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Intrinsic and Commissural Connections of the Rat Inferior Colliculus

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ABSTRACT

This study analyzes the distribution of the intrinsic and commissural fiber plexuses originating in the central nucleus of the inferior colliculus in the rat. The anterograde tracer *Phaseolus vulgaris*-leucoagglutinin (PHA-L) was injected iontophoretically at different places along the tonotopic axis of the central nucleus and visualized immunohistochemically. In coronal sections the terminal fields of axons originating at each injection site are seen to create four well-defined bands across the rostrocaudal extent of the inferior colliculus, two in the ipsilateral and two in the contralateral side. The "ipsilateral main band" extends dorsomedially and ventrolaterally from the injection site, in register with the known isofrequency contours of the central nucleus, spanning this nucleus and extending into the dorsal cortex of the inferior colliculus. The "ipsilateral external band" is located in the external cortex, where it is oriented dorsoventrally, slightly oblique to the pial surface. In caudal sections, the ventral portion of these two bands appear to join. The two bands in the contralateral inferior colliculus occupy a symmetric position to those of the ipsilateral side, forming a mirror-like image. The position of the four bands changes as the position of the injection site is varied along the frequency gradient axis of the central nucleus. After ventromedial (high frequency area) injections, the main band is ventral and medial, and the external band ventral and lateral. After more dorsolateral (lower frequency) injections, the main band is more dorsal and lateral, whereas the external band is more dorsal but more medial. Thus, the change in the position of the external band is separate and opposite to that of the main band. We suggest that the main bands represent isofrequency contours. Since the projection from the central nucleus to the external cortex of the inferior colliculus also appears to be tonotopic, we also propose a tonotopic organization for the external cortex. The main bands overlap the terminal field of the lemniscal fibers in the central nucleus; thus, it is concluded that the intracollicular fibers contribute to the formation of the known fibrodendritic laminae of the central nucleus.

A possible role in preservation of frequency information and integration of other different acoustic parameters is proposed for the main bands. The external bands could participate in polysensory integration, and the commissural connections could be involved in hitherto unknown stages of binaural processing of sound.

Based on our results, several modifications are proposed for delineating the subdivisions of the inferior colliculus.

Key words: auditory midbrain, acoustic tectum, tonotopy, cytoarchitecture, *Phaseolus vulgaris*-leucoagglutinin (PHA-L)

The mammalian inferior colliculus (IC) is a critical structure for processing auditory information. Located in the most dorsal and caudal regions of the midbrain, this voluminous and complex tectal center receives synapses from virtually all ascending and descending auditory pathways. Thus, the IC is reached by the lateral lemniscus, which contains ascending fibers from different neuron types in the cochlear nuclei, the superior olivary complex, and the nuclei of the lateral lemniscus (Beyerl, '78; Adams, '79; Coleman and Clerici, '87). The IC also receives substantial input from the contralateral IC via the commissure of the IC (CoIC) (Aitkin and Phillips, '84; Coleman and Clerici, '87). Additionally, the IC is subjected to cortical control by means of a direct descending projection from the auditory cortex (Andersen et al., '80a; Druga and Syka, '84b; Faye-Lund, '85; Herbert et al., '91); these cortical

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fibers enter the IC via the brachium of the IC. Besides being an auditory relay station, the IC is the target of projections from somatosensory structures (RoBards et al., '76; Aitkin et al., '78, '81; RoBards, '79; Tokunaga et al., '84; Coleman and Clerici, '87) and, to a lesser extent, from visual structures (Itaya and van Hoesen, '82; Paloff et al., '85). Furthermore, recent reports show that the inferior colliculus is also innervated by non-sensory structures such as the substantia nigra (Olazábal and Moore, '89) and the globus pallidus (Yasui et al., '90).

The complexity of the input to the IC contrasts with the apparent simplicity of its output. The IC contributes to the descending auditory pathway (Faye-Lund, '86) and the already mentioned projection to the contralateral IC. It also innervates the deep layers of the ipsilateral superior colliculus (Edwards et al., '79; LeDoux et al., '85). However, the main targets of collicular neurons are the various subdivisions of the thalamic medial geniculate body (Kudo and Niimi, '78, '80; Andersen et al., '80b; Oliver, '84a). It is therefore reasonable to assume that much elaboration and integration of auditory information takes place within the IC before being sent to higher centers.

The morphology of the collicular neuron types and the afferent fibers that innervate the IC has been extensively studied optically and ultrastructurally, making the IC one of the best described regions of the acoustic pathway (Morest, '64a; Geniec and Morest, '71; Rockel and Jones, '73a,b,c; Henkel and Spangler, '83; Morest and Oliver, '84; Oliver, '84a,b, '85, '87; Oliver and Morest, '84; Faye-Lund and Osen, '85; Meininger et al., '86; Ribak and Roberts, '86; Shneiderman and Henkel, '87; Shneiderman et al., '88; Paloff et al., '89; Shneiderman and Oliver, '89). The intrinsic collicular connections, however, have received relatively little attention. This is surprising because the intracollicular connections may be responsible, at least in part, for the processing of ascending auditory information, the integration between ascending and descending inputs, and the integration between auditory and other kinds of sensory information. Although recent attempts have been made to elucidate the intrinsic collicular connections in the mouse and the cat (González-Hernández et al., '86; Herrera et al., '88, '89; Oliver et al., '91), very little is known about intrinsic collicular fibers in the rat. Similarly, little is known about the connections between the two IC. Studies using retrograde transport of horseradish peroxidase (HRP) have provided data concerning the topography of the neurons projecting to the contralateral IC in a number of species (Aitkin and Phillips, '84; Tokunaga et al., '84; González-Hernández et al., '86; Coleman and Clerici, '87; Hutson et al., '91), but very little information is available about the distribution of the commissural fibers in the IC.

The IC is not a homogeneous structure. Since the pioneering studies of Cajal ('03, '11), it has generally been accepted that it consists of a densely celled central nucleus (CNIC) with a laminar organization (Morest, '64a), which is surrounded dorsally, laterally and caudally by layered cortical structures. Studies of the anatomy and physiology of the IC have lead to a number of different cytoarchitectural models (Rockel and Jones, '73a,c; Morest and Oliver, '84; Oliver and Morest, '84; Faye-Lund and Osen, '85; Meininger et al., '86). Given the lack of pre-existing information, none of the proposed models has used the intracollicular connections as an important element or criterion to complement the details of cellular anatomy, afferent inputs and physiology of the IC. Therefore, we set forth to analyze in detail both the intrinsic and commissural projections of the IC in an effort to improve our understanding of the general functional organization of the IC.

In the present study, *Phaseolus vulgaris*-leucoagglutinin (PHA-L) was used as an anterograde tracer. This lectin was chosen because of its extreme sensitivity, specificity, and reliability as a nearly purely anterograde tracer (Gerfen and Sawchenko, '84; Groenewegen and Wouterlood, '90). Specifically, PHA-L was injected in the CNIC of adult rats to examine 1) the distribution of the intrinsic collicular fibers, 2) the distribution of the commissural collicular fibers, and 3) the possible tonotopic organization of the intrinsic and commissural collicular fiber systems.

This report is the first of a series describing the intrinsic and efferent connections of the rat IC. Detailed accounts of the morphology and trajectories of the intrinsic and commissural collicular fibers, as well as the descending and ascending projections of the IC, are forthcoming. Some of the data reported herein have been presented in abstract form (Saldaña et al., '88; Saldaña, '91).

MATERIALS AND METHODS

PHA-L technique

Experimental animals and tracer injection. Twelve young adult, female Wistar rats (body weight 190–210 g) received injections of *Phaseolus vulgaris*-leucoagglutinin (PHA-L) into the right CNIC. Nine of the animals received single injections and three received two injections along the tonotopic axis of the CNIC. Prior to surgery, rats were deeply anesthetized by intramuscular injection of 1 ml/kg body weight of a 4:3 mixture of ketamine 10% (Aescoket®, Aesculaap B.V., Boxtel, The Netherlands) and xylazine 10% (Rompun®, Bayer, Brussels, Belgium) and mounted in a stereotaxic frame. The PHA-L (Vector Labs., Burlingame, CA; 2.5% in 0.05 M Tris-buffered saline, TBS, pH 7.4) was injected iontophoretically at various coordinates in the CNIC as defined by Faye-Lund and Osen ('85) and Paxinos and Watson ('86). The injections were made through glass micropipettes through which a pulsed 5–6 μA DC positive current was applied (7 seconds on/7 seconds off) for 15–30 minutes.

Fixation and sectioning. Seven to ten days after surgery, the animals received intraperitoneally an overdose of 6% sodium pentobarbital (Nembutal®, Ceva, Paris, France). The brains were fixed by transcardial perfusion with perfusates applied at a hydrostatic pressure of 30 KPa (Jonkers et al., '84). The perfusion was started with 100–150 ml of a calcium-free variant of the Ringer solution saturated with 95% O_2 and 5% CO_2 to pH 6.9 (38°C), which was followed immediately by 1,000 ml of a fixative composed of 4% freshly depolymerized paraformaldehyde, 0.1% glutaraldehyde, and 0.2% saturated picric acid in 0.125 M sodium phosphate buffer (pH 7.3, room temperature). The brains were left in situ for 2 hours at room temperature, removed from the skull, placed into 0.125 M phosphate buffer for another 2 hours, and then cryoprotected in 30% sucrose in phosphate buffer at 4°C for 60 hours. Blocks containing the inferior colliculi and the thalamus were sectioned on a freezing microtome at a thickness of 40 μm in the coronal plane, and the sections were collected in TBS, pH 7.6.

PHA-L immunostaining. The transported PHA-L was visualized by immunocytochemistry on free-floating sections (unlabeled antibody method of Sternberger, '79). After several rinses in TBS, pH 7.6, with 0.5% Triton X-100

(TBS-TX, Triton X-100®, Sigma Chemical Co., St. Louis, MO), the sections were incubated in a goat anti-PHA-L antiserum (Vector Labs., Burlingame, CA.; dilution 1:5,000 in TBS-TX) for 60 hours (4°C, in the dark, under gentle agitation). Following extensive rinsing in cold TBS-TX, the sections were then incubated in immunoglobulin G raised in donkey against goat (DoAG/IgG H + L, Nordic Immunochemicals, Tilburg, The Netherlands; dilution 1:50 in TBS-TX) for 2 hours, at room temperature and under gentle agitation. The sections were again thoroughly rinsed with TBS-TX and incubated with a goat peroxidase anti-peroxidase complex (goat PAP, Nordic Immunochemicals; dilution 1:800 in TBS-TX) for 90 minutes, at room temperature and under gentle agitation. Following this step, the sections were rinsed three times with TBS-TX, rinsed two times with 0.05 M Tris-HCl buffer, pH 7.6, and treated for 20–60 minutes with a solution of 0.04% 3,3′ diaminobenzidine tetrahydrochloride (DAB, Sigma Chemical Co., St. Louis, MO) and 0.015% H_2O_2 in 0.05 M Tris-HCl, pH 7.6. The precipitation of colored reaction product was monitored by visual inspection of the sections at intervals during the DAB-reaction. When satisfactory staining was obtained, the reaction was terminated by rinsing several times in the Tris-HCl buffer. After the immunostaining, the sections were mounted on glass slides, allowed to dry, and coverslipped with Entellan (Merck, Darmstadt, Germany). Every fourth section was first counterstained with Cresyl violet before dehydrating and coverslipping.

Definitions

Intrinsic and commissural fibers. In the present experiments, the morphology of the PHA-L-labeled axons and axon terminals is interpreted as follows. The occurrence of smooth fibers possessing only few ramifications and en passant or terminal varicosities is indicated as "fiber labeling." Fibers that run laterally from the injection site and enter the brachium or the lateral lemniscus on their way to the thalamus or the descending pathway, respectively, belong to this category and will not be analyzed in this study. When fibers form a plexus and ramify profusely into short branches with numerous en passant and terminal varicosities, they are referred to as "terminal labeling." The distribution of the terminal labeling is the primary focus of this paper.

All labeled fibers with terminal appearance observed in either right or left colliculus are considered "intracollicular fibers." The term "intrinsic collicular fibers" refers to the terminal labeling within the IC that received the PHA-L injection; the expression "commissural collicular fibers" refers to the terminal labeling in the IC contralateral to the injection site. Although in this study two small terminal fields of fibers from the CNIC were consistently seen within the CoIC itself, one on each side of the midline, they will not be considered further in this account; they will be the subject of a forthcoming publication. Similarly, the trajectories and morphology of the intracollicular fibers will be described in detail elsewhere.

Cytoarchitecture of the IC. In the present account, the cytoarchitectural boundaries of the rat IC proposed by Faye-Lund and Osen ('85) and detailed by Paxinos and Watson ('86) are basically adopted. According to this scheme, the IC consists of a central nucleus (CNIC), an external cortex (ECIC), and a dorsal cortex (DCIC). The CNIC occupies the medial two thirds of the caudal two thirds of the IC. The ECIC is located lateral, rostral, ventral and ventrocaudal to the CNIC. The DCIC is located dorsomedial and dorsocaudal to the CNIC. However, some adjustments in the limits between the three subdivisions have been made (Fig. 1). These modifications, based on our observations of numerous experimental animals, will be explained in the Discussion. The ECIC has been extended dorsally, to include the lateral part of the DCIC as defined by Faye-Lund and Osen ('85). In this way, the border between the DCIC and the ECIC is actually in register with the limit between the CNIC and the ECIC. In the text, the expression "medial IC" refers to CNIC + DCIC; the term "lateral IC" refers to the ECIC.

The subdivisions and nomenclature proposed for the IC of other mammals may vary considerably. The homologies and equivalences between the different cytoarchitectural models proposed so far have been recently reviewed and discussed by Huffman and Henson ('90).

Isofrequency and tonotopic axis of the IC. It is well known that the IC possesses a tonotopic (or cochleotopic) organization, which seems to be continuous through the CNIC and DCIC; the neurons with low best or characteristic frequency (CF) are located dorsolaterally, and the neurons with high CF are located ventromedially (Rose et al., '63). Studies using extracellular recording of single units and/or 2-deoxyglucose (2-DG) uptake in animals stimulated with pure tones have described detailed "functional maps" of the tonotopic organization of the IC in varied species, including cat (Rose et al., '63; Merzenich and Reid, '74; Semple and Aitkin, '79; Servière et al., '84) and rat (Clopton and Windfield, '73; Huang and Fex, '86; Ryan et al., '88). By connecting points with the same CF, isofrequency contours have been defined (Merzenich and Reid, '74), which in the coronal plane are oriented from dorsomedial to ventrolateral. Three-dimensional reconstructions of the isofrequency contours suggest that each contour represents a section through a three-dimensional sheet which slopes from dorsomedial to ventrolateral and spans most of the IC rostrocaudally (Semple and Aitkin, '79; Servière et al., '84; Huang and Fex, '86; Ryan et al., '88).

In this study the *isofrequency axes* of the IC are assumed to coincide with the orientation of the isofrequency contours of the rat IC as recently described by Ryan and co-workers ('88) by the 2-DG method. The *tonotopic axis,* approximately orthogonal to the isofrequency axes, would be represented by a line connecting the region of low CF to the region of high CF, i.e., from dorsolateral to ventromedial. Along the tonotopic axis the CF would increase progressively, while along a given isofrequency axis the CF would remain constant.

Our experiments did not include electrical recordings at the injection site, but the tracer was systematically delivered in regions of the CNIC previously characterized functionally by others. Thus, the terms low, medium, and high frequencies used throughout the text refer to the presumed CF at the injection site, based on the previously mentioned studies.

RESULTS

Each PHA-L injection into the CNIC gives rise to four major laminar plexuses of labeled fibers, two in the ipsilateral and two in the contralateral IC. On each side, one plexus is located in the medial IC (CNIC + DCIC), while the other is located in the lateral IC (ECIC). These four laminar plexuses, ordered in a topographic array, form an

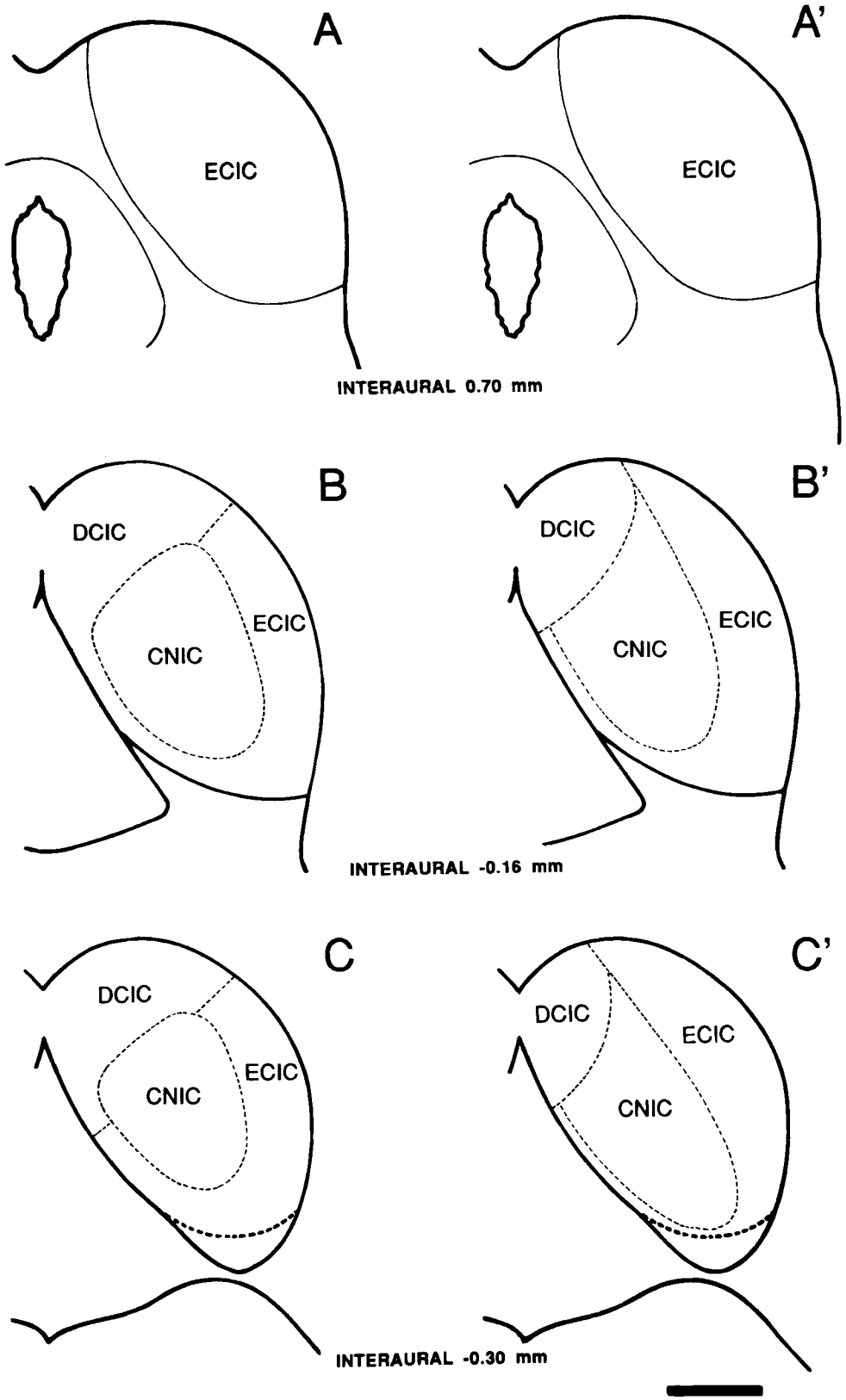


Fig. 1. Subdivisions of the rat IC in coronal sections. The left hand column (sections A, B, and C) depicts the subdivisions proposed by Faye-Lund and Osen ('85) as illustrated by Paxinos and Watson ('86) at three different rostrocaudal levels. The right hand column (sections A', B', and C') represents the modified subdivisions suggested by our results. In the caudal two thirds of the IC, the lateral ECIC has been extended dorsally, to include the lateral third of the former DCIC. Thus, the border between the DCIC and the ECIC is actually in register with the border between the CNIC and the ECIC. The CNIC has been extended dorsally and approaches the dorsal surface of the IC. For the rationale underlying these modifications, see Discussion. CNIC, central nucleus; DCIC, dorsal cortex; ECIC, external cortex. Calibration bar, 1 mm.

intracollicular projection system that conforms to a general pattern of organization within the IC. In some of our cases a fifth, minor laminar plexus is found at the ventromedial border of the ipsilateral IC. This minor plexus represents an entity that seems to be separate from the other laminar plexuses and does not conform to the same topographic order. These two groups of intracollicular projection systems are described separately below.

General pattern

In coronal sections the four major plexuses appear as bands (Fig. 2A) composed of labeled fibers, numerous collateral branches, varicosities, and terminal specializations (Fig. 3A,B). The first ipsilateral band, herein termed "ipsilateral main band," extends both dorsomedially and ventrolaterally from the injection site, spanning most of the CNIC. This band is clearly in register with the known fibrodendritic laminae of the CNIC (Morest, '64a, Oliver and Morest, '84; Faye-Lund and Osen, '85), although unlike these, the main band extends dorsally and penetrates the DCIC (Fig. 2A), to such an extent that in some instances the labeled fibers occupy all three layers of the DCIC and approach the dorsal surface of the IC (Fig. 2C). This band is, therefore, parallel to the isofrequency axis of the IC. In general, the ipsilateral main band is the most dense of the four.

The second ipsilateral band, referred to as the "ipsilateral external band," is situated in the ECIC. This band runs dorsoventrally and its dorsal end is closer to the lateral surface of the IC than its ventral end (Fig. 2A). Most of this band appears to be located in layer 2 of the ECIC, but its ventral portion bends towards the deeper layer 3. The superficial layer of the ECIC (layer 1) is devoid of terminal labeling.

The two bands in the contralateral IC occupy positions symmetrical to those of the ipsilateral bands, forming a mirror-like image. The band symmetric to the main band is referred to as "contralateral main band," while the band symmetric to the external band is called the "contralateral external band." Like the ipsilateral main band, the contralateral main band crosses the three layers of the DCIC dorsoventrally before entering the CNIC (Fig. 2B). The mirror-like image is always found in coronal sections, although the contralateral bands are formed of fewer elements than the ipsilateral bands (Fig. 2A).

In serial coronal sections, the position, orientation, and topographic relationships of the bands are seen to vary throughout the rostrocaudal extent of the IC. To illustrate these changes, case 1, in which the injection site is located approximately in the rostrocaudal center of the IC, has been selected (Fig. 4). Figure 4D illustrates the position of the four bands in a section including the injection site. As one proceeds rostrally from the level of the injection site, the four bands become progressively shorter at their ventral ends (Fig. 4C) and appear less dense (Fig. 4A,B). The loss of density is most apparent in the contralateral bands, and in the ipsilateral side, is more noticeable in the external band. Rostrally the four bands penetrate the rostral ECIC (Fig. 4A,B). As one proceeds caudally from the injection site, the main and external bands approach each other until they merge at their ventral ends (Fig. 4F). Close to the caudal pole of the IC, labeled fibers are still present, although the banded pattern becomes disorganized (Fig. 4G). In each section, the two contralateral bands are approximately as thick as their corresponding ipsilateral band. On each side, the main band is somewhat thicker than the external band. Unlike the main bands, the external bands are patchy in appearance (Fig. 4).

From the observation of serial coronal sections, it can be concluded that the bands represent slices through three-dimensional, laminar fibrillar plexuses which span the IC rostrocaudally. These plexuses possess a dorsoventral elongation, evident in all sections, and a rostrocaudal elongation, evident from the fact that each plexus appears in many consecutive sections. In rostral sections, the plexus that forms the main bands is vertically oriented and at caudal levels it becomes progressively more horizontal. The plexus that forms the lateral bands is tilted from dorsomedial to ventrolateral at rostral and middle levels, and becomes progressively more vertical caudally, until it merges with the other plexus (Fig. 4). The distance between this plexus and the surface of the IC increases at caudal levels. The two laminar plexuses join caudoventrally in a plow-like formation that seems to circumvent the caudal margin of the incoming lemniscal fibers. The same applies for the two contralateral plexuses.

Differences between cases. The distribution pattern of the intrinsic and commissural terminal fields, with four bands in each section, two per side, that merge caudally and ventrally, was consistent in all the cases studied. In contrast, the mediolateral and dorsoventral positions of the bands change when the injection site is shifted in position. This variation is especially pronounced when the injection site is shifted along the tonotopic axis of the CNIC.

In case 1, the tracer was injected in the ventromedial part of the CNIC (high frequencies region). As described, four bands can be distinguished (Fig. 4). The main band runs dorsoventrally and mediolaterally close to the medial border of the IC. Rostrally this band is tilted 60° with respect to the horizontal plane (Fig. 4C); at the level of the injection site the angle is 40–45° (Fig. 4D), and only 30–35° at caudal levels (Fig. 4F). The external band is almost parallel to the lateral surface of the IC, with its dorsal end situated approximately 40 μm beneath the surface (Fig. 4C,D). This band, with a dorsomedial to ventrolateral inclination, lies mostly in layer 2 of the ECIC along the rostrocaudal extent of the IC, and only in caudal sections becomes deeper, particularly at its ventral end (Fig. 4C–F). At caudal levels, when the main and the external bands merge at their ventral ends, they create a "V"-shaped structure, that can be followed nearly to the caudal pole of the IC (Fig. 4E,F).

In case 2, the tracer was injected in the center of the CNIC (region of medium frequencies). In this case (Fig. 5) the main band is more lateral and dorsal than in case 1 (compare Figs. 4 and 5). Rostrally, the angle between the band and the horizontal plane is 55° (Fig. 5A); at the level of the injection site, the angle is 40° (Fig. 5C); more caudally, at the level illustrated in Figure 5E, the inclination decreases to 30° and is reduced even further in Figure 5F. The external band is more medial than in case 1 and, particularly in caudal sections, also more dorsal. The distance between the external band and the surface of the IC is 150 μm at its dorsal end, and 250–280 μm at its ventral end (Fig. 5D). Caudally, this band is tilted but becomes almost vertical after joining the main band (Fig. 5F). When the main and external bands merge, they define a "Y"-shaped structure with its concavity oriented dorsomedially and its base ventrolaterally (Fig. 5E,F). It should be noted that the two bands merge at a level slightly more rostral than in case 1.

Case 3 received the PHA-L injection in the dorsolateral part of the CNIC (Fig. 6). The main band is situated more lateral and dorsal than in the two previous cases. As in other cases, this band is more vertical rostrally: at the level of Figures 6C,D the angle between the main band and the horizontal plane is 45°, and this angle decreases progressively until, when the main and external bands merge, the main band is nearly horizontal (Fig. 6E,F). Moreover, the main band is shorter than the main band in the two previous cases. The external band is located more dorsal and medial than in cases 1 and 2. At the level of the section illustrated in Figure 6D, the distance between the ventral end of the external band and the surface of the IC is approximately 400 μm. Moreover, the external band in this case is notably shorter than the external band in the previous cases. Rostrally, the external band has a dorsomedial to ventrolateral inclination (Fig. 6C,D). Caudally, the inclination of this band becomes progressively more vertical (Fig. 6E) and, after joining the main band, its angle relative to the midline appears inverted (Fig. 6F). The main and external bands merge caudally at their ventral ends, forming a dorsally open semicircle situated in the dorsomedial part of the IC (Fig. 6F). It should be noted that in this case, the merging of the main and external bands occurs more rostrally than in the previous cases (Fig. 6D,E).

Other cases (n = 6) were obtained in which PHA-L injections were made in various locations within the CNIC. In general, the pattern of distribution of the ipsi- and contra-lateral bands was predictable, i.e., injections placed in positions intermediate between those illustrated above resulted in bands whose position and orientation were also intermediate to those described.

To examine the distribution of bands resulting from double injections, three additional animals received two injections along the tonotopic axis of the IC. In case 4, one injection site occupies a position intermediate between cases 1 and 2, and the other is intermediate between cases 2 and 3. As shown in Figure 7, each injection gives rise to the expected four bands. The ipsilateral two main bands, one arising from each injection, are not perfectly parallel. At rostral levels, the two main bands are nearly parallel and closer to each other than at the level of the injection site (Fig. 7B–E). At the rostralmost end of the IC, the separation between the main bands disappears, and the fibers of one band intermingle with the fibers of the other, particularly in the dorsal portions of the bands (Fig. 7A,B). Caudal to the injection site, the two main bands separate progressively, particularly at their ventral ends, adopting divergent trajectories (Fig. 7F,G). Similarly, the external bands produced by the two injections are not perfectly parallel. In rostral sections they are closer to each other and at caudal levels, when the main bands and the external bands merge, the distance between the two external bands increases (Fig. 7C–F).

Summary of results. Figure 8 shows an idealized representation of the distribution of the terminal labeling in cases 1, 2, and 3. Comparing the distribution of the bands in these three cases, other similar ones, and the cases with two injections, several conclusions regarding the topography of the intrinsic and commissural collicular projections can be drawn:

1. The more ventromedial the injection site, the more *ventral and medial* is the main band. By contrast, the more ventromedial the injection site, the more *ventral and lateral* is the external band.
2. The more ventromedial the injection site, the more *vertical* is the main band. The more ventromedial the injection site, the more *tilted from dorsal and medial to ventral and lateral* is the external band.
3. The more ventromedial the injection site, the *longer* are the bands. This difference is particularly noticeable in caudal sections.
4. Globally considered (main band + external band), the bands arising after injecting the tracer in regions of higher frequencies encompass ventrally the regions that would contain the bands resulting from injections in lower frequency regions. Specifically, the bands arising after ventromedial injections (high frequency regions) encompass ventrally those resulting from central injections (medium frequency regions), and these, in turn, encompass those arising from dorsolateral injections (low frequency regions).
5. The main bands produced by different injections along the tonotopic axis of the IC are closer rostrally than caudally; the same applies for the external bands.

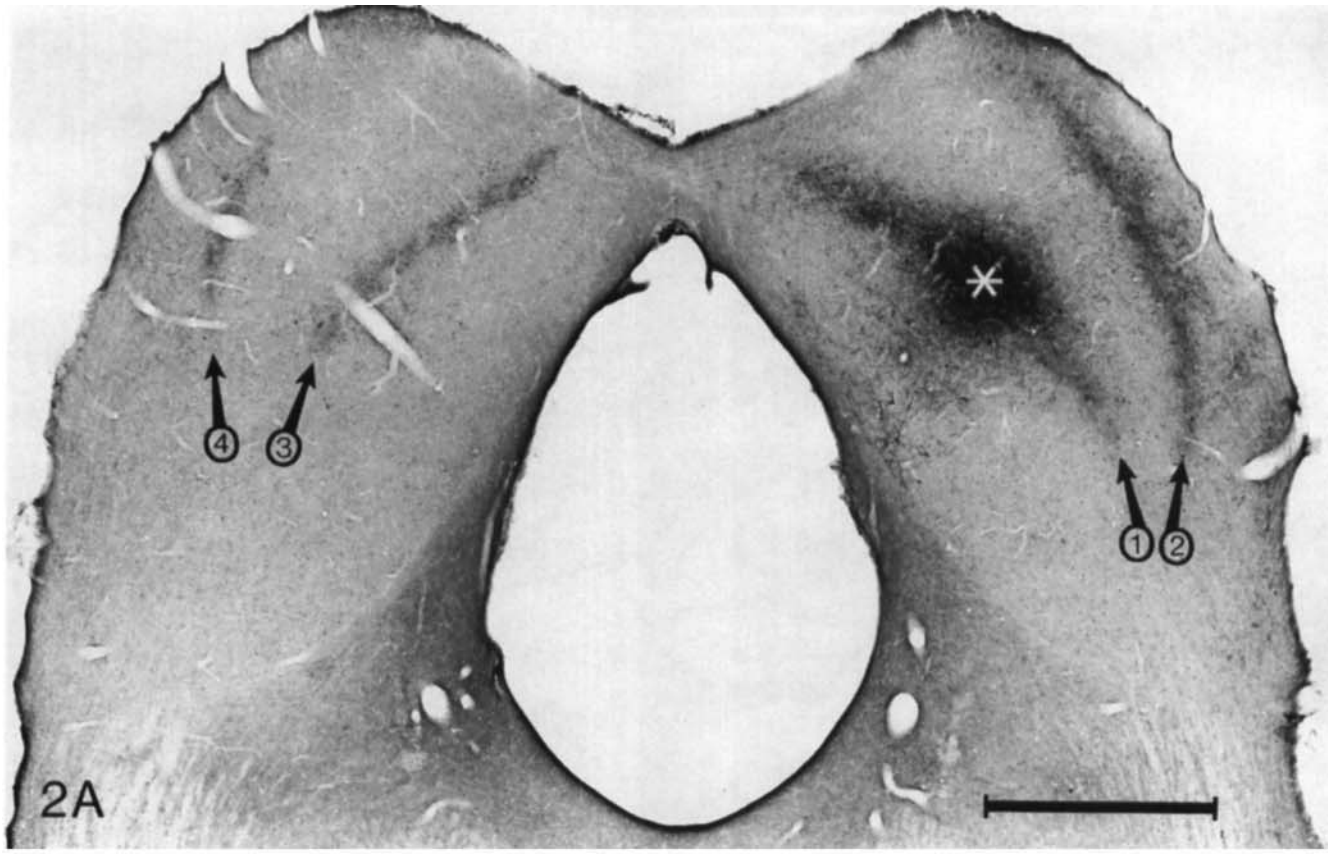
Labeling at the ventromedial border of the IC

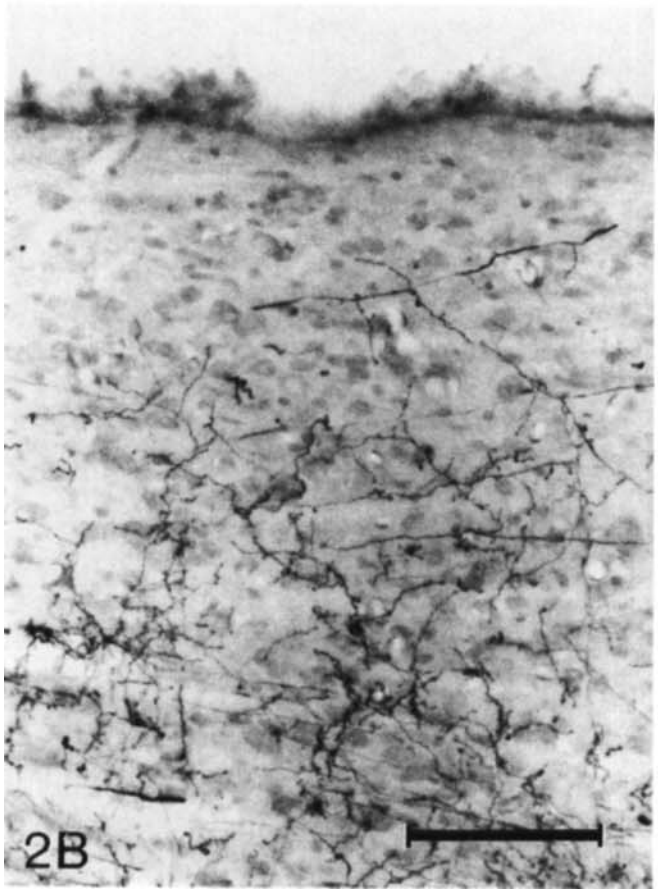
In some of our experiments, a fifth plexus of labeled fibers is found at the ventromedial border of the ipsilateral IC. In coronal sections, this plexus appears as a band composed of en passant and terminal fibers located consistently at the border between the IC and the adjacent periaqueductal gray (Fig. 2A). This band is always far less dense than the ipsilateral main and external bands (Figs. 2A, 4, 5). This minor band is best appreciated when the injection site is close to the ventromedial border of the IC and becomes less evident and shorter as the injection site is shifted dorsolaterally. Among the cases illustrated in this article, this plexus is clearly observed in case 1 (Fig. 4C–E), less noticeable in case 2 (Fig. 5C,D), and not visible in case 3 (Fig. 6). These results indicate that neurons situated in the ventromedial and central (high-medium frequency) parts of the CNIC but not in more dorsolateral (lower frequency) regions of the CNIC, project to the ventromedial border of the IC. Only in case 1, which resulted in the strongest labeling at the ventromedial border of the ipsilateral IC, were a few labeled fibers seen in the corresponding region of the contralateral IC.

DISCUSSION

Our results demonstrate that the rat IC contains a system of laminar axonal plexuses that connect each point

Fig. 2. **A:** Panoramic view of a coronal section including a PHA-L injection site (asterisk) into the central zone of the CNIC (case 2). Note the four fibrillar bands, indicated by numbered arrows: 1) Ipsilateral main band, 2) Ipsilateral external band, 3) Contralateral main band, and 4) Contralateral external band. Note on each side the convergence of the main and the external band in the ventral direction and the extension of the ipsi- and contralateral main bands into the DCIC. A camera lucida drawing of this section is illustrated in Figure 5C. Calibration bar, 1 mm. **B** and **C:** Details of the dorsal end of the contralateral (B) and ipsilateral (C) main bands in a section approximately 500 μm more rostral than that shown in Figure 2A. Note the numerous ramifications and varicosities of the terminal fibers, and the presence of some fibers near the pial surface. This section was counterstained with Cresyl violet and photographed with a Kodak Wratten number 46 blue filter. Calibration bar in B, 100 μm, applies also to C.





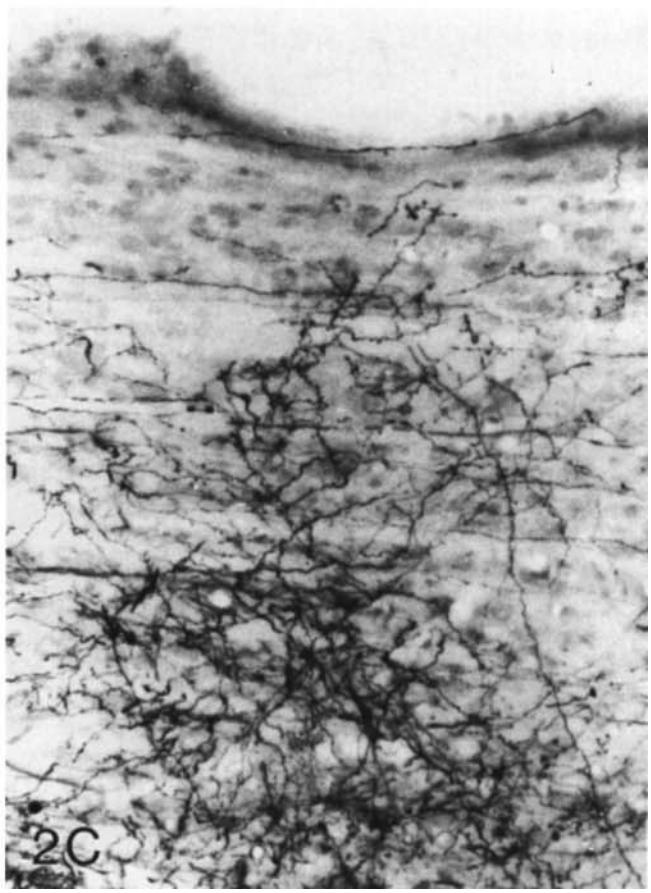
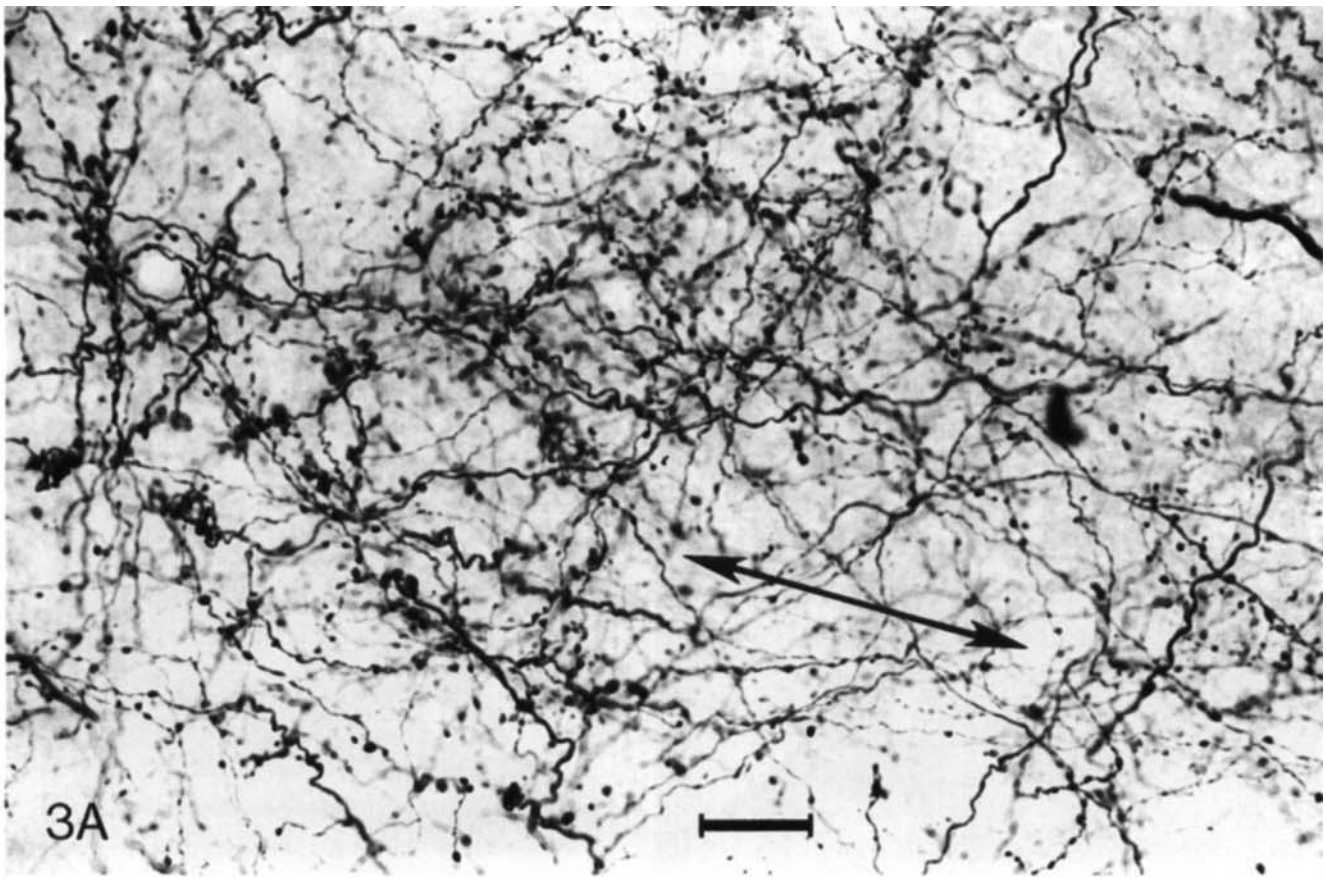


Figure 2



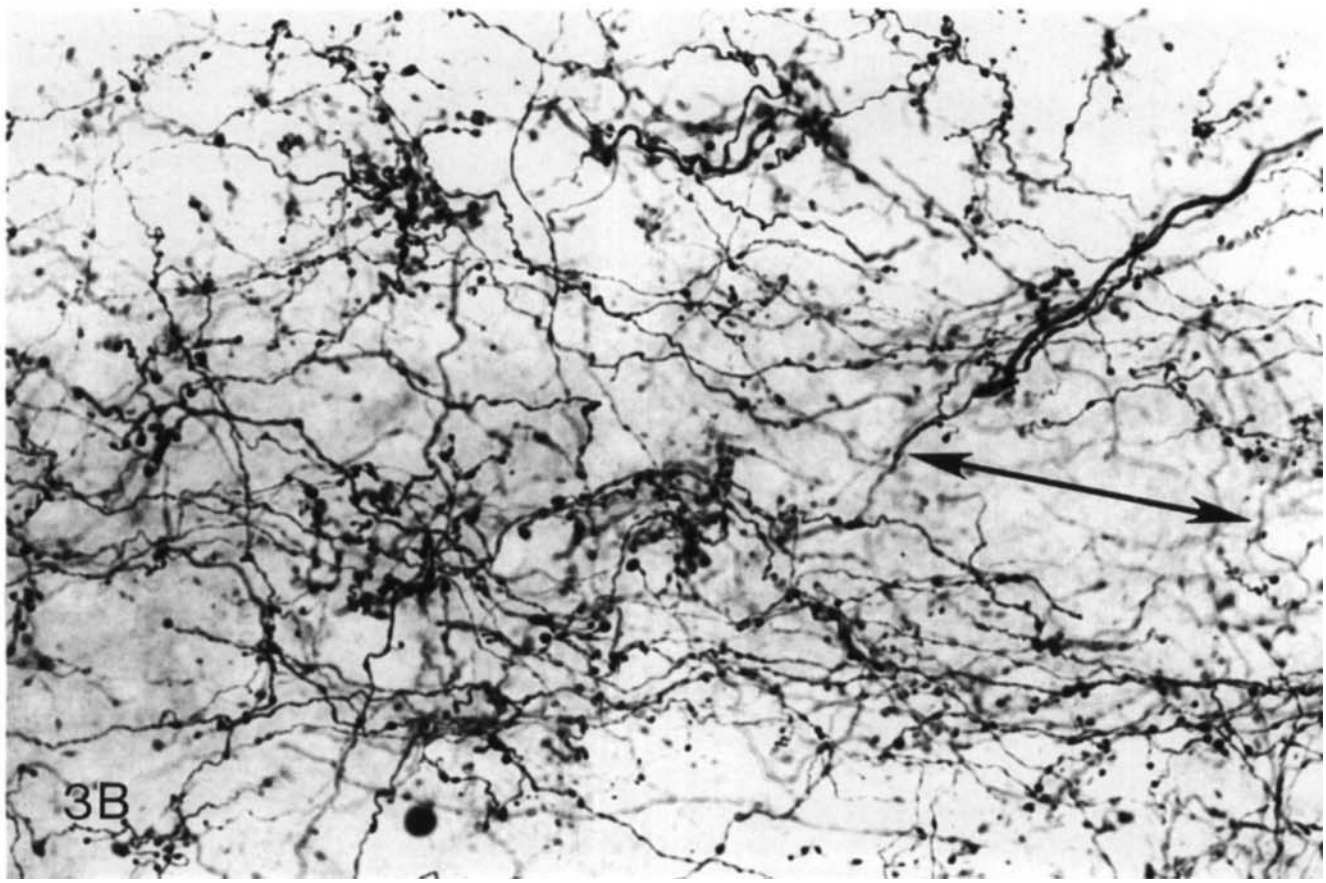
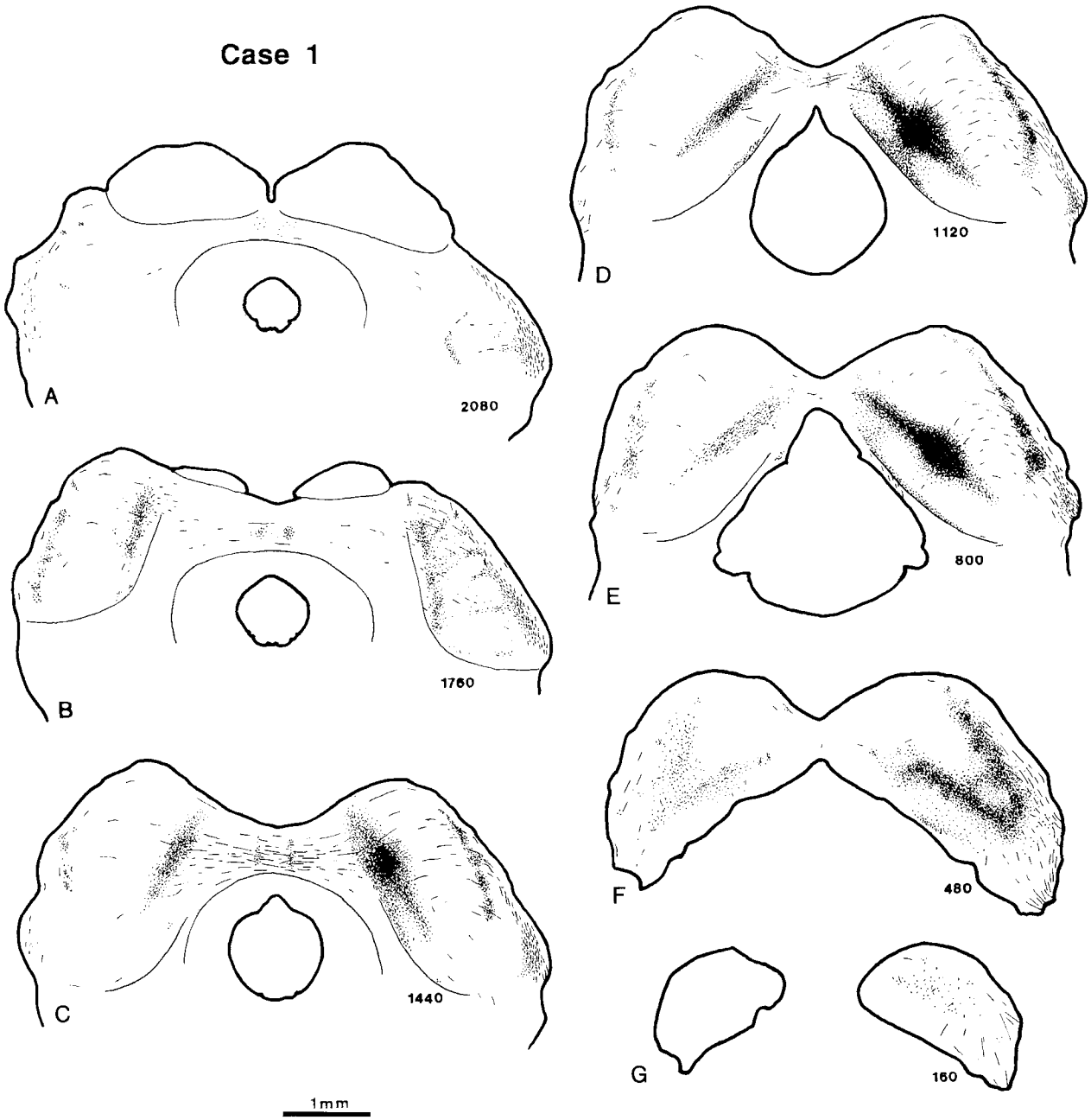


Figure 3



Figs. 4–7. Camera lucida drawings of coronal sections of the two IC illustrating the pattern of labeling after PHA-L injections into different regions of the right CNIC. The serial sections are labeled with capital letters, A indicating the most rostral section. The numbers on the right side of each section indicate the distance from the caudal pole of the IC in micrometers. Within the IC, the lines represent fibers of passage, and the stippling represents terminal fields.

Fig. 4. Single PHA-L injection into the ventromedial (high frequency) region of the right CNIC (case 1). The injection site is located approximately at the level of section D. Note the symmetry of the intracollicular terminal fields, and the lower density of the labeling in the contralateral IC. The four bands of terminal labeling span most of the IC rostrocaudally, and penetrate into the rostral ECIC. The main bands penetrate also into the DCIC. Note the ventral mergence of the main and external bands at caudal levels. Note also in C, D, and E the patchy appearance of the external bands. Two small terminal fields within the CoIC, one on each side of the midline, can be seen in sections A–D.

Fig. 3. Details of the fibrillar composition of the ipsilateral main (**A**) and external (**B**) bands. Field A was taken from the dorsal half of the main band, and field B corresponds to the center of the external band. In both cases, most fibers run parallel to the main axis (indicated by the double-headed arrows) of the band which they form. The numerous en passant and terminal boutons give the bands their unequivocal terminal field appearance. Calibration bar in A, 20 μm, applies also to B.

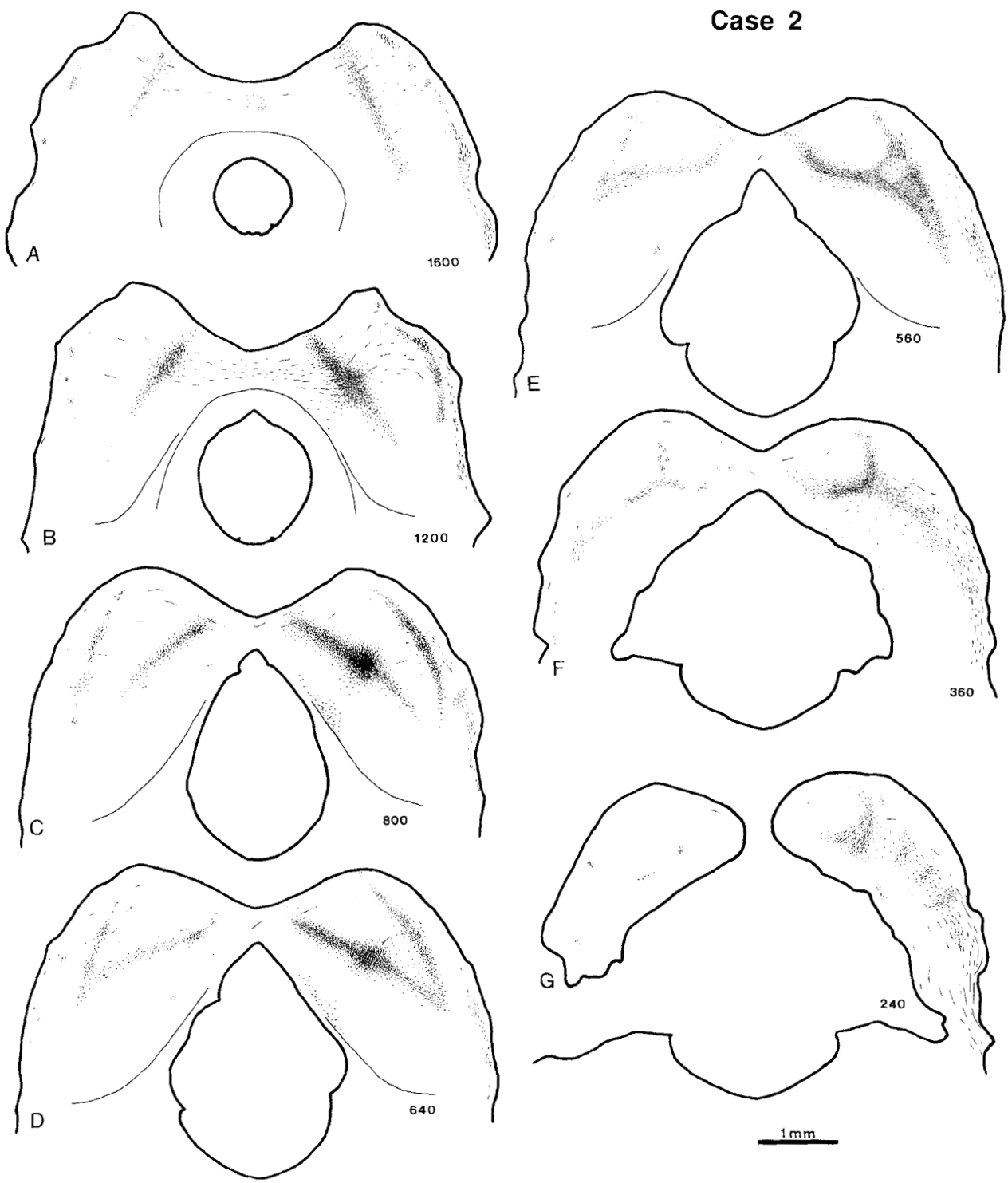


Fig. 5. Single PHA-L injection into the central (medium frequency) region of the right CNIC (case 2). The injection site is located at the level of section C. The main band is more lateral and the external band more medial than in case 1 (Fig. 4). In this case the plane of section is slightly tilted from dorsal and caudal to ventral and rostral. Note the patchy appearance of the external band (sections B–E) and the terminal fields within the CoIC (section A).

of the CNIC with extensive regions in the three subdivisions of the IC on both sides. There are several new and interesting aspects of this system of intracollicular projections. First, every point of the CNIC projects heavily to a laminar region within the same CNIC and the adjacent DCIC. Second, the CNIC projects heavily to the ipsilateral ECIC. Third, the commissural collicular projection is symmetric to the ipsilateral one. These three aspects of the intracollicular connections share a common feature: the precise topographic arrangement, so that points of the

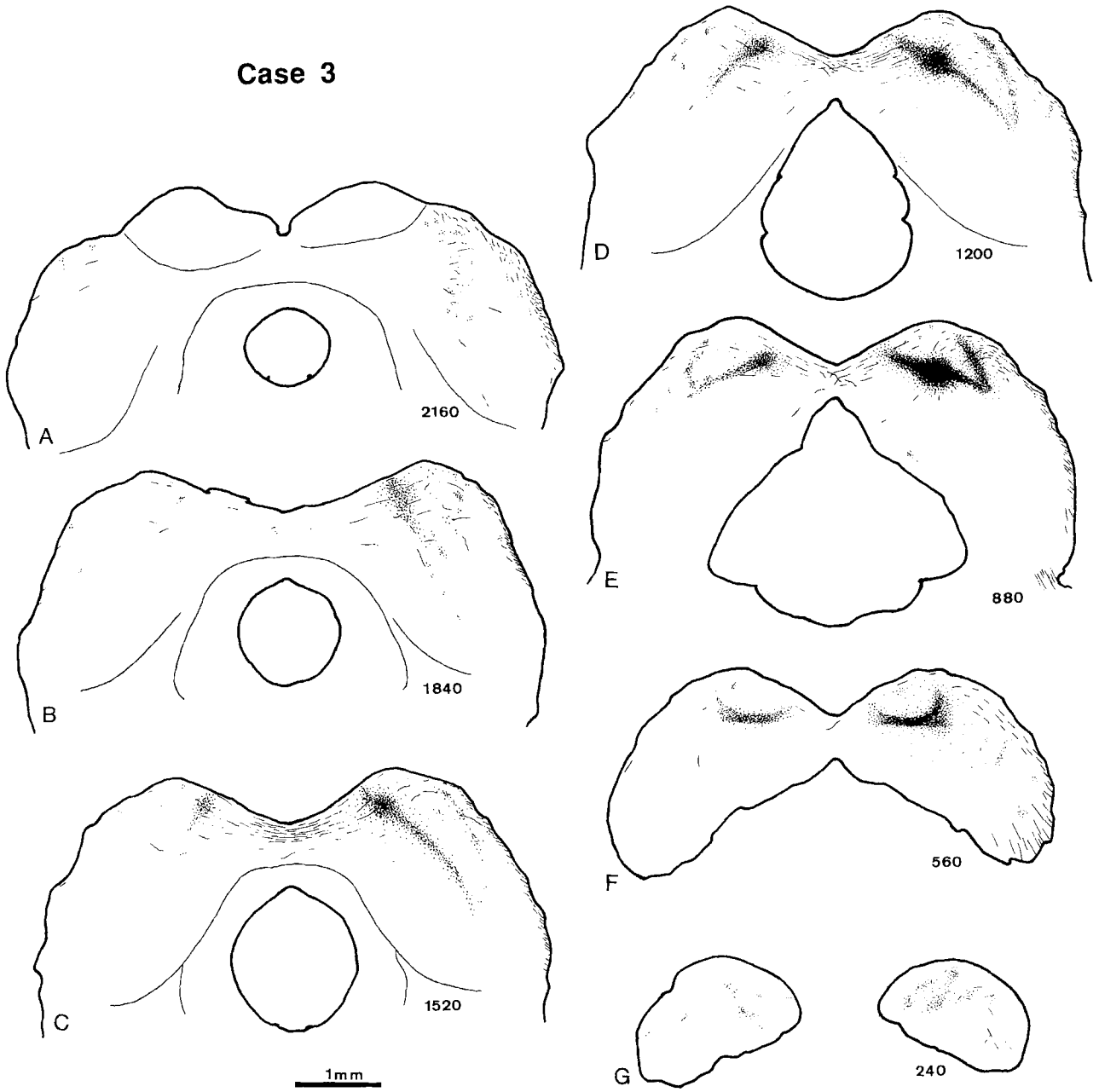


Fig. 6. Single PHA-L injection into the dorsolateral (low frequency) region of the right CNIC (case 3). The injection site is located approximately at the level of section E. The main band is more lateral and the lateral band more medial than in cases 1 and 2. The main and the external bands merge at a level more rostral and dorsal than in cases 1 and 2 (Figs. 4 and 5). Note that at caudal levels the terminal labeling is restricted to the dorsomedial regions of the IC.

CNIC with different CF innervate different regions in each one of the three subdivisions of the two colliculi.

The most parsimonious explanation for the fact that such major projections had been scarcely appreciated or overlooked is, perhaps, the lower resolution of the methods previously employed. Earlier attempts to elucidate the intracollicular connections were hampered by the limited resolution of techniques such as Golgi method (Herrera et al., '88, '89), degeneration (Moore and Goldberg, '63; van Noort, '69; Rockel and Jones, 73a,c; Morest and Oliver, '84), autoradiography (Andersen et al., '80b; Kudo and Niimi, '80; Hutson et al., '91), and tracing with HRP-conjugates (Willard and Ryugo, '83; Aitkin and Phillips, '84; González-Hernández et al., '86; Hutson et al., '91). The intracellular injections recently reported by Oliver and co-workers provide a wealth of data about the distribution of intrinsic axons within the CNIC and DCIC, but they do not reveal the projection to the ECIC, or the commissural projection (Oliver et al., '91). The PHA-L tracer, by virtue of its extreme selectivity and sensitivity (Groenewegen and Wouterlood, '90), has proven in this study to be an excellent tool for the analysis of intrinsic connections.

One could argue that our findings are unique to the rat. Although we have not yet made PHA-L injections into the

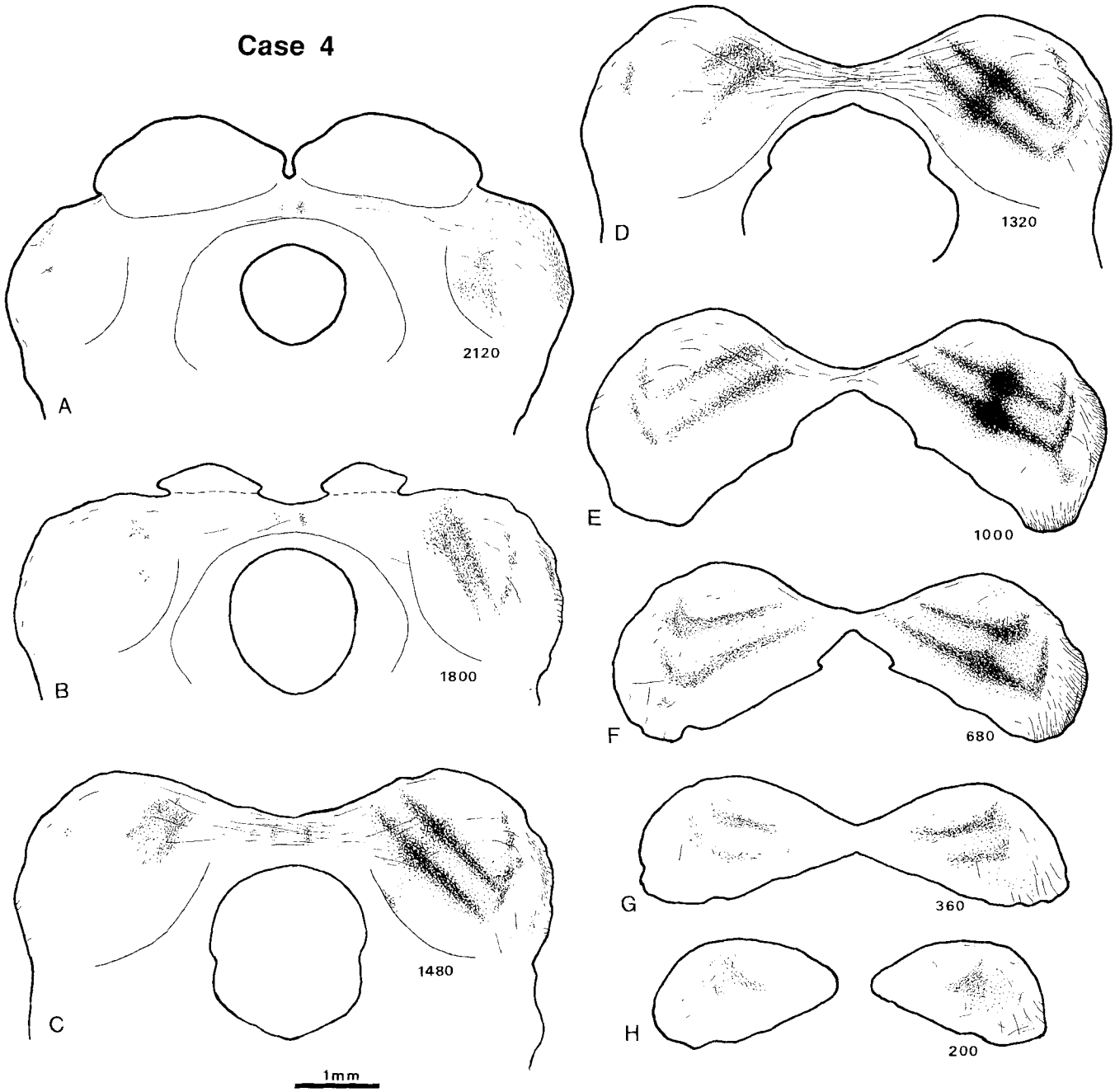


Fig. 7. Distribution of labeling after two separate PHA-L injections along the tonotopic axis of the right CNIC (case 4). Note the mirror-like image of the bands in every section. Also, the bands produced by the two injections diverge somewhat at caudal levels. The bands produced by the more ventromedial injection (higher frequency zone) enclose ventrally the territory occupied by the bands resulting from the more dorsolateral (lower frequency zone) injection. In this case the sections are slightly tilted from dorsal and rostral to ventral and caudal. Two small terminal fields within the CoIC, one on each side of the midline, are visible in sections A–C.

IC of other experimental animals, and therefore cannot make direct comparisons, the occurrence of a symmetric, banded pattern for the intrinsic and commissural collicular projections has been previously reported for the mouse following HRP injections (González-Hernández et al., '86). Our data generally confirm the banded aspect of these projections, but several discrepancies between our results and those of González-Hernández and co-workers are noted (i.e., the number and topography of the bands on each side). These probably reflect the different methods used, rather than real interspecies variations. To test this hypothesis, we have made small HRP injections into the rat CNIC (unpublished observations). Alternate sections were stained with DAB and TMB. The same PHA-L pattern could be identified in the HRP material, but never with the clarity provided by the PHA-L. While the ipsilateral main band

was readily distinguished, the ipsilateral external band and the contralateral main band were less defined, and partially obscured by the retrogradely labeled neurons, and the contralateral external band was hardly revealed. The retrograde labeling observed with HRP poses an additional problem in the interpretation of the origin of labeled fibers.

Numerous illustrations of injection sites of different tracers in the CNIC of various mammals can be found in the literature. In many instances, the injection sites are elongated in the direction of the fibrodendritic laminae, thus resembling our ipsilateral main bands. Such images have been published for the IC of the opossum (Willard and Martin, '84; see their Fig. 1), the mouse (Ryugo et al., '81; their Figures 1a,b), and the cat (Brunso-Bechtold et al., '81; their Figures 2b,c). Furthermore, injections of HRP in the CNIC of the horseshoe bat (Vater and Feng, '90) and PHA-L in the CNIC of the bush baby (Thompson and Thompson, '89) produced a band of anterogradely labeled fibers whose morphology and orientation closely coincide with those of our main band. Although these data do not allow one to draw definite conclusions about the pattern of intracollicular connections in species other than the rat, they strongly suggest that the pattern reported here might be, at least to some extent and with some interspecies variations, present in most mammals.

In recent years it has been generally accepted that, at the midbrain level and above, the auditory system is composed of different ascending pathways: a central, "core" pathway, involved in the processing and transmission of purely auditory information, whose final target is the primary sensory area of the auditory cortex, and multiple peripheral, "belt" pathways, involved in polysensory association and integration, which are possibly related to the acoustico-motor system, and whose final target are the auditory cortical belt areas (for a review, see Brodal, '81; Calford and Aitkin, '83; Huffman and Henson, '90). According to this line of thought, the CNIC is the origin of core connections, while the cortical areas of the IC, particularly the ECIC, which lacks a significant direct ascending auditory input, appear to be the origin of connections to the peripheral, non-laminated regions of the auditory thalamus; the ECIC, in addition, projects to the deep layers of the superior colliculus. For the sake of clarity, in the remainder of the Discussion the general pattern of intracollicular projections will be dealt with in two separate blocks. We will first deal with the projections from the CNIC to the CNIC and DCIC on both sides, because these seem to participate in the "core" auditory pathway. We will then focus on the projection from the CNIC to the ECIC, which may constitute one of the first steps of the "belt" ascending auditory pathway. The minor ventromedial ipsilateral band seen in some of our cases will be briefly addressed at the end of the Discussion.

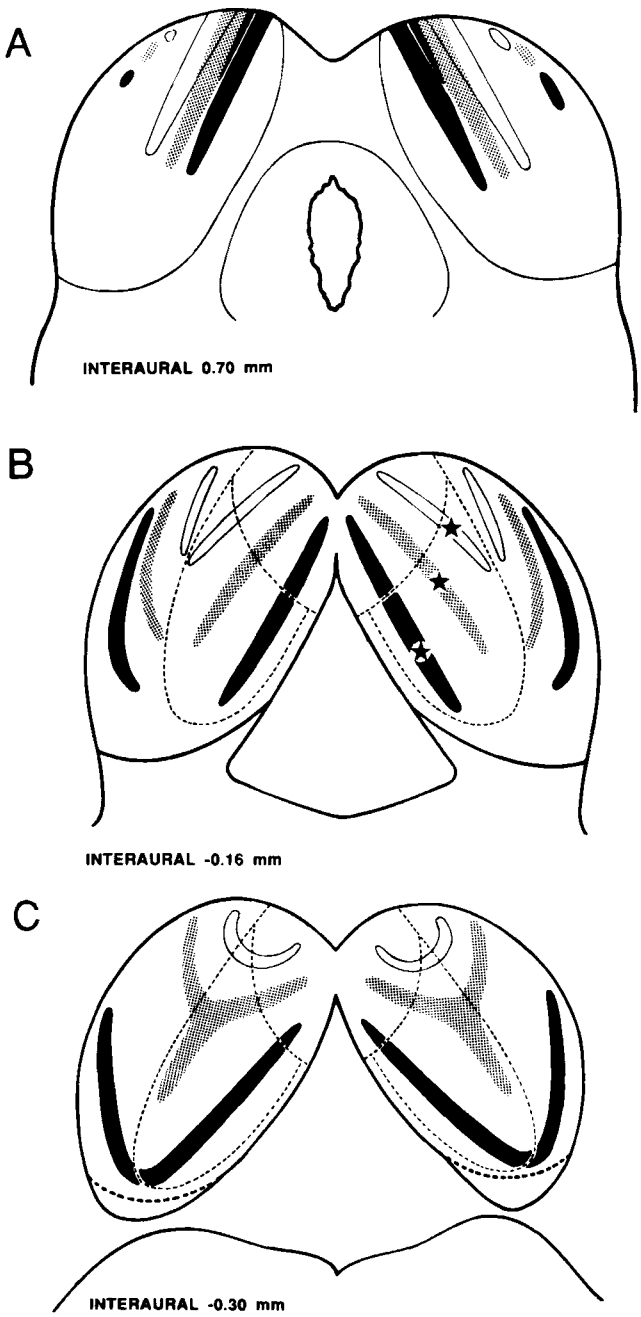


Fig. 8. Schematic representation of the distribution of intracollicular terminal fields in cases 1, 2, and 3. A is rostral; C is caudal. The individual injection sites in the right IC are indicated by stars in B. Black areas: distribution of the terminal fields after injection into the ventromedial (high frequency) portion of the CNIC. Grey areas: idem, after injection into the central (medium frequency) zone of the CNIC. White areas: idem, after injection into the dorsolateral (low frequency) regions of the CNIC. For the sake of clarity, the external bands in A and B have been clearly separated from one another. However, in reality, some convergence could occur between the dorsal end of the different external bands in B, while in A considerable overlapping may take place. The subdivisions of the IC and the rostrocaudal coordinates of the sections are the same as in Figure 1, right column. The intracollicular connections are arranged in a banded, tonotopic pattern. The contralateral terminal fields are a mirror-like image of the ipsilateral ones. The intracollicular fibers suggest that the IC consists of fibrocellular laminae (appearing as bands in coronal sections) that cross the subdivision boundaries and involve most of the IC. These fibrocellular laminae are roughly curved around a point located dorsally and caudally. The fibrodendritic laminae of the CNIC described by the Golgi method could constitute only a minor, central field of these major laminae. This scheme is comparable, although not identical, to the one proposed by Rockel and Jones ('73a,c).

Intrinsic projection to CNIC and DCIC

The main bands reported here represent an intrinsic plexus that connects every point of the CNIC with a

laminar region within the same CNIC and the DCIC. While a projection from CNIC to the ipsilateral DCIC has been shown in the rat by retrograde transport of HRP (Tokunaga et al., '84; Coleman and Clerici, '87), this is the first description of its topography. The main bands have a ventrolateral-to-dorsomedial orientation which, as already mentioned, parallels the fibrodendritic laminae of the CNIC of the rat (Faye-Lund and Osen, '85). However, the main bands extend beyond the limit of the laminated CNIC into the non-laminated DCIC. No inflections or deviations of the main bands were observed at the limit between these two subdivisions, or when the bands crossed the different layers of the DCIC or penetrated the rostral ECIC. At caudal levels the bending of the main bands and their mergence with the external bands are in line with previous descriptions of the organization of the cat IC based on Golgi impregnation. Rockel and Jones ('73a) compared the medioventral zone of the CNIC with an onion composed of concentric, curved shells around a dorsolaterally located center (see also González-Hernández et al., '89). Oliver and Morest ('84), on the other hand, described a different fish bone-like angulation of the fibrodendritic laminae at the transition of their *pars lateralis* and *pars centralis* of the CNIC. The latter pattern was described also by Meininger et al. ('86) in the IC of the mouse. A corresponding shift in dendritic orientation was observed by Faye-Lund and Osen ('85) and Malmierca ('91) at the border between the CNIC and ECIC in the rat. These observations most probably correspond to the sharp angle of bands observed ventrally and caudally by the PHA-L method.

According to the cytoarchitectural models based on Golgi impregnations, the fibrodendritic laminae of the CNIC are formed by the ascending lemniscal axons which run parallel to and intermingle with the flattened dendritic trees of the oriented principal or disc-shaped neurons (Morest, '64a; Rockel and Jones, '73a; Morest and Oliver, '84; Oliver and Morest, '84; Faye-Lund and Osen, '85). There are no reports of the distribution of specific lemniscal projections in the IC of the rat. In the cat, these projections have been described as either diffuse or focused. The diffuse inputs arrive from periolivary regions and the ipsilateral ventral nucleus of the lateral lemniscus (Whitley and Henkel, '84). The focused inputs, which come mainly from the contralateral cochlear nuclei (Oliver, '84b, '87), the ipsilateral medial superior olive (Henkel and Spangler, '83), both lateral superior olives (Shneiderman and Henkel, '87) and both dorsal nuclei of the lateral lemniscus (Shneiderman et al., '88; Hutson et al., '91), terminate in discrete terminal fields or bands which have the same ventrolateral-to-dorsomedial direction as our main bands. Few studies have addressed whether the bands of lemniscal afferents of different origin are segregated (for a review, see Oliver and Shneiderman, '91). In any case, it seems reasonable that, taken together, focused and diffuse lemniscal afferents cover the whole extent of the CNIC. Consequently, the intrinsic collicular fibers (as well as the commissural fibers) must intermingle with lemniscal fibers and, therefore, contribute to the formation of the fibrodendritic laminae. Thus, we conclude that the intracollicular fibers, both intrinsic and commissural, are an essential element of the fibrodendritic laminae. That intrinsic fibers contribute to the formation of the laminae has already been proposed based on the morphology of single intrinsic collicular axons impregnated with Golgi (Herrera et al., '88, '89) or filled with HRP injected intracellularly (Oliver et al., '91). While these studies did not provide information about the overall quantitative importance of the intrinsic connections, our data show that the main bands (both ipsi- and contra-lateral) are so dense, and the labeled fibers bear so many presumptive synaptic boutons, that the collicular neurons are likely to be a major source of inputs to other collicular cells. The intracollicular projections seem to be at least as dense as any afferent projections from subcollicular nuclei. The intracollicular fibers probably also intermingle with the corticocollicular fibers in the DCIC (Diamond et al., '69; Rockel and Jones, '73a; Andersen et al., '80a; Faye-Lund, '85; Herbert et al., '91), and the bands formed by the neocortical fibers in the medial DCIC (Andersen et al., '80a) could be the counterpart of the dorsal portion of our main bands (see below).

The commissural projection to DCIC and CNIC

Very few studies have focussed on the intercollicular connections. In an attempt to retrogradely label all the collicular neurons projecting through the collicular commissure (CoIC), HRP has been deposited into a cut made in the CoIC (Aitkin and Phillips, '84; Hutson et al., '91). These studies show that, in the cat, the commissural neurons are concentrated ventrolaterally at posterior levels, and more dorsally at anterior levels. According to the authors, the collicular interconnections are "complementary," since regions of the IC supplying fibers to the commissure are not topographically coincident with their target areas. By contrast, other HRP studies in rat, cat, and bat have defined the commissural projection as "reciprocal," because after small injections in the CNIC, labeled neurons in the contralateral IC were seen to occupy a position homotopic to the injection site (Adams, '80; Schweizer, '81; Zook and Casseday, '82; Druga and Syka, '84a; Tokunaga et al., '84; Coleman and Clerici, '87). This reciprocity has been questioned by González-Hernández et al. ('86), who found that in the mouse the position of the ipsi- and contra-lateral intracollicular terminal fields is constant irrespective of the location of the HRP injection. By contrast, our results show that the position of the intracollicular terminal fields changes according to an orderly, topographic pattern. Furthermore, our data confirm the reciprocity of the commissural projection, since after every PHA-L injection the labeled fibers reach the area of the contralateral IC homotopic to the injection site. However, the crossed collicular projection is more than just reciprocal or homotopic, because the territory innervated in the contralateral IC includes areas homotopic to the entire ipsilateral bands and, therefore, is much larger than the area homotopic to the injection site.

Various degeneration and autoradiographic studies have concluded that, at least in the cat, the main, if not the only, recipient of commissural fibers is the DCIC, and that the DCNIC, with the exception of its dorsomedial part, is poorly innervated (Moore and Goldberg, '63; van Noort, '69; Rockel and Jones, '73a,c; Hutson et al., '91). This notion is supported by the HRP experiments of Brunso-Bechtold et al. ('81), who found that the number of retrogradely labeled cells in the contralateral IC is highest when the tracer is delivered in the dorsomedial area of the IC. Our results confirm the projection to the contralateral DCIC, but they also demonstrate a considerable input from the CNIC to the contralateral CNIC. In some of our cases, the contralateral main band spans the whole dorsoventral extent of the IC and can be found along nearly the entire rostrocaudal

extent of the IC, indicating that practically no region of the DCIC or CNIC is devoid of commissural input. Taking into account the contralateral external band, it is reasonable to suppose that the whole IC is subjected to commissural influence. In partial agreement with previous studies, it seems that the contralateral main bands are more dense dorsally. Further experiments are being conducted in our laboratory to elucidate the exact topography of the commissural projections after injections placed at different dorsoventral and rostrocaudal levels. Preliminary data indicate that the symmetric pattern is consistently found, regardless of the level of the injection site, thus suggesting that all regions of the CNIC contribute to the commissural projections.

Most fibers within the CoIC are likely to belong to the intercollicular system. However, the CoIC contains other fibers (see Hutson et al., '91 for a recent review); there are axons projecting from the IC to the contralateral medial geniculate body (Kudo and Niimi, '78; Aitkin and Phillips, '84; Oliver, '84a; Hutson et al., '91) or from the auditory neocortex to the contralateral IC (Rockel and Jones, '73a; Andersen et al., '80a; Faye-Lund, '85; Herbert et al., '91). The CoIC also contains fibers originating from the DCIC and ECIC (Coleman and Clerici, '87), whose morphology and distribution are unknown. The fact that different subdivisions of the IC project to different regions of the medial geniculate body (Andersen et al., '80b; Kudo and Niimi, '80; Calford and Aitkin, '83) raises the question of whether their commissural projections could also differ. The resolution of this problem will require further study.

Functional implications

Intracollicular projections and tonotopic organization. The most prominent feature of the intracollicular projections, apart from their density, is their topographical organization. The lateromedial and dorsoventral position of the main bands changes when injecting the tracer in points of the CNIC known to differ in their CF, indicating a close relationship between the distribution of the intracollicular fibers and the spatial representation of frequencies within the IC. Electrophysiological studies have shown that the isofrequency contours are continuous through the CNIC and DCIC of the mouse (Harnischfeger, '78; Willot and Urban, '78; Stiebler and Ehret, '85), cat (Semple and Aitkin, '79; Schreiner and Langner, '88), and monkey (FitzPatrick, '75), thus proving that CNIC and DCIC share a common tonotopic arrangement. Similarly, studies with radiolabeled 2-deoxyglucose (2-DG) uptake have demonstrated that stimulation with pure tones results in bands, or isofrequency contours, which span the entire CNIC and penetrate the DCIC (Servière et al., '84; Webster et al., '84; Martin et al., '88). Thus, there is a coincidence between the position and extent of the PHA-L main bands and those of the isofrequency contours, since both are continuous through the CNIC and DCIC. Comparison of our main bands with the isofrequency contours of the rat IC (Ryan et al., '88) reveals that both have exactly the same ventrolateral-to-dorsomedial orientation. Furthermore, like the isofrequency contours, our main bands are more vertical when they occupy a more ventromedial position (higher frequency), and for the same experiment, they are more vertical in more rostral sections. Like isofrequency contours, the main bands of different frequencies are closer together rostrally and are not perfectly parallel, particularly in caudal sections. Moreover, the dorsolaterally located main bands are shorter than the ventromedial bands, just as the low frequency contours are shorter than the higher frequency ones. Finally, both the main bands and the isofrequency contours represent a slice of a longitudinal sheet that spans the IC rostrocaudally. The isofrequency planes reconstructed by some authors (FitzPatrick, '75; Semple and Aitkin, '79; Servière et al., '84; Webster et al., '84; Stiebler and Ehret, '85; Huang and Fex, '86; Martin et al., '88; Ryan et al., '88) are in register with our laminar plexuses. These similarities, together with the fact that the position of the main band changes when the injection site is shifted along the tonotopic axis of the CNIC, lend support to the idea that the ipsilateral and contralateral main bands actually represent isofrequency contours or regions.

The coincidence in the extension of the isofrequency contours and the main bands is particularly interesting. The notion put forth by Morest ('64a,b) that the fibrodendritic laminae are related to the representation of frequencies in the IC has been generally accepted. The isofrequency contours, however, extend into the non-laminated DCIC. This may be engendered by the innervation of the dorsal collicular regions by lemniscal fibers (Servière et al., '84; Webster et al., '84, Martin et al., '88). Although there is increasing evidence that the deep DCIC is reached by fibers from several subcollicular nuclei (Oliver, '84b, '87; Whitley and Henkel, '84; Shneiderman et al., '88), this innervation is far less dense than the one reaching the CNIC and could hardly account for the strong 2-DG labeling observed in the DCIC. Another possible explanation could be that the isofrequency contours in the DCIC reflect the corticocollicular innervation (Andersen et al., '80a), yet as noted by Servière et al. ('84), the physiological data (Semple and Aitkin, '79) do not indicate long latency responses of such strength as to produce the 2-DG contours. Given the density and the position of the main bands seen with PHA-L, a further possibility is that the 2-DG contours reflect the activity of the intrinsic fibers and, to a lesser extent, the commissural fibers. The 2-DG labeling is known to be associated more with active synapses than discharging neurons (Nudo and Masterton, '86), but these synapses are not solely formed by afferent fibers, but also by intrinsic fibers. This could satisfactorily explain the extension of the isofrequency contours into the DCIC. This could also explain why the 2-DG bands are consistently more dense in the CNIC than in the DCIC (Servière et al., '84), since in the CNIC the 2-DG labeling would be the result of the combined synaptic activity of the lemniscal plus the intracollicular fibers.

It could be argued that the main bands obtained with PHA-L could include a wide group of adjacent frequencies. We concede this point, but we note that inferences similar to ours have previously been made on the basis of labeling with 2-DG, and the "isofrequency" bands seen in such experiments were often as thick as the bands seen with PHA-L (Servière et al., '84; Webster et al., '84; Ryan et al., '88; Martin et al., '88). New PHA-L experiments are being conducted in our laboratory in an attempt to determine the relationship between the size of the injections and the width of the bands, and the limit of resolution of the technique when very small volumes of tracer are delivered.

The association between frequency representation and intracollicular connections is reinforced by the fact that in our experiments little, if any, terminal labeling was seen outside the bands. Consequently, points of the CNIC project predominantly, if not exclusively, to points of both

colliculi that lie within its own frequency, which is in line with the observation that most units in the CNIC are narrowly tuned (Aitkin et al., '75; Semple and Aitkin, '79; Schreiner and Langner, '88). Taken together, it seems that within the inferior colliculi the information about frequency is maintained. However, the intracollicular projections could be responsible for significant changes of frequency specific signals (see below).

Intracollicular projections and other acoustic parameters. Another consideration arises from the possibility that the neurons connected by intrinsic fibers may be heterogeneous. Within a given isofrequency plane, neurons differ in several response properties, including tone response threshold, sharpness of frequency tuning curves, onset latencies to tones, binaural interaction type and best modulation frequency for amplitude modulated tones (Stiebler and Ehret, '85; Horikawa and Murata, '88; Schreiner and Langner, '88). Furthermore, some of these parameters appear to have their own topographic representation within isofrequency planes (Wenstrup et al., '86; Schreiner and Langner, '88). Thus, it is reasonable to assume that a possible function of the intracollicular projections is to process, integrate or even generate other parameters of acoustic information, while preserving frequency specificity.

Intrinsic and commissural collicular fibers: Excitatory or inhibitory? A relevant question which still remains is whether the fibers composing the main bands are excitatory or inhibitory. Adams and Wenthold ('79) have shown that considerable amounts of putative inhibitory neurotransmitters and glutamate decarboxylase (GAD), the synthesizing enzyme for GABA, are present throughout the CNIC. Although subcollicular centers like the dorsal nucleus of the lateral lemniscus provide a GABAergic innervation for the CNIC (Adams and Mugnaini, '84; Shneiderman and Oliver, '89), the detected high levels of GABA also fit with the abundance of GABA-immunopositive and GAD-immunopositive neurons in the IC (Vetter and Mugnaini, '84; Mugnaini and Oertel, '85; Moore and Moore, '87; Roberts and Ribak, '87a,b; Caspary et al., '90). The role of GABA as an inhibitory neurotransmitter in the IC has recently been tested by Faingold and co-workers ('89a, '91), who proposed that collicular interneurons are an important source of GABA-mediated inhibition.

The CNIC also contains considerable amounts of putative excitatory neurotransmitters (Adams and Wenthold, '79), part of which could have an extrinsic origin. However, numerous cells of the CNIC show moderate to high glutamate-like immunoreactivity (Ottersen and Storm-Mathisen, '84), indicating that excitatory amino acids could be synthesized locally. Most efferent fibers of the CNIC appear to be excitatory, based on the morphology of their boutons in the medial geniculate body (Morest, 1975), ventral nucleus of the trapezoid body (Saldaña and Mugnaini, unpublished observations), and cochlear nuclei (Kane, '77). This supports the notion that some of the intrinsic connections may be excitatory, since some of the efferent fibers give off local collaterals (Oliver et al., '91). This also indicates that some of the commissural connections may be excitatory, since some collicular neurons have been shown to innervate both the ipsilateral medial geniculate body and the contralateral IC (González-Hernández et al., '91). Finally, there is pharmacological evidence supporting an important role of excitatory amino acids as afferent neurotransmitters in neurons of the CNIC (Faingold et al., '89b).

Glutamate decarboxylase-positive fibers and terminals have been seen within the rat CoIC, but their origin is uncertain (Vetter and Mugnaini, '85). Using the brain slice technique, Smith ('90) has studied the response of neurons in the rat DCIC after shock stimulation of the CoIC. The stimulation produced an early IPSP followed by a slightly longer latency EPSP. Pharmacological evidence suggests that the early IPSP is due to a direct, commissural GABAergic input, while the EPSP represents a direct, crossed input that activates non-NMDA type glutamate receptors. These data strongly suggest that the commissural projection has excitatory as well as inhibitory components. However, these in vitro studies do not reveal the origin of the stimulated fibers, and the recordings were made from very dorsal regions of the contralateral IC. Thus, the possibility that the stimulated fibers come from regions other than the CNIC or that they do not participate in the system described here cannot be ruled out. The stimulated fibers could, for instance, provide a few collaterals in the contralateral DCIC on their way to the contralateral medial geniculate body without further contributions to the innervation of the contralateral IC.

In summary, the previous data combine to suggest that both the ipsi- and contra-lateral main bands are made up of a mixture of excitatory and inhibitory fibers. Since the intracollicular connections are frequency specific, they could increase the output of some frequency-selective signals while decreasing others. This dynamic balance between excitation and inhibition could constitute the local basis for an improvement in the signal to noise ratio. It is, therefore, tempting to speculate that the IC could play a relevant role in phenomena of selective attention. Assuming that the pitch of the sound is an important clue to select the source of sound to which attention is paid (Meddis, '92), the IC could be one of the first places where such a selection takes place.

The commissural projection and the representation of auditory space. It has been shown in the barn owl that the commissural collicular projection contributes to the representation of both ipsilateral and contralateral auditory hemifields in the CNIC. This is due to a direct projection from the so called core of the CNIC, where the representation of ipsilateral space is found, to the lateral portion of the shell of the contralateral CNIC (Takahashi et al., '89). As in the owl, the projection from CNIC to the contralateral CNIC in the rat is tonotopic. However, there are notable anatomical differences between the auditory systems of birds and mammals. In avians, the IC receives ascending inputs from fewer centers than in mammals, and some of these inputs are clearly segregated within the IC (Leibler, '75; Takahashi and Konishi, '88). Furthermore, in the owl the collicular commissural projection reaches heterotopic regions in the contralateral IC, whereas in mammals it is homotopic (Takahashi et al., '89; present results). These anatomical differences raise doubts as to whether the CoIC plays a similar role in mammals and birds. Physiological and behavioral studies have generally indicated that the mammalian IC contains a representation of contralateral space (Jenkins and Masterton, '82; Calford et al., '86) but have yet to find a significant contribution from CoIC. Using behavioral testing paradigm, Moore et al. ('74) have shown that transection of the CoIC and/or the corpus callosum has little or no effect on the cat's ability to localize sound. While these tests suggest that the CoIC is not necessary for sound localization, the possibility that it plays a role in sound localization that was not detected in the behavioral studies or is involved in different, hitherto unknown, aspects of binaural processing should be kept in mind. It

would be interesting to know the effect of the commissural transection on individual binaural collicular neurons, and the effect of electrophysiological stimulation of one IC on neurons of the opposite IC.

In summary, many questions still remain concerning the role of the CoIC in mammals. Given its relatively large volume, and the fact that the CoIC is the last site where the auditory information crosses the midline before reaching the cerebral cortex, it is logical to assume that the CoIC subserves important, yet unknown and largely unexplored functions.

The projection from CNIC to ECIC

The external bands seen in this study demonstrate a dense, direct projection from the CNIC to the ECIC. These results confirm previous studies with lesions or injections of tritiated amino acids in the cat CNIC (Rockel and Jones, '73a,c; Andersen et al., '80b; Kudo and Niimi, '80; Hutson et al., '91). Moreover, our experiments reveal two significant aspects of this projection: its exquisite topographic arrangement, and the projection to the contralateral ECIC, which is also topographically organized and symmetric to the ipsilateral one (see also Aitkin et al., '81; Coleman and Clerici, '87).

Topography of the CNIC-ECIC projection. The topographic arrangement of the CNIC-ECIC projection is somewhat surprising, since previous anatomical studies had failed to provide clear evidence of topography (Andersen et al., '80b; Kudo and Niimi, '80; Aitkin et al., '81) and the possible tonotopy of the ECIC had been debated (Aitkin et al., '81; Stiebler and Ehret, '85). As shown in this study, points of the CNIC with different CF project to different areas of ECIC, suggesting that this projection could be tonotopically organized. When the injection site is displaced along the tonotopic axis of CNIC, the lateromedial displacement of the external bands is opposite to that of the main bands: high frequency injections give rise to a medially located main band and a laterally located external band, while after low frequency injections the main band is more lateral and the external band more medial. This arrangement resembles the topography of the corticocollicular projection, which in the cat is known to be tonotopic. In autoradiographic studies, injections of tritiated amino acids in physiologically defined areas of the cat auditory cortex (Andersen et al., '80a) revealed two bands of labeled terminals in the ipsilateral IC, a medial band in the DCIC and a lateral band in the ECIC, and a third band in the contralateral DCIC symmetric to the ipsilateral one. Following injections in the high frequency areas of the auditory cortex, the medial band was located medially in the DCIC, the lateral band laterally in the ECIC. After injections into the low frequency cortical area the first band was located more laterally and the second more medially. Each medial band was found to be oriented in register with an isofrequency contour in the CNIC with the same best frequency as the injection site. When two injections are made at widely separated places, with different characteristic frequency, the two lateral bands are much closer together than the two medial ones. The medial and lateral bands of Andersen et al. ('80a) appear to be the counterpart of the dorsal part of our main and external band, respectively. In further agreement with the present findings, the medial and lateral bands of Andersen et al. ('80a) also converge in the ventrocaudal direction. The topographic arrangement of the corticocollicular and CNIC-ECIC projections suggests that the ECIC could have its own tonotopic organization, which is separate and opposite to that of the CNIC, and in which the orientation of the isofrequency contours would coincide with that of our external bands. Some electrophysiological recordings support the notion of a reverse tonotopic sequence for the IC of the cat (Rose et al., '63; Merzenich and Reid, '74; Aitkin et al., '75; Roth et al., '78) and the gerbil (Kitzes, '84). The studies of the tonotopic organization of the rat IC by Clopton and Windfield ('73) are not conclusive in this regard, and the recordings of Stiebler and Ehret ('85) suggest a tonotopic organization of the mouse ECIC separate but rather parallel, instead of opposite, to that of the CNIC. In a recent study Schreiner and Langner ('88) have shown that in the *pars lateralis* of the cat CNIC the isofrequency contours have a dorsolateral to ventromedial orientation, i.e., they are nearly perpendicular to those of *pars centralis* and *pars medialis,* and more interestingly, the isofrequency contours of these two regions merge ventrally in a way that resembles the ventral mergence between the main and the external bands seen with PHA-L, supporting the notion that the external bands could represent isofrequency contours. One may also speculate that the ventrolateral nucleus and the *pars lateralis* of the CNIC of the cat are homologous to the deep part of the rat ECIC, as already suggested by Faye-Lund and Osen ('85).

In the rat, little, if any, ascending auditory input to ECIC arrives from outside the inferior colliculi (Tokunaga et al., '84; Coleman and Clerici, '87). Similarly, in the cat the external nucleus does not appear to receive significant input from any subcollicular auditory nuclei (Aitkin et al., '81; Henkel and Spangler, '83; Oliver, '84b, '87; Shneiderman et al., '88). However, the *pars lateralis* of the feline CNIC receives a tonotopically organized projection from the lateral superior olive (LSO), with lower frequency regions of LSO projecting more dorsally into the *pars lateralis* (Shneiderman and Henkel, '87. Compare their Figs. 2, 5, and 6). This fact is in agreement with the distribution of frequencies suggested by the PHA-L experiments and corroborates the similarity between *pars lateralis* of the cat CNIC and the deep part of the rat ECIC. A homologous projection from LSO to the deep ECIC, however, remains to be demonstrated in the rat.

New electrophysiological studies are needed to clarify the functional organization of the ECIC of mammals. In view of the orientation of the external bands, better understanding of the possible tonotopic ordering of the ECIC would require electrophysiological studies with oblique penetrations in the laterodorsal-ventromedial direction rather than the typical oblique parasagittal (Merzenich and Reid, '74; Aitkin et al., '75, '78, '81), caudorostral (Semple and Aitkin, '79), or vertical (Stiebler and Ehret, '85) penetrations.

Implications for the cytoarchitectural subdivisions of the IC. The distribution of the intracollicular fibers within the lateral IC has led us to refine the limits between the subdivisions of the IC. According to the cytoarchitectural model of the rat IC proposed by Faye-Lund and Osen ('85) and Paxinos and Watson ('86) (Fig. 1, left column), the dorsal end of our external bands penetrates into their DCIC. This, on the one hand, would imply that the DCIC possesses two regions, medial and lateral, that clearly differ in their afferent connections. On the other hand, this could mean that within the DCIC there is a double tonotopic organization, the representation of frequencies in the medial two thirds being a reversal of the representation of frequencies in the lateral third. Furthermore, the DCIC would have an overrepresentation of low frequencies, especially at caudal levels, where the dorsomedially located low

frequency contours would lie completely within the limits of DCIC, while the more ventrally situated high frequency contours would barely reach the border of the DCIC. With the techniques employed by Faye-Lund and Osen ('85) (Nissl and Golgi methods, and cell-myelin staining), the borderlines between the DCIC and the ECIC are "everywhere difficult to define," leading these authors to trace this limit only tentatively. We propose that the border between DCIC and ECIC be displaced dorsally, in register with the border between CNIC and ECIC (Fig. 1B',C'). With this change, the external bands lie completely within the limits of the ECIC (Fig. 8B,C). Recent studies by Malmierca ('91) using computer-assisted three-dimensional (3-D) reconstructions of Golgi impregnated neurons of the rat IC support the notion that the border between DCIC and ECIC is more dorsal and medial than shown by Faye-Lund and Osen ('85). A dorsal extension of the ECIC is also consistent with the distribution of acetylcholinesterase and nicotinamide adenine dinucleotide phosphate (NADPH) histochemistry (Paxinos and Watson, '86; Herbert et al., '91) and GAD immunoreactivity (Vetter and Mugnaini, '84) in the different layers of the ECIC. Also interesting is the fact that in coronal sections stained for acetylcholinesterase (Paxinos and Watson, '86) or GAD (Vetter and Mugnaini, '84) layer 2 of the ECIC has a patchy appearance that resembles the patches observed in our external bands. Our results also indicate that the CNIC reaches further dorsally than claimed by Faye-Lund and Osen ('85), so that the low frequency part of the CNIC actually approaches the dorsal surface of the IC. This change is justified by some of our experiments with dorsal injections (i.e., case 3, Fig. 6) and is in agreement with the cytoarchitectural analysis of Malmierca ('91) (see his Figs. 30 and 39). The dorsal extension of the CNIC is also supported by functional 2-DG experiments, in which stimulation with pure tones of very low frequency resulted in a band which occupied the dorsolateral margin of the CNIC, in the vicinity of the dorsal surface of the IC (Ryan et al., '88, see their Fig. 4).

The extension of the main bands into the rostral ECIC (Fig. 8A) suggests that the CNIC and/or DCIC could reach further rostrally than proposed by the Golgi models. However, more anatomical, connectional, and functional evidence is needed to clarify the nature of this poorly known region of the IC.

Projection from CNIC to ECIC: Morphofunctional considerations. Many questions remain concerning the function of the ECIC. The most widely accepted hypothesis is that the ECIC is an area for auditory and somatosensory integration (RoBards et al., '76; Aitkin et al., '78, '81; RoBards, '79; Willard and Ryugo, '79; McCown and Breese, '91), a role shared in part by other auditory structures such as the cochlear nuclei (Itoh et al., '87; Weinberg and Rustioni, '87) and the medial geniculate body (Ledoux et al., '87). The somatosensory inputs arise mainly from the contralateral cuneate and gracile nuclei, as well as the contralateral spinal trigeminal nucleus (Feldman and Kruger, '80; Aitkin et al., '81; Tokunaga et al., '84; Coleman and Clerici, '87; Weinberg and Rustioni, '89). The ECIC also receives a descending projection form the ipsilateral somatosensory cortex (RoBards, '79). The main auditory input arises from the IC itself. Most "auditory" neurons in the ECIC have been defined as having much broader tuning curves than neurons of the CNIC (Aitkin et al., '75; Willot and Urban, '78), which concurs with the presumed lack of tonotop in the CNIC-ECIC projection (Aitkin et al., '81). While our results clearly demonstrate a strict topographic arrangement for this projection, the broader tuning curves still make sense: a change in the position of the PHA-L-injection site along the tonotopic axis of the CNIC has a greater effect on the lateromedial position of the main bands than of the external bands. Therefore, as suggested by our results, the isofrequency contours seem to be much closer in the ECIC than in the CNIC and DCIC, and therefore the ECIC frequency receptive fields are likely to be less well defined. This could be true particularly in rostral and dorsal regions of the ECIC, where much convergence or even overlapping of inputs from different points of the CNIC could take place (see Fig. 8). It would be interesting to ascertain whether dendritic fields of individual ECIC neurons span across different isofrequency planes. The large neurons of layer 3, some of which have dendrites extending laterally towards the surface of the IC (Cajal, '03, '11; Faye-Lund and Osen, '85; Malmierca, '91), could receive inputs from different isofrequency regions of the CNIC. Despite the relatively poor auditory properties of ECIC neurons (broad, irregular tuning curves, high acoustic threshold, and rapid habituation to tones), the acoustic representation within ECIC appears to be comparatively more refined than the tactile representation, because the ECIC somatic receptive fields are large (Aitkin et al., '78) and no topographical representation of the body surface has yet been found in the ECIC (RoBards, '79; Aitkin et al., '81).

The acoustico-tactile integration in the ECIC could play a role in the enhancement of complex sound-evoked behavioral responses. Given the poor auditory properties and the large tactile receptive fields of the ECIC, it has been suggested that this nucleus probably subserves the initial stages of such integration. Moreover, direct or indirect projections from the ECIC to integrative centers like the superior colliculus or cerebellum suggest that the ECIC is an important relay station in the acousticomotor pathways (see review in Huffman and Henson, '90).

The projection from CNIC to the ventromedial border of the IC

Our PHA-L experiments demonstrate a sparse ipsilateral projection from high and medium frequency regions of the CNIC to the ventromedial border of the IC. There seems to be a gradient in the density of this projection, with regions of the CNIC closer to the ventromedial border contributing more fibers than more distant regions. This convergent innervation of the ventromedial border of the IC seems to be the exception among the otherwise strictly tonotopic intracollicular projections and suggests that the ventromedial border of the IC may be involved in processing of acoustic information within a wide range of frequencies in the upper half of the rat's auditory spectrum. While no electrophysiological data are available comparing the sharpness of frequency tuning curves between this and other collicular territories, a preliminary study with 2-DG has shown that this region has unique functional characteristics (Silverman, '79). According to this report, in barbiturate anesthetized animals that are not acoustically stimulated, the ventromedial border of the IC shows a band of increased 2-DG activity bilaterally. Unilateral stimulation with a wide variety of pure tones substantially diminishes or eliminates the band ipsilaterally but not contralaterally.

These results led Silverman ('79) to conclude that this ipsilateral suppression is broadly tuned.

The ventromedial border of the IC also appears different from the rest of the IC in that it receives an important contingent of fibers from the auditory cortex. Studies with anterograde degeneration or transport of HRP conjugates show that, at least in the rat, this collicular region is the target of projections from various neocortical regions, particularly those that project to medial regions of the DCIC (Faye-Lund, '85, see her Figs. 3B, 5B, and 7B; Herbert et al., '91, their Figs. 3, 10B, 11, and 12).

With the little data available at present, it is difficult to speculate about the possible biological role of this collicular region. Future detailed cytoarchitectural, ultrastructural, connectional and functional studies, as well as across-species comparisons, are needed to understand its significance.

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