Development of tonotopy in the inferior colliculus. I. Electrophysiological mapping in house mice

Raymond Romand¹ and Günter Ehret²

¹Laboratoire de Neurobiologie, Université Blaise Pascal, Aubière, France and ²Fakultät für Biologie, Universität Konstanz, Konstanz, F.R.G.

(Accepted 6 March 1990)

Key words: Tonotopic organization; Auditory development; Inferior colliculus; Frequency mapping; Mouse

The development of the size and tonotopy of the mouse inferior colliculus (IC) was studied at postnatal ages of 9–20 days. During that time, the size of the IC remained constant in all 3 planes (rostrocaudal, mediolateral, dorsoventral). At day 10, the first low-frequency responses without tonotopy could be recorded from neurons in the rostral and central parts of the central nucleus sparing its caudal part, very medial portions, the medial part (M) of the central nucleus, the dorsal cortex and the lateral nucleus. Then, an extension of the frequency responsiveness occurred towards (1) the caudal pole which was reached by about day 14, (2) the dorsal surface reached between days 12 and 14, (3) the ventral border of the IC reached by about day 15. The high-frequency nucleus of the IC (M part of the central nucleus) remained unresponsive to tones up to day 13. Between days 10 and 20, there was a constant increase of highest characteristic frequencies (CFs) measurable of neurons in the IC. During that time, lowest measurable CFs remained rather constant. Neurons at a given constant collicular depth of more than about 400 μm showed a clear shift of CF from low to high, that is, they were tuned to the higher frequencies the older the animals were. Cochlear and collicular origins of this observed shift of tonotopy are discussed.

INTRODUCTION

The development of hearing in mammals takes several days or even weeks until, after the first sensation of a sound, the adult levels of functioning of the auditory system and of sound perception are reached^{9,20}. The first behavioral activities to acoustical stimulation comprise various types of startle and orientation responses^{17–19,21,22,40,46} such as twitching of pinnae, eyelids and facial muscles, stop moving, head turns, changes of heart rate which all seem to be mediated at the level of the midbrain or below^{13,23,25,27,32,70}. Thus, strategies of coding auditory information in the inferior colliculus (IC) may directly show up in behavioral responses. An example will be presented in the Discussion.

Although neuronal activities in the IC of young mammals have been studied in some detail^{2,42,47,59,61,73} only few notes on the tonotopic development are available^{1,2,10,63}. Hence, we are far from understanding the functional maturation within the highly convergent collicular neuronal network with regard to both the underlying anatomical and physiological processes and the consequences for auditory perception.

Since tonotopy is the basic characteristic and general principle of ordering frequency information in all audi-

tory centers, which may give rise to frequency-related sensations, we decided to study the development of frequency representation in the IC of two mammals, mouse and cat, for which many other data on various aspects of auditory development are available for comparison. In the present paper, we deal with electrophysiological mapping of frequency representation in the IC and with the development of collicular size of the house mouse.

MATERIALS AND METHODS

Subjects

House mice of both sexes (*Mus musculus*, outbred strain NMRI or F₁-hybrids of NMRI $\times$ feral) bred in the University of Konstanz were used. The animals were taken from litters with 8–12 offspring which were well nourished and cared for by their mothers. Mother and offspring were housed in plastic cages (20 $\times$ 25 cm) under a constant 12 h light/dark cycle at temperatures of 20–22 °C. Food and water were available ad libitum.

We recorded from animals of the following postnatal ages (numbers of animals in parentheses): 9 days (4), 10 days (5), 11 days (7), 12 days (7), 13 days (8), 14 days (7), 15 days (6), 16 days (7), 18 days (7), and 20 days (7). Usually, the young were born during the night. The day following that night was counted as postnatal day 1.

Sound system

Experiments were run in a sound-proof and anechoic room. Tone

Correspondence: G. Ehret. Present address: Abteilung Vergleichende Neurobiologie, Universität Ulm (M 25/5), Postfach 40 66, D-7900 Ulm, F.R.G.

bursts (50 ms duration, 5 ms rise and fall times, 1/s rate in animals younger than 15 days, 3/s rate in animals of 15 days and older) were delivered free-field to the subject by either a dynamic speaker for frequencies below 12 kHz or an electrostatic speaker³⁶ for higher frequencies. Speakers were mounted 60 cm from the subject. Maximum sound pressure levels (SPLs re. 20 μ Pa) available without distortion (harmonics at least 55 dB down) were 116 dB for the dynamic speaker and 108 dB for the electrostatic speaker. The sound field at the left ear of the subject was calibrated with a condenser microphone (Brüel & Kjær 4135 plus measuring amplifier 2606) and the SPL continuously measured in every recording session.

Tones of known frequencies (Kontron counter/timer 6001) were produced in a generator (Wavetek 132) fed through an electronic switch (generation of bursts), attenuator (Hewlett-Packard 350D), voltage amplifier (Hewlett-Packard 465 A) and a power amplifier (Filtronic DA-200 or Exact 170) to the dynamic speaker or to the electrostatic speaker respectively.

Frequency mapping

Animals were anesthetized with sodium pentobarbital (Nembutal, 70 mg/kg i.p.) for surgery. The skin on top of the skull was removed. In subjects younger than 15 days a small metal bar was glued (Loctite) on the nasal/frontal bones. The bar was then fixed in a clamp that allowed the orientation of the head for recording. Older animals were fixed in a head-holder (a clamp between the palate and nasal/frontal bones). The head of the subject was always adjusted so that the surface of the skull was in a horizontal position. Animals were placed on a feedback-controlled heating pad which kept the temperature of the subject at $37 \pm 1^\circ\text{C}$.

The inferior colliculus of the right side was exposed. Steel electrodes (impedance 1.3–1.5 M Ω) were placed stereotactically, with reference to the λ -point of the skull, on the visible dorsal surface of the IC and advanced into the IC by a remote-controlled hydraulic microdrive (Trent Wells, Mark III). The electrode output was amplified (WPI DAM 5A and Hewlett-Packard 465 A) 10,000 times, filtered (Krohn-Hite 3500, bandwidth 0.3–6 kHz) and sent to an oscilloscope and audiometer. Single- or multi-unit responses were recorded in predetermined depths of 100, 200, 300, . . . 1000, 1200, 1400 . . . μm until responses to tone bursts could no longer be detected. At every depth the characteristic frequency (CF) and the lowest excitation threshold of a unit or a group of units were determined audiovisually as that frequency and SPL at which a tone-related increase of activity above the spontaneous level was just noticeable.

Penetrations were advanced into the IC as long as the physiological state (breathing, heart rate) of the subject remained stable, usually 4–8 h within which 5–11 penetrations could be sampled. One electrode track was always placed in the center of the visible IC surface. At least one penetration in a given IC was terminated with an electrolytic lesion at the most ventral point with a clear tone response for later histological verification. Anesthesia was maintained by injections of Nembutal (10 mg/kg) when necessary (about every 2–3 h).

Histology

At the end of a recording session, the animal received an overdose of Nembutal, the brain was quickly removed, frontal frozen sections (20 μm) of the unfixed brain were made on a freezing microtome (Reichert-Jung 2700), and stained with Cresyl violet. The sections served two purposes: first, nucleus regions within the IC and electrode tracks could be identified and served together with the stereotactic coordinate system for the reconstruction of penetrations and the determination of recording sites within the nuclei of the IC. Second, the size of the IC could be determined in the dorsoventral, mediolateral and rostrocaudal plane. For this purpose, brains of additional animals were cut as described above, so that sections of 4 brains per age with good frontal orientation were available for size measurements.

The size of a given IC was determined 100, 300, 500, 700 and 900

μm from the posterior pole with a calibrated ocular micrometer inserted in a photomicroscope (Zeiss). The rostrocaudal extension was defined as the distance (measured as the number of 20 μm sections) between the caudal pole and the first appearance of cells of the superior colliculus which were always clearly visible. The thus defined rostrocaudal extension does not include the most rostral part of the IC and its rostral pole which, however, could often not unambiguously be determined in the Cresyl violet sections. The data presented for the rostrocaudal extension of the IC, therefore, are accurate and homogeneous for the comparative purposes intended (age-related changes) but do not comprise the total extension of the IC in that plane. The mediolateral extension in a given section was defined as the distance between the midline and the most lateral point of the IC. The dorsoventral extension was measured as the distance between the most dorsal and most ventral point of the IC. These points usually had different medio-lateral coordinates. The ventral border of the IC could easily be determined in caudal sections (demarcation against the cerebellum). In more rostral sections the border between IC and lateral lemniscus was estimated in order to determine the ventralmost point of the IC.

RESULTS

Development of collicular size

The development of the size of the mouse inferior colliculus is shown in Fig. 1a–c for postnatal ages of 9–20 days. The mediolateral and dorsoventral extensions were determined 100, 300, 500, 700 and 900 μm from the posterior pole of the IC. These rostrocaudal planes are the parameters in Fig. 1a, b. The mediolateral (m-l) and dorsoventral (d-v) extensions of the IC obviously remain rather constant at the postnatal ages measured. Significant differences of values among postnatal days (*U*-test) and trends of decreases or increases of size in this postnatal period (regression analysis) did not occur at any rostrocaudal plane. Furthermore, the rostrocaudal extension did not show significant changes (Fig. 1c). This means that the size of the inferior colliculus is constant between postnatal days 9 and 20 during which time the tonotopic development was assessed. Hence, frequency maps from the various postnatal ages can directly be compared without factors of adjustment for size or shape differences necessary.

Differences between the NMRI strain and the hybrids were not observed.

Development of frequency representation: single animals

Examples of frequency mapping of mice of postnatal days 10–16, 18 and 20 are shown in Fig. 2. The insets to the figures show how isofrequency lines develop. These lines connect points of representation of a certain frequency in a given frontal plane.

At day 9, we could not measure any tone-related responses at the highest available SPLs, although spontaneous activity of neurons in the IC, especially in the ventral part was regularly present.

At day 10, responses to low frequencies, usually in the

range of 3–7 kHz were present in the rostral three quarters of the central nucleus (CN) of the IC (Fig. 2a). Responses could not be detected in the most caudal part of the CN, in the medial part (M) of the CN, the dorsal cortex (DC) and lateral nucleus (LN) of any animal tested. At that age, a tonotopic organization with a regular pattern of isofrequency planes was absent (see inset to Fig. 2a).

At day 11, animals were rather inhomogeneous in that two of them showed a pattern very much like that of 10-day-old mice, two showed a progress in development as indicated in Fig. 2b with highest CFs of about 15 kHz, and another three showed an even more advanced pattern with CFs as high as 24 kHz. At this age, tone responses could be detected in the whole IC (at least in the most advanced animals) except in the medial part of the central nucleus (M) and in the upper layers of the dorsal cortex (DC) where tone responses were absent in all animals. First signs of tonotopy occurred in that a regular increase of CF from dorsal to ventral was present in about the first 800 μ m (400 μ m) depth of the rostral (central) IC. In the caudal IC, neurons had CFs between about 8 and 15 kHz but a regular order of CFs was absent even in the most advanced animals.

The typical day 12 pattern is shown in Fig. 2c. CFs up to about 30 kHz were measurable in the CN of the IC. The M part of the central nucleus was still without tone responses. A regular frequency increase from dorsal to ventral up to a depth of about 1200 μ m was present in most penetrations in the center of the IC at any rostrocaudal position. The most caudal part of the central nucleus was included in the general tonotopic pattern.

At day 13, for the first time tone responses in the M part of the ventral nucleus became detectable in 5 of 8 animals tested (Fig. 2d). However, tone responses did not pertain the whole M but only its dorsal layers. CFs as high as 40 kHz were measured. The regularity of CF increase with increasing depth as seen at day 12 (Fig. 2c) was lost in some penetrations of several mice in the CF range of 15–25 kHz (e.g. penetrations 2, 6, 10 in Fig. 2d). In the mouse shown (Fig. 2d) penetrations only about 200 μ m from the caudal pole were sampled (No. 4, 7, 8) which show that tone responses were not present in the pericentral region of the IC close to its caudal pole at that age.

At day 14, responsiveness of neurons proceeded

Fig. 1. Development of average ($n = 4-6$) collicular size in the mediolateral (a), dorsoventral (b), and rostrocaudal (c) dimension. Values for the right (open circles) and left (closed circles) IC are presented separately. The distance from the caudal pole of the IC (0.1–0.9 mm) is the parameter in a and b. Examples of S.D.'s are shown which are characteristic also of the other means.

Fig. 2. a-f. (For legend, see next page.)

Fig. 2. a-i. Camera lucida drawings of frontal sections with reconstructed electrode tracks from inferior colliculi of mice of various ages. The most caudal section is either about 200 μ m (d), 300 μ m (a-c, e, h, i), or 400 μ m (f, g), the central section about 500 μ m (b-e), or 600 μ m (a, f-h) or 700 μ m (i), the most rostral section about 800 μ m (a-h) or 900 μ m (i) from the caudal pole of the respective IC. Dashes in the electrode tracks indicate recording sites, those without dashes were without response. Tracks without dashes were without response all through the IC. The dashed lines mark borders of subnuclei in the IC which were clearly distinguishable. The insets show the same frontal sections with some isofrequency lines or fields of similar frequencies indicated. CN, central nucleus; M, medial part of the central nucleus; DC, dorsal cortex; LN, lateral nucleus.

further ventralward in the M part of the central nucleus but still did not reach its ventral border in most penetrations (Fig. 2e). In the great majority of electrode tracks a regular and steady increase of CFs from dorsal to ventral was found as indicated by the isofrequency lines (inset). Highest CFs measured at that age were 30-46 kHz.

At day 15 (Fig. 2f), responses to tone bursts could be obtained through the total dorsoventral extension of the

Fig. 3. Schematic dorsal view on the IC. Areas marked I-V are those within which data from different electrode tracks were averaged for the calculation of values in Figs. 4-13 (see text for further description); m, medial; l, lateral; c, caudal; r, rostral.

Figs. 4-7. (For legend, see next page.)

IC including M in most penetrations. The highest CFs detected increased further reaching a maximum of 56 kHz in M (Fig. 2f, penetration 11).

The trend of increasing CFs represented in M was continued at days 16, 18 and 20 reaching maximum values of more than 70 kHz in some animals (Fig. 2g-i). Common characteristics of frequency representation at ages 15–20 days were: (1) Neurons with CFs in the high ultrasonic range (above 45 kHz) were always found in the M part of the central nucleus. (2) Deviation from a regular CF increase from dorsal to ventral occurred in very medial penetrations probably outside the central nucleus (Fig. 2f, penetration 4; Fig. 2h, 3–5) and in tracks through the lateral part of the central nucleus and the lateral nucleus (Fig. 2f, 2, 8, 9; Fig. 2g, 2; Fig. 2h, 2, 9; Fig. 2i, 2, 7).

Development of frequency representation: general patterns

Fig. 2 demonstrates the development of tonotopy in

Figs. 4–8. Average frequencies represented in the inferior colliculus as a function of postnatal age with collicular depths (0.1 mm. . .), from which the frequencies were averaged, as parameter. Fig. 4 shows values from the center of the IC (area I in Fig. 3). Figs. 5–8 show values from areas II–V (Fig. 3). In Fig. 6 (area III) data only from the central nucleus without LN and DC were included, because the border between these nuclei and the central nucleus could often not exactly be delimited. Some characteristic S.D.'s are also shown.

the IC of the mouse only by examples of single animals. To include the mapping data of all animals, we determined average values of CFs per age and recording site in the IC. As explained in Materials and Methods, we placed one penetration in the center of the visible surface of each IC. Since the coordinates of these points differed with regard to the λ -points of the respective skulls, we could not refer the coordinates of electrode tracks of all animals of one age to a common λ -point. Therefore, we calculated the average rostrocaudal and mediolateral coordinates of the central tracks of all ICs separately for each age and used the coordinates of these average IC centers as reference points for comparing the locations of penetrations of different animals of a certain age. Thus, the original coordinates of all penetrations were adjusted to the coordinates of the average center of the IC separately for each age. After this adjustment, we could define areas on the average IC surface within which CF values were averaged. These areas are indicated in Fig. 3. Electrode tracks in areas II and III were at least 400 μ m, and those in areas IV and V 200 μ m from the center (I).

Based on the described procedure, the average frequency representation in the 5 areas of the IC (center, medial, lateral, caudal, rostral) was evaluated as a function of postnatal age. Figs. 4–8 show the results. The figures demonstrate shifts of the average CF as a function of age with collicular depth as parameter. In general, such shifts were from low to high frequencies and increased with increasing distance from the dorsal surface of the IC (depth). Three periods of development became obvious: a first period of CF increase with increasing age for a given depth, a second period of rather constant CFs, and a third period of again increasing CFs which predominantly took place in the deeper layers of the IC, i.e. the medial part of the central nucleus. The first period of increasing CFs started at day 10 and lasted until day 12 or 14 in the medial IC (Fig. 5), until day 14 in the center (Fig. 4), until day 14 or 16 in the lateral (Fig. 6) and rostral IC (Fig. 8), and until day 16 in the caudal part (Fig. 7). After the initial frequency increase, representation of adult CF values was reached already in area III, IV and V for depths of less than 1400 μ m or 1800 μ m. For more ventral regions of the IC, a third period of again increasing CFs followed the second period of rather constant CFs. Such a period was also found in areas I and II (Fig. 4, 5). The third period started at day 16 in the center area (Fig. 4) and at day 14 in the medial area (Fig. 5) for depths of 600 μ m and more. Altogether, we demonstrate clear shifts of characteristic frequencies of neurons with increasing age of the animals in all parts of the IC except for the very dorsal 100–200 μ m. Adult patterns of frequency representation are achieved in

Figs. 9-12. (For legend, see next page.)

Figs. 9–13. Modification of the frequency-place relationship as a function of age. In each figure there are three pairs of functions showing the average most dorsal (filled triangles) and most ventral (filled upside down triangles) points of frequency responsiveness in the IC, the depths of the average lowest (filled squares) and highest (filled circles) frequencies in the IC and the average lowest (open triangles) and highest (open upside down triangles) frequencies, all as a function of postnatal age. Left ordinates (depth) refer to filled symbols, right ordinates (frequency) to open symbols. Data are presented separately for the five areas (I–V) shown in Fig. 3 (Figs. 9–13, respectively).

different parts and dorsoventral locations of the IC at different postnatal days.

Another view on the developmental changes in the IC of the mouse is presented by Figs. 9–13. Three sets of curves are shown in each figure. The first set delimitates the average extension of neurons responsive to tone bursts towards the dorsal and ventral borders of the IC. In the medial and lateral IC (areas II and III, responses in the lateral nucleus disregarded) the ventral border of tone responsiveness remained at a rather constant depth of 1300 μm or 1600 μm respectively over the whole postnatal observation time despite strong daily variations (Figs. 10, 11). These depths mark the average ventral border of the central nucleus in these areas indicating that the ventral part of the central nucleus is responsive to tones right from the beginning of such a responsiveness. Similarly, Figs. 9, 12 and 13 show a rather constant

average ventral border of responsiveness in the center (area I, Fig. 9), caudal (area IV, Fig. 12) and rostral IC (area V, Fig. 13), however, only for the first 13–14 days. After that age, a rapid further ventralward extension of tone responsiveness occurred in these areas, i.e. the M part of the central nucleus became active and the activity moved ventralwards in this nucleus with increasing age (compare Fig. 2).

Looking at the dorsal layers of the IC, tone responses were initially absent in the most dorsal 200–400 μm (Figs. 9–13). Up to postnatal day 14 or 16, tone responsiveness shifted close to the dorsal surface. Obviously, there is a development from ventral to dorsal in the dorsal cortex of the IC.

In addition to the maturation of the extension of tone responsive areas in the IC, Figs. 9–13 demonstrate the development of average highest and lowest CFs and of their average loci of representation in the IC. There is very little change in the lowest CFs from postnatal day 10 onwards. That is, right from the beginning of responsiveness lowest CFs of the adult hearing range are represented in the IC, however, not close to the dorsal margin but in deeper layers. Adult patterns of representation of the low-frequency range seems to be accomplished by day 16 at the latest.

In contrast to low frequencies, the highest recorded CFs increased continuously from day 10 onwards. The slope of increase was about 5 kHz/day in all areas except area III (lateral part of the central nucleus) with about 2.5 kHz/day (Figs. 9–13). During the same time, the places of representation of these highest CFs shifted ventralwards in all areas except area III (Figs. 9–13). This expansion of high frequencies towards the ventral border with increasing age was most pronounced in the center (Fig. 9) and caudal (Fig. 12) and rostral areas (Fig. 13). These are the areas where the M part of the central nucleus is present the responsiveness of which started late (Figs. 2, 9, 12, 13) so that CFs in the high ultrasonic range were not represented before day 14, on the average.

Although CF increases and ventralward extensions of responsiveness of neurons with increasing age run parallel in some areas of the IC (Figs. 9, 12, 13) the two phenomena must not necessarily be coupled in their origin because clear CF increases can be demonstrated for neurons at constant dorsoventral coordinates in the IC (Figs. 4–8), and CF increases are observed in the absence of a shift of the locus of representation of highest CFs (e.g. area I from 15 days onwards, Fig. 9; area III, Fig. 11).

The lateral nucleus

Most of the data presented, especially those shown in

Figs. 4–13, are related to the central nucleus, its medial part and the dorsal cortex of the IC. Since we did not regularly sample the lateral nucleus (LN) in its total dorsoventral extension, we cannot give quantitative results on the development of frequency representation in this nucleus.

We did not record tone responses in the LN of 10-day-old animals (Fig. 2a). First responses were seen at day 11 (Fig. 2b) with an average lowest CF of 9.1 kHz and highest CF of 12.6 kHz ($n = 4$). Average lowest CFs remained between 4.2 and 6.4 kHz at all postnatal ages, average highest CFs increased to 24.8 kHz at day 14 remained rather constant up to day 16 (26.6 kHz) and showed a final increase to average 29.9 and 29.1 kHz at day 18 and 20 respectively. Only in one mouse (Fig. 2i), frequencies higher than 35 kHz were recorded at a place probably belonging to the lateral nucleus.

Differences between NMRI mice and hybrids

All the reported dynamics of frequency representation in the IC were found equally in both groups of mice. Qualitative differences were not observed in any of the measurements made or relationships described. The only quantitative difference found concerns the highest CF recorded at a given age. There were no significant differences of highest CFs between the two groups of mice at any single postnatal day. However, if we compare the highest CFs obtained in all 36 NMRI mice with those of the 25 hybrids on a daily basis and sum up the differences, then we find that altogether hybrids have significantly higher CFs than the NMRI strain ($P < 0.01$, sign test).

DISCUSSION

Collicular size

The present results demonstrate dynamics of frequency representation in the IC of the mouse after inception of hearing. The observed development of tonotopy takes place on the background of a constant collicular size. It is remarkable that the volume of the IC does not change in the period between postnatal days 9 and 20 during which the dorsal and ventral cochlear nucleus of mouse and rat dramatically increase in size^{14,72}. The auditory midbrain is obviously faster than auditory brainstem nuclei in reaching the adult external shape, at least in the mouse. This does not mean that the mouse IC is mature with regard to the internal organization at postnatal day 9. Growth and differentiation of neurons, synaptogenesis and myelination most probably continue within the IC after first responses to sounds can be observed^{41,64}. However, too little is known about postnatal anatomical changes in the IC to discuss possible

consequences of such changes with regard to auditory function.

In the following discussion of tonotopic development, therefore, we shall assume that the basic properties of cellular arrangement and dendritic lamination in the IC of the young mouse (9–20 days old) do not differ substantially from those shown for adults^{39,74}. This assumption is supported by data from kittens obtained during comparable periods showing that the organization of dendritic trees of neurons in laminae is present at birth²⁴ and adult orientation is reached already after the first postnatal week³⁸.

Tonotopic development

The first responses occurred at day 10 in the rostral and central parts of the central nucleus sparing its caudal part, very medial portions, the medial part (M) of the central nucleus, the dorsal cortex and the lateral nucleus (Fig. 2a). After the onset of responsiveness a series of changes of frequency representation took place.

(1) *Central-to-caudal and medial gradient of responsiveness.* Responsiveness to tone burst (Fig. 2a) and first signs of a regular tonotopy (Fig. 2b) started in the central and rostral part of the central nucleus and proceeded caudalwards and medialwards. The pericentral nucleus near the caudal pole of the IC was still unresponsive at day 13 (Fig. 2d).

(2) *Ventral-to-dorsal gradient of frequency responsiveness.* At postnatal days 10 and in most animals also at day 11 the dorsal cortex (DC) of the IC as defined by Willard and Ryugo⁷⁴ excluding layer IV of the DC (nomenclature of Meininger et al.³⁹) was without tone response (Fig. 2a, b). Responsiveness proceeded dorsalwards as shown in Figs. 9–13. Since lowest characteristic frequencies (CFs) were represented most dorsally in the IC of the adult mouse^{68,69} and average lowest CFs did not change during development (Figs. 9–13), there was a ventral-to-dorsal expansion of frequency responsiveness in general and of lowest CFs of the mouse hearing range in the dorsal layers of the IC.

(3) *Dorsal-to-ventral gradient of frequency responsiveness.* The medial part (M) of the central nucleus remained unresponsive to tone bursts up to day 13 (Fig. 2a–c). Then responsiveness shifted ventralwards and reached the ventral border of M at about day 15 (Figs. 2d,e, 9, 12, 13). This developmental pattern shows that an anatomically distinct nucleus, the medial part of the central nucleus, became responsive to sound later than the central nucleus and the dorsal cortex and has its own dorsal-to-ventral progression of activity. In gerbils, a dorsal-to-ventral progression of responsiveness in the ventral IC within 3 postnatal days (16–19) was also observed⁶³.

(4) *Increase of characteristic frequency.* Figs. 9–13 demonstrate almost constant increases of CFs in all parts of the IC from the beginning of responsiveness up to 20 days of age. At that age, highest CFs reached are comparable to those measured in adult mice of our strains^{68,69}. Such expansions of the CF-range towards higher frequencies were observed in all mammalian midbrains from which respective recordings exist (mice^{66,73}, bats^{10,59}, gerbil⁶³). This feature, therefore, seems to be a general one of auditory maturation.

(5) *Shift of frequency representation.* The progression of frequency responsiveness ventralwards in the M nucleus and the increase of CFs with increasing age does not mean that the CF-increase is accomplished just by the initiation of activity in more and more ventral parts of the IC as found by Schäfer and Rübsamen⁶³. Our present data (Figs. 2a–i, 4–8) and those of cats² demonstrate a clear shift of CF from low to high for a given constant depth (dorso-ventral position) in the IC. Such a shift is seen for all depths of more than about 400 μm . That is, the frequency-place relationship in the IC, including the central nucleus and its medial part has dynamic properties. Neurons at a given place first respond best to rather low frequencies and then to increasingly higher frequencies. This is an important result because it shows that the dendritic laminae and ascending fibers in the central nucleus of the IC^{39,43,45,51,74} are tuned to different frequencies at different postnatal days. Whether this has consequences for auditory perception will be discussed below.

Possible causes of the tonotopic development

Studies using electrical stimulation of the auditory nerve in mammals have shown that the whole afferent auditory pathway is functioning well before sound entering the ear can elicit responses from the VIIIth nerve^{44,56}. Thus it appears that mechanisms centrifugal of auditory nerve fibers are responsible for the onset of hearing and probably for many developmental changes observed thereafter. A large variety of such peripheral maturational changes that can be related to the onset of hearing and the improvement of auditory perception have been described for the mouse and other mammals (for reviews see^{19,50,52,56,57,65}). In the context of the present study we shall refer only to those factors which may contribute to the measured gradients and shifts in the collicular response development.

The central-to-caudal and medial progression of responsiveness may be explained at least in part by a gradient of increasing response thresholds of collicular neurons in these directions. Present recordings (Ehret and Romand, to be published) and a study in adult mice⁶⁷ showed that neurons with lowest thresholds (highest

sensitivities) to tone bursts were located in the center of an isofrequency plane. Neurons in the caudal and medial parts of the same isofrequency plane have up to about 50 dB higher response thresholds. This means that in 10- and 11-day-old animals the response threshold of those medial and caudal neurons may not be reached with the SPLs available because thresholds in the center of the IC are already higher than 85 dB at day 10 and higher than 50 dB at day 11.

The ventral-to-dorsal gradient of frequency responsiveness in the dorsal cortex and the dorsal-to-ventral gradient in the medial part of the central nucleus may have a common origin in the maturation pattern of the cochlea. Anatomical studies on the development of the mammalian cochlea^{3,8,9,31,48,49,54,55} including the mouse^{4,5,29,40,58} showed that structural maturation proceeds from the basal coil towards the apex. Besides this very general pattern, another trend was reported by authors who differentiated maturation within the basal cochlear coil (studies in man⁹, cat^{37,54}, mouse and rat^{11,12,40} opossum³¹). They found that cochlear structures including hair cells and myelination of spiral ganglion cells develop from the basal or mid-basal turn also towards the hook region at the absolute base. Since cochlear maturation in the mouse is not complete before day 14^{6,29,40} and for some structural details continues even further³⁰ until at day 18 the last mitoses are seen in the region of the organ of Corti⁵⁸, we may relate the expansion of responsiveness dorsalward in the dorsal cortex and ventralward in the medial part of the central nucleus to the described cochlear maturation. Cochlear nerve fibers innervating the apical and hook region may start later with sound-related activity than those from the other regions. Therefore, representation sites of very low and very high frequencies in the adult IC, the dorsal cortex and M nucleus, may not receive appropriate input activity from their respective tonotopically related cochlear places as early as the central nucleus. The delay is 1–2 days for the dorsal cortex and 2–3 days for the M nucleus. It is interesting to note that in mice the adult arrangement of hair cells is seen earlier near the apex compared with the base¹² which corresponds well with the observed difference of delays of responsiveness between DC and M nucleus. Since the absolute speed of maturation varies dramatically (differences of more than 10 days were reported) even among different strains of mice or rats^{11,12} valid comparisons of anatomy and physiology for this parameter may be allowed only among animals of the same strain.

In order to explain the constant increase of highest CFs represented in the IC (Figs. 9–13) and the shift of frequency representation at a given locus across dendritic laminae (Figs. 4–8) further developmental processes

within the cochlea have to be considered. The first concerns a medial-to-lateral gradient of hair cell maturation. That is, inner hair cells and their relationship to the tectorial membrane and to efferent and afferent nerve fibers appear mature earlier than outer hair cells of the first row which in turn precede those of the third row in mice and rats^{5,29,34}. Similar results exist for other mammals (see refs. 52,53). Thus one could assume that during periods of immaturity of outer hair cells they would contribute less to sound encoding in the auditory nerve than in adult animals and one would record only high-threshold low-frequency responses⁵³. Recordings from auditory nerve fibers revealed high thresholds and broad tuning in the low-frequency range (tail of tuning curve) without the sharply tuned and most sensitive tip region at higher frequencies if these fibers originated at places with outer hair cell damage³⁵. Taking this evidence together, cochlear function may start in the basal turn without major contributions of outer hair cells and thus generating high thresholds and shallow tuning in VIIIth nerve fibers and a restriction of response ranges to frequencies lower than would be appropriate for the basal coil of the adult cochlea. This high-threshold low-frequency response is sent according to the topography of cochlear projections to high-frequency auditory regions of the brain. The delayed maturation of outer hair cells thus leads to responsiveness to comparably low frequencies at places in the IC which, in adult animals, would respond best to higher frequencies at lower thresholds.

This lateral gradient of maturation could explain several of our present observations: First, the continuous increase of highest CFs represented in the IC by the appearance of high-frequency tuning-curve tips in neurons at more and more ventral loci of the central nucleus and its medial part. Second, the discrepancy between the depth of the most ventral frequency response and the depth of the highest CF measured (Figs. 9–13). If differences exist between these two measures the position of the most ventral frequency response is always deeper than that of the highest CF. Since in adult mice both measures coincide^{68,69} one can argue that neurons close to the ventral border of responsiveness in the IC have not yet developed their appropriate high CF because place-adequate cochlear tuning is missing and, therefore, neurons situated more dorsally in the IC receiving farther developed cochlear output can have higher CFs than those at a more ventral position.

Third, the shift in frequency representation at a given collicular depth (Figs. 4–8). If the CF of a cochlear nerve fiber is increased by increasing influences of outer hair cells on its tuning curve so that the tip is added to the tail, neurons in higher auditory centers receiving projections

of this nerve fiber would show comparable CF increases. This is what our present data on the IC of mouse show. Comparable changes of tonotopic maps have been reported before for the cochlear nucleus⁶⁰ and lateral superior olive⁶² of the gerbil and the IC of the cat^{1,2} and bat¹⁰.

A change of frequency representation in the IC could also be predicted if developmental changes of traveling wave displacement would lead to related shifts of tonotopy in the cochlea. It is known that the mechanical properties of the basilar membrane and the organ of Corti of the mouse show significant changes that continue up to at least postnatal day 15^{11,30}. Whether such changes in mechanical properties not directly related to the actions of outer hair cells might contribute to shifts of tonotopy as measured in the developing cochlea of the gerbil^{7,16,26,75} is not yet clear. As stated before, changes in cochlear tonotopy could be explained by the lateral gradient of maturation related mainly to outer hair cells.

Consequences of tonotopic development for tone perception

If the neuronal activity induced by a given tone frequency at a certain place of the tonotopic map of an auditory center leads to the sensation of a related pitch, that is if pitch has a place code, then tones must sound different during the development of hearing. This has to be postulated from the developmental changes of frequency representation as presently discussed for the IC. Young animals soon after the inception of hearing should perceive mid-frequencies of their hearing range as high pitched tones because such frequencies are first represented in high-frequency areas of the auditory pathway. This is exactly what happened in a psychophysical test with rats. Hyson and Rudy²⁸ used 8 kHz tone bursts to condition 15- and 18-day-old rats. Frequency generalization of the conditioned response was tested 0.5 and 72 h later. Rats of 15 days old, whose auditory system is still developing^{15,33}, showed strongest responses to the conditioned tone of 8 kHz 0.5 h later; 75 h later, however, they responded most to 12 kHz. Three days after conditioning, the rats obviously perceived 12 kHz as equivalent to 8 kHz. If 18-day-old rats were conditioned, that is at the end of the auditory development, no such shift of frequency perception occurred when they were tested at day 21.

This test has two interesting consequences. It shows that young animals during the period of auditory development, have a different sensation of the sound quality compared with adults and that frequency perception is related to a place code of frequency representation in the auditory pathway. In other words, the place of neural activity for example on the tonotopic map of the inferior colliculus, is decisive for the valuation of the tonal quality not the frequency of the sound input into the ear.

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