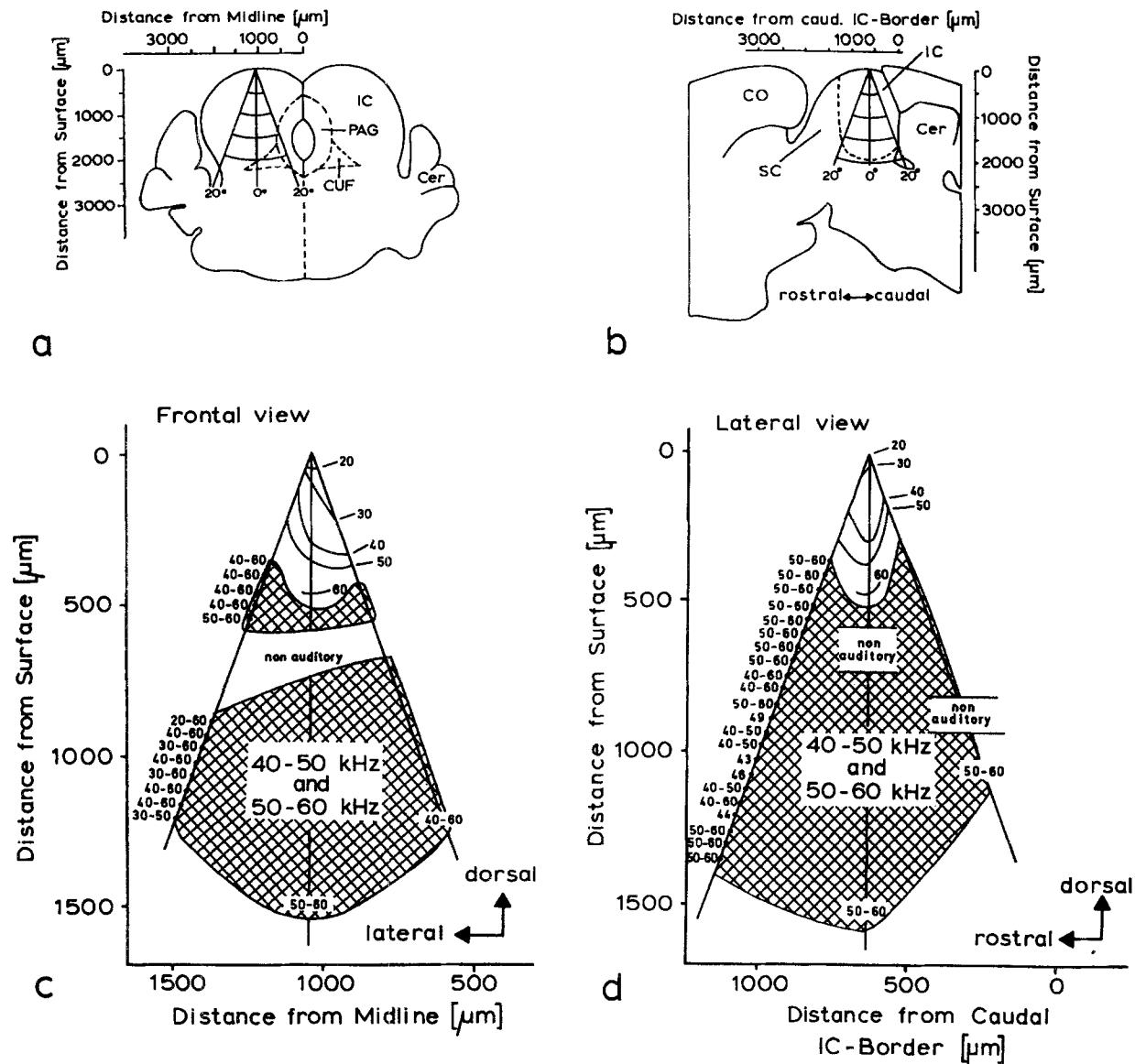
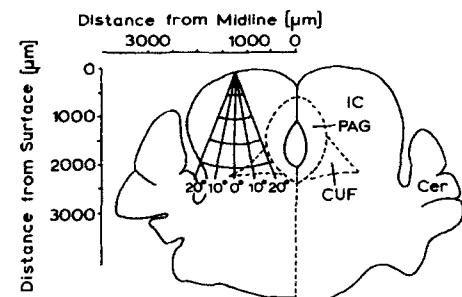


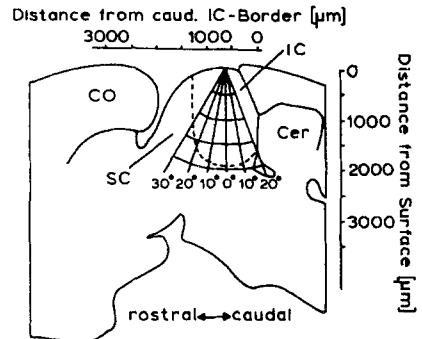
Fig. 2a-d. Tonotopy in the inferior colliculus of a 1-week-old *H. speoris* (HS19). **a** Position of electrode tracks in the IC in a frontal view to the midbrain and **b** in a lateral view. *Cer*, cerebellum; *CO*, cortex; *CUF*, cuneiform nucleus; *PAG*, periaqueductal grey; *SC*, superior colliculus; *IC*, inferior colliculus. **c** Contours of isofrequency sheets in the frontal view, reconstructed from stereotaxic recordings of multiunit ensembles. The 3 electrode tracks have a fan-like arrangement originating from a single point at the dorsal surface of the IC. Solid curved lines show the isofrequency contours in 10-kHz steps, as indicated at the right. The hatched area shows regions characterised by broad-band excitability, mainly in the range of 40–60 kHz.

harmonic and pure tone isolation calls. In HS11 the fundamental frequency of 41.2 ± 2.5 kHz was accompanied by two harmonics at 83.1 ± 5.1 kHz and 127.8 ± 4.1 kHz. The mean pulse duration was 5.8 ± 3.7 ms ($n=10$). In this specimen, the electrode penetrations began at the dorsal IC, 1300 μ m later-

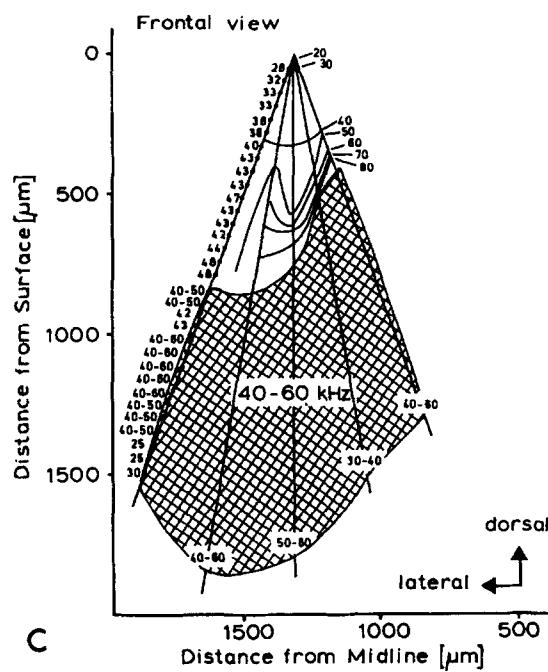
al to the midline (Fig. 3a) and 600 μ m rostral to the posterior IC border (Fig. 3b). The collicular frequency maps in this 2-week-old bat revealed that the tonotopically organised and more sensitive dorsal sheets of the IC had expanded ventrally to a depth of 750–800 μ m, and the BFs in the most



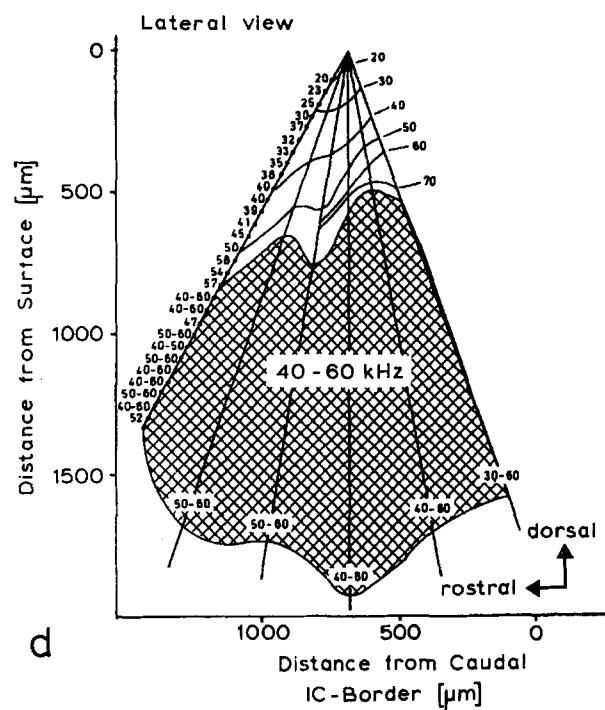
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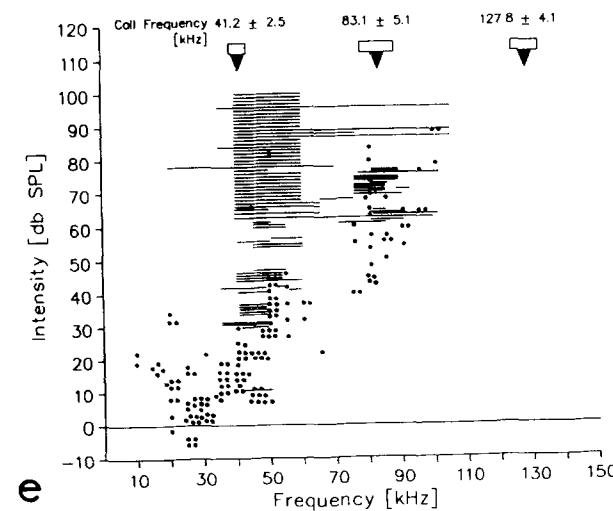
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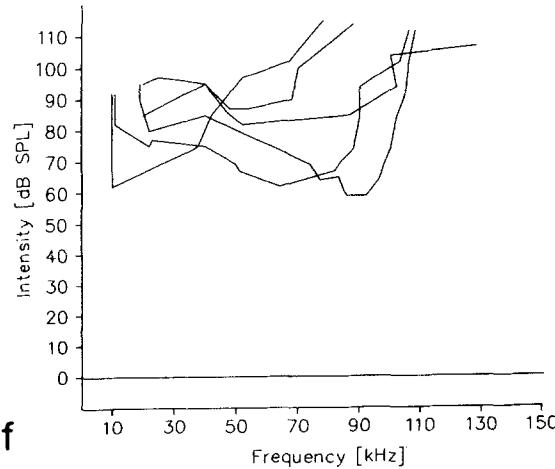
c



d



e



f

ventral layers of this area reached a maximum of 98 kHz (Fig. 3c, d). The isofrequency contours in the dorsal IC were inclined to the rostral and lateral sides. In HS11, as in more mature specimens (see below), the borderline between the sensitive and the insensitive regions of the IC followed the contour of the isofrequency sheet with the highest frequency tuning.

During the 2nd week a threshold decrease was observed in the dorsal tonotopically organised area. For BFs between 25 and 30 kHz thresholds dropped to +10 to -5 dB SPL. For BFs up to 98 kHz thresholds dropped to 60 dB SPL (Fig. 3e). In the rest of the IC unit clusters showed weakly tuned responses in the range of 40–60 kHz or 40–90 kHz, measured 10 dB above threshold. The absolute threshold values mostly varied between 60 and 100 dB SPL (Fig. 3e). Broad frequency tuning in these ventral parts of the IC was also found in single unit recordings (Fig. 3f).

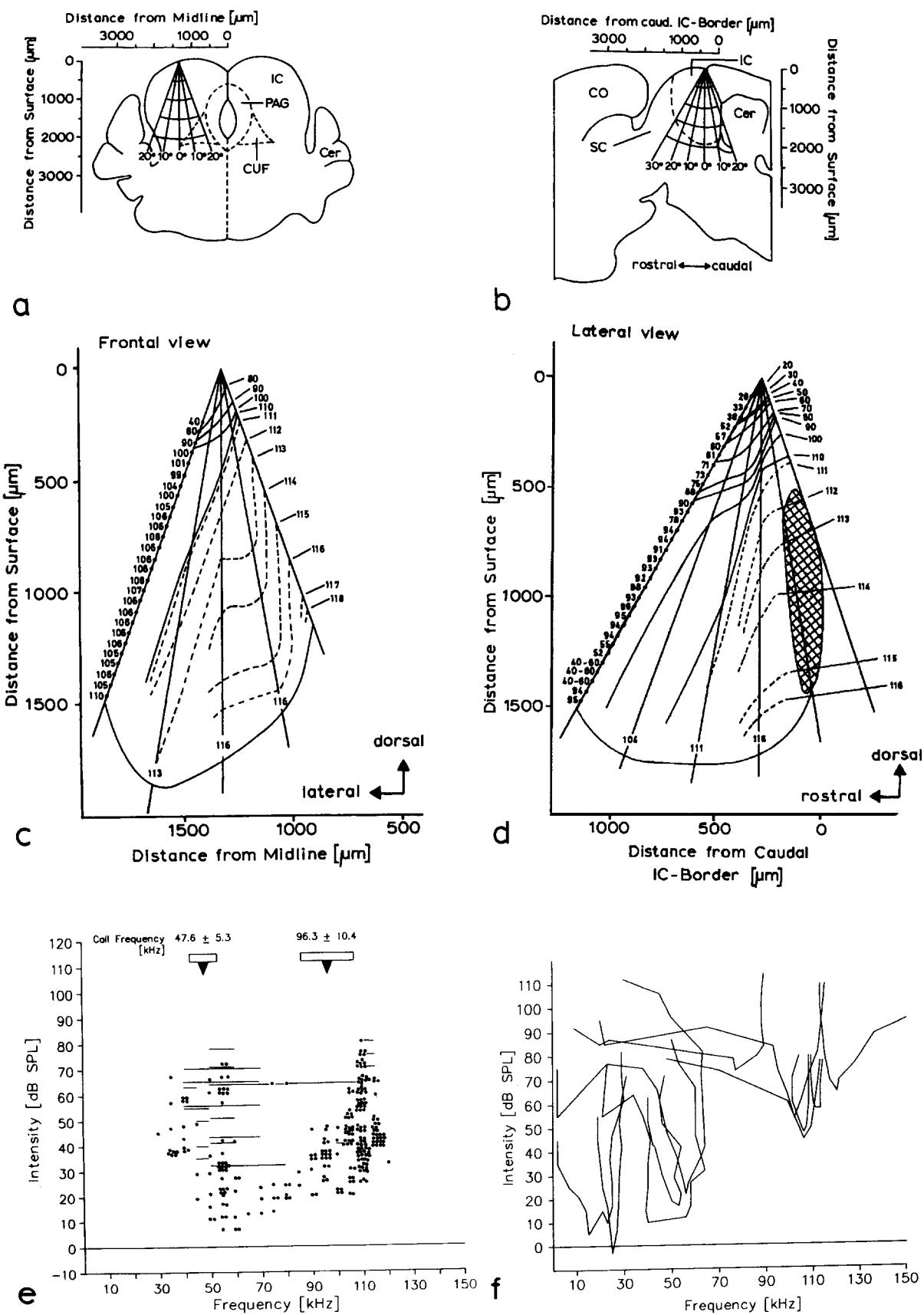
Up to the 4th week of postnatal development, the tonotopically arranged regions continued to expand further ventrally and caudally and progres-

sively replaced the insensitive and broadly tuned areas of the IC. By this process, progressively higher frequencies were incorporated into the tonotopy. Data from specimens of this developmental stage (HS12 and HS8, 3rd week; HS9 and HS13, 4th week; Table 1) disclosed that this phase of development was nearly complete by the beginning of the 5th postnatal week, when the IC showed a coherent tonotopic organisation throughout its entire extent with the exception of the caudal pole (HS13; Fig. 4). Thus, the previously nontonotopic and insensitive part of the IC had been transformed to contain obliquely oriented isofrequency sheets with BFs progressing dorsoventrally from 104 to 118 kHz (Fig. 4c, d). However, this organisation is still not that of an adult bat. While the thresholds of these units are high, ranging from 25 to 80 dB SPL (Fig. 4e), the neurons are more narrowly tuned than those recorded in younger bats (Fig. 4f). Although the frequency range from 104 to 118 kHz is vastly overrepresented, it still does not correspond to the foveal frequency range of adult bats. Also, the vocalised call frequencies are below the adult ranges: in HS13 the pulse CF-components were emitted at 47.6 ± 5.3 kHz (1 harmonic) and 96.3 ± 10.4 kHz (2 harmonic; mean pulse duration 5.2 ± 2.0 ms; $n=10$). The second harmonic of these calls falls partly into the range of the auditory fovea, but the mean frequency is about 10 kHz below the foveal centre frequency (Fig. 4e).

At the end of the 5th and the beginning of the 6th postnatal week, the IC of *H. speoris* shows a complete and coherent tonotopic arrangement extending from the surface to the most ventral layers. Just as in adult *H. speoris* (Rübsamen et al. 1988), the IC consists of a dorsal cap with the frequencies below 100 kHz represented in a condensed tonotopic organisation. The large ventro-medial and caudal part of the IC is fully occupied by the dorsoventrally progressing tonotopic representation of a narrow frequency band above 100 kHz, which thus is enormously overrepresented. However, in the two bats tested during their 5th week, the 'foveal frequency range' that filled up the ventral IC still ranged below the adult values. In HS15 the overrepresented frequency band comprised 110–123 kHz, and in HS16, 105–125 kHz; in each of these specimens the upper limit of this band was also the upper limit of the audible frequency (Table 1).

During the 4th and 5th postnatal weeks the tuning curves of single units with BFs up to 70 kHz have the same shape as those of adult specimens, with Q_{10} dB values ranging between 2 and 25. The

Fig. 3a–f. Tonotopy and tuning curves in the inferior colliculus of a 2-week-old *H. speoris* (HS11). **a** Position of electrode tracks in the IC seen in a frontal view to the midbrain and **b** in a lateral view. *Cer*, cerebellum; *CO*, cortex; *CUF*, cuneiform nucleus; *PAG*, periaqueductal grey; *SC*, superior colliculus; *IC*, inferior colliculus. **c** Course of isofrequency sheets in the frontal view reconstructed from stereotaxic recordings of multiunit ensembles. The 5 electrode tracks have a fan-like arrangement originating from a single point at the dorsal surface of the IC. Solid curved lines show the isofrequency contours in 10-kHz steps, as indicated at the right side. The hatched area shows regions characterised by broad-band excitability, mainly in the range of 40–60 kHz. For the leftmost electrode track, the complete sequence of BF measurements is given on the left side. For all other electrode tracks only the most ventral BFs are indicated. Position of the fan of electrode tracks within the IC as shown in **a**. **d** Contours of isofrequency sheets in the lateral view, reconstructed from stereotaxic recordings of multiunit ensembles. The 6 electrode tracks have a fan-like arrangement originating from a single point at the dorsal surface of the IC. Depiction of the BFs same as in **c**. For the rostral-most electrode track the complete sequence of BF measurements is given on the left side. Position of the fan of electrode tracks within the IC as shown in **b**. **e** Audiogram of HS11 resulting from recordings of 320 multiunit ensembles. Corresponding BF and threshold values are indicated by dots. For the regions which lack definable BF values, the excitatory band ca. 10 dB above threshold is shown by horizontal bars. Mean frequency values and standard deviations of the bat's harmonically structured vocalisations are shown at the top of this graph (1 harmonic: 41.2 ± 2.5 kHz; 2 harmonic: 83.1 ± 5.1 kHz; 3 harmonic: 127.8 ± 4.1 kHz, $n=10$). **f** Frequency tuning of 5 typical single units in the ventral part of the IC (hatched areas in **c** and **d**) recorded during the 2nd postnatal week



tuning characteristics of auditory fovea units, however, differ from those found in adults, in that their frequency selectivity is lower. The Q_{10} dB values never exceeded 30, and many units were characterised by pronounced 'low frequency tails' never found in adult auditory fovea units (Fig. 4f).

In their 5th postnatal week the young bats were found flying inside the cave, providing evidence that they were able to exploit a functional echolocation system for orientation, even though their hearing capacities – as noted above – differed somewhat from those of adult bats.

During the 6th and 7th weeks, when the young bats were observed flying outside the cave, maturational changes in auditory processing were confined to the domain of the collicular auditory fovea. The oldest adolescent bats tested were from the 7th postnatal week (HS18 and HS23; Fig. 5). The frequency ranges represented in their auditory fovea were 110–128 kHz (HS18; Table 1) and 110–127 kHz (HS23; Fig. 5c, d). The bandwidth of the auditory fovea remained constant in the range of about 15 kHz from the 4th through the 7th week. However, the foveal mid-frequency increased by about 10 kHz (compare Figs. 4e, 5e) during this period, and by about 20 to 25 kHz up

Fig. 4a–f. Tonotopy and tuning curves in the inferior colliculus of a 5-week-old *H. speoris* (HS13). **a** Position of electrode tracks in the IC seen in a frontal view and **b** in a lateral view. *Cer*, cerebellum; *CO*, cortex; *CUF*, cuneiform nucleus; *PAG*, periaqueductal grey; *SC*, superior colliculus; *IC*, inferior colliculus. **c** Contours of isofrequency sheets in the frontal view, reconstructed from stereotaxic recordings of multiunit ensembles. The 5 electrode tracks have a fan-like arrangement originating from a single point at the dorsal surface of the IC. Solid curved lines show the isofrequency contours in 10-kHz steps and dashed lines the isofrequency contours in the domain of the auditory fovea in 1-kHz steps, with frequency values indicated at the right. For the leftmost electrode track the complete sequence of BF measurements is given on the left side. For all other electrode tracks only the most ventral BFs are indicated. Position of the fan of electrode tracks within the IC as shown in **a**. **d** Contours of isofrequency sheets in the lateral view, reconstructed from stereotaxic recordings of multiunit ensembles. The 6 electrode tracks have a fan-like arrangement originating from a single point at the dorsal surface of the IC. Solid curved lines show the isofrequency contours in 10-kHz steps and dashed lines the isofrequency contours in the domain of the auditory fovea in 1-kHz steps. Position of the fan of electrode tracks within the IC as shown in **b**. **e** Audiogram of HS13 resulting from recordings of 360 multiunit ensembles. Arrangement of the graph same as in Fig. 3e. Mean frequency values and standard deviations of the bat's harmonically structured vocalisations are shown at the top of this graph (1 harmonic: 47.6 ± 5.3 kHz; 2 harmonic: 96.3 ± 10.4 kHz; $n=10$). **f** Frequency tuning of single units recorded during the 5th postnatal week

to the adult stage (see Fig. 5a in Rübsamen et al. 1988). Because during this development neither the position nor the spatial extent of the auditory fovea representation in the IC changed, it is concluded that at each given IC site the frequency tuning of the units changed.

Single units recorded in the foveal range during the 7th week showed little increase in sensitivity (50–60 dB SPL) compared with younger bats, but the excitatory bandwidths generally were much narrower, characterized by Q_{10} dB values up to 60. 'Low frequency tails' were rarely found, and a number of units exhibited upper thresholds at 100 to 110 dB SPL (Fig. 5f).

The two bats tested during the 7th week vocalised solely CF-FM echolocation calls (Table 1). In both specimens there was a mismatch between auditory fovea frequencies (110–127 kHz in HS23) and the CF components of their calls (133.3 ± 1.0 kHz in HS23; Fig. 5e).

Apparently the frequency representation in the IC of *H. speoris* matures in three steps (Fig. 6): (1) In the beginning only the dorsal part of the IC representing frequencies below those of the echolocation signals are tonotopically organised, and the medial and ventral part, which later represents the narrow frequency band of the echolocation signals, is undifferentiated (Fig. 6a, b).

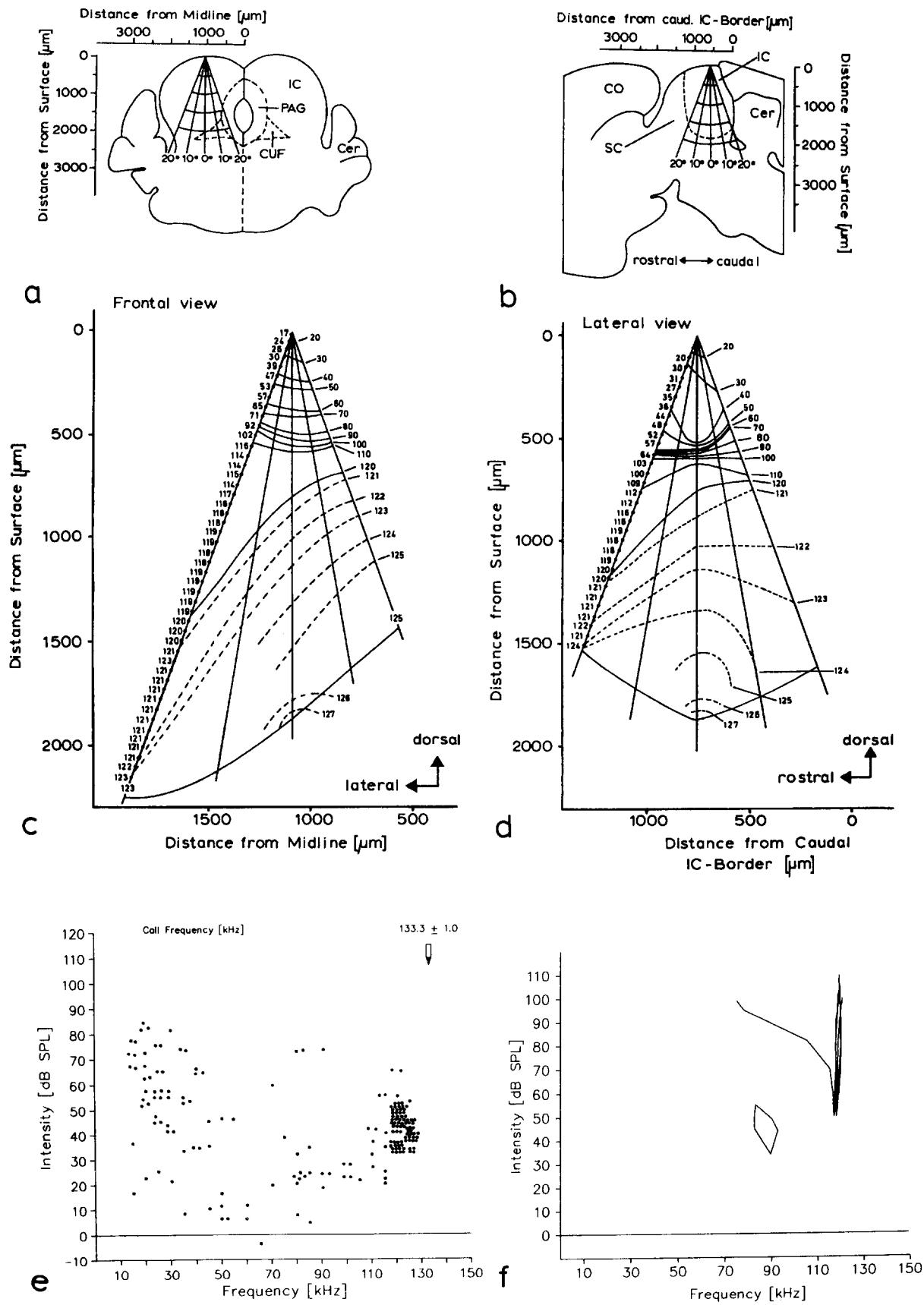
(2) From the 2nd to the end of the 4th postnatal weeks, tonotopic organisation slowly progresses through the undifferentiated medial and ventral region and transforms it into an expanded, 'foveal' representation of a high frequency band (bandwidth 15–20 kHz), which, however, is not yet tuned to the adult frequency range (Fig. 6c, d).

(3) From the 5th week to adulthood the frequency range of the large foveal region shifts from 105–120 kHz to 125–145 kHz, the latter being the overrepresented frequency band in adult *H. speoris*.

If the rule holds that the tonotopy in the auditory brain centres is determined by cochleotopic order, these results indicate that the frequency representation on the basal, foveal part of the basilar membrane must shift its frequency band from 105–120 kHz to 125–145 kHz in late adolescence, a change of 20–25 kHz.

Discussion

This study demonstrates that a diffuse frequency representation of 40–60 kHz initially occupies the major part of the IC but is replaced gradually by a dorsoventrally organised tonotopy from 110 to 128 kHz. These results strongly support the hy-



pothesis that during the postnatal development of audition the frequency-place code shifts from lower to higher frequencies (Rubel and Ryals 1983; Rubel et al. 1984).

This hypothesis is based on cochlear experiments in birds and mammals (Rubel et al. 1984; Harris and Dallos 1984), which showed that in the earliest postnatal stages audition is generally restricted to the species-specific low and middle frequency ranges. These findings seem to be at odds with the morphological maturation of the cochlea which progresses apically starting from the basal half-turn, where in adults high frequencies are represented (Kraus and Aulbach-Kraus 1981; Vater 1988). Rubel's model assumes that during postnatal development a given place on the basilar membrane gradually becomes tuned to higher frequencies; thus, the frequencies represented at a basal place on the basilar membrane will shift from a lower frequency in young stages to a higher one in the adult specimen. This contention was supported by lesion experiments in the chick (Rubel and Ryals 1983) and by place-specific recordings of cochlear microphonics in the gerbil (Harris and Dallos 1984; Arjmand et al. 1988). As a consequence of the shifting frequency representation in the cochlea, the tonotopy in the ascending auditory pathway should also shift from low to high frequencies. Such shifts have been reported in the chicken (Lippe 1987).

Recently, this model of the ontogeny of tonotopic organisation has met opposition. Cotanche et al. (1987) questioned whether lesion experiments give relevant results, and Manley et al. (1987) did not find any tonotopic shift in the chicken. These conflicting results may be due to the fact that the auditory range of the chicken is limited to low fre-

Fig. 5a-f. Tonotopy and tuning curves in the inferior colliculus of a 7-week-old *H. speoris* (HS23). **a** Position of electrode tracks in the IC seen in a frontal view and **b** in a lateral view. *Cer*, cerebellum; *CO*, cortex; *CUF*, cuneiform nucleus; *PAG*, periaqueductal grey; *SC*, superior colliculus; *IC*, inferior colliculus. **c** Contours of isofrequency sheets in the frontal view, reconstructed from stereotaxic recordings of multiunit ensembles. Depiction of BFs same as in Fig. 4c. Position of the fan of electrode tracks as shown in **a**. **d** Course of isofrequency sheets in the lateral view, reconstructed from stereotaxic recordings of multiunit ensembles. Depiction of the BFs same as in Fig. 4d. Position of the fan of electrode tracks as shown in **b**. **e** Audiogram of HS23 resulting from recordings of multiunit ensembles in 320 different IC sites. Mean frequency values and standard deviations of the second harmonic of the bat's echolocation calls are shown at the top of this graph (133.3 ± 1.0 kHz; $n = 10$). **f** Frequency tuning of single units in ventral parts of the IC recorded during the 7th postnatal week

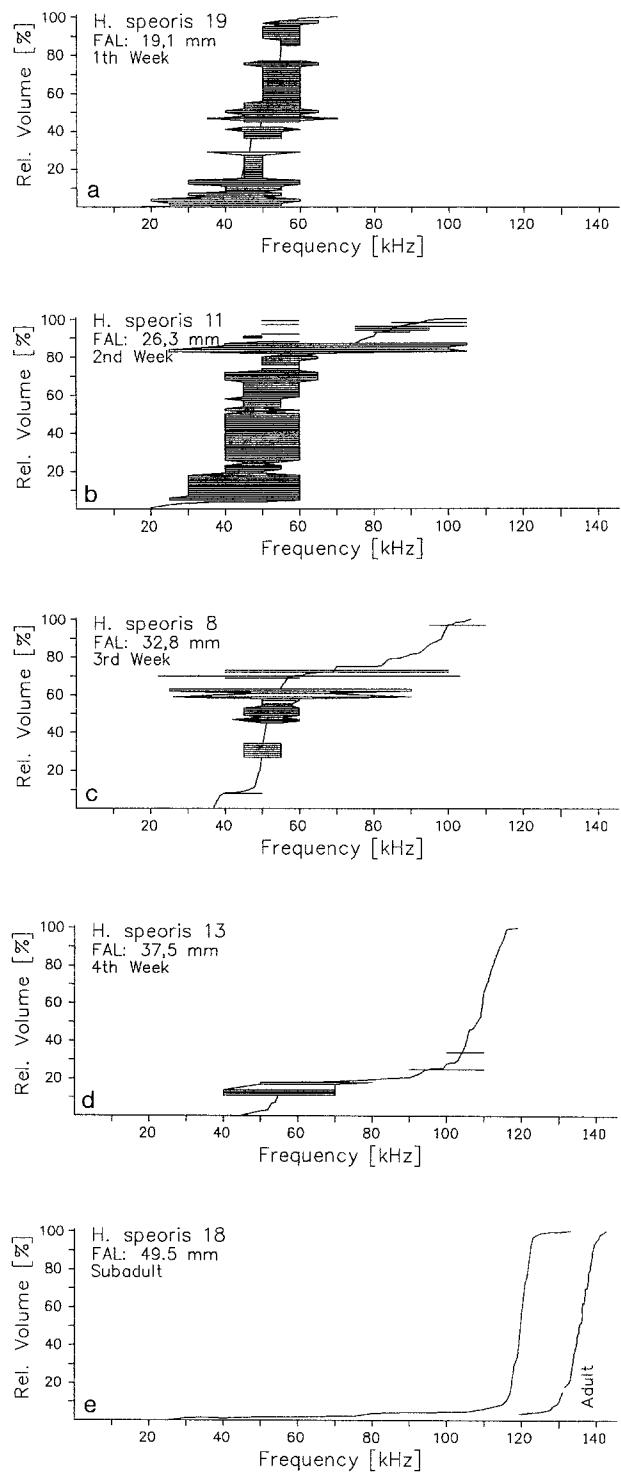


Fig. 6a-e. Postnatal development of the absolute frequency range of audition and of the composition of relative volumetric frequency representation in the inferior colliculus (IC). Cumulative frequency distributions are measured for bats in the **a** first, **b** second, **c** third, **d** fourth, and **e** seventh week. For comparison the cumulative frequency distribution within the IC is shown for an adult *H. speoris* (data from Rübsamen et al. 1988). The cumulative frequency distribution within the IC is calculated on basis of multiunit recordings. Broad-band sensitivities indicated by horizontal bars centered around the midfrequency

quencies from 0.15 kHz to 4 kHz, the overall length of the basilar membrane is not more than 3 mm, and the auditory nuclei are small in size. Therefore, detection and verification of small shifts in frequency representation might be very difficult to achieve.

Such experimental problems are avoided in studies with echolocating bats, which have hypertrophied auditory nuclei and hear a wide range of frequencies from a few kHz to 150 kHz. Hipposiderid and rhinolophid bats offer an additional advantage for studying the ontogeny of tonotopy because their auditory foveae represent an extremely narrow frequency band in an expanded fashion (Schuller and Pollak 1979; Neuweiler et al. 1984). In *H. speoris*, the species studied here, the overrepresented frequency range from 120 to 140 kHz occupies about 60% of the IC volume in adult bats (Rübsamen et al. 1988). In this species the basal part of the cochlea features morphological specialisations which are commonly associated with the cochlear representation of the foveal frequency band (Kraus 1983), and in *H. lankadiva* the existence of an auditory fovea within the cochlear frequency map has been experimentally demonstrated by neurotracer techniques (Peters 1987).

In *H. speoris*, the maturation of tonotopy proceeds in a distinctly different manner for frequencies within the auditory fovea than for those below the foveal frequency range. We first consider the auditory fovea. The first auditory responses of IC neurons appear in the 1st postnatal week. At that time, only the dorsolateral nuclear layers are tonotopically organised and represent frequencies from 20 to 60 kHz. In the 2nd postnatal week, tonotopy extends further ventrally and includes frequencies up to about 100 kHz. However, the major portion of the IC below this dorsal cap, which in adult specimens represents the foveal frequency range from 125–145 kHz, responds only weakly to stimuli between 40–60 kHz, without any apparent tonotopy. Only in the 4th postnatal week is this main part of the IC invaded by higher frequencies in a dorsoventral tonotopic organisation. As in adult bats, the foveal area in the IC of 5-week-old bats represents a frequency band 15–20 kHz wide. Its frequency tuning, however, is 20–25 kHz lower than in the adult and slowly rises from 100–118 kHz in the 5th postnatal week to 110–128 kHz in the 7th week. At this stage the frequency range is still about 10 kHz below that of adults.

Throughout this period of continuous frequency shift, the tonotopic organisation in the 'foveal' area of the IC remains coherent and consis-

tent. Because the afferent innervation of the mammalian auditory system is most likely complete at the time when air-conducted hearing starts (Tokimoto et al. 1977; Schweitzer and Cant 1984), we conclude that the observed frequency shift must be due to a corresponding shift in tuning of the basilar membrane. We assume that during maturation the basal part of the basilar membrane, which represents the frequencies of the auditory fovea (Peters 1987), transduces gradually higher frequencies.

Recent HRP-tracing experiments in the cochlea of young *Rhinolophus rouxi* provide direct evidence that such shifts in the cochlear frequency representation do indeed occur (Vater and Rübsamen 1988). Evoked potential recordings in these horseshoe bats disclosed that, as in *Hipposideros speoris*, the auditory fovea appears with some delay after the onset of hearing. Sensitivity to the foveal frequency band first developed in the 3rd postnatal week, and the tuning shifted from an initial value of 58 kHz to 77 kHz in the adults (Rübsamen 1987).

Thus, in both bat species examined, the development of frequency representation within the foveal frequency range is consistent with Rubel's model. This, however, does not apply to the representation of frequencies below those for echolocation. Beginning in the 1st and 2nd postnatal weeks, the low frequencies have the same collicular representation as that found in the adult (Rübsamen et al. 1988). In young bats the isofrequency contours up to 90 kHz occupy a superficial sheet in the IC, and they remain in this position throughout development up to the 7th week. This demonstrates that the central representation of *low frequencies* does not follow the concept of altered frequency-place code; thus, maturation of the central frequency representation as a whole cannot be described adequately by a model based on a single developmental principle.

Apparently, the maturation of audition in *H. speoris* involves two distinct processes, which may be to some extent superimposed. Up to the 4th week, tonotopy progressively develops following a dorsolateral to ventromedial gradient. In the 4th to 7th weeks, development is restricted to the auditory fovea, for which the overall frequency tuning increases. Based on recent experiments in *R. rouxi* we suggest that the developmental shift to higher frequencies occurring in the foveal region is due to hydromechanical changes in the basal part of the cochlea. Basal parts of the basilar membrane would thereby gradually increase their tuning to higher frequencies (Vater and Rübsamen 1988),

and this same frequency shift would be reflected in all high frequency sites within the cochleotopically organised central auditory nuclei.

However, the maturation of IC tonotopy for frequencies up to 100 kHz remains puzzling. If this maturation is caused by development of the cochlea, it follows that frequency processing on the basilar membrane matures from low to high frequency sites, i.e. topologically from the apical to the basal part – a conclusion which is contradictory to the morphological analyses. Light microscopic observations of basilar membrane development in *R. rouxi* have revealed that postnatal maturation proceeds from basal to apical (Vater 1988).

Alternatively, restriction of auditory sensitivity to frequencies below 100 kHz in the first 3 postnatal weeks might also be explained by the maturation of the middle ear. Developmental changes of middle ear admittance were reported in rodents (Relkin and Saunders 1980) and in chicks (Saunders et al. 1986). These changes might be related to progressive ossification of the middle ear oscicles (Stephens 1972; Webster 1975). These studies suggest that in newborn animals the middle ear acts as a low pass filter which successively increases its upper cut-off frequency during development. As a consequence, apical parts of the basilar membrane would be stimulated prior to basal parts. However, in bats, the effect of middle ear maturation on audition remains to be tested.

Postnatal development of collicular tonotopy can be described in accordance with the experimental data if we assume that maturation of both the cochlea *and* the middle ear contribute to this development: the maturational shifts of the central frequency-place code might reflect the cochlear component of auditory development described above. The low-to-high frequency gradient in the development of tonotopy, a process which seems to run contrary to the shift in the frequency-place code, might represent the middle ear component of maturation. The successively increasing upper frequency limit of transmission by the middle ear might cause a basally directed extension of cochlear stimulation and thereby a concomitant increase in centrally represented frequencies. From this it would follow that the delayed emergence of the central foveal representation in the 4th week is not due to delayed maturation of the basal cochlea itself but rather to some other mechanism, possibly middle ear maturation.

Successive recordings of single units in the period up to the 7th postnatal week corroborate the findings based on multiunit studies and give further insight into the maturation process. High sen-

sitivity and narrow tuning emerged during the 2nd postnatal week in units with BFs between 20 and 60 kHz. In contrast, units recorded from the foveal region of the IC were insensitive (thresholds 90–100 dB SPL) and broadly tuned up to the 3rd week of life (Fig. 3f). In the 4th and 5th postnatal weeks, tuning curves of more and more units recorded from the foveal region showed a narrowly tuned sensitivity peak in the frequency range of 100–120 kHz. During successive development this high frequency sensitivity increases, and the low frequency tails are reduced until, by the 7th week, most units have become extremely narrowly tuned (Q_{10} dB-values up to 60). By then some units have already developed upper thresholds (compare Fig. 4f and Fig. 5f).

Although the increasing sensitivity of IC neurons might be explained by changes in the middle ear filter characteristics, as described above, it could to some extent also be influenced by developmental changes in basilar membrane structure. In *R. rouxi* cell layers covering the tympanic membrane are gradually removed during postnatal development. In young stages these covering layers are prominent and, as an additional mass loading, might damp the mechanical responsiveness of the basilar membrane (Vater 1988; Vater and Rübsamen 1988).

The single unit recordings from young *H. speoris* are in accordance with data obtained from rodents and cats, in which narrow tuning characteristics and low thresholds are always first found in neurons with BFs in the species-specific low to mid-frequency ranges (Willott and Shnerson 1978; Moore and Irvine 1979). The apparent contradiction found in the report of Woolf and Ryan (1985) might be explained by their arbitrary correlation of the terms 'low' and 'high frequency' to the absolute frequency scale.

In echolocating bats audition is vital. It is possible to relate our physiological evidence of developmental change with behavioural development. In the 5th postnatal week, when the foveal frequency representation in the IC has just begun to emerge, the first young *H. speoris* were found flying inside the dark cave and actively tried to escape from catching nets. Seven-week-old bats were found flying outside the cave, even though their auditory system had not reached the full frequency range of adults. At that age the auditory foveae were still tuned to 110–125 kHz, even though the same young bats already emitted echolocation signals with a pure tone component of 133 to 137 kHz. In spite of this apparent mismatch, the bats were actively echolocating and flew skillfully inside and

outside the cave. Nevertheless, even in adult hippociderids there is a tendency for the emitted frequencies of the pure tone components to be slightly higher than the centre frequency of the fovea. This tolerance of the specific, foveal echolocation system to a frequency mismatch might have two explanations. (1) The auditory fovea in *H. speoris* is much broader than in horseshoe bats and incorporates a large part of the frequency range emitted in the final FM-sweep (Rübsamen et al. 1988). (2) Hipposiderid bats do not maintain the frequency of Doppler-shifted echoes with the same precision and consistency as many horseshoe bats do (Habersetzer et al. 1984). The frequency emitted by an individual hippociderid bat may vary by 3–4 kHz from day to day (Peters 1987). Due to this mismatch between audition and vocalisation hippociderid bats seem less suitable for the study of the postnatal development of audiovocal interaction. We have already started to investigate this topic in young rufous horseshoe bats (*R. rouxi*; Rübsamen 1987). In this species we can investigate the question whether the auditory or the vocalisation system presets the frequency to which the echolocation system becomes matched (Rübsamen and Schäfer, unpublished).

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