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Time of Origin of Neurons of the Rat Inferior Colliculus and the Relations Between Cytogenesis and Tonotopic Order in the Auditory Pathway

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Summary. Groups of pregnant rats were injected with two successive daily doses of ^{3}H-thymidine from gestational day 12 and 13 (E12+13) until the day before parturition (E21+22) in order to label in their embryos the proliferating precursors of neurons. At 60 days of age the proportion of neurons generated (or no longer labelled) on specific embryonic days was determined quantitatively in six vertical strips of the inferior colliculus. It was established that the neurons of the inferior colliculus are produced between days E14 and the perinatal period in an orderly sequence: the earliest generated cells are situated rostrally, laterally and ventrally in the principal nucleus, the latest generated cells are situated caudally, medially and dorsally in the pericentral nucleus. This cytogenetic gradient suggested that the cells are produced dorsally in the caudal recess of the embryonic aqueduct and are deployed in an "outside-in" pattern.

This study has brought to a conclusion our datings of neuron production in the central auditory pathway of the rat. The results revealed that in those structures in which a cytogenetic gradient could be recognized, the orientation of this gradient and the regional tonotopic order (demonstrated mostly in species other than the rat) tended to be aligned. Moreover, with the exception of the medial trapezoid nucleus and the dorsal nucleus of the lateral lemniscus (which receive contralateral input from the cochlear nuclei), sites with early-produced neurons correlated with units responding preferentially to high frequency tones and vice versa. This suggested that the orderly production of neurons within different components of the auditory system is a factor in their subsequent topographic organization. A comparison of the temporal order of neuron production in different components of the auditory pathway suggested that the establishment of orderly topographic relations between some of the structures (e.g., the medial geniculate body and the primary auditory cortex) takes place before this spatial relationship could be specified as a cochleotopic order.

Key words: Inferior colliculus – Neurogenesis – Thymidine-radiography – Auditory system development

In previous studies dealing with the development of the brain stem and the diencephalon of the rat we have determined the birth dates of neurons in the following components of the central auditory pathway: the cochlear nuclei, the superior olivary nuclei, and the trapezoid nuclei (Altman and Bayer 1980a), the dorsal and ventral nuclei of the lateral lemniscus (Altman and Bayer 1980b), and the medial geniculate body (Altman and Bayer 1979a). In these, as in other studies of the series, we have utilized thymidine-radiography with the progressively delayed cumulative labelling procedure – a technique that permits the exact specification of the proportion of neurons produced (no longer labelled) on a particular embryonic day. These investigations have revealed an unexpected precision in the temporal order of neuron production throughout the central nervous system. This raised the question whether or not this precise chronology of neurogenesis is a factor in the establishment of orderly connections in the central nervous system.

The present study is concerned with neurogenesis in the inferior colliculus. It completes our datings of the time of origin of neurons in the major subcortical components of the auditory pathway in the rat. This provides us with the opportunity to examine to what extent the temporal order of neuron production in the auditory system might be a mechanism in pro-

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moting the establishment of its precise topographic (cochleotopic or tonotopic) organization.

The morphological organization of the inferior colliculus has been extensively investigated in the cat (Rockel and Jones 1973a, b, c) and man (Geniec and Morest 1971). Rockel and Jones distinguished three components: the external nucleus, the pericentral nucleus and the central nucleus, and subdivided the latter into the larger principal (or ventrolateral) and the smaller dorsomedial nuclei. In the principal nucleus the cells, dendrites, and axons are arranged in a lamellar series of curved shells; here terminate most of the fibers of the lateral lemniscus. Afferents have been traced to this region from the contralateral dorsal and ventral cochlear nuclei (Woolard and Harpman 1940; Barnes et al. 1943; Fernandez and Karapas 1967; Osen 1972; Beyerl 1978), the contralateral dorsal nucleus of the lateral lemniscus, the ipsilateral ventral nucleus of the lateral lemniscus, and a bilateral projection from the lateral superior olive (Adams 1979). All these projections appear to be topographically organized (Osen 1972; Beyerl 1978; Adams 1979). The other nuclei of the inferior colliculus appear to receive primarily cortical and commissural afferents (Rockel and Jones 1973c). Physiological studies have established that in the central core of the inferior colliculus there is a continuous tonotopic representation with high frequencies dorsomedially and low frequencies ventrolaterally (Rose et al. 1963; Aitkin et al. 1970; Clopton and Winfield 1973; Merzenich and Reed 1974; FitzPatrick 1975; Roth et al. 1978). The tuning curves in the pericentral nucleus are very broad, and neurons of the dorsomedial nucleus are not activated by tones (Merzenich and Reid 1974).

Methods

Purdue-Wistar pregnant rats were injected subcutaneously with two successive daily doses of ^{3}H-thymidine (specific activity, 6.0 c/mM; dose, 5 μc/g body weight) between 9:00–11:00 a.m. on the following gestational ages E12+13, E13+14 . . . E21+22. (The day of sperm positivity was counted as E1.) The progeny of at least two dams/injection group were killed at the constant postnatal age of 60 days by cardiac perfusion with 10% neutral formalin. The brains were embedded in paraffin, sections were cut at 6 μm serially in the 3 planes and every fifteenth section was saved. Successive sections were stained with cresyl violet and hematoxylin-eosin for examination without nuclear emulsion or were prepared for autoradiography. The latter procedure has been described elsewhere (Altman 1964). Briefly, deparaffinized sections were coated with Kodak NTB-3 emulsion in the dark, exposed for 90 days with a dessicant, developed with D-19, and stained with hematoxylin-eosin.

Sagittal sections from 6 male rats per injection group were used for quantitative purposes; the additional material was used for qualitative assessments. The number of labelled and unlabelled neurons was counted at three sagittal planes (lateral, intermediate and medial) in an anterior and a posterior level in strips traversing the inferior colliculus from dorsal to ventral. In all instances a minimum of 100 (up to several hundred) cells were classified in every strip in each animal. The estimation of the proportion of cells differentiating (ceasing to divide) on a particular day was based on the progressively delayed comprehensive labelling procedure. The rationale of this procedure is that as long as virtually all the cells of a selected brain region can be labelled (in the populations studied here nearly 100% of labelling can be accomplished with two successive daily injections) all the cells are considered to be primitive precursors that have not started to differentiate. When with delayed onset of injections all cells can no longer be tagged, the proportion of cells that can no longer be labelled as a result of a single day delay is taken to be the complement that differentiated on the previous day. As an example, the cells arising on day E15 are determined as follows: E15 = (E15+16) – (E16+17).

Previous examination of our quantitative results indicated that the sequence of neuron generation in varous nuclei was often more clearly indicated in the data from individual animals than in the pooled data. This apparent variability between animals might be due to differences in the exact age of the pooled individuals or their exact developmental stage (differences were often noted within littermates). Accordingly, we employed a statistical procedure, the sign test (Conover 1971), to determine the consistency of sequential neuron production in pairs of inferior collicular strips regardless of the chronological grouping of the individual animals. This test is based on paired comparisons (X, Y) within individual animals. The comparisons are grouped into 3 categories: (1) $X > Y$, "–" comparison; (2) $X < Y$, "+" comparison; (3) $X = Y$, "0" comparison. The zero comparisons are discarded and, depending on the total number of remaining "–" and "+" comparisons, either a binomial distribution or a normal approximation is used to calculate probabilities (p's).

Results

The core of the inferior colliculus of the rat is occupied by the ventrolateral part of the central nucleus, the principal nucleus (Fig. 1A). The cells of the principal nucleus are mostly of two types: one is small in size and round-to-oval in shape, the other large and multipolar (Fig. 2C). The packing density of cells of the principal nucleus is relatively low. The dorsomedial part of the central nucleus (Fig. 2B) is transitional in cell composition in relation to the principal nucleus ventrally, the pericentral nucleus medially (Fig. 2A), and the external nucleus laterally (Fig. 2D). The pericentral nucleus has a high proportion of small cells with little cytoplasm, the granule cell type, and the external nucleus has many small spindle-shaped cells. Posteriorly (Fig. 1B) the principal nucleus disappears and boundaries between the external, dorsomedial and pericentral nuclei become unclear.

The cells of the inferior colliculus are produced late with respect to the underlying tegmentum (Altman and Bayer 1980c) and cytogenesis continues for several days after cell production has ceased in the

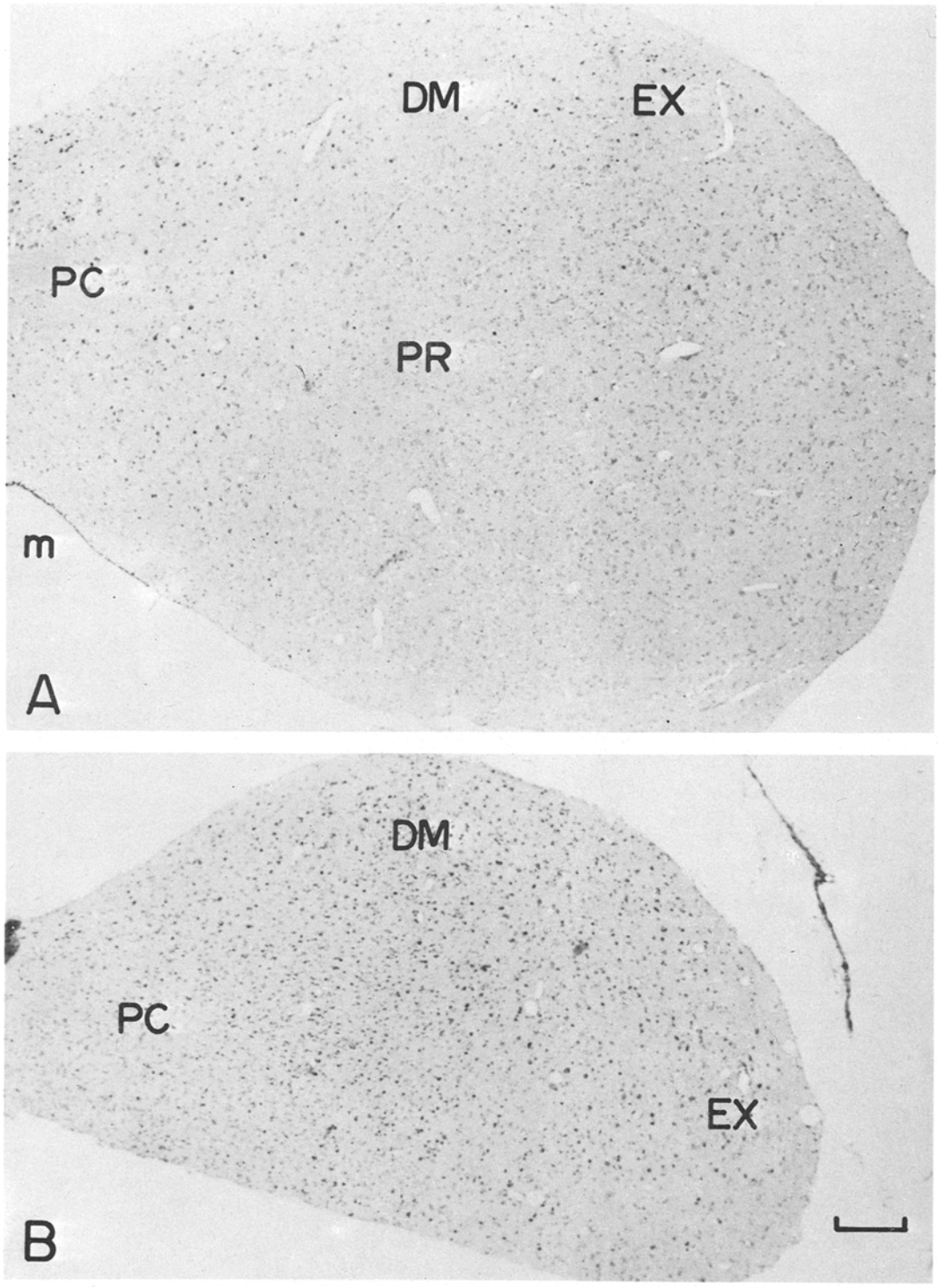

Fig. 1A, B. Thymidine-radiograms of the inferior colliculus from an adult rat in which proliferating cells were tagged on days E17+18. Coronal sections from a rostral (**A**) and a caudal (**B**) level. Scale: 200 μm

superior colliculus (unpublished observations). Within the inferior colliculus differences were noted in cell labelling in relation to cell type and cell position. In general, the earliest group of neurons that ceased to be labelled were the large and small cells of the principal nucleus. All of these cells were labelled in the day E15+16 injection group; some were no longer labelled in the day E16+17 group

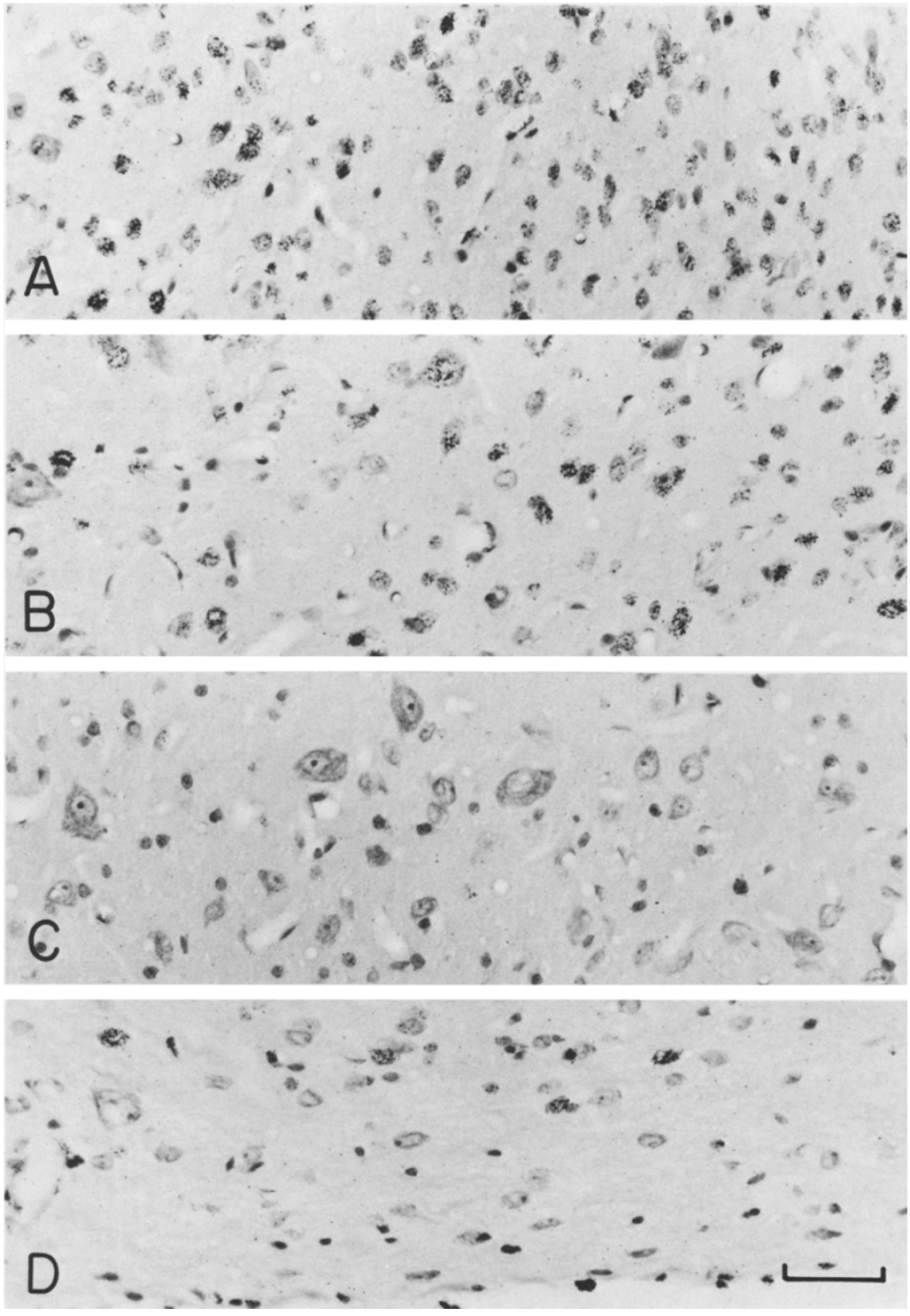

Fig. 2A–D. Labelling pattern of neurons in different regions of the inferior colliculus from a rat tagged on days E17+18. **A** pericentral nucleus; **B** dorsomedial nucleus; **C** principal nucleus; **D** external nucleus. Scale: 50 μm

(Fig. 3) and very few were labelled in the E17+18 group (none as seen in Fig. 2C).

This suggested that the two typical cells of the principal nucleus are generated between days E16 and E17. However, essentially all the cells were labelled in the E17+18 group in the pericentral nucleus (Fig. 2A) except ventrally; many of the cells labelled in the dorsomedial nucleus, including some

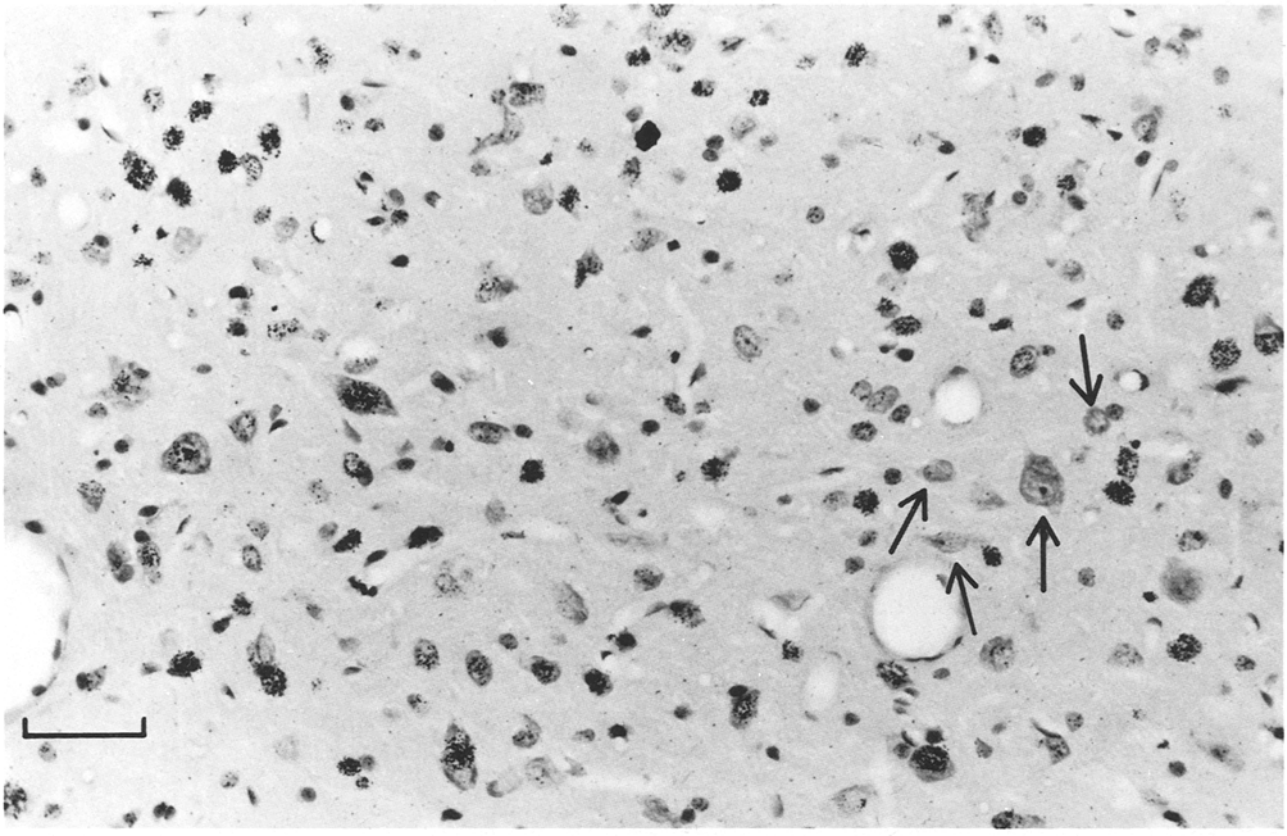

Fig. 3. Some unlabelled large and small neurons (arrows) in the principal nucleus. From a rat tagged on days E16+17. Scale: 50 μm

large cells (Fig. 2B); and many cells were labelled in the external nucleus (Fig. 2D), except again ventrally. The concentration of labelled cells remained high in the day E18+19 group in the pericentral nucleus (Fig. 4A), the dorsomedial nucleus (Fig. 4B) and the external nucleus, except in the ventral aspects of these nuclei. These observations suggested that in addition to differences in the time of origin of neurons in the different subdivisions of the inferior colliculus there is a complex cytogenetic gradient: from lateral to medial, from rostral to caudal, and from ventral to dorsal. Cell labelling persisted in the group injected on days E21+22. The labelled cells were seen in the pericentral nucleus caudally and ventrally in the vicinity of the inferior collicular recess of the aqueduct adjacent to the anterior cerebellar vermis (Fig. 4). The latest-generated cells were typically small granule-type neurons.

Because of the uncertain boundaries of the pericentral, dorsomedial and the external nuclei, cell counts were made in vertical strips in matched sagittal sections in three planes (lateral, intermediate, and medial) and at two levels (anterior and posterior), as shown in Fig. 5. The lateral strips (Fig. $5A_1$) traversed essentially through the anterior and posterior aspects of the central nucleus; the medial strips (Fig. $5C_1$) through the anterior and posterior parts of the pericentral nucleus. The results indicate significant differences between anterolateral and posterolateral strips (Fig. 5A; $p < 0.049$), anterior intermediate and posterior intermediate strips (Fig. 5B; $p < 0.0001$), and anteromedial and posteromedial strips (Fig. 5C; $p < 0.0001$). The differences between individual strips at lateral, intermediate and medial levels were all significant ($p < 0.0001$). This demonstration of a rostral-to-caudal and a lateral-to-medial gradient in the inferior colliculus indicates that this tectal structure is produced in an outside-in pattern in relation to the inferior collicular recess of the aqueduct (Fig. 6). In addition to these gradients we observed a clear ventral-to-dorsal gradient throughout the inferior colliculus, suggesting a dorsal locus in the posterior aqueduct for the production of cells of the inferior colliculus.

Discussion

In preceding publications (Altman and Bayer 1979a, 1980a, b) we determined the time of origin of

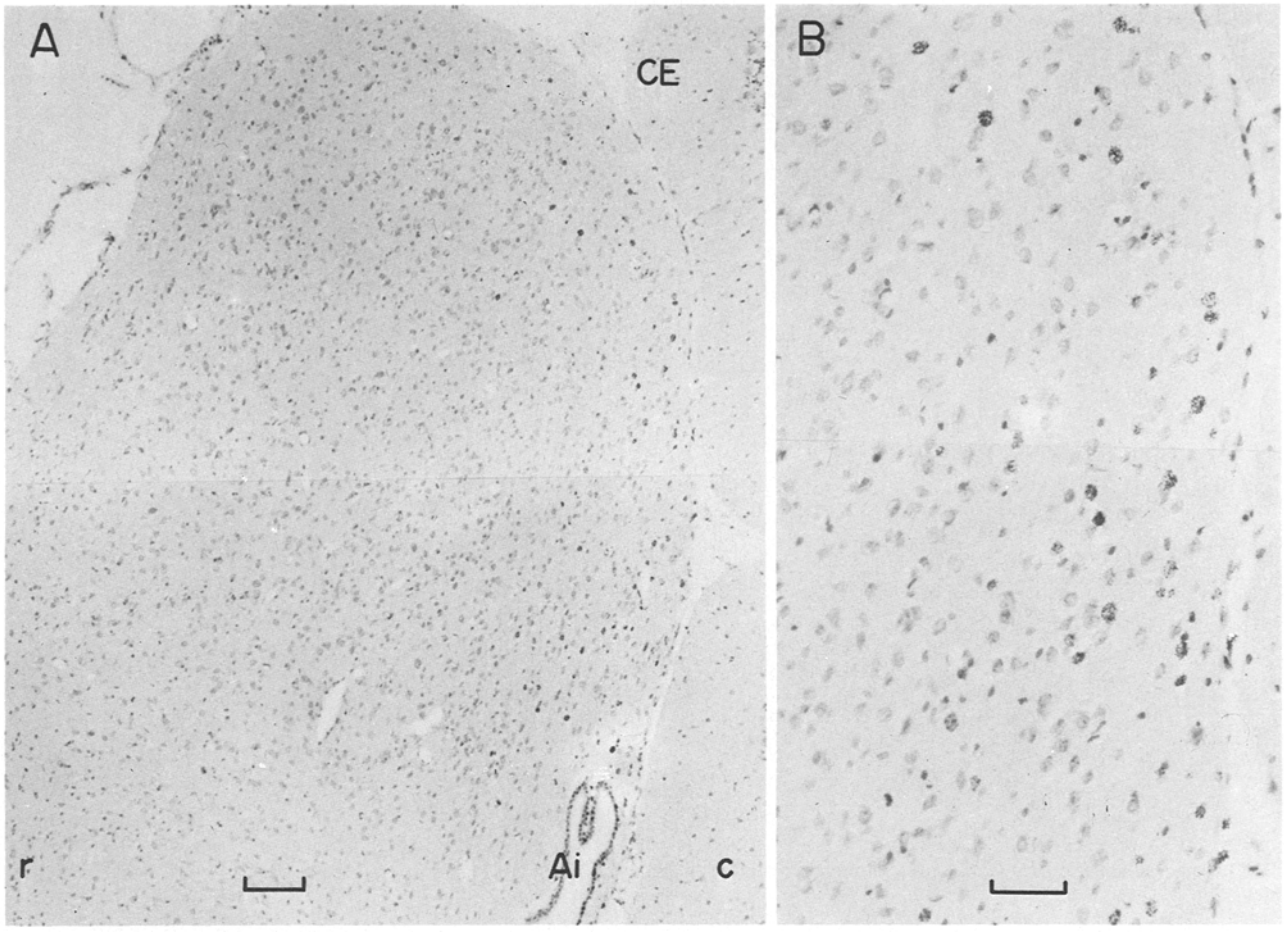

Fig. 4. A Labelled neurons in the caudal aspect of the inferior colliculus in a medial sagittal section (pericentral nucleus); from a rat tagged on days E21+22. **B** Region of labelled cells at higher magnification. Scales: **A** 100 μm, **B** 50 μm

neurons in 10 discrete components of the auditory pathway (Fig. 7). Materials from the same animals were used in these studies with a thymidine-radiographic procedure that permits the specification of the exact proportion of cells generated (no longer labellable) on specific embryonic days. The results indicate considerable differences in the dates when neurons are produced in different auditory structures and in the time span of cell production. The earliest produced central auditory region is the lateral trapezoid nucleus (described variously as the ventral trapezoid nucleus, retro-olivary nucleus or medial periolivary nucleus; see Altman and Bayer 1980a) which is one of the sites of origin of the efferent olivocochlear bundle (Rasmussen 1960; Shute and Lewis 1965; Brown and Howlett 1972). The latest forming structure is the inferior colliculus with the exception of the granule cells of the cochlear nucleus which are produced last, through the early days of the postnatal period (Altman and Das 1966). We have not investigated in this study the time of origin of neurons of the primary auditory cortex: available studies in the rat (Berry and Rogers 1965; Hicks and D'Amato 1968; Bisconte and Marty 1975) indicate that neuron production in the cerebral cortex in general spans the period between days E15 and E21.

The heterogeneous chronology of neuron production in the different components of the auditory system is undoubtedly related to their origins in different sites of the embryonic neuroepithelium, extending from the region of the fourth ventricle to the lateral ventricle. The differences in the duration of cell production may at least partly be related to differences in the cytological heterogeneity of the various structures; for instance, we found that in the dorsal cochlear nucleus, where cell production begins on day E12 and continues until the early postnatal period, the cells of different layers are produced at different times (Altman and Bayer 1980a).

At first glance it should appear unlikely that, with such a varied chronology of cell production, the time of origin of neurons in the different auditory brain

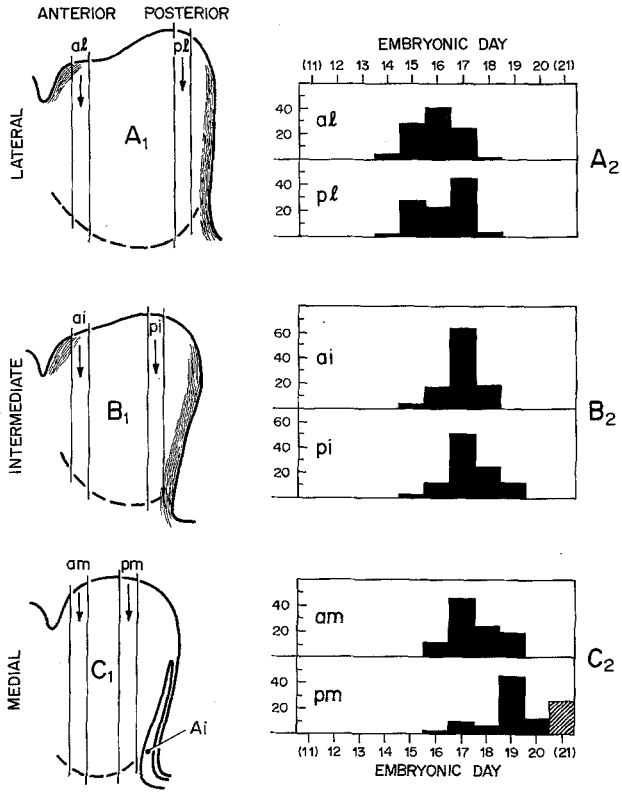

Fig. 5. Proportion of neurons generated (no longer labellable) on specific embryonic days in six vertical strips traversing the inferior colliculus. Abbreviations: al, anterior lateral; pl, posterior lateral; ai, anterior intermediate; pi, posterior intermediate; am, anterior medial; pm, posterior medial

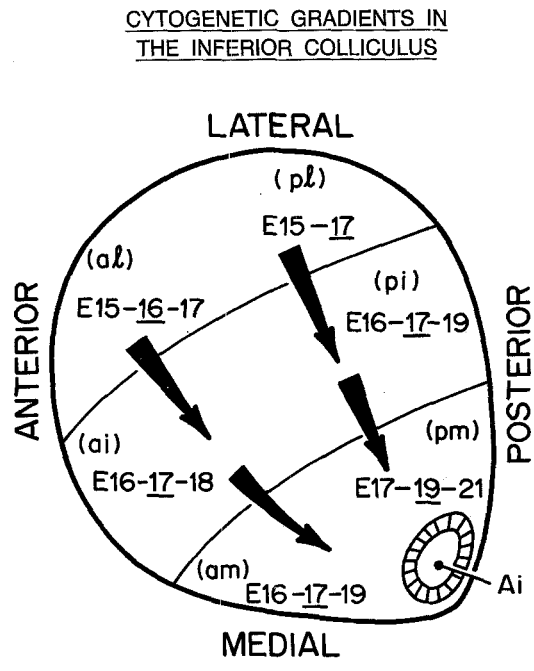

Fig. 6. Dorsal view of the inferior colliculus. Arrows indicate the cytogenetic gradients, based on data presented in Fig. 5; the gradients point to a neuroepithelial source in the posteromedial aspect of the nucleus and an outside-in pattern of cell settling. Underlined days represent times of peak cell production

structures could be a determinant of the cochleotopic or tonotopic arrangement of cells and their orderly alignment throughout the neuraxis. But we shall present the argument that the precise timing of neuron production is not only the first step but an important factor in the topographic organization of the auditory pathway.

Time of Origin of Neurons in the Auditory System in Relation to Tonotopic Organization

Tonotopic, or cochleotopic, organization is present in most components of the central auditory pathway (Fig. 8B). In all three nuclei of the cochlear nuclear complex, with minor species differences, cells responding best to low frequency tones (or receiving fibers from the apical turn of the cochlea) are situated ventrolaterally, and those responding best to high frequency tones (or receiving fibers from the basal cochlea) are situated dorsomedially (Rose et al. 1960; Powell and Cowan 1962; Webster 1971; Moskowitz and Liu 1972). In the medial superior olivary nucleus (Goldberg and Brown 1968) and in the lateral superior olivary nucleus (Tsuchitani and Boudreau 1966) high-to-low frequencies are represented roughly ventral-to-dorsal. In both the ventral and the dorsal nuclei of the lateral lemniscus high-to-low frequencies are also represented ventral-to-dorsal (Aitkin et al. 1970). Tonotopic representation in the inferior colliculus was first studied in detail by Rose et al. (1963) in the cat and their results were subsequently confirmed in the rabbit (Aitkin et al. 1972), rat (Clopton and Windfield 1973) and monkey (FitzPatrick 1975). A recent detailed investigation in the cat (Merzenich and Reid 1974) indicated that in the central nucleus high frequency is represented posteroventrally and low frequency anterodorsally in strips or laminae crossing the entire mediolateral width of the inferior colliculus. The neurons in the dorsomedial and external nuclei were difficult to drive with simple tones and had a different tonotopic organization. In the principal nucleus of the medial geniculate body high-to-low frequencies are represented lateral-to-medial (Aitkin and Webster 1971). Finally, in the primary auditory cortex (AI) a tonotopic organization has been demonstrated in the cat (Hind et al. 1960; Goldstein et al. 1970), the

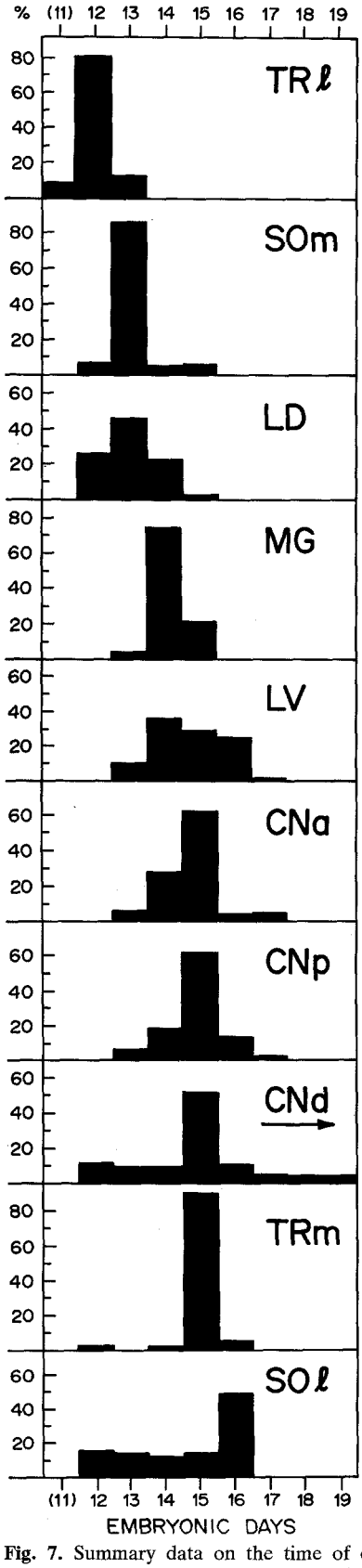

Fig. 7. Summary data on the time of origin of neurons in 10 components of the auditory pathway. (Based on data in Altman and Bayer 1979a, 1980a, 1980b.) Compare with Fig. 5

monkey (Woolsey and Walzl 1944; Walzl 1947; Merzenich and Brugge 1973), and the rabbit (Galli et al. 1971). A recent study in the cat (Merzenich et al. 1975) describes a high-to-low frequency order from rostral-to-caudal in the koniocortex of the primary auditory area. Different frequencies occupy three dimensional strips across and through the depth of the cortex, similar to that described for the inferior colliculus (Merzenich and Reid 1974) and also implicated for the medial geniculate body (Aitkin and Webster 1971).

Tonotopic organization throughout the nuclei of the auditory pathway implies that the spatial order of the spinal ganglion cells along the cochlea is maintained throughout the neuraxis from the level of the medulla to the cerebral cortex. There are several conceivable morphogenetic mechanisms that could assure the segregation of auditory afferents through many synaptic junctions both ipsi- and contralaterally. The purpose of this series of studies was to examine the possible role of the precise temporal order of neurogenesis in the auditory system in the establishment of this spatial order. We have asked two specific questions. First, is the temporal order of cell production within the different auditory nuclei relatable to their tonotopic organization. If this were so, it would support the hypothesis that it is the order in which cells are produced, and presumably arrive at their settling sites, that determines which contingent of auditory afferents will innervate them. The second question was whether components of the auditory nuclei are produced in a sequential order. If this were the case, it would be possible that the topographic order is impressed serially on successively produced nuclei by those already so specified. The results we have obtained partially support both hypotheses but they also point to some important qualifications and modifications.

In Fig. 8A small arrows represent the alignment and directions of cytogenetic gradients in the components of the auditory system, and in Fig. 8B their tonotopic order. The cytogenetic arrows point from the loci occupied by early-produced cells toward the loci of late-produced cells; the tonotopic arrows arbitrarily point from high frequency regions to low frequency regions. It may be seen that the alignment of tonotopic gradients is roughly in register in all the structures for which we have cytogenetic gradients. The exceptions are the cochlear nuclear complex, and the ventral nucleus of the lateral lemniscus, in which we were not able to establish a cytogenetic gradient, and the small medial superior olivary nucleus. In the primary auditory cortex the tonotopic order is from rostral-to-caudal, and there is similar cytogenetic gradient in the cortex of the rat (unpub-

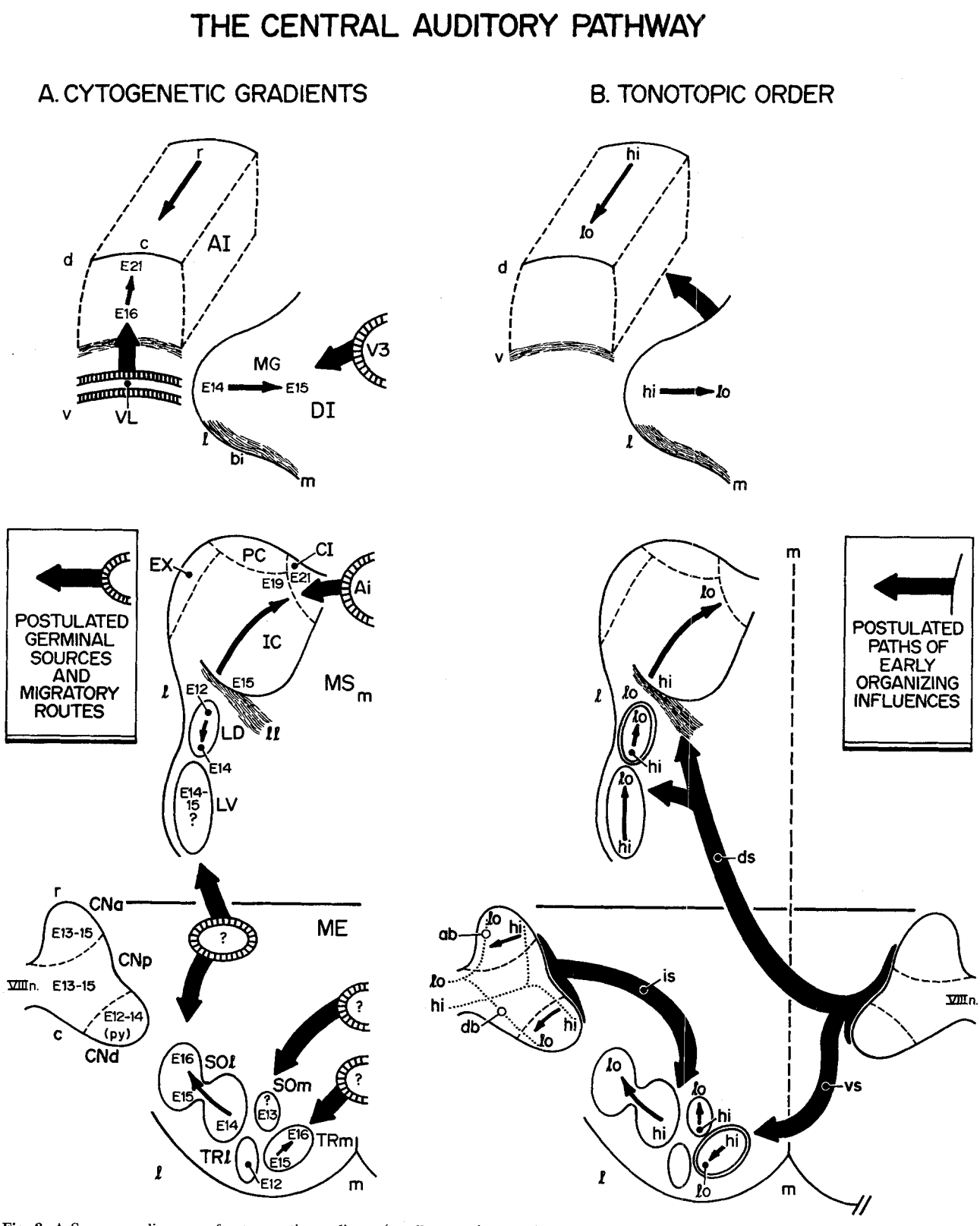

Fig. 8. A Summary diagram of cytogenetic gradients (small arrows), postulated germinal sources (striped double curves) and migratory routes (large arrows) throughout the entire central auditory system. Neuroepithelial sources with a question mark have not been identified in embryonic material. The source of the cochlear nuclear complex, which probably derives from more than one neuroepithelial site, is not indicated. **B** Corresponding tonotopic order (small arrows) and postulated paths of early embryonic organizing influences (large arrows). The two structures in which the cytogenetic gradient and tonotopic order point in opposite directions (LD and TRm) are shown with double boundaries. They receive predominantly contralateral input from second-order auditory neurons

lished observations). In the medial geniculate body the tonotopic order is lateral-to-medial, and the same holds for the cytogenetic gradient (Altman and Bayer 1979a). In the principal nucleus of the inferior colliculus both gradients are roughly ventral-to-dorsal, and in the lateral superior olivary nucleus both gradients are ventromedial-to-dorsolateral. The two structures in which the cytogenetic and tonotopic gradients were parallel but of opposite signs (according to the convention adopted) are the dorsal nucleus of the lateral lemniscus and the medial trapezoid nucleus. As we noted earlier (Altman and Bayer 1980a) the medial trapezoid nucleus is distinguished from most other nuclei of the superior olivary complex, which receive ipsilateral or bilateral cochlear nuclear afferents, in receiving only contralateral input, as determined anatomically (Stotler 1953; Strominger and Strominger 1971; Harrison and Howe 1974) and physiologically (Galambos et al. 1959; Goldberg and Brown 1968). Although our knowledge of the connections of the nuclei of the lateral lemniscus is somewhat sketchy, input to them has been described as predominantly contralateral from the cochlear nuclei both in the cat (Fernandez and Karapas 1967) and the monkey (Strominger and Strominger 1971). The possibility that the contralateral cochlear input to the medial trapezoid nucleus and the dorsal nucleus of the lateral lemniscus may account for their reversed cytogenetic and tonotopic gradients is complicated by two facts: (1) physiological studies have indicated that the neurons of the dorsal nucleus of the lateral lemniscus respond binaurally (Aitkin et al. 1970; Brugge and Imig 1970) and (2) that the cochlear nuclear input to the inferior colliculus is also mainly contralateral (Fernandez and Karapas 1967; Osen 1972; Beyerl 1978). The inferior colliculus, of course, receives ipsilateral and bilateral inputs through its rich connections with the various nuclei of the superior olivary complex (Beyerl 1978).

In summary, our results indicate that *within* all the auditory structures in which we were able to identify a cytogenetic gradient, there was an alignment in the orientation of this gradient with the tonotopic order. Moreover, with the exception of two structures, the regions of high frequency units tended to overlap with the sites of early generated neurons and vice versa. Accordingly, the answer to the first question whether the temporal order of cell production in the auditory structures of the neuraxis are relatable to their selective topographic (cochleotopic) organization may be, with some qualifications, an affirmative one. This raises the question how the orderly production of neurons in separate structures of the auditory system can assure the establishment of selective connections by their axons. Since we do not at present have embryonic data on the time of settling of the migrating cells of the auditory system, when their axons begin to grow towards their targets, and when they make synaptic contacts, we have to content ourselves with the more general question as to what is the temporal relation in the production of neurons *between* different auditory structures such that the cochleotopic order of the spiral ganglion cells might be impressed on successive structures at ascending levels of the neuraxis from the medulla to the cortex.

It is evident from our radiographic datings (Fig. 7) that the initial establishment of orderly topographic relations between the medial geniculate body and the primary auditory cortex must be independent of organizing influences emanating from lower levels of the auditory system. Auditory input to the medial geniculate body has a synaptic relay in the inferior colliculus (Barnes et al. 1943; Moore and Goldberg 1963; Erulkar 1975). However, not only are the bulk of medial geniculate neurons produced several days before those of the inferior colliculus but our embryonic evidence suggested (Altman and Bayer 1979b) that by day E15 the medial geniculate body is a recognizable structure and that thalamocortical fibers may be present by that time. It is quite likely, therefore, that the fibers of the medial geniculate nucleus make contacts with cortical cells before organizing influences could be exerted on them by the later forming inferior colliculus. The inferior colliculus itself could receive organizing influences from the first way-station in the central auditory pathway, the cochlear nuclear complex. The pyramidal cells of the dorsal cochlear nucleus, which are a major source of afferents to the inferior colliculus (Osen 1972; Beyerl 1978; Adams 1979), are produced on days E12–14 (Altman and Bayer 1980a). Their axons, by way of dorsal acoustic stria (stria of Monakow), could arrive at the site of the inferior colliculus contralaterally before the earliest contingent of inferior collicular neurons. However, the possibility that the early-forming pyramidal cells exert the cochleotopic order on the inferior colliculus, and on the nuclei of the superior olivary and trapezoid nuclear complexes, is counterindicated by our inability to recognize a cytogenetic gradient in the cochlear nucleus as a whole (Altman and Bayer, 1980a).

Thus, the datings of the time of origin of neurons in the central auditory pathway indicate that the initial topographic organization in the forebrain (thalamocortical) components of the system proceeds independently of the alignment of its brain stem (medullo-collicular) components. This suggests that topographic organization and tonotopic specification

are separate development events. We noted that at least in some structures of the auditory pathway there is evidence of anatomical segregation of cells into strips or laminae, as in the inferior colliculus (Rockel and Jones 1973a) and the medial geniculate body (Morest 1964). This morphological organization could be based on the chronological factor of neurogenesis that we have referred to as the "first come-first serve" principle (Bayer 1980; Bayer and Altman 1980b). It is interesting in this context that at least in two auditory structures in which the location of the influx of afferents is known – the lateral lemniscus in the case of the inferior colliculus and the brachium of the inferior colliculus in the case of the medial geniculate body – the early produced cells are located proximal to these fiber tracts. We have noted previously this arrangement in many brain regions, for instance, the cells of the pontine gray in relation to the pyramidal tract (Altman and Bayer 1978a), the cells of the medial and lateral septal nuclei in relation to the fornix (Bayer 1980), and the motor neurons of the trochlear and oculomotor nuclei in relation to the medial longitudinal fasciculus (Altman and Bayer 1980c). This also holds for the cerebral cortex, where cells of layers VI to II settle proximodistally in relation to the white matter. However, in the cerebral cortex this gradient results in an inside-out pattern (Angevine and Sidman 1961) in contradistinction to the outside-in pattern obtained in most nuclear structures. We do not know whether or not this is a general principle and holds also for the other components of the auditory pathway. If this were the case, then the pattern of settling of migratory cells in relation to the arrival of fibers with which they establish contact could be the first step in the morphogenesis of topographically ordered circuits. The second step of transforming this unspecified topographic order into a cochleotopic or tonotopic order could start with the physiological maturation of the cochlea peripherally, and the establishment of contacts between the inferior colliculus and the medial geniculate body centrally. We have at present no data about the latter event. Studies in the cat (Pujol and Marty 1970; Pujol et al. 1978) suggest that cochlear maturation is a late, perinatal event and that it may be the last step in the wiring of the auditory system prior to its functional postnatal maturation.

Intranuclear Cytogenetic Gradients as Indicators of the Site of Origin and Settling Patterns of Auditory System Neurons

In this study the datings of the time of origin of neurons were obtained in mature brains. In order to test the hypothesis that the chronology of cytogenesis in central auditory structures has an organizing influence on the establishment of a topographic order throughout the auditory pathway, it is essential to examine the intervening morphogenetic steps in embryonic brains. These steps include cell migration, the organization of afferents where the cells settle, the onset of axonogenesis and the tracing of the course of efferents. Our thymidine-radiographic evidence provides the first clue as to where cells originate and what paths they may follow to reach their final settling sites. In general, in brain structures with nuclear (or ganglionic) organization cells tend to settle in an outside-in pattern (Angevine 1970), that is, the earliest produced cells are located farthest and the latest cells closest to their germinal sources. Applying this principle, and having identified structures with discrete cytogenetic gradients, we infer a minimum of 6 distinct "cytogenetic zones" in the auditory pathway. We have recently introduced (Altman and Bayer 1980a) the terms "cytogenetic zone" or "cytogenetic system" to designate a group of brain structures, one brain structure or a part of it which appear to derive from a single germinal source (presumably a single cell line) in the embryonic neuroepithelium. The criteria for a cytogenetic zone are that its cells are produced in a similar temporal pattern (over a rapid or a protracted period) and that they form a single, continuous cytogenetic gradient. For instance, in the hypothalamus (Altman and Bayer 1978b) the supraoptic and paraventricular nuclei constitute a single cytogenetic zone, and the dorsomedial and ventromedial nuclei another. In correlated embryological investigations we were able to identify the probable neuroepithelial sources, or "neuroepithelial zones", of these two set of structures on the basis of the dates and gradients suggested by thymidine-radiography.

The six cytogenetic zones of the auditory pathway (Fig. 8A) are (1) the medial geniculate body, (2) the inferior colliculus, (3) the nuclei of the lateral lemniscus, (4) the lateral superior olivary nucleus, (5) the medial superior olivary nucleus and the lateral trapezoid nucleus, and (6) the medial trapezoid nucleus. The probable embryonic source of neurons of the medial geniculate body from the dorsal neuroepithelial lobe of the third ventricle has been described (Altman and Bayer 1979b). We have embryonic evidence (unpublished observations) of the derivation of cells of the inferior colliculus from the long-persisting neuroepithelium in the posterior recess of the aqueduct. The dorsal and ventral nuclei of the lateral lemniscus with their similar pattern of cell production and single dorsal-to-ventral cytogenetic gradient, seem to derive from an unidentified site

in the medulla. The cells of the lateral superior olivary nucleus may derive from a contiguous source but migrating in the opposite direction. The lateral trapezoid nucleus and the medial superior olivary nucleus have a single cytogenetic gradient (Fig. 8A) and a similar rapid pattern of cell production (Fig. 7) may derive from a midline medullary site. An adjacent site that is active later may be the source of the neurons of the medial trapezoid nucleus. These 6 cytogenetic zones do not include the primary auditory cortex because we do not have any evidence that it is cytogenetically different from the rest of the neocortex, or the cochlear nuclear complex because it is probably derived from more than one neuroepithelial source and would, therefore, constitute more than one cytogenetic zone.

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Abbreviations

ab, cochlear nerve, ascending branch
Ai, aqueduct, inferior collicular recess
AI, primary auditory cortex
bi, brachium of inferior colliculus
c, caudal
CE, cerebellum
CI, central nucleus, inferior colliculus
CNa, anteroventral cochlear nucleus
CNd, dorsal cochlear nucleus
CNp, posteroventral cochlear nucleus
d, dorsal
db, cochlear nerve, descending branch
DI, diencephalon
ds, dorsal acoustic stria (stria of Monakow)
DM, dorsomedial nucleus, inferior colliculus
EX, external nucleus, inferior colliculus
IC, inferior colliculus
is, intermediate acoustic stria (stria of Held)
l, lateral
LD, dorsal nucleus of lateral lemniscus
ll, lateral lemniscus
LV, ventral nucleus of lateral lemniscus
m, medial
ME, medulla
MG, medial geniculate body
MS, mesencephalon
PC, pericentral nucleus, inferior colliculus
PR, principal nucleus, inferior colliculus
py, pyramidal cells, dorsal cochlear nucleus
r, rostral
SOl, lateral superior olivary nucleus
SOm, medial superior olivary nucleus
TRl, lateral trapezoid nucleus
TRm, medial trapezoid nucleus
v, ventral
VL, lateral ventricle
vs, ventral acoustic stria (trapezoid body)
V3, third ventricle
VIIIn, cochlear nerve

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