Hearing Research, 34 (1988) 1–26
Elsevier

HRR 01052

Layer V in rat auditory cortex: Projections to the inferior colliculus and contralateral cortex

Kate D. Games and Jeffery A. Winer

Department of Physiology-Anatomy, University of California, Berkeley, California, U.S.A.

(Received 16 July 1987; accepted 17 January 1988)

This study compares the form and distribution within layer V of cells projecting to the inferior colliculus with that of commissural cells of origin in adult rat auditory cortex after horseradish peroxidase injections in the ipsilateral inferior colliculus or auditory cortex. The goal of this work was to determine whether every part of layer V participates equally in both projections, and if the cortical neurons in each pathway were similar. The types of neurons were defined in Golgi-Cox preparations and matched with the profiles of retrogradely labeled cells from architectonically defined cortical area 41. Inferior colliculus and commissural neurons form two populations that differ in their distribution in layer V, in somatic area, and in the form of their apical dendritic arbors.

Corticocollicular neurons include the largest pyramidal cells, whose robustly filled apical dendrites ascend into layer II or farther. Commissural cells are smaller and have a more heterogeneous form. Their apical dendrites do not usually extend above layer IV, and a few of these cells may be non-pyramidal. Small pyramidal cells and inverted pyramidal cells project to the opposite cortex, but not to the inferior colliculus. Medium-sized pyramidal cells project in both systems. In addition, certain callosal cells of origin in layers V and III were morphologically similar.

More than one-third of the commissural cells originate in the superficial part of layer V, where only 7% of the inferior colliculus projection neurons arise. Most corticocollicular cells lie deeper in layer V, where there are fewer commissural neurons.

These findings suggest that the efferent systems projecting to telencephalic and mesencephalic targets are morphologically distinct and spatially segregated in layer V. However, the commissural projection includes similar cells in different cortical layers. The types of these efferent neurons may be more closely related to their target than to their laminar origin.

Interhemispheric projection; Sensory neocortex; Layer V; Pyramidal cell; Commissural projection system; Laminar organization

Introduction

Corticofugal neurons in the sensory neocortex are generally thought to have one primary laminar origin, for example, layer III is largely commissural (Jones and Wise, 1977), layer IV projects mainly in ipsilateral corticocortical pathways (Winguth and Winer, 1986), while many layer VI neurons send their axon to the thalamus (Gilbert and Kelly, 1975). In rat auditory cortex, some layer V

Correspondence to: Jeffery A. Winer, Department of Physiology-Anatomy, University of California, Berkeley, California 94720, U.S.A.

Abbreviations: Aq, cerebral aqueduct; BIC, brachium of the inferior colliculus; BSC, brachium of the superior colliculus; BV, blood vessel; Cb, cerebellum; CN, central nucleus of the inferior colliculus; CP, cerebral peduncle; D, dorsal division of the medial geniculate body; DAB, 3,3′-diaminobenzidine; DC, dorsal cortex of the inferior colliculus; DCN, dorsal cochlear nucleus; Ex, external nucleus of the inferior colliculus; HRP, horseradish peroxidase; LL, lateral lemniscus or nuclei of the lateral lemniscus; IA, inter-aural level; ICP, inferior cerebellar peduncle; M, medial division of the medial geniculate body; ML, medial lemniscus; MLF, medial longitudinal fasciculus; Mo5, trigeminal motor nucleus; MRF, mesencephalic reticular formation; PVCN, posteroventral cochlear nucleus; R, rhinal sulcus; SC, superior colliculus; SN, substantia nigra; SPN, suprapeduncular nucleus; Sp5, spinal trigeminal nucleus; TMB, 3,3′,5,5′-tetramethylbenzidine; TrB, trapezoid body; V, ventral division of the medial geniculate body; VCN, ventral cochlear nucleus; wm, white matter; I-VI, cortical layers; 41,20,36, auditory cortical areas; 17,18a, visual cortical areas. *Orientation of sections*: C, caudal; D, dorsal; L, lateral; M, medial; R, rostral; V, ventral.

cells are commissural (Jacobson and Trojanowski, 1974), while others project to the inferior colliculus (Beyerl, 1978). It is not known whether these cells of origin have a uniform distribution in layer V, or if they have a particular concentration that could serve as a basis for subdividing this layer. There is evidence in layers III (Code and Winer, 1985) and V (R.A. Code and J.A. Winer, unpublished observations) of cat primary auditory cortex that the somata of commissural cells are selectively distributed within these layers. As a prelude to further connectional studies of layer V, the first goal is to define the principal types of neurons and their primary architectonic features. A second goal is to compare systematically the spatial distribution of anatomically characterized layer V cells projecting to the auditory telencephalon and mesencephalon.

A third objective is to identify the principal cell types in each pathway, since both non-pyramidal and pyramidal neurons project into the cat corpus callosum (Code and Winer, 1985). There might be morphological differences between corticocollicular and commissural neurons. Finally, the question arises as to whether commissural neurons in layers III and V are comparable in their structure and distribution, or if they represent distinct classes of cells.

The following account presents evidence for two efferent systems arising in layer V, each with a characteristic neuronal architecture and laminar distribution. Preliminary accounts of these results have appeared (Games and Winer, 1983, 1986).

Method

Animal preparation

Adult male albino rats from the Sprague-Dawley outbred strain, weighing 275–300 g, were studied. Eight animals received horseradish peroxidase (HRP) injections in the ipsilateral inferior colliculus, seven had similar injections in the contralateral auditory cortex, and three had both injections. Normal reference material from the brains of twenty-seven other animals was prepared by the Golgi-Cox technique and from five frozen-sectioned, Nissl-stained brains.

Anesthesia was by intraperitoneal injection of sodium pentobarbital (50 mg/kg) supplemented by a 1 : 1 mixture of ketamine hydrochloride (66 mg/kg) and xylazine (13 mg/kg) to maintain areflexia. Atropine sulfate (0.05 mg/kg, i.m.) reduced respiratory congestion. The rat was placed in a stereotaxic frame, the head stabilized, and the corneas were covered with ophthalmic ointment. A heating pad maintained body temperature at 37–38°C. For cortical injections, the temporal muscles were retracted and a craniotomy exposed auditory areas 41, 20, and 36, as well as parts of the visual and somatic sensory cortex (Krieg, 1946; Patterson, 1976; Zilles, 1985). The injection track was oblique to the cortical surface to maximize the involvement of area 41. A direct approach to the inferior colliculus is complicated by the confluence of the superior sagittal and transverse sinuses over the tectum. A horizontal penetration through the cerebellum, parallel to the floor of the brain stem, minimized cortical damage. The caudal occipital shelf was removed in these animals. In animals receiving both cortical and tectal injections, the cortex contralateral to the inferior colliculus was injected. Injections consisted of 0.05–1.9 μl of 30% HRP (Sigma Chem. Co., type VI) dissolved in physiological saline or combined with tritiated leucine (New England Nuclear, NET-135H, sp. act. 40–60 Ci/mmol). The leucine was reconstituted in physiological saline after evaporation of the carrier in nitrogen, and the injection was made with a 1.0 μl, 28 gauge Hamilton syringe with a beveled tip. The syringe was left in place for 10 min after the end of the injection to minimize the spread of tracer.

Perfusion

The rat was reanesthetized 24–48 h later, perfused with 250 ml of 0.12 M phosphate-buffered wash, pH 7.4, with 0.002% $CaCl_2$ and 0.05% lidocaine HCl, at 37°C. This was followed by 500–750 ml of 1.0% paraformaldehyde and 1.5% glutaraldehyde in the same buffer. In some animals, 300–400 ml more of fixative with 10% sucrose was perfused at 4°C. After 3–4 h of postfixation, the brain was blocked stereotaxically in the frontal plate at an angle approximating that in the atlas of Paxinos and Watson (1982, 1986), then removed from the skull, placed in 0.12 M phosphate buffer with 30% sucrose, and stored overnight at 4°C.

Histology

Frozen sections were cut the next day in a continuous, transverse, 60-μm-thick series from the caudal pole of the cochlear nucleus through the rostral pole of the lateral geniculate body. The tetramethylbenzidine (TMB; Mesulam, 1978) and/or the diaminobenzidine (DAB; Graham and Karnovsky, 1966) chromogens were used to visualize the horseradish peroxidase reaction product. In experiments using mixtures of both HRP and tritiated amino acid, every third section was cut at 30 μm and reserved for autoradiographic studies in progress, so that each set of sections was 30-, 60-, and 60-μm-thick, respectively. Sections were mounted onto gelatinized slides, air-dried for 48 h, dehydrated, counterstained with neutral red (TMB) or cresyl violet (DAB), then cleared and coverslipped.

Analysis

Profiles of labeled somata were traced through planapochromatic lenses and the drawing tube of a Zeiss Universal microscope. Only TMB-processed neurons whose soma and proximal dendritic arbors were filled with reaction product and whose nucleus could be seen were studied or measured. Neurons with a few sparse or scattered granules were not classified.

The varieties of layer V neurons were identified independently in Golgi preparations (Figs. 2C, 3) to relate experimentally labeled cells (Figs. 2B, 4B, 5B, 6B, 9A–C) to the classes of morphologically defined neurons (Fig. 3B). The somatic and dendritic profiles of labeled cells and their laminar distributions were compared to those of the Golgi-impregnated neurons. Cell areas were measured with an electronic digitizer (Numonics model 1224). Measurements of normal neuronal somata were taken from the Nissl-stained material.

The perimeter of the injection site was reconstructed from serial sections. For cortical injection sites, sections were projected onto a scaled lateral reconstruction of the rat cerebral cortex and the extent of the tracer determined.

The ventral, dorsal, and medial divisions of the medial geniculate body have been identified in previous studies (Winer and Larue, 1987; J.A. Winer et al., in preparation). Tissue stained with the Nissl method, Golgi-impregnated material, and horseradish peroxidase and autoradiographic tract-tracing experiments was also available for study. The pattern of retrograde labeling in the medial geniculate body confirmed that the injection site was located in auditory cortex. The distribution of anterogradely transported, tritiated leucine served as an independent check since the corticothalamic projections largely recapitulate the distribution of thalamocortical afferents revealed by horseradish peroxidase (Larue and Winer, 1985; Winer and Larue, 1987). Experiments with retrogradely labeled neurons in the ventral division of the medial geniculate body were used to analyze the commissural cells of origin; sometimes cells were also labeled in the dorsal and medial divisions of the auditory thalamus. Neurons in the lateral geniculate body and ventrobasal complex of the thalamus were never labeled in these experiments.

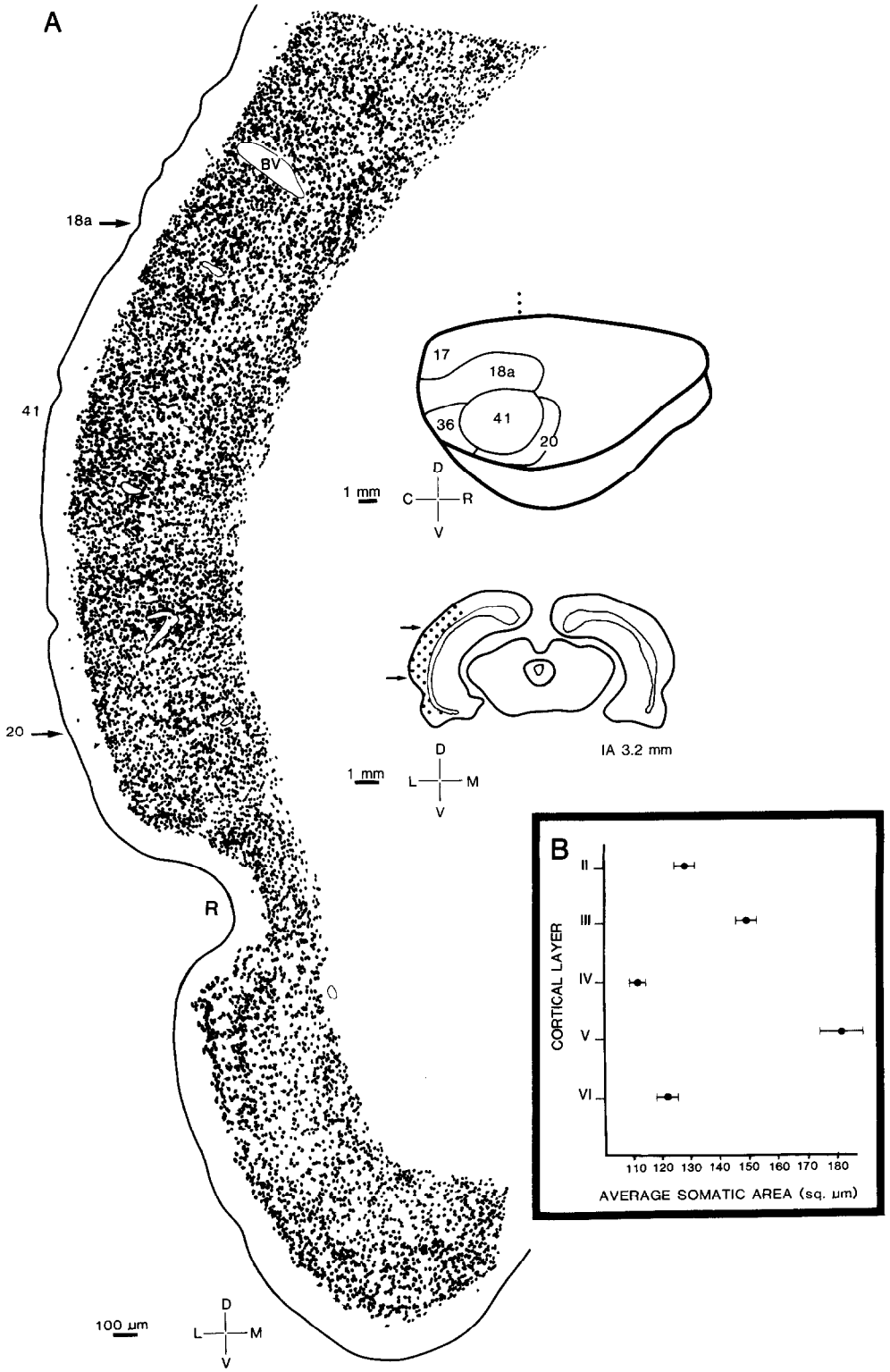
Many cells in the contralateral dorsal and ventral cochlear nuclei were labeled by midbrain injections. Neurons were labeled bilaterally in the dorsal nucleus of the lateral lemniscus and in the lateral superior olive. Anterograde transport of horseradish peroxidase to the ventral, dorsal, and medial divisions of the medial geniculate body was also routinely observed.

Every cortical section with labeled cells was studied; illustrations of labeled neurons were taken from representative sections and show patterns commonly encountered. To reconcile each series of sections with the stereotaxic coordinates in the atlas of Paxinos and Watson (1982, 1986), a correction factor was calculated for the variations in frozen section thickness. The caudal poles of the inferior and superior colliculi or the caudal extremity of the superior colliculus and the posterior commissure served as landmarks to compute the distance from interaural zero (±IA). Low power drawings were made on a microprojector through Zeiss Luminar objectives.

Results

Auditory cortical cytoarchitectonics and neuronal architecture

As a framework for our experimental results, the limits and laminar subdivisions of auditory cortex were defined in Nissl and Golgi material


A
BV
18a
41
20
R
100 µm
D
L
M
V
17
18a
36
41
20
1 mm
D
C
R
V
1 mm
D
L
M
V
IA 3.2 mm
B
II
III
IV
V
VI
CORTICAL LAYER
110 120 130 140 150 160 170 180
AVERAGE SOMATIC AREA (sq. µm)

(Figs. 1A, 2A,C, 3). This region occupies the caudodorsal extremity of the temporal cortex, and approaches, but does not invade, the rhinal sulcus (Fig. 1A). The present analysis included the chief part of area 41 as defined by Krieg (1946); usually, the dorsal part of area 20 and the rostral portion of area 36 were also studied, although the present findings are limited to area 41. These subdivisions approximate all of the core cortex and most of the dorsal half of the belt cortex according to Patterson (1976), and largely overlap the three temporal cortical zones (Te1-3) of Zilles (1985). Horseradish peroxidase injections in area 41 always produced both anterograde and retrograde labeling in corresponding regions of the contralateral hemisphere and in the ventral division of the medial geniculate body. Neurons in the dorsal and medial divisions were sometimes labeled, suggesting that architectonic fields beyond area 41 were involved (Winer and Larue, 1987).

Definition of auditory cortical layers

In frozen-sectioned, Nissl-stained material and in Golgi preparations, six layers were distinguished in area 41 (Figs. 1A, 2A,C, 3A). The average somatic area of neurons in different layers was measured from a total of 325 cells (Fig. 1B).

Layer I had few neurons and extended some 140 μm below the pial surface. It formed 13% of the total thickness of the cortex (averaging 1100 μm).

Layer II contained many small, densely packed, polymorphic cells with an average somatic area of 128 μm^2 (s.d. ± 24.6 μm^2). It was 125 μm thick, representing 11% of the cortical thickness.

The layer II-III boundary showed a sharp decrease in packing density and larger somata. The heterogeneous form of layer III cells was apparent in the orientation and shapes of their dendritic fields (see below). Both pyramidal and non-pyramidal neurons occurred, and the average somatic area was 149 μm^2 (s.d. ± 20.5 μm^2). Layer III was 190 μm thick and formed 17% of the cortical thickness.

The layer III-IV border was conspicuous in Nissl preparations. There was a slight increase in the packing density of the small stellate cells, the chief neuronal population of layer IV; their average somatic area of 111 μm^2 (s.d. ± 18.6 μm^2) was much smaller than that of cells in layers III or V (Fig. 1B; see below), while their spherical or oblate profiles were readily distinguished from the more angular shapes of layer III neurons. Only about 105 μm thick, layer IV represented 10% of the cortical depth.

Layer V began approximately midway through the cortical depth and had a lower neuronal packing density and larger (Figs. 1A, 2A) cells than did layer IV. The pyramidal neurons were conspicuous (Figs. 2, 3, 4B, 6B, 7C, 9A); large pyramidal cells were more numerous in the lower (Vb) than in the upper half (Va) of layer V. Their average somatic area was 182 μm^2 (s.d. ± 47.9 μm^2). Layer V was 270 μm thick, forming 26% of the cortical thickness.

Layer VI contained closely packed, somewhat flattened neural somata with an average perikaryal area of 122 μm^2 (s.d. ± 23.7 μm^2). Both pyramidal and non-pyramidal cells were present, and layer VI was 245 μm thick, representing 22% of the cortical depth.

Neuronal architecture of layer V

In Golgi impregnations from area 41, we noted several distinctive patterns of dendritic organization among layer V neurons. This summary will serve as a basis for the subsequent identification of experimentally labeled cells on the basis of their somatic shape and dendritic profiles.

Fig. 1. Cytoarchitecture of rat auditory cortex. (A) Boundaries of area 41 and low power view of cortical layers. Nissl-stained, 30-μm-thick section embedded in low-viscosity nitrocellulose. Planachromat, N.A. 0.35, $\times 200$. Upper inset (lateral view) depicts area 41 and the boundaries of neighboring cortical areas. Row of dots shows the plane of section for panel A. Coarse stipple (lower inset, transverse view) corresponds to the region drawn in panel A; arrows show the dorso-ventral extent of area 41. In this and following figures, the distance rostral (numbers without a sign) or caudal (negative numbers) to inter-aural (IA) zero is given. (B) Mean somatic areas and standard errors of neurons in layers II-VI, from frozen-sectioned, Nissl-stained material. For abbreviations, see list of Abbreviations.

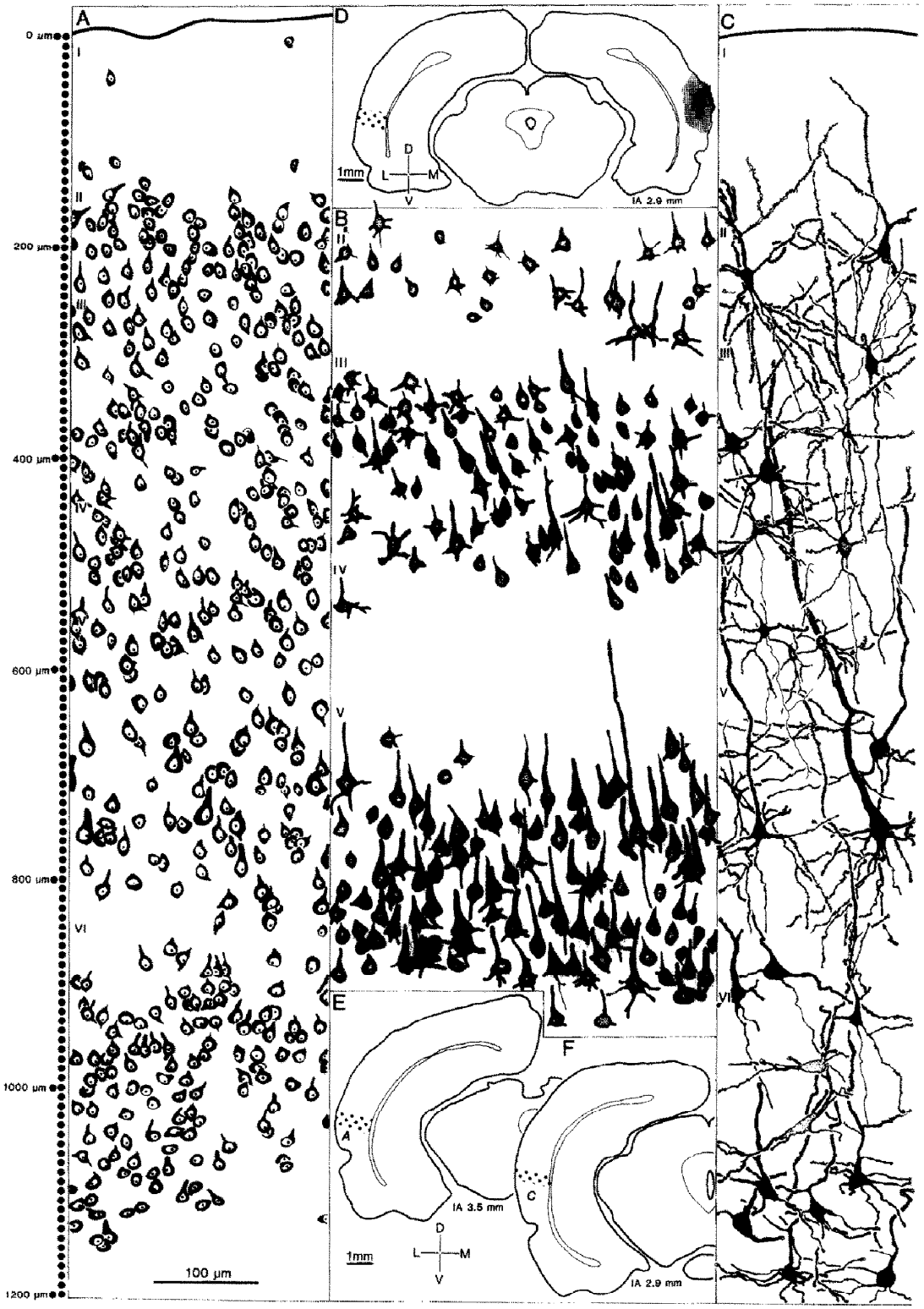

A
B
C
D
E
F
0 µm
200 µm
400 µm
600 µm
800 µm
1000 µm
1200 µm
I
II
III
IV
V
VI
100 µm
1mm
D
L
M
V
IA 2.9 mm
IA 3.5 mm
A
C

TABLE I

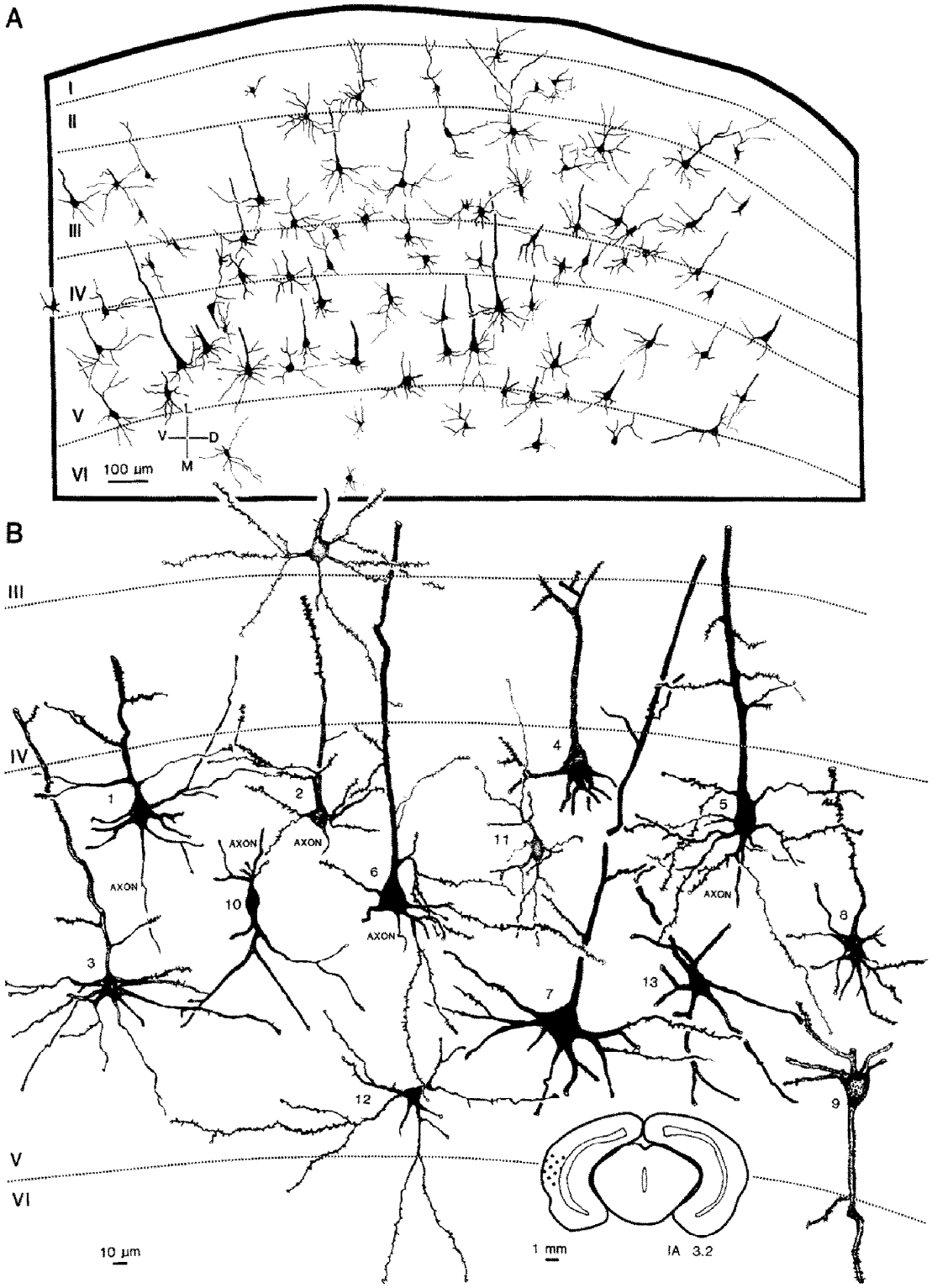
SUMMARY OF LAYER V CELL TYPES IN RAT AREA 41

Neuron type	Primary dendrites	Somatic shape and size *	Commissural cell of origin	Corticotectal cell of origin
Small pyramidal cell	Fine basal arbors, narrow apical dendrite	Flask-shaped or rounded 10–12×12–18 μm	+	–
Medium-sized pyramidal cell	Extensive basal dendritic arbors, long apical dendrite	Triangular and elongated 12–18×15–20 μm	+	+
Large pyramidal cell	Thick basal roots, stout apical dendrite	Triangular 15–22×18–30 μm	–	+
Inverted pyramidal cell	Inverted orientation of apical dendrite and axon	Triangular 12–20×15–30 μm	+	–
Star pyramidal cell	Thin apical dendrite, delicate basal dendrites	Stellate or star-shaped 12–15×15–20 μm	0	0
Bipolar or bitufted cell	Polarized primary dendrites; hour glass-shaped field	Oval and elongated 10–15×15–25 μm	0	–
Multipolar cell	Radial origins, spherical dendritic field	Stellate or star-shaped 12–20×15–25 μm	0	–

* width by length +: positive –: negative 0: unknown

Pyramidal cells. Small pyramidal cells occurred throughout layer V and were common in the superficial half (Va; Fig. 3B: *1,2,3*; Table I). The cell body was triangular or flask-shaped with primary dendrites originating usually from the basal and apical poles, although occasionally they were dis-

Fig. 2. Laminar boundaries and neurons in the auditory cortex from Nissl-stained, HRP-labeled and Golgi-impregnated material. (A) Nissl-stained cells in layers I-VI. Note the smaller cells and lower density of neurons in the upper half of layer V and larger cells in the lower half. 30-μm-thick frozen section. (B) HRP-labeled cells after combined injections in the contralateral auditory cortex and ipsilateral inferior colliculus. Layer V has many robustly labeled pyramidal cells. Note the scattered, labeled cells in layer II, and the scant labeling in layer IV. 60-μm-thick frozen sections, TMB chromogen. (C) Golgi-impregnated neurons in layers II-VI. See also Fig. 3. Golgi-Cox method, 160-μm-thick sections. For panels A–C: Planapochromat, N.A. 0.65, ×800. (D,E,F) Sections from which panels B, A, and C were drawn. Representative cortical injection site is shown in panel D (fine stipple). In this and other injection sites, the center of the injection is black, the zone of diffusion is stippled.


A
I
II
III
IV
V
VI
100 µm
L
V
D
M
B
III
IV
V
VI
AXON
10 µm
1 mm
IA 3.2

tributed more evenly (Fig. 3B: *3*). The thin basal dendrites typically had two to five trunks arising from the ventral half of the cell body, forming slender, tapering branches before ending in layer V or, more rarely, in adjacent layers (Fig. 3B: *1*). They often had dendritic appendages on their apical shaft (Fig. 3B: *2*), but not always (Fig. 3B: *1*). The number of dendritic spines on the thicker apical shaft and on secondary branches increased with distance from the initial root. Sometimes the apical dendrite branched within layer V (Fig. 3B: *2*) or layer IV (Fig. 3B: *1*); second-order dendrites usually did not cross the IV-III border. Other small pyramidal cells sent apical dendrites well into layer III. The axon usually emerged from a basal dendrite or the ventral somatic pole (Fig. 3B: *1,2*).

The medium-sized pyramidal cell was the most common neuron and occurred throughout layer V. It resembled the small pyramidal neuron, but was larger and had a longer soma (Table I) which was triangular (Fig. 3B: *4,6*) or, less often, somewhat oval (Fig. 3B: *5*). The number and extent of the basal dendrites ranged from relatively sparse and limited (Fig. 3B: *6*) to richly branched and broad (Fig. 3B: *5*). Apical dendrites ramified both within layer V (Fig. 3B: *6*) and beyond (Fig. 3B: *5*), sometimes as far as layer II. The axon typically emerged from a basal dendrite (Fig. 3B: *5*) or the ventral somatic pole (Fig. 3B: *6*). Most dendritic appendages lay on secondary branches remote from the cell body, and near their terminations. Spine size and density among this class of neurons was varied (Fig. 3B: *4,5,6*).

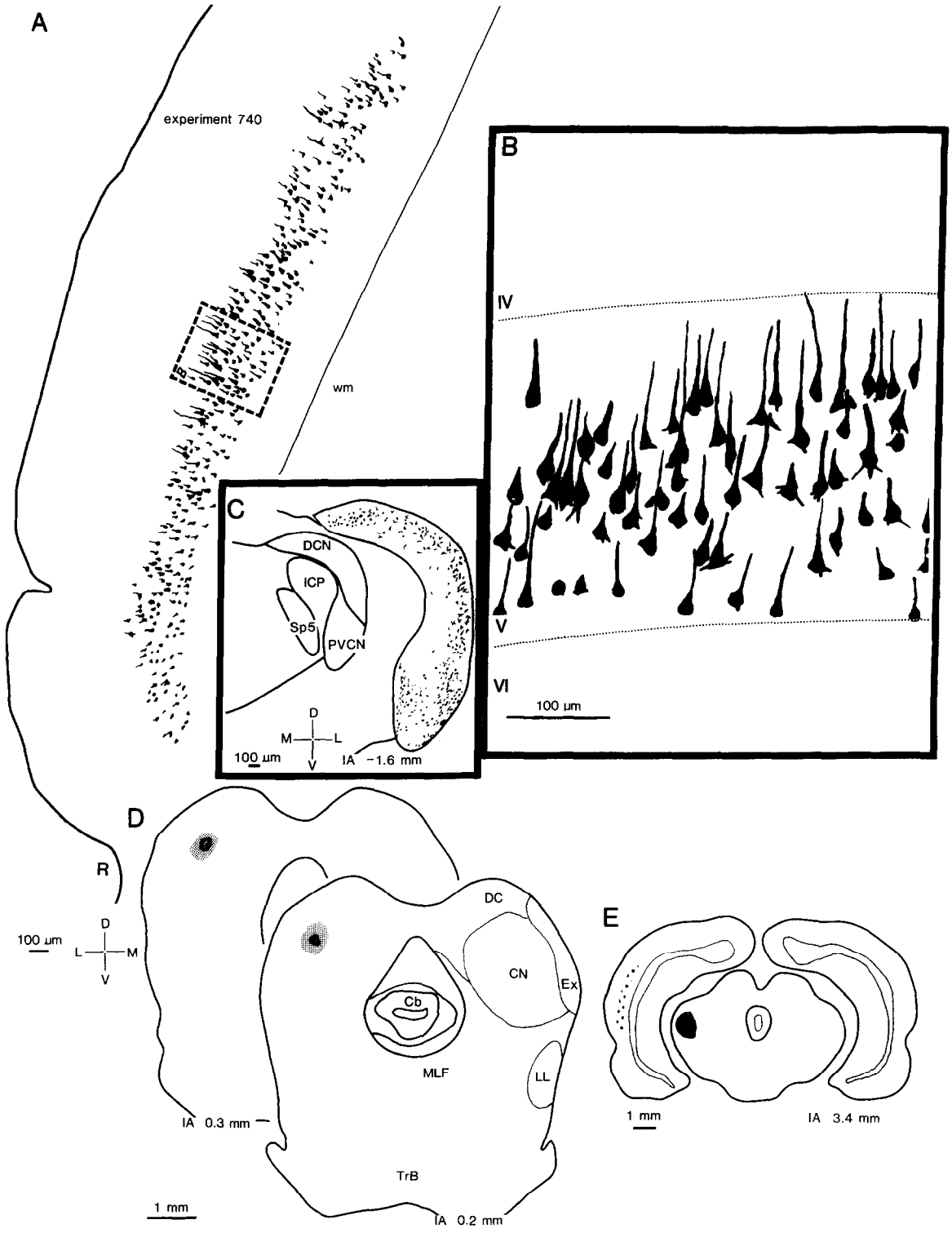
The large pyramidal cell (Fig. 3B: *7*) had a larger soma and thicker dendrites than did the medium-sized pyramidal cell, though the arbors themselves were less elaborate. Distal branches ramified laterally both in layer V and other layers. The apical dendrite often ascended to the upper part of layer II or farther. In contrast to the small pyramidal cell, this neuron was concentrated in the middle and lower half of layer V (Vb).

The star pyramidal cell was less common than other pyramidal cells. It had features common to both pyramidal and multipolar neurons (see below). The soma had less obvious basal and apical poles than pyramidal cells, and the cell body was star-shaped (Fig. 3B: *8*). The primary dendrites resembled those of the multipolar cell and radiated irregularly from the soma (compare with Fig. 3B: *13*). This neuron was classified as a pyramidal cell by the slightly thicker, longer apical dendrite which was directed towards the pia, and which usually had some dendritic spines. In size, this neuron resembled small and medium-sized pyramidal cells (Table I).

The inverted pyramidal cell was distinguished from other pyramidal cells by its orientation (Fig. 3B: *9*). The apex of the large, angular soma and the single, thick "basal" dendrite were directed towards the white matter, while the axon frequently arose from the apical somatic pole. The basal dendrite entered layer VI, with branches in both layers V and VI. The apical dendrites ramified in layer V and were mostly contained within it. This cell occurred throughout layer V and was similar in size to the medium-sized pyramidal cell.

Non-pyramidal cells. Bitufted neurons occurred throughout layer V. The vertically elongated perikaryon ranged from small to large, the long axis approximating that of the medium-sized pyramidal cell (Table I). Typically, a thick primary

Fig. 3. Golgi-impregnated auditory cortex neurons. (A) Low power composite of four sections, each 160 μm thick. Note small stellate cells in layer IV and many layer V pyramidal cells. Planapochromat, N.A. 0.32, ×125. (B) Higher power drawing of characteristic types of Golgi-impregnated layer V cells drawn from a composite of five 160-μm-thick sections, each from the region shown in the inset. Small pyramidal cells (*1,2,3*) predominated in layer Va; their thin apical dendrites rarely ascended beyond layer IV. Medium-sized pyramidal cells (*4,5,6*) were common throughout layer V; their apical dendrites pass through layer IV. The large pyramidal cells (*7*) had a thick apical dendrite which often extended to layer II and beyond. These cells were numerous in layer Vb. Star pyramidal cell dendrites (*8*) radiated from any part of the soma, and they have a prominent apical dendrite. The basal dendrite of the inverted pyramidal cell (*9*) ramified in layer VI; apical dendrites were largely confined to layer V. The bitufted cell (*10,11*) had a fusiform cell body and two primary arbors projecting from apical and basal poles. Multipolar cells (*12,13*) had equally thick dendrites emerging from the soma. The apparent thinness of layer I is an artifact of shrinkage from the low-viscosity nitrocellulose embedding. Planapochromat, N.A. 0.65, ×500.


A
experiment 740
wm
B
IV
V
VI
100 µm
C
DCN
ICP
Sp5
PVCN
D
M
L
V
100 µm
IA −1.6 mm
D
R
100 µm
D
L
M
V
DC
CN
Ex
Cb
MLF
LL
IA 0.3 mm
TrB
1 mm
IA 0.2 mm
E
1 mm
IA 3.4 mm

dendrite arose from the upper and lower somatic poles, divided into smooth branches, and formed polarized dendritic fields which flared vertically (Fig. 3B: *10,11*). The axon often arose from the apical pole (Fig. 3B: *10*).

Multipolar cells were found throughout layer V; their primary dendrites emerged from various parts of the cell body. Three to six spinous (Fig. 3B: *12*) or smooth (Fig. 3B: *13*), equally thick dendrites formed a wide, spherical dendritic field. Most somata were medium-sized (diameter < 20 μm; Table I), although larger ones occurred. The axon arose from the soma or from a primary dendrite.

Distribution of neurons projecting to the inferior colliculus

Inferior colliculus injections retrogradely labeled cells in layer V of area 41, throughout the dorso-ventral (Fig. 4A) and rostro-caudal extent of the ipsilateral auditory cortex. The pattern of retrograde labeling in brain stem auditory nuclei served as a check for the locus of the injection site (see below).

The largest injections labeled about half of the cells in layer V; most such cells had triangular perikarya and were medium-sized to large, with a somatic area of 150–250 μm^2 (Fig. 9A). TMB-reacted neurons usually had robustly labeled apical dendrites (Figs. 4B, 9A) ascending to layer II (Fig. 7C) and beyond. Cells projecting to the inferior colliculus corresponded in shape, size, and laminar distribution to the medium-sized (Fig. 3B: *4,5,6*) and large pyramidal (Fig. 3B: *7*) cells identified in Golgi preparations. Both types and sizes were observed throughout layer V, although the largest neurons were in the lower half. Smaller, fusiform, and unequivocally non-pyramidal somata were not labeled (Fig. 4B), nor were inverted pyramidal cells marked by such injections. In heavily labeled sections, patches of 5–10 labeled cells were interspersed among unlabeled cells, and solitary, labeled neurons were rare. The same pattern of cortical retrograde transport resulted, on a lesser scale, from smaller injections. In the most heavily labeled sections, up to 10% of the labeled cells lay in layer VI.

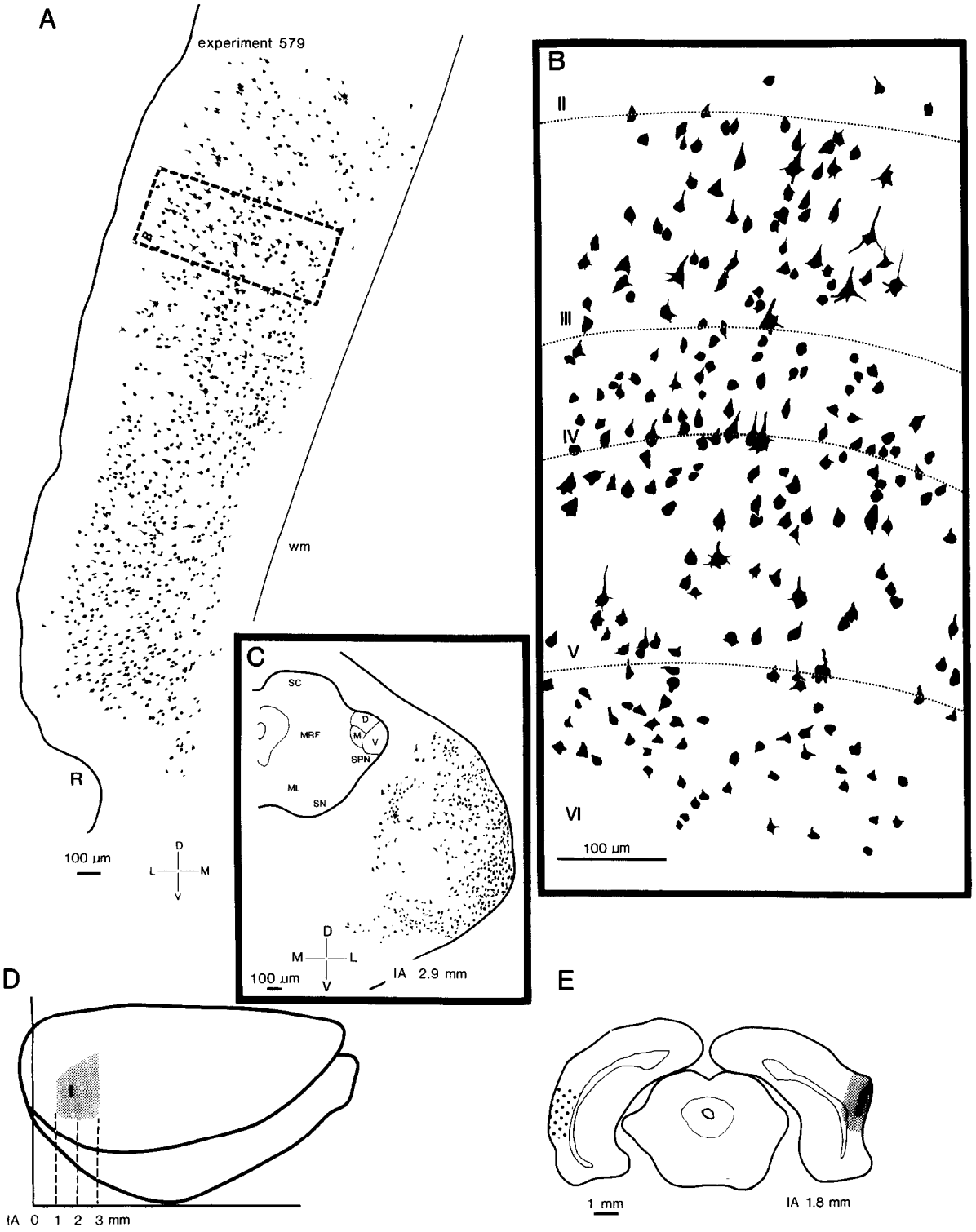
To examine the distribution of inferior colliculus cells of origin within layer V, we divided it into successive sublayers whose borders ran parallel to the pial surface. The superficial boundary of sublayer 1 was formed by the IV-V border, and the lower limit, sublayer 4, was defined by the V-VI junction (Fig. 8). We plotted the position of tectal cells of origin within each sublayer across layer V (Fig. 8B,C) in nine representative sections from three experiments. Sublayer 1 contained only 7% of the total number of cells of origin (Fig. 8D). The number of labeled cells increased to 33% in sublayer 2, while sublayers 3 and 4 contained 31% and 29%, respectively, of such cells.

Photomicrographs of TMB-processed sections confirmed that many heavily labeled pyramidal cells project to the inferior colliculus (Fig. 7C). The dense retrograde filling of the apical dendrites and their projection toward the supragranular layers were striking, as was the virtual absence of labeled non-pyramidal profiles defined by somatic shape and dendritic origins (see also Fig. 9A).

Patterns of brain stem labeling

Representative labeled cells in the cochlear nucleus after horseradish peroxidase injections in the inferior colliculus are shown in Fig. 4C. Labeled cells were observed in: (1) the subdivisions of the contralateral cochlear nuclear complex (dorsal, anteroventral, and posteroventral nuclei; Fig. 4C; Fig. 6C, bottom); (2) bilaterally, in the lateral superior olivary nuclei, and in the ipsilateral medial superior olive; (3) bilaterally, in

Fig. 4. Neurons labeled by HRP injections in the ipsilateral inferior colliculus. 60-μm-thick frozen sections, TMB chromogen. (A) Low power view of cortex. The cells of origin are almost entirely confined to layer V; neurons enclosed in the box are drawn at higher magnification in panel B. Planapochromat, N.A. 0.14, $\times 50$. (B) All inferior colliculus cells of origin are pyramidal; note the large size and the extensive filling of the apical dendrite in most cells. See also Fig. 7C. Planapochromat, N.A. 0.45, $\times 313$. (C) Labeled cells in the contralateral dorsal and posteroventral cochlear nuclei. Left side: subdivisions of the cochlear nucleus; right side: labeled cochlear nucleus cells. Planapochromat, N.A. 0.14, $\times 50$. (D) The center of the injection site in the inferior colliculus. (E) The cortical region containing the illustrated cells (dots) is depicted; anterograde HRP transport to the ipsilateral medial geniculate body (dark stipple) is also shown.


A
experiment 579
B
wm
R
100 µm
D
L
M
V
B
II
III
IV
V
VI
100 µm
C
SC
MRF
D
M
V
SPN
ML
SN
D
M
L
V
100 µm
IA 2.9 mm
D
IA 0 1 2 3 mm
E
1 mm
IA 1.8 mm

the dorsal nuclei of the lateral lemniscus, and in the ipsilateral ventral nucleus of the lateral lemniscus; and (4) in the contralateral inferior colliculus [(2)–(4) are not illustrated in the present account]. Labeled cells in the ipsilateral cochlear nucleus and in the contralateral medial superior olive were rare. Anterograde transport was seen in all three major divisions of the ipsilateral medial geniculate body (ventral, dorsal, and medial; Fig. 6C, left).

Distribution of commissural cells of origin

Horseradish peroxidase injections retrogradely labeled cells in homotopic regions of the opposite auditory cortex (Fig. 5E). In contrast to inferior colliculus cells of origin, which were principally in layer V (Fig. 4A,B), commissural cells of origin arose from layers II-VI (Fig. 5A,B). After large horseradish peroxidase injections, their somata were distributed continuously throughout the rostro-caudal and dorso-ventral cortical axes. Up to half of the neurons projected in the most heavily labeled sectors of layer V. These cells (Figs. 5B, 9B) were varied in form and included neurons distinct from the typical large pyramidal profile of cells projecting to the inferior colliculus (Figs. 4B, 7C, 9A). Some cells resembled the small or medium-sized layer V pyramidal cells identified in the Golgi impregnations (Figs. 3B: *1–6*, 9B: *1,2*) while others were similar to inverted pyramidal cells (Fig. 3B: *9*; Fig. 9B: *3,4*).

The apical dendrites of commissural neurons had far fewer horseradish peroxidase granules (Fig. 9B) than those of pyramidal cells projecting to the midbrain (Figs. 7C, 9A), even after the largest injections. Groups of 3–10 commissural neurons interdigitated with single cells or small groups of unlabeled neurons. The varied form of the labeled commissural cells and the lack of larger pyramidal cells with robustly filled apical dendrites (Figs. 5B, 7B) were conspicuous. Many labeled neurons had a primary, apical dendrite oriented toward the pia; the primary dendrites of other cells were too lightly labeled to classify them with confidence. In contrast to cells projecting to the midbrain, very few commissural neurons had retrogradely filled apical dendrites extending above layer IV (Figs. 5B, 7B).

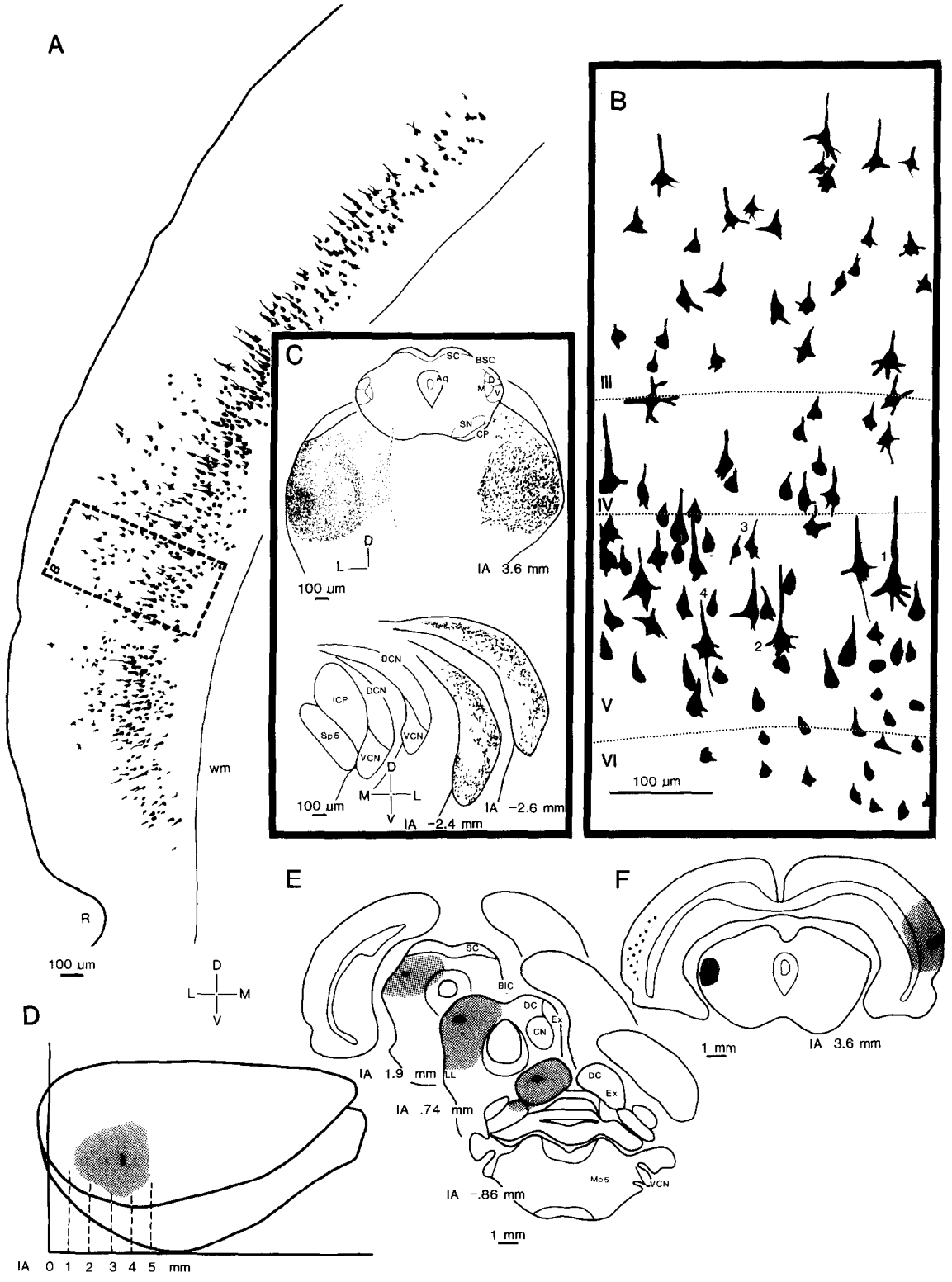
Patterns of thalamic labeling

After horseradish peroxidase or combined [^{3}H]leucine/horseradish peroxidase injections in the auditory cortex, transport to the ventral division of the ipsilateral medial geniculate body had a systematic relationship to the locus of the injection site (Fig. 5, experiment 579). An injection in the caudal half of the auditory cortex, in a zone associated with a tonotopic sequence representing low frequencies (Sally and Kelly, 1988, and personal communication), produced retrogradely labeled cells mainly in the lateral part of the ventral division of the medial geniculate body. Although no tonotopic maps of the rat medial geniculate body are available, the comparable region in the cat is devoted to low frequencies (Aitkin and Webster, 1972). Retrogradely labeled cells were also found in the dorsal and medial divisions of the medial geniculate body. Anterograde labeling was dense among the retrogradely labeled cells in the medial geniculate body (see also Winer and Larue, 1987), in the opposite auditory cortex, and there was anterograde labeling in the dorsomedial division of the ipsilateral inferior colliculus.

Combined inferior colliculus and auditory cortex experiments

Horseradish peroxidase injections in both the ipsilateral inferior colliculus and the contralateral auditory cortex labeled cells in all auditory cortical areas. Their distribution (Fig. 6A,B) combined the patterns of retrograde labeling from inferior

Fig. 5. Labeled neurons in layers II-VI after injections in the contralateral auditory cortex. 60-μm-thick frozen sections, TMB chromogen. (A) Low power view of contralateral cortical layers II-VI; cells in the box appear at higher magnification in panel B. Planapochromat, N.A. 0.14, ×50. (B) Layer V commissural neurons are smaller and more varied in shape than inferior colliculus cells of origin (compare with Fig. 4B). Most apical dendrites of pyramidal cells are sparsely labeled. Planapochromat, N.A. 0.45, ×313. (C) Thalamocortical relay cells occur in all three divisions of the medial geniculate body. Left side: medial geniculate subdivisions; right side: labeled cells. Planapochromat, N.A. 0.14, ×50. (D) Lateral view of the cortex showing the injection site. (E) The center of the injection site (stipple) and the region containing the illustrated cells (dots) in the contralateral hemisphere.


A
B
C
D
E
F
wm
R
100 µm
D
L
M
V
SC
BSC
Aq
SN
CP
IA 3.6 mm
DCN
ICP
Sp5
VCN
IA −2.6 mm
IA −2.4 mm
III
IV
V
VI
1
2
3
4
BIC
DC
Ex
CN
LL
IA 1.9 mm
IA .74 mm
IA −.86 mm
Mo5
1 mm
IA 0 1 2 3 4 5 mm

colliculus (Fig. 4) or cortical (Fig. 5) injections alone. In contrast to inferior colliculus injections, however, cells in layers II-VI were labeled (Fig. 6A). The same cell types labeled by tectal or cortical injections were marked: large, robustly filled layer V pyramidal cells, like those labeled by inferior colliculus injections, were scattered among smaller cells typically marked by cortical injections (Figs. 2B, 6B, 7D, 9C). Labeled cells filled the depth of layer V, and included up to 80% of the neurons.

These cells formed a more or less continuous distribution across layer V (Fig. 7D). Smaller cells, probably commissural cells of origin, lay among large pyramidal cells. The latter fill the gap in the lower half of layer V seen after cortical injections (Fig. 7B) and might project to the inferior colliculus.

Comparison of commissural and tectal cells of origin

The somatic sizes of commissural and midbrain cells of origin revealed that different populations of neurons represent each (Fig. 9C). The former had significantly smaller somata ($\bar{X} = 118\ \mu m^2$, s.d. $\pm 30\ \mu m^2$, t-test, $P < 0.001$; df = 364) than cells projecting to the inferior colliculus ($\bar{X} = 197\ \mu m^2$, s.d. $\pm 47\ \mu m^2$). The size overlap between the two populations primarily involved medium-sized cells with somatic areas of 100–200 μm^2 (Fig. 9C). The largest cells did not project commissurally, while most of the smallest cells did.

Comparison of the average somatic area of layer V commissural cells ($\bar{X} = 120\ \mu m^2$, s.d. $\pm 32\ \mu m^2$) to that of such layer III cells ($\bar{X} = 115\ \mu m^2$, s.d. $\pm 25\ \mu m^2$) showed no significant difference (t-test, $P > 0.1$, df = 196) between them (Fig. 9B). No inverted pyramidal cells occurred in layer III in either Golgi (Fig. 2C) or horseradish peroxidase material (Figs. 5B, 6B), nor was there any apparent difference in the form of labeled commissural neurons in layers V and III, besides their laminar origin.

Laminar distribution of layer V efferent cells

The position of commissural and inferior colliculus cells of origin within layer V was compared (Fig. 8C). The contrast between these populations was greatest in the upper quarter, where many more commissural neurons occurred (36% of the labeled cells) (Fig. 8D). Sublayers 2, 3, and 4 contained fewer commissural cells (25, 21, and 18% of the total, respectively), and overlapped with inferior colliculus cells of origin. In some heavily labeled sections, commissural cells were more evenly distributed across the four sublayers (Fig. 8A, lower part).

The contrast in laminar disposition and dendritic branching between tectal and commissural cell distributions was striking under darkfield illumination (Fig. 7B,C), demonstrating the smaller size and lightly labeled apical dendrites of commissural layer V pyramidal cells. The absence of labeled cells in the lower half of layer V after cortical injections corresponds to the region filled with large pyramidal cells projecting to the inferior colliculus (compare Fig. 7B,C).

Discussion

Layer V contains at least two efferent systems, each with characteristic cell types and a distinct distribution. We first consider differences between the corticocollicular and commissural systems, then conclude with speculations on the functional and laminar organization of sensory neocortex.

Technical considerations

The incomplete retrograde filling of dendrites

Fig. 6. Labeled neurons in layers II-VI after HRP injections in both the inferior colliculus and contralateral auditory cortex. 60-μm-thick frozen section, TMB chromogen. (A) Low power view of the auditory cortex showing extensive layer V labeling. Cells enclosed in the box are shown at higher power in panel B. Planapochromat, N.A. 0.14, ×50. (B) Both large pyramidal (*1,2*) and smaller layer V neurons are labeled (*3,4*). Note the differences in retrograde filling of the apical dendrites. Planapochromat, N.A. 0.45, ×313. (C) Upper part: medial geniculate body labeling. Left side: ipsilateral anterograde transport from the inferior colliculus; right side: retrograde labeling from the cortical injection; center: medial geniculate body subdivisions. Lower parts: retrograde labeling in subdivisions of the cochlear nuclear complex; left side: architectonic subdivisions. Planapochromat, N.A. 0.14, ×50. (D) The cortical injection filled most of area 41. Compare with Fig. 5D and Fig. 1 (upper inset). (E) The HRP injection saturated the ipsilateral inferior colliculus. (F) The injection site in the contralateral cortex (stipple), the cortical region containing labeled cells (dots), and the anterograde transport to the medial geniculate body (dark stipple) from the injection shown in panel E.

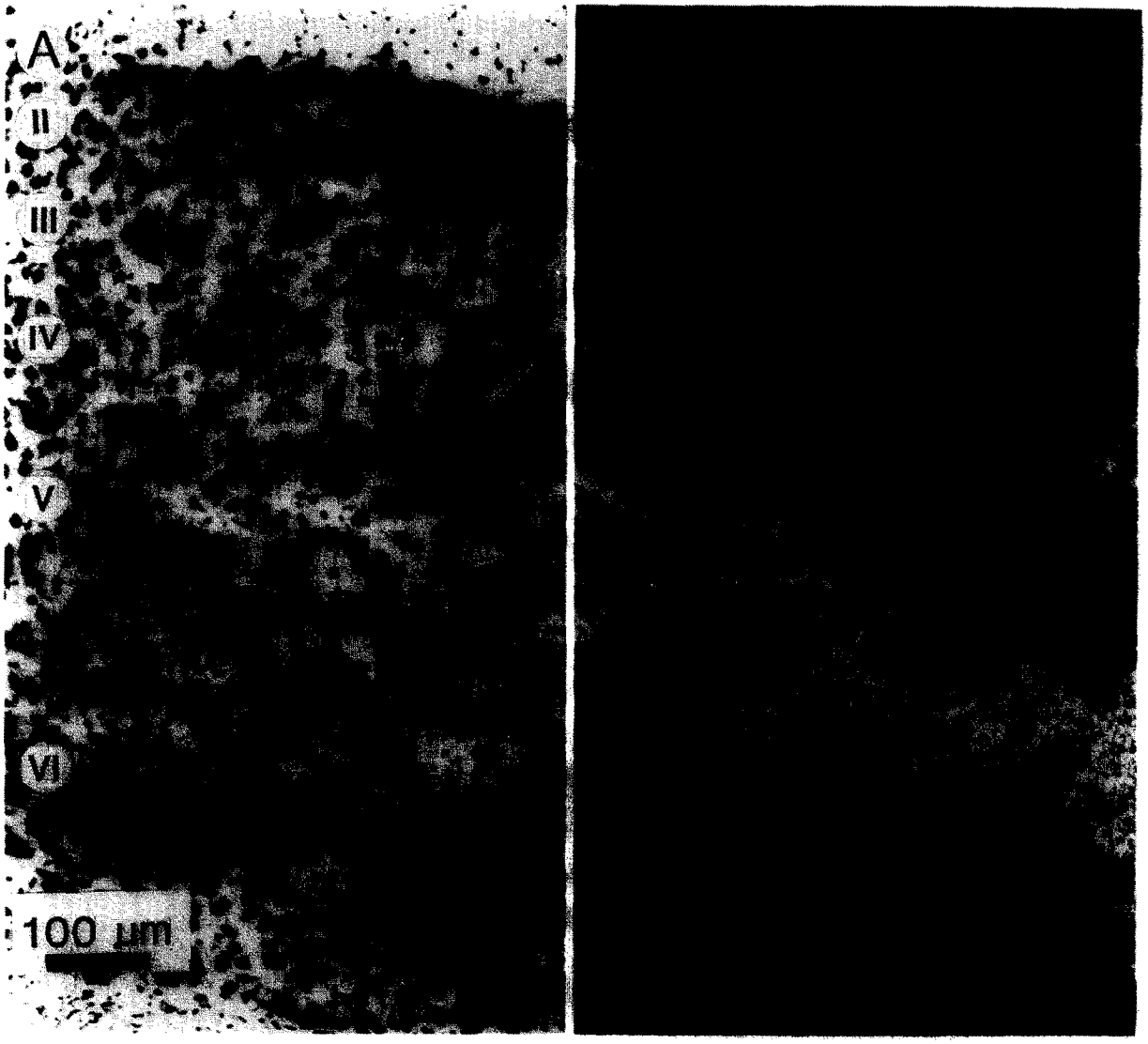


Fig. 7. Photomicrographs of Nissl stained tissue and of auditory cortex labeling. (A) Nissl preparation from normal material (brightfield). Note the range of cell sizes and the lower cellular packing density in the superficial part of layer V. For panels A–D: planapochromat, N.A. 0.32, ×125. B–D, TMB-processed sections (darkfield). White lines: layer V borders. (B) Commissural cells predominate in layer Va, and there is a gap in layer Vb. (C) Large layer Vb pyramidal cells with robustly labeled apical dendrites; their axons project to the inferior colliculus (compare with panel B). (D) Neurons labeled by injections in the inferior colliculus and contralateral cortex. The two efferent populations occupy the depth of layer V.

with horseradish peroxidase complicates any comparison of their profiles with those of Golgi-impregnated neurons, especially for the smallest cells in which, for reasons considered below, the degree of dendritic labeling is modest. Several criteria were used to match labeled cells with their Golgi-impregnated counterparts, including somatic size and shape, location and orientation of primary dendrites, and position in layer V. The concordance between methods supports our main conclusions about the varieties of neurons. Whether all large pyramidal cells, or some subset (Schofield et al., 1985) project to the midbrain is unknown, nor is it certain that every medium-sized pyramidal neuron is a commissural cell of origin.

A further issue is the reliability of morphologi-

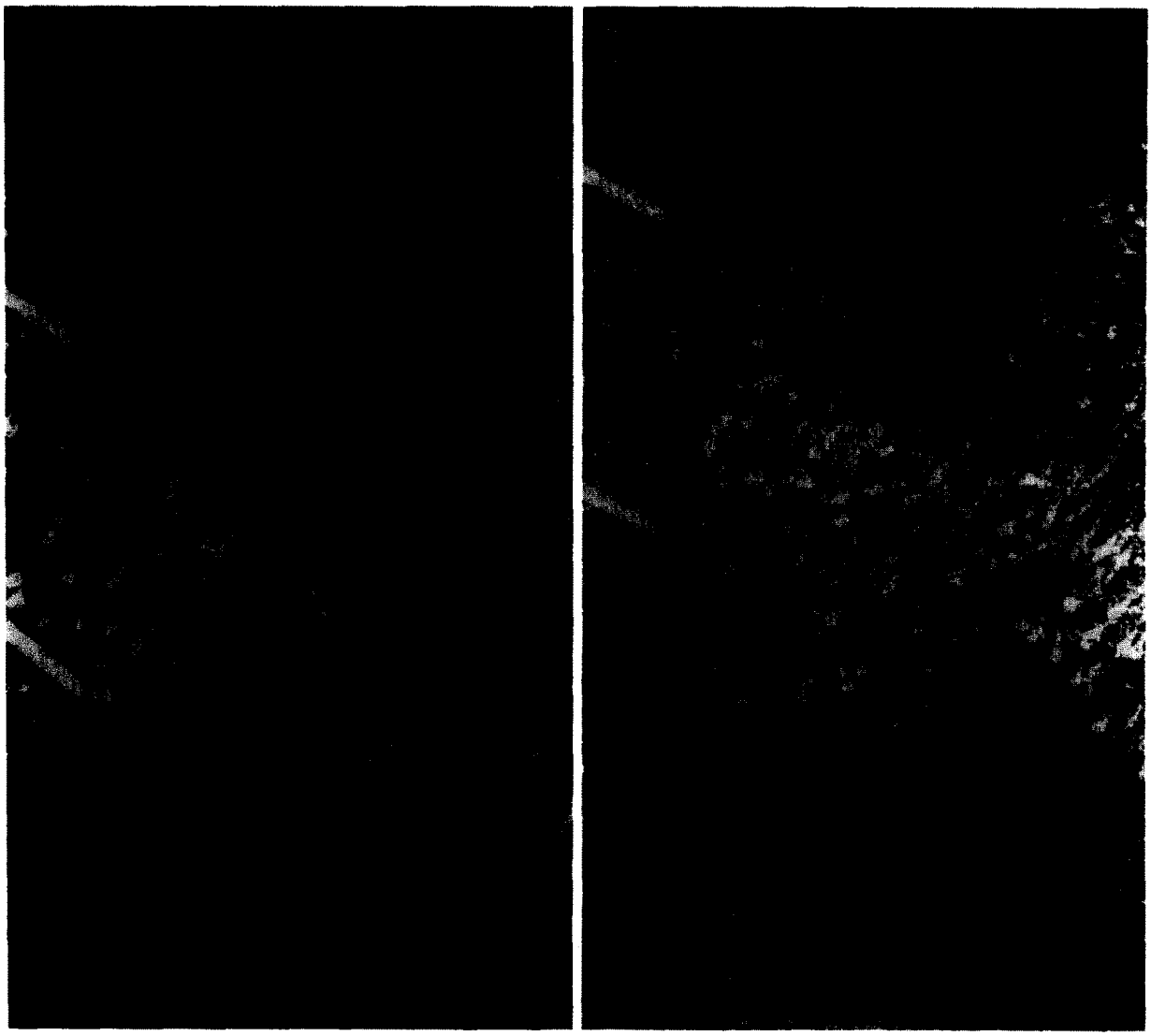


Fig. 7 (continued).

cal methods for defining cortical boundaries. Therefore, the loci of the inferior colliculus and auditory cortex injections were compared with the retrograde labeling of nuclei in the brain stem auditory pathway. The contralateral cochlear nucleus, superior olivary complex, and dorsal nuclei of the lateral lemniscus each project to the inferior colliculus in both the rat (Druga and Syka, 1984) and cat (Warr, 1966, 1969; Roth et al., 1978; Adams, 1979; Brunso-Bechtold et al., 1981). Inferior colliculus injections labeled cells in all of these structures.

The rat auditory cortex receives projections from the medial geniculate body (Ryugo and Killackey, 1974; LeDoux et al., 1985) and has descending connections with the dorsal and medial aspects of the inferior colliculus (Coleman et al., 1984; Faye-Lund, 1985) and with all subdivisions of the medial geniculate complex (Winer and Larue, 1987). In the present study cortical regions receiving input from the medial geniculate body are also labeled by injections in the inferior colliculus. To ensure that labeled cortical neurons and cells identified in Golgi preparations were

taken from corresponding areas, all the observations were remote from the borders of area 41.

Efferent systems in layer V

Morphological and laminar differences between layer Va commissural neurons and layer Vb cells projecting to the inferior colliculus reveal a selective pattern of connections. The corticocollicular system consists exclusively of pyramidal cells and includes the largest auditory cortex neurons. Their apical dendrites contain many horseradish peroxidase granules and are easily followed to layer II. This projection arises almost entirely from layer V and has few or no supragranular components. Layer V pyramidal cells in many different architectonic fields project subcortically, for example, to the superior colliculus (Holländer, 1974), thalamus (Jones and Wise, 1977), pontine nuclei (Albus and Donate-Oliver, 1977), and to the spinal cord (Murray and Coulter, 1981). Many of these neurons have apical dendrites that form bundles projecting as far as layer I (Escobar et al., 1986; Peters and Kara, 1987; see also Roney et al., 1979). Since there is little evidence that the axons of such layer V neurons project superficially, this sharply distinguishes these cells from layer VI neurons, whose axons project to supragranular layers but whose dendrites do not (Divac et al., 1987). On the other hand, visual cortex layer VI projection cells have a laminar segregation within layer VI such that those projecting corticocortically (either ipsi- or contralaterally) lie more superficially than those projecting subcortically (McCourt et al., 1986). No non-pyramidal layer V cells project subcortically (present results).

In contrast, the commissural projection involves many small cells; few apical dendrites contain enough horseradish peroxidase to trace past layer IV, and, as in cat primary auditory cortex (Code and Winer, 1985), this projection could include some bitufted and multipolar neurons.

The resemblance between commissural cells in layers V and III suggests that infragranular and supragranular cells of origin involve similar classes of neurons, insofar as somatic shape and size and dendritic arrangement are considered. Cell type, as well as laminar location, may define a corticocortical pathway. Some neurons, such as the inverted pyramidal cell in layer V of cat primary auditory cortex (R.A. Code and J.A. Winer, unpublished observations) and in rat auditory cortex (present results), may project exclusively in the commissural system.

The unusual form, orientation, and connections of the inverted pyramidal cell, as well as its specific laminar position within layer V (Winer, 1988; R.A. Code and J.A. Winer, unpublished observations) distinguish it from pyramidal cells and suggest a special functional role in the commissural system. Commissural input to the basal (inverted primary) dendrite might convey information from layers V and VI to the opposite cortex, while other layer V pyramidal cells might be expected to receive little such input to their apical dendrites. Perhaps the view that the inverted pyramidal cell is simply a conventional pyramidal cell which has been misaligned during development (Van der Loos, 1965) should be re-examined.

The callosal system includes efferent neurons in layers II and III. While this projection is well documented in rat auditory (Jacobson and Trojanowski, 1974) and somatic sensory cortex (Wise, 1975), in opossum parietal cortex (Foster et al., 1981), and in monkey (Jouandet et al., 1984) and rat neocortex (Granger et al., 1985), few studies have compared the location and form of layer V commissural and subcortical projection neurons. In hamster striate cortex, Klein et al. (1984) found that layer V callosal cells had smaller somata and more limited axonal and dendritic arborizations than neurons projecting to the thalamus and tectum. The commissural cells also had more varied receptive field properties than did corticofugal cells. Non-pyramidal layer V auditory cortex cells project commissurally (R.A. Code and J.A. Winer, unpublished observations) and have ipsilateral corticocortical targets (Winguth and Winer, 1986) in the cat (though it is unknown if any cells project in both pathways simultaneously). The different cell types and the participation of both infragranular and supragranular layers distinguish the commissural and subcortical projections.

Contrasting the commissural and corticocollicular systems

Layer V commissural and corticocollicular neurons differ in somatic size and shape and in the extent of retrograde filling of their apical den-

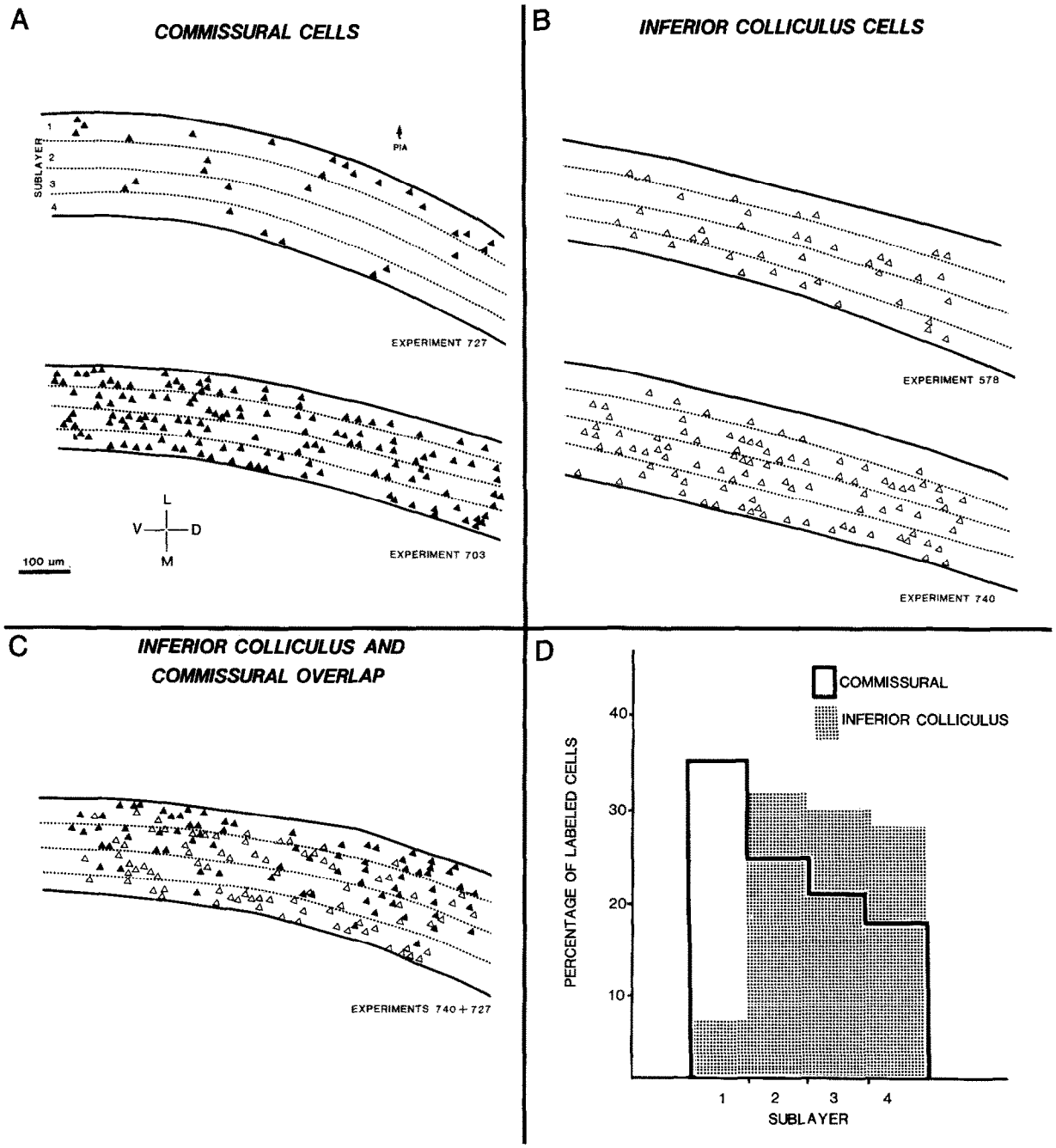
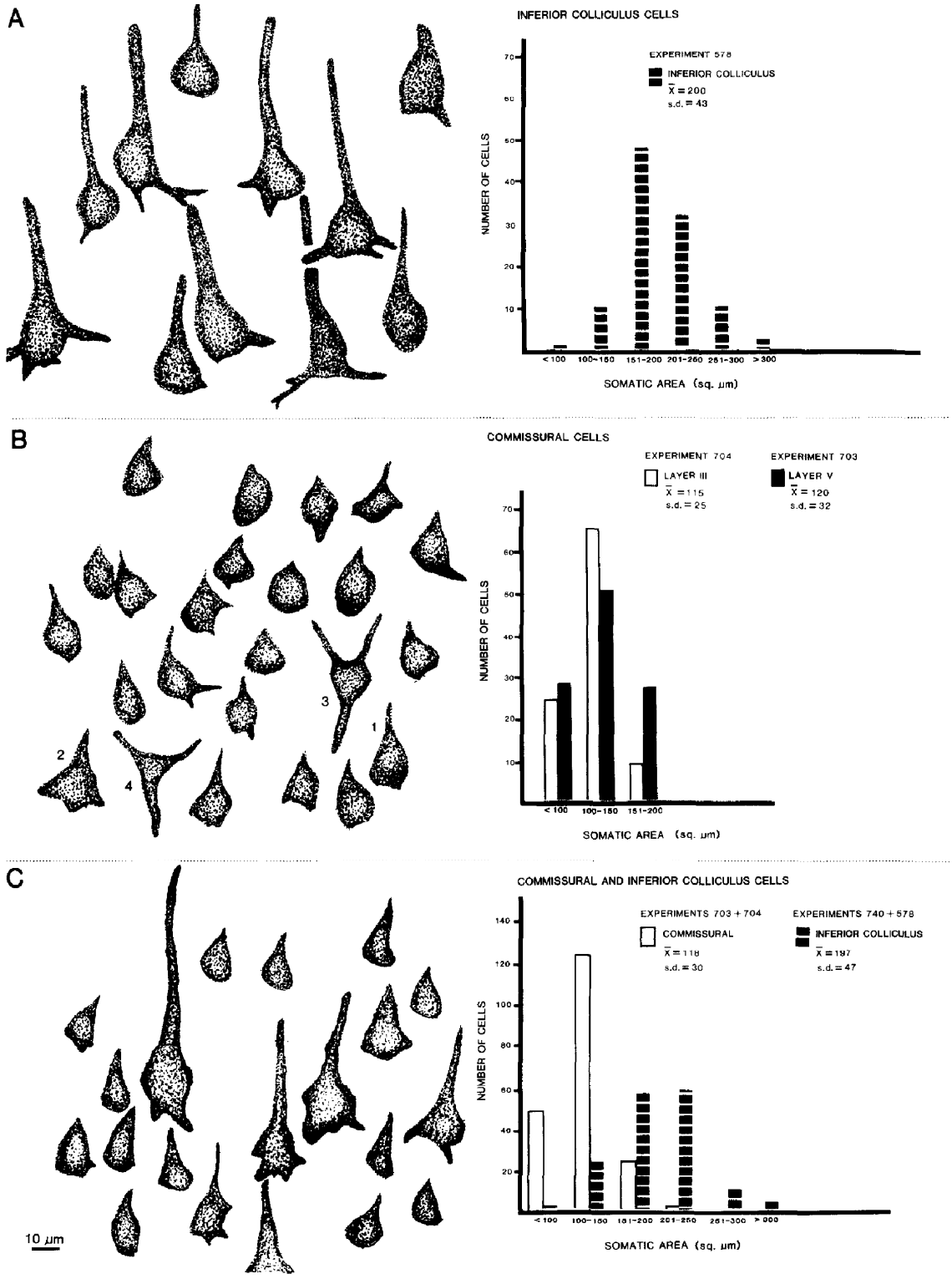


Fig. 8. Distributions of commissural and inferior colliculus cells of origin. Layer V is divided into four sublayers; 1 is the most superficial, 4 the deepest. In panels A and B, lightly (upper) and heavily (lower) labeled sections are illustrated. (A) Sublayer 1 contains many commissural neurons; in the most heavily labeled sections they are equally distributed across layer V. (B) Many corticocollicular cells occur in the lower three sublayers of layer V, especially in the most heavily labeled experiments. Sublayer 1 still has few inferior colliculus projection cells (compare with panel A) but these are chiefly in the lower part of the sublayer. (C) Overlap of commissural and corticocollicular cells from combined injections. Few corticocollicular cells arise in sublayer 1. (D) Percentages of commissural and inferior colliculus cells of origin across each sublayer in layer V. The greatest difference in their distribution is in sublayer 1, which contains many more commissural than corticocollicular projections.


A
INFERIOR COLLICULUS CELLS
EXPERIMENT 578
INFERIOR COLLICULUS
X̄ = 200
s.d. = 43
NUMBER OF CELLS
< 100
100–150
151–200
201–250
251–300
> 300
SOMATIC AREA (sq. μm)
B
COMMISSURAL CELLS
EXPERIMENT 704
LAYER III
X̄ = 115
s.d. = 25
EXPERIMENT 703
LAYER V
X̄ = 120
s.d. = 32
NUMBER OF CELLS
1
2
3
4
SOMATIC AREA (sq. μm)
C
COMMISSURAL AND INFERIOR COLLICULUS CELLS
EXPERIMENTS 703 + 704
COMMISSURAL
X̄ = 118
s.d. = 30
EXPERIMENTS 740 + 578
INFERIOR COLLICULUS
X̄ = 197
s.d. = 47
NUMBER OF CELLS
10 μm
SOMATIC AREA (sq. μm)

drites. The patterns of labeling might reflect (1) axonal terminal field size differences, (2) divergent projections from single cells, (3) variation in axonal diameter and transport velocity, or (4) structural differences among apical dendrites.

One distinction between the two populations was the small, morphologically varied commissural cells. If their terminal axonal fields were broadly distributed, then large or combined injections might saturate many of their terminals and thus label more of these cells than a small or a single injection. However, the number of small, labeled commissural neurons and the laminar distributions of midbrain and commissural cells of origin in these experiments remained the same. It is unlikely that remote or uninjected pyramidal cell axon collaterals underlie these morphological differences, unless they have a very broad distribution. Electrophysiological and double-labeling studies in several systems suggest that few commissural cells have extensive collateral branches projecting to remote, non-homotypic cortical areas (Schwartz and Goldman-Rakic, 1982; Segraves and Innocenti, 1985) or subcortically (Swadlow et al., 1978; Wong and Kelly, 1981). In cat and rat sensory-motor cortex (Miller, 1975; Catsman-Berrevoets et al., 1980) and rabbit visual cortex (Swadlow and Weyand, 1981), no neurons with both callosal and subcortical projections were identified.

In other areas more fully studied, some subcortically projecting cells have axonal collaterals. An electrophysiological and anatomical investigation found that up to 50% of hamster visual cortex cells projecting to the superior colliculus sent axon collaterals to the thalamus (Klein et al., 1986). Immature neurons in the rat occipital cortex with transient pyramidal tract axons maintain subcortical collaterals but do not form ipsilateral cortical or callosal projections (O'Leary and Stanfield, 1985). Intracellular horseradish peroxidase injections show that corticofugal neurons send collaterals to the striatum (Donoghue and Kitai, 1981). Commissural cells may thus have comparatively restricted and localized terminal fields, while subcortical efferent axons have more highly branched and widespread endings. The contrast between the laminar origins of inferior colliculus and commissural cells of origin should not obscure the fact that their concentration is roughly equal (though a declining percentage) in the lower three-quarters of layer V (Fig. 8D).

Types of projection neurons

The different cell types labeled suggest that each class of efferent neuron is not equally represented in both projection systems. We have attempted to match the profiles of these retrogradely labeled neurons with those of Golgi-impregnated cells. Inverted pyramidal cells, small pyramidal cells, and non-pyramidal cells are excluded from the corticocollicular system (Table I). Others, such as the large pyramidal cells, do not project in the commissural system, while the medium-sized pyramidal cells project in both.

The primacy of the pyramidal cell in subcortical projections and the morphological diversity of neurons in the commissural system suggest that phylogenetically older, subcortical pathways are dominated by pyramidal cells, while the newer, neocortical systems might include non-pyramidal neurons. Cells projecting in both, such as the medium-sized pyramidal cell, may contain functional and anatomical subsets which remain to be identified.

How far these conclusions extend to other species and sensory systems is uncertain; however, the major morphological classes of neurons so far recognized also occur in the cat (Winer, 1988). Differences between commissural and cortico-

Fig. 9. High power illustrations of retrogradely labeled layer V neurons and histograms of the somatic size of commissural and corticocollicular cells. Semi-apochromat, N.A. 1.25, ×787. (A) Layer V pyramidal cells projecting to the inferior colliculus are the largest of the labeled cells. (B) Commissural layer V cells are smaller and polymorphic. Some resemble small and medium-sized pyramidal cells (*1,2*), while others were similar to inverted pyramidal cells (*3,4*). The somatic areas of layer V commissural cells do not differ significantly from those in layer III. (C) After HRP injections in the ipsilateral inferior colliculus and contralateral auditory cortex, both large pyramidal cells with well-filled apical dendrites and smaller cells occur in layer V. Layer V corticocollicular cells are significantly larger than commissural cells in layers V and III.

collicular pyramidal cells occur in layer V of the rat visual cortex (Hallman et al., 1986). The somatic shape and dendritic profiles of many commissural (Code and Winer, 1985) and ipsilateral corticocortical (Winguth and Winer, 1986) neurons from layers II and III of cat auditory cortex resemble commissural cells from layer V in the rat. Candidates for these several connectional roles in auditory neocortex are the small and medium-sized pyramidal cells, inverted pyramidal cells, and the bitufted and multipolar classes of non-pyramidal cells.

Physiological correlates

Commissural and corticofugal pathways have distinct physiological properties. Fast- and slow-conducting commissural auditory axons are reported in the cat (Mitani and Shimokouchi, 1985), and both myelinated and unmyelinated callosal axons project in the primate splenium (Swadlow et al., 1980). Differences in axonal conduction velocity and structure may reflect the heterogeneity of commissural cells. In cat motor cortex, pyramidal tract responses were related to morphological differences in their projection cells (Deschênes et al., 1979). Fast-conducting neurons had larger somata and their dendrites had more extensive tangential arbors than those of the slow-conducting pyramidal tract cells. The latter study also noted morphological differences in their apical dendritic shafts. Some electrophysiological properties, such as regular spiking patterns, may be common to many pyramidal cells outside layer I, while other response patterns, for example, bursting, may be restricted to layer IV-V pyramidal cells (McCormick et al., 1985). These studies suggest that there may be a physiological segregation within efferent pathways.

Efferent layer V neurons in the auditory cortex have widespread targets and probably diverse physiological roles. These include motor-related projections to the spinal cord (Walberg and Brodal, 1953), input to the external nucleus of the inferior colliculus (Rasmussen, 1964; Cooper and Young, 1976; Faye-Lund, 1985), and corticopontocerebellar projections (Azizi et al., 1985), as well as projections to extrapyramidal motor centers (Reale and Imig, 1983). These descending influences could affect acoustic startle (Leaton and Supple, 1986) or audiogenic reflexes (Browning et al., 1985), or coordinate multisensory inputs to brain stem motor systems (Tawil et al., 1983). While the synaptic and local neuronal circuits mediating these pathways are largely unknown, layer V projection neurons may have a role in these sensory-motor behaviors insofar as they represent the efferent limb of the auditory cortex.

Speculations

Our results raise certain questions about neocortical cell types and the nature of corticofugal projections. Do projections from smaller, less obviously pyramidal cells terminate entirely in the telencephalon? Do corticofugal projections to subcortical loci always involve large pyramidal cells?

The origins of these efferent systems might embody a basic pattern of laminar and functional organization in sensory neocortex. In both layers III and V, the upper part of each layer contains smaller cells than the lower half, while the latter has many pyramidal cells. Cells in the upper part of layer V may project more locally or have cortical targets (Foster et al., 1981), while deeper cells would project to more distant, subcortical locations (Wise and Jones, 1977). This pattern prevails for the commissural and inferior colliculus projection cells in rat auditory cortex. If it also applies to layer III, then more local, intracortical projections might arise in layer IIIa, while more distant, commissural connections would be more numerous in layer IIIb. Such a pattern occurs in cat primary auditory cortex (Code and Winer, 1985). Layers without large pyramidal cells, such as II and IV, may not be similarly subdivided, nor do they have such extensive long-distance projections. By analogy, however, perhaps there is an internal order for ipsilateral local versus remote corticocortical projections arising from layer II, so that cells with a more local target might arise from the upper part of layer II, while those with more distant associations would originate more deeply. Whether this idea can be extended to layers IV and VI is uncertain. If it applies generally in sensory neocortex, then within each layer more superficial cells will be largely devoted to intrinsic or corticocortical projections, while deeper-lying neurons would project more distantly. Whether

thalamic and corticocortical axon terminals in auditory cortex preserve these patterns is unknown. The superficial part of layer I has few corticofugal projections and neuronal somata (Sousa-Pinto et al., 1975); most of its cells and efferent connections are concentrated in the lower half. There are both confirmatory examples of the above patterns and some exceptions to them, the latter generally in areas outside the sensory neocortex, for example, in retrosplenial periallocortex (Sripanidkulchai and Wyss, 1987).

Another issue is the reciprocal connectivity between afferent and efferent systems. In the cat, retrogradely labeled cell bodies in layer III and anterogradely marked axon terminals largely overlap after horseradish peroxidase injections in the opposite hemisphere (Imig et al., 1982; see also Code and Winer, 1986). Vaughan (1983) noted many commissural auditory cortex terminals in the superficial portion of layer V in the rat, while deep layer V received neither commissural nor thalamocortical terminals. That the superficial part of layer V also contains many commissural cells of origin implies that the degree of commissural reciprocity is not uniform throughout the layer. Dense commissural input zones correspond with physiological summation or ipsilateral dominance and suppression zones (Imig and Brugge, 1978); thus, patterns of commissural input may be physiologically and anatomically distinct. The non-uniformly distributed layer V commissural system may have functional significance: superficially situated neurons would receive commissural terminals on their somata and dendrites, while deep-lying pyramidal cells might have such input preferentially on their apical dendrites.

Acknowledgements

This research was supported by United States Public Health Service Grant R01 NS16832, by NIH Training Grant GM07379, and by a Dissertation Year Fellowship Award. We thank David T. Larue for technical assistance and we are grateful to Aileen K. Kim for typing the manuscript. We are indebted to an anonymous reviewer who constructively criticized an earlier version of the manuscript, and to Drs. Jack B. Kelly and Brett R. Schofield for valuable comments. Stuart J. Cheff generously shared his experimental results with us.

References

Adams, J.C. (1979) Ascending projections to the inferior colliculus. J. Comp. Neurol. 183, 519–538.

Aitkin, L.M. and Webster, W.R. (1972) Medial geniculate body of the cat: organization and responses to tonal stimuli of neurons in ventral division. J. Neurophysiol. 35, 365–380.

Albus, K. and Donate-Oliver, F. (1977) Cells of origin of the occipito-pontine projection in the cat: functional properties and intracortical location. Exp. Brain Res. 28, 167–174.

Azizi, S.A., Burne, R.A. and Woodward, D.J. (1985) The auditory corticopontocerebellar projection in the rat: inputs to the paraflocculus and midvermis. An anatomical and physiological study. Exp. Brain Res. 59, 36–49.

Beyerl, B.D. (1978) Afferent projections to the central nucleus of the inferior colliculus in the rat. Brain Res. 145, 209–223.

Browning, R.A., Nelson, D.K., Mogharreban, M., Jobe, P.C. and Laird II, H.E. (1985) Effect of midbrain and pontine tegmental lesions on audiogenic seizures in genetically epilepsy-prone rats. Epilepsia 26, 175–183.

Brunso-Bechtold, J.K., Thompson, G.C. and Masterton, R.B. (1981) HRP study of the organization of auditory afferents ascending to central nucleus of inferior colliculus in cat. J. Comp. Neurol. 197, 705–722.

Catsman-Berrevoets, C.E., Lemon, R.N., Verburgh, C.A., Bentivoglio, M. and Kuypers, H.G.J.M. (1980) Absence of callosal collaterals derived from rat corticospinal neurons. Exp. Brain Res. 39, 433–440.

Code, R.A. and Winer, J.A. (1985) Commisural neurons in layer III of cat primary auditory cortex (AI): pyramidal and non-pyramidal cell input. J. Comp. Neurol. 242, 485–510.

Code, R.A. and Winer, J.A. (1986) Columnar organization and reciprocity of commissural connections in cat primary auditory cortex (AI). Hear. Res. 23, 205–222.

Coleman, J., Rainer, R. and Clerici, W.J. (1984) Projection patterns of rat auditory cortex. Proc. Soc. Neurosci. 10, 247 (Abstract).

Cooper, M.H. and Young, P.A. (1976) Cortical projections to the inferior colliculus of the cat. Exp. Neurol. 51, 488–502.

Deschênes, M., Labelle, A. and Landry, P. (1979) Morphological characterization of slow and fast pyramidal tract cells in the cat. Brain Res. 178, 251–274.

Divac, I., Marinkovic, S., Mogensen, J., Schwerdtfeger, W. and Regidor, J. (1987) Vertical ascending connections in the isocortex. Anat. Embryol. 175, 443–455.

Donoghue, J.P. and Kitai, S.T. (1981) A collateral pathway to the neostriatum from corticofugal neurons of the rat sensory-motor cortex: an intracellular HRP study. J. Comp. Neurol. 201, 1–13.

Druga, R. and Syka, J. (1984) Ascending and descending projections to the inferior colliculus in the rat. Physiol. Bohemoslav. 33, 31–42.

Escobar, M.I., Amienta, H., Caviness, Jr., V.S., Jacobson, M., Crandall, J.E. and Kosik, K.S. (1986) Architecture of apical

dendrites in the murine neocortex: dual apical dendritic systems. Neuroscience 17, 975–989.

Faye-Lund, H. (1985) The neocortical projection to the inferior colliculus in the albino rat. Anat. Embryol. 173, 53–70.

Foster, R.E., Donoghue, J.P. and Ebner, F.F. (1981) Laminar organization of efferent cells in the parietal cortex of the Virginia opossum. Exp. Brain Res. 43, 330–336.

Games, K.D. and Winer, J.A. (1983) Projections of layer V in auditory cortex to the rat inferior colliculus: cells of origin and neuronal architecture. Proc. Soc. Neurosci. 9, 954 (Abstract).

Games, K.D. and Winer, J.A. (1986) Two efferent populations in layer V of rat auditory cortex: cells projecting to the inferior colliculus and contralateral cortex. Proc. Soc. Neurosci. 12, 1275 (Abstract).

Gilbert, C.D. and Kelly, J.P. (1975) The projections of cells in different layers of the cat's visual cortex. J. Comp. Neurol. 163, 81–106.

Graham, Jr., R.C. and Karnovsky, M.J. (1966) The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney: ultrastructural cytochemistry by a new technique. J. Histochem. Cytochem. 14, 291–302.

Granger, E.M., Masterton, R.B. and Glendenning, K.K. (1985) Origin of interhemispheric fibers in a callosal opossum (with a comparison to callosal origin in rat). J. Comp. Neurol. 241, 82–98.

Hallman, L.E., Schofield, B.R. and Lin, C.-S. (1986) Morphology and axon collateralization of layer V cells in primary visual cortex of hooded rats that project to the superior colliculus, pons or contralateral cortex. Proc. Soc. Neurosci. 12, 129 (Abstract).

Holländer, H. (1974) On the origin of the corticotectal projections in the cat. Exp. Brain Res. 21, 433–439.

Imig, T.J. and Brugge, J.F. (1978) Sources and terminations of callosal axons related to binaural and frequency maps in primary auditory cortex of the cat. J. Comp. Neurol. 182, 637–660.

Imig, T.J., Morel, A. and Kauer, C.D. (1982) Covariation of distributions of callosal cell bodies and callosal axon terminals in layer III of cat primary auditory cortex. Brain Res. 251, 157–159.

Jacobson, S. and Trojanowski, J.Q. (1974) The cells of origin of the corpus callosum in rat, cat and rhesus monkey. Brain Res. 74, 149–155.

Jones, E.G. and Wise, S.P. (1977) Size, laminar and columnar distribution of efferent cells in the sensory-motor cortex of monkeys. J. Comp. Neurol. 175, 391–438.

Jouandet, M.L., Garey, L.J. and Lipp, H.-P. (1984) Distribution of the cells of origin of the corpus callosum and anterior commissure in the marmoset monkey. Anat. Embryol. 169, 45–59.

Klein, B.G., Mooney, R.D., Jacquin, M.F. and Rhoades, R.W. (1984) Comparison of the structural and functional properties of lamina V striate callosal neurons with those of cells which project to the superior colliculus and lateral posterior nucleus. Proc. Soc. Neurosci. 10, 730 (Abstract).

Klein, B.G., Mooney, R.D., Fish, S.E. and Rhoades, R.W. (1986) The structural and functional characteristics of striate cortical neurons that innervate the superior colliculus and lateral posterior nucleus in hamster. Neuroscience 17, 57–78.

Krieg, W.J.S. (1946) Collected Papers Relating to the Cerebrum. Charles C Thomas, Springfield.

Larue, D.T. and Winer, J.A. (1985) Reciprocity of auditory corticothalamic and thalamocortical projections: study with autoradiographic and horseradish peroxidase methods in the rat medial geniculate body. Proc. Soc. Neurosci. 11, 33 (Abstract).

Leaton, R.N. and Supple, Jr., W.F. (1986) Cerebellar vermis: essential for long-term habituation of the acoustic startle response. Science 232, 513–515.

LeDoux, J.E., Ruggiero, D.A. and Reis, D.J. (1985) Projections to the subcortical forebrain from anatomically defined regions of the medial geniculate body in the rat. J. Comp. Neurol. 242, 182–213.

McCormick, D.A., Connors, B.W., Lighthall, J.W. and Prince, D.A. (1985) Comparative electrophysiology of pyramidal and sparsely spiny stellate neurons of the neocortex. J. Neurophysiol. 54, 782–806.

McCourt, M.E., Boyapati, J. and Henry, G.H. (1986) Layering in lamina 6 of cat striate cortex. Brain Res. 364, 181–185.

Mesulam, M.-M. (1978) Tetramethyl benzidine for horseradish peroxidase neurohistochemistry: a non-carcinogenic blue reaction-product with superior sensitivity for visualizing neural afferents and efferents. J. Histochem. Cytochem. 26, 106–117.

Miller, R. (1975) Distribution and properties of commissural and other neurons in cat sensorimotor cortex. J. Comp. Neurol. 164, 361–374.

Mitani, A. and Shimokouchi, M. (1985) Neuronal connections in the primary auditory cortex: an electrophysiological study in the cat. J. Comp. Neurol. 235, 417–429.

Murray, E.A. and Coulter, J.D. (1981) Organization of corticospinal neurons in the monkey. J. Comp. Neurol. 195, 339–365.

O'Leary, D.D.M. and Stanfield, B.B. (1985) Occipital cortical neurons with transient pyramidal tract axons extend and maintain collaterals to subcortical but not intracortical targets. Brain Res. 336, 326–333.

Patterson, H.A. (1976) An anterograde degeneration and retrograde axonal transport study of the cortical projections of the rat medial geniculate body. (Dissertation, Boston University).

Paxinos, G. and Watson, C. (1982) The Rat Brain in Stereotaxic Coordinates. Academic Press, New York.

Paxinos, G. and Watson, C. (1986) The Rat Brain in Stereotaxic Coordinates. Second ed., Academic Press, Sydney.

Peters, A. and Kara, D.A. (1987) The neuronal composition of area 17 of rat visual cortex. IV. The organization of pyramidal cells. J. Comp. Neurol. 260, 573–590.

Rasmussen, G.L. (1964) Anatomic relationships of the ascending and descending auditory systems. In: W.S. Field and B.R. Alford (Eds.), Neurological Aspects of Auditory and Vestibular Disorders, Charles C Thomas, Springfield, p. 5.

Reale, R.A. and Imig, T.J. (1983) Auditory cortical field pro-

jections to the basal ganglia of the cat. Neuroscience 8, 67–86.

Roney, K.J., Scheibel, A.B. and Shaw, G.L. (1979) Dendritic bundles: survey of anatomical experiments and physiological theories. Brain Res. Rev. 1, 225–271.

Roth, G.L., Aitkin, L.M., Andersen, R.A. and Merzenich, M.M. (1978) Some features of the spatial organization of the central nucleus of the inferior colliculus of the cat. J. Comp. Neurol. 182, 661–680.

Ryugo, D.K. and Killackey, H.P. (1974) Differential telencephalic projections of the medial and ventral divisions of the medial geniculate body of the rat. Brain Res. 82, 173–177.

Sally, S.L. and Kelly, J.B. (1988) Organization of auditory cortex in the albino rat: sound frequency. J. Neurophysiol. (submitted).

Schofield, B.R., Hallman, L.E. and Lin, C.-S. (1985) Physiological and morphological properties of corticotectal cells in the hooded rat. Proc. Soc. Neurosci. 11, 227 (Abstract).

Schwartz, M.L. and Goldman-Rakic, P.S. (1982) Single cortical neurones have axon collaterals to ipsilateral and contralateral cortex in fetal and adult primates. Nature 299, 154–155.

Segraves, M.A. and Innocenti, G.M. (1985) Comparison of the distributions of ipsilaterally and contralaterally projecting corticocortical neurons in cat visual cortex using two fluorescent tracers. J. Neurosci. 5, 2107–2118.

Sousa-Pinto, A., Paula-Barbosa, M. and Matos, M.D.C. (1975) A Golgi and electron microscopical study of nerve cells in layer I of the cat auditory cortex. Brain Res. 95, 443–458.

Sripanidkulchai, K. and Wyss, J.M. (1987) The laminar organization of efferent neuronal cell bodies in the retrosplenial granular cortex. Brain Res. 406, 255–269.

Swadlow, H.A. and Weyand, T.G. (1981) Efferent systems of rabbit visual cortex: laminar distribution of the cells of origin, axonal conduction velocities, and identification of axonal branches. J. Comp. Neurol. 203, 799–822.

Swadlow, H.A., Weyand, T.G. and Waxman, S.G. (1978) The cells of origin of the corpus callosum in rabbit visual cortex. Brain Res. 156, 129–134.

Swadlow, H.A., Waxman, S.G. and Geschwind, N. (1980) Small-diameter nonmyelinated axons in the primate corpus callosum. Arch. Neurol. 37, 114–115.

Tawil, R.N., Saade, N.E., Bitar, M. and Jabbur, S.J. (1983) Polysensory interactions on single neurons of cat inferior colliculus. Brain Res. 269, 149–152.

Van der Loos, H. (1965) The "improperly" oriented pyramidal cell in the cerebral cortex and its possible bearing on problems of neuronal growth and cell orientation. Bull. Johns Hopkins Hosp. 117, 228–250.

Vaughan, D.W. (1983) Thalamic and callosal connections of the rat auditory cortex. Brain Res. 260, 181–189.

Walberg, F. and Brodal, A. (1953) Pyramidal tract fibres from temporal and occipital lobes. An experimental study in the cat. Brain 76, 491–510.

Warr, W.B. (1966) Fiber degeneration following lesions of the anterior ventral cochlear nucleus of the cat. Expl. Neurol. 14, 453–474.

Warr, W.B. (1969) Fiber degeneration following lesions of the posteroventral cochlear nucleus of the cat. Expl. Neurol. 23, 140–155.

Winer, J.A. (1988) Neuronal organization of layer V in cat primary auditory cortex (AI). J. Comp. Neurol. (to be submitted).

Winer, J.A. and Larue, D.T. (1987) Patterns of reciprocity in auditory thalamocortical and corticothalamic connections: study with horseradish peroxidase and autoradiographic methods in the rat medial geniculate body. J. Comp. Neurol. 257, 282–315.

Winguth, S.D. and Winer, J.A. (1986) Corticocortical connections of cat primary auditory cortex (AI): laminar organization and identification of supragranular neurons projecting to area AII. J. Comp. Neurol. 248, 36–56.

Wise, S.P. (1975) The laminar organization of certain afferent and efferent fiber systems in the rat somatosensory cortex. Brain Res. 90, 139–142.

Wise, S.P. and Jones, E.G. (1977) Cells of origin and terminal distribution of descending projections of the rat somatic sensory cortex. J. Comp. Neurol. 175, 129–158.

Wong, D. and Kelly, J.P. (1981) Differentially projecting cells in individual layers of the auditory cortex: a double-labeling study. Brain Res. 230, 362–366.

Zilles, K. (1985) The Cortex of the Rat. A Stereotaxic Atlas. Springer-Verlag, Berlin.