THE JOURNAL OF COMPARATIVE NEUROLOGY 404:332–358 (1999)

Layer VI in Cat Primary Auditory Cortex: Golgi Study and Sublaminar Origins of Projection Neurons

JORGE J. PRIETO[1]* AND JEFFERY A. WINER[2]
[1]Department of Histology, Institute of Neurosciences, University Miguel Hernández, 03550 San Juan, Alicante, Spain
[2]Division of Neurobiology, Department of Molecular and Cell Biology, University of California at Berkeley, Berkeley, California 94720–3200

ABSTRACT

The organization of layer VI in cat primary auditory cortex (AI) was studied in mature specimens. Golgi-impregnated neurons were classified on the basis of their dendritic and somatic form. Ipsilateral and contralateral projection neurons and the corticogeniculate cells of origin were labeled with retrograde tracers and their profiles were compared with the results from Golgi studies. Layer VI was divided into a superficial half (layer VIa) with many pyramidal neurons and a deeper part (layer VIb) that is dominated by horizontal cells. Nine types of neuron were identified; four classes had subvarieties. Classical pyramidal cells and star, fusiform, tangential, and inverted pyramidal cells occur. Nonpyramidal neurons were Martinotti, multipolar stellate, bipolar, and horizontal cells. This variety of neurons distinguished layer VI from other AI layers. Pyramidal neuron dendrites contributed to the vertical, modular organization in AI, although their apical processes did not project beyond layer IV. Their axons had vertical, intrinsic processes as well as corticofugal branches. Horizontal cell dendrites extended laterally up to 700 μm and could integrate thalamic input across wide expanses of the tonotopic domain. Connectional experiments confirmed the sublaminar arrangement seen in Nissl material. Commissural cells were concentrated in layer VIa, whereas corticocortical neurons were more numerous in layer VIb. Corticothalamic cells were distributed more equally. The cytological complexity and diverse connections of layer VI may relate to a possible role in cortical development. Layer VI contained most of the neuronal types found in other layers in AI, and these cells form many of the same intrinsic and corticofugal connections that neurons in other layers will assume in adulthood. Layer VI, thus, may play a fundamental ontogenetic role in the construction and early function of the cortex. J. Comp. Neurol. 404:332–358, 1999.

Indexing terms: pyramidal cells; nonpyramidal cells; connections; cortical circuits; columnar organization

Layer VI has a special role in sensory neocortex because of its diverse connections. It is a source of corticofugal projections to the thalamus (Kelly and Wong, 1981), a target of thalamic input (Sousa-Pinto, 1973), a significant origin for and terminus of the ipsilateral corticocortical pathways (Winguth and Winer, 1986), an important component in the commissural system (Code and Winer, 1985), and it participates in intrinsic interlaminar connections (Usrey and Fitzpatrick, 1996 [tree shrew]). Such a wide range of connections distinguishes it from layer IV, which receives specific thalamocortical input (Davis and Sterling, 1979) and the powerful projections from which are limited to the ipsilateral corticocortical system (Meyer and Albus, 1981) and to local interlaminar circuits (Mitani et al., 1985). The more restricted connectivity of layer IV is complemented by a neuronal architecture in which only a few types of primarily nonpyramidal cells occur (Winer, 1984a). Far more data are available on layer IV (Lund et al., 1981 [macaque monkey]) than on layer VI (Tömböl,

Grant sponsor: Dirección General de Investigación en Ciencia y Tecnología (DGICYT); Grant numbers: PB93–0928 and PM96–0082; Grant sponsor: National Institutes of Health; Grant number: R01 DC02319–18.

*Correspondence to: Jorge J. Prieto, M.D., Ph.D., Department of Histology, Institute of Neuroscience, University Miguel Hernández, Carretera Valencia s/n, 03550 San Juan, Alicante, Spain. E-mail: jorge.prieto@umh.es

Received 13 April 1998; Revised 11 September 1998; Accepted 17 September 1998

1984), and this difference accentuates how little is known about the role of layer VI in cortical function.

The present study analyzes layer VI neurons in cat primary auditory cortex (AI). As the chief origin of the corticothalamic system, layer VI projections in other systems may influence receptive field dynamics (Sillito et al., 1993), the augmenting response (Deschênes and Hu, 1990), the timing of relay cell discharge (Sillito et al., 1994), and the control of sensory gating (Crick, 1984), to name just a few of the thalamic processes whose physiological basis might entail cortical control. The role of layer VI in ipsilateral corticocortical projections and in the commissural system is largely unknown. Our goal is to identify the resident neuronal populations and to relate these to specific projections. These data will enhance our perspective on the contribution of the infragranular layers to cortical and subcortical function.

MATERIALS AND METHODS

The procedures used in this study have been described previously in detail (Winer, 1984a–c, 1985, 1992). Procedures were approved by and administered under the auspices of the appropriate institutional animal care and use committee. Adult cats of either sex and free of middle ear disease were used. Sodium pentobarbital (40 mg/kg, i.p.) or isofluorane (1–4%, inhalant) were used to maintain stage III, plane ii of general anesthesia through perfusion or surgery, respectively.

Golgi material

Neurons were impregnated with the Golgi-Cox method (Cox, 1891) and by using the on-the-slide variation (Ramón-Moliner, 1970). Cells from more than 20 complete hemispheres, consisting of serial sections from the visual cortex through the somatic sensory cortex, were available. Neurons were classified by their laminar location, somatic form, and dendritic arborization (Table 1). Axons rarely were impregnated.

Histology

Tract-tracing experiments. Cortical injections were made into areas identified by their sulcal pattern and corroborated later by architectonic analysis. Wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP; 5%; Sigma Chemical Co., St. Louis, MO) in distilled water was injected by pressure through a glass micropipette coupled to a Unimetrics syringe (Unimetric, Shorewood, IL). Cortical tracer injections (six 0.2-μl deposits) into AI or the second auditory cortical area (AII; four 0.2-μl injections) labeled commissural and ipsilateral corticocortical projection cells, respectively. Thalamic coordinates were taken from standard atlases, and the volume of these deposits was 0.15 μl. The total number of experiments available was eight, including studies of corticocortical (n = 3), commissural (n = 3), and corticothalamic (n = 2) projection systems. The technical procedures are presented in detail in earlier studies of the commissural (Code and Winer, 1985, 1986) and ipsilateral corticocortical (Winguth and Winer, 1986) connections.

After a 3-day survival, the animals were reanesthetized and perfused. Brains were sectioned on a freezing microtome at 60 μm, and the sections were either developed for tetramethylbenzidine and counterstained with neutral red or incubated with diaminobenzidine and counterstained by using the Nissl method.

Immunocytochemistry. The protocols for γ-aminobutyric acid (GABA) and glutamate immunostaining have been presented in detail in prior studies of cortical GABAergic neurons (Prieto et al., 1994a).

Data analysis

Only neurons that were encountered commonly in Golgi material were drawn and analyzed. Retrogradely labeled neurons were classified by laminar location, somatic size and shape, and dendritic arborization. The labeled neurons were plotted and counted. A sample of 20 sections/experiment through the region of maximum labeling was used.

Abbreviations

AAF	anterior auditory field
aes	anterior ectosylvian sulcus
AI	primary auditory cortex
AII	second auditory cortical area
Bp	bipolar cell
BV	blood vessel
CG	central gray
CP	cerebral peduncle
D	dorsal nucleus of the medial geniculate body
DD	deep dorsal nucleus of the medial geniculate body
DS	dorsal superficial nucleus of the medial geniculate body
EPD	posterior ectosylvian gyrus, dorsal part
EPI	posterior ectosylvian gyrus, intermediate part
EPP	posterior ectosylvian gyrus, posterior part
EPV	posterior ectosylvian gyrus, ventral part
FHP	fusiform horizontal pyramidal cell
FVP	fusiform vertical pyramidal cell
GABA	γ-aminobutyric acid
Glu	glutamate
H	horizontal cell
Hyp	hypothalamus
I–VIa,b	cortical layers
Ins	insular cortex
IP	inverted pyramidal cell
LGN	lateral geniculate body
LM	large multipolar cell
LP	lateral posterior nucleus *or* large pyramidal cell
M	medial division of the medial geniculate body
Ma	Martinotti cell
MGBm	medial geniculate body, medial division
MGBv	medial geniculate body, ventral division
MM	medium-sized multipolar cell
MP	medium-sized pyramidal cell
OT	optic tract
P	posterior auditory field
PC	posterior commissure
RN	red nucleus
SF/daz	suprasylvian fringe/dorsal auditory zone
Sg	suprageniculate nucleus
SM	small multipolar cell
SmP	small pyramidal cell
StP	star pyramidal cell
Te	temporal cortex
TP	tangential pyramidal cell
V	ventral auditory field *or* ventral division of the medial geniculate body
Vb	ventrobasal nucleus of the thalamus
VP	ventral posterior auditory field
wm	white matter

TABLE 1. Summary of Layer VI Cell Types in Cat Primary Auditory Cortex

Cell type	Subtypes	Sublayer(s)[1]	Somatic shape and size[2]	Number of primary dendrites	Dendritic field shape	Dendritic field size[2]	Figure(s)
1. Pyramidal	a. Small	VIa, VIb	Triangular; 14 × 18 μm	4–5	Vertically elongated	280 × 730 μm	4:1a; 15A–C:1a
	b. Medium-sized	VIa, VIb	Triangular; 18 × 28 μm	5–9	Cylindrical	310 × 800 μm	4:1b; 5; 7:1b; 15A–C:1b
	c. Large	VIa	Triangular; 36 × 40 μm	7–9	Cylindrical	680 × 1,100 μm	4:1c; 15A–C:1c
2. Star pyramidal	—	VIa, VIb	Round; 20 × 18 μm	6–8	Vertically elongated	240 × 510 μm	6:2; 15A:2
3. Fusiform pyramidal	a. Vertical	VIa, VIb	Fusiform; 10 × 25 μm	2–4	Fusiform	180 × 700 μm	6:3a; 15A–C:3a
	b. Horizontal	VIb	Fusiform; 28 × 14 μm	2–3	Fusiform	730 × 150 μm	6:3b; 15B:3b
4. Tangential pyramidal	—	VIb	Round or oval; 20 × 18 μm	4–5	Horizontally elongated	660 × 360 μm	8:4; 15A, B:4
5. Inverted pyramidal	—	VIa, VIb	Triangular; 20 × 30 μm	4–5	Conical (inverted)	270 × 600 μm	7:5, 8:5/i; 8:5/ii; 15 A, B:5
6. Martinotti	—	VIa	Triangular; 16 × 25 μm	3–5	Conical (inverted)	190 × 540 μm	9:6/i 9:6/ii
7. Multipolar	a. Small	VIa, VIb	Round or oval; 13 × 14 μm	5–8	Stellate	180 × 200 μm	10:7a
	b. Medium sized	VIa, VIb	Polygonal; 16 × 18 μm	5–10	Stellate	340 × 320 μm	10:7b/i; 10:7b/ii
	c. Large	VIa	Oval or polygonal; 27 × 36 μm	6–9	Stellate; vertically elongated	450 × 600 μm	11:7c/i; 11:7c/ii
8. Bipolar	—	VIa	Fusiform; 15 × 35 μm	2	Vertical; poorly branched	150 × 540 μm	12:8/i; 12:8/ii
9. Horizontal	a. Bitufted	VIb	Oval; 28 × 20 μm	6–10	Horizontal; tufted	700 × 240 μm	13:9a
	b. Bipolar	VIb	Fusiform; 35 × 15 μm	2–3	Horizontal; poorly branched	380 × 210 μm	13:9b

[1]The preferential locus is in underlined.
[2]Width by height.

Figure 14 was computer edited. Negatives were made on a photomicroscope (Leica DMRB; Heidelberg, Germany) by using black-and-white film (Kodak T-Max 100; Eastman-Kodak, Rochester, NY), then scanned with a 35-mm film scanner (Nikon LS-1000; Tokyo, Japan), and edited to adjust contrast (Adobe Photoshop version 4.0; Adobe Systems, Mountain View, CA). Composition and labeling (Adobe Illustrator version 7.0) preceded printing on a dye-sublimation color printer (Fargo Pictura 310e, Fargo Electronics Incorporated, Eden Prairie, MN). Digital editing did not change materially the content of the images, nor did it alter their meaning.

RESULTS

Cytoarchitecture and myeloarchitecture

In Nissl material layer VI was ≈300 μm thick and began ≈1,400 μm beneath the pia (Fig. 1). Its neurons were smaller than layer V cells, because layer VI contained few large pyramidal cells. Neurons with horizontally oriented somata (Fig. 2A,B) and laterally disposed dendrites (Fig. 13) dominated the lower one-third of layer VI; these were not present in layer V. Small clusters of cells were prevalent in layer VI (Fig. 2A). The neuropil also differed in layers V and VI (Fig. 2B). The white matter contained massive fascicles of myelinated axons with various orientations. As fibers ascended, they branched between and among the cell clusters; some axons lost their myelin as they reached layer V. They formed bundles at regular intervals in both layer VI and layer V; in layer V, cell-poor and neuropil-rich zones were larger and were concentrated in both halves (Fig. 1).

Layer VI had a bilaminar arrangement, with fewer neurons in layer VIa. Layer VIa contained many fusiform and inverted pyramidal cells, of which most were glutamatergic (Fig. 3A), whereas horizontal cells dominated the deepest part of layer VIb. Their laterally arranged primary dendrites (Fig. 2B, open profiles) crossed the path of the ascending axons. Many neurons were found 200–300 μm beneath layer VI, deep in the white matter.

Neuronal architecture

Classical and modified pyramidal cells were impregnated. The former had triangular somata, a principal dendrite that arose at the top of the soma, and tufted basolateral dendritic arbors. The so-called modified neurons lacked one or more of these attributes and were rarer.

Pyramidal cells. The large (Fig. 4:1c) and medium-sized (Fig. 4:1b) pyramidal cells differed in perikaryal size, dendritic organization, and sublaminar position; both classes were glutamate immunoreactive (Fig. 3A). The large neurons were rarer in layer VI than in layer V, and they were limited to those layers. Large pyramidal cells occurred only in layer VIa. The perikaryon was the largest in this survey (Table 1:2), and the apical dendrite extended 200–250 μm. The trunk formed three of four thinner processes in layers IV or IIIa and ended in oblique branches. The six to ten basolateral dendrites made compact parallel bundles, which distinguished them from medium-sized and small pyramidal neurons. The basolateral dendritic arbor was disk shaped, whereas the arbors of medium-sized (Fig. 5) and small (Fig. 4:1a) pyramidal cells were spherical or oval. Spines increased in number after the initial 20–40 μm, and these appendages varied in shape; they were similar for all pyramidal cells (see below).

The axon (not illustrated in this example) was rarely impregnated after the initial segment. It arose beneath the soma with an initial segment that was 30–45 μm long and ≈4–5 μm thick. It divided into three to six branches that projected horizontally in layers Vb and VI, and then

Fig. 1. Cytoarchitecture and laminar arrangement of primary auditory cortex (AI). The limits of AI were determined independently in each hemisphere. In the main figure, the layer Vb/VI border (white line) was denoted by the higher density of layer VI neurons, their slightly smaller size, their greater heterogeneity, and the virtual absence of large layer VI cells. The layer VIa/VIb border was marked by an abrupt decrease in cell density in layer VIb and the appearance of many neurons with somata oriented laterally, parallel to the pia. The junction with the white matter was prominent in plastic-embedded material (wm; see Fig. 2B). Neurons displaced deep into the white matter may be a residual feature of the genesis and subsequent regression of the cortical subplate (Valverde and Facal-Valverde, 1988). **Top inset**: Lateral view of auditory and periauditory cortex. **Bottom inset**: Transverse section showing the origin (stippled area) of the observation in the main figure. Arrows indicate the borders between cortical fields. Celloidin-embedded, 50-μm-thick section. For abbreviations, see list. In this and all subsequent figures, the crossed axes lettered C, D, L, M, R, and V indicate the caudal, dorsal, lateral, medial, rostral, and ventral cardinal planes, respectively. Planapochromat, N.A. 0.65; ×500.

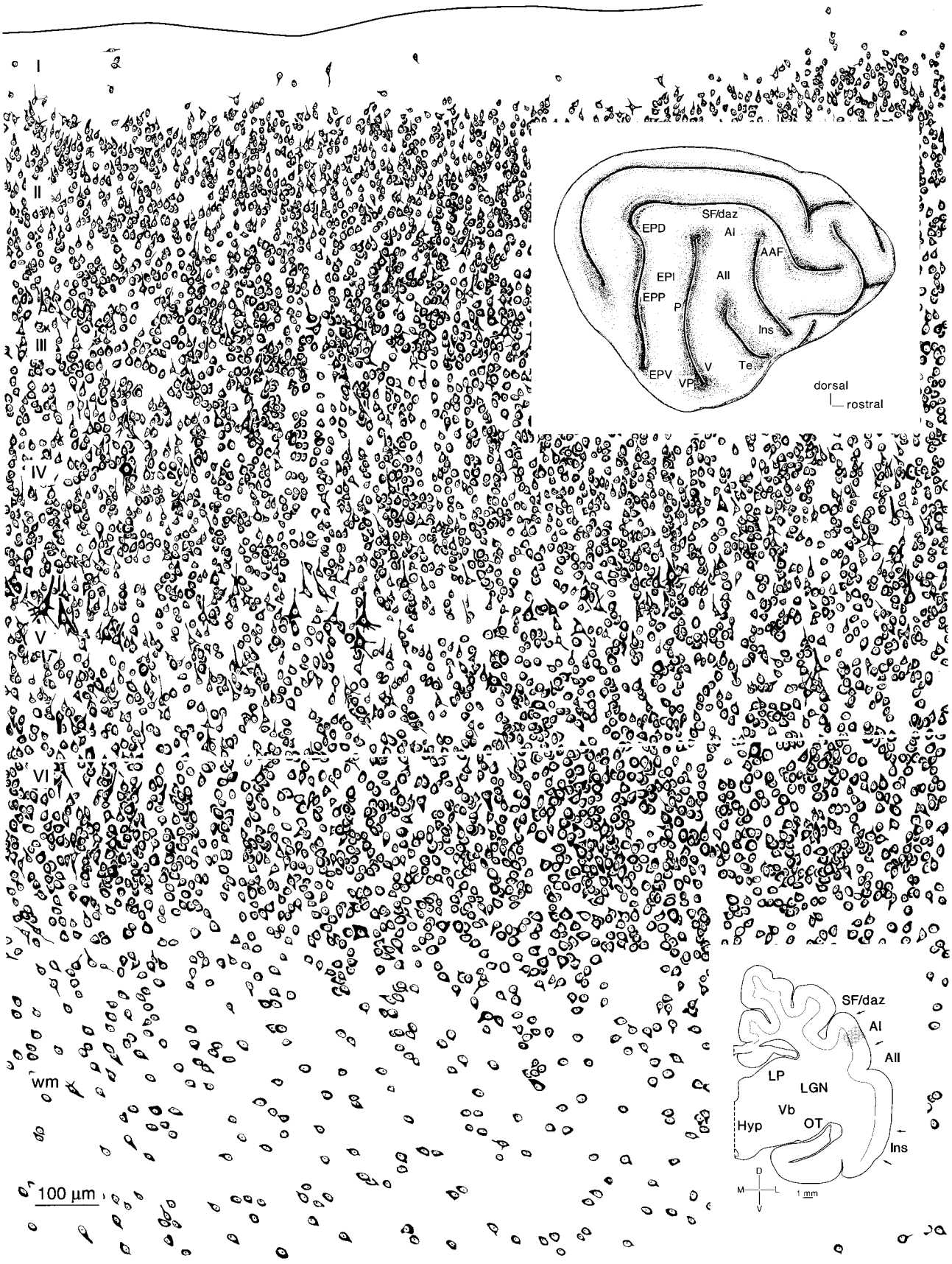
I
II
III
IV
V
VI
wm
100 μm
SF/daz
EPD
AI
AAF
EPI
AII
EPP
P
Ins
EPV
V
Te
VP
dorsal
rostral
SF/daz
AI
AII
LP
LGN
Vb
Hyp
OT
Ins
D
M
L
V
1 mm

Figure 1

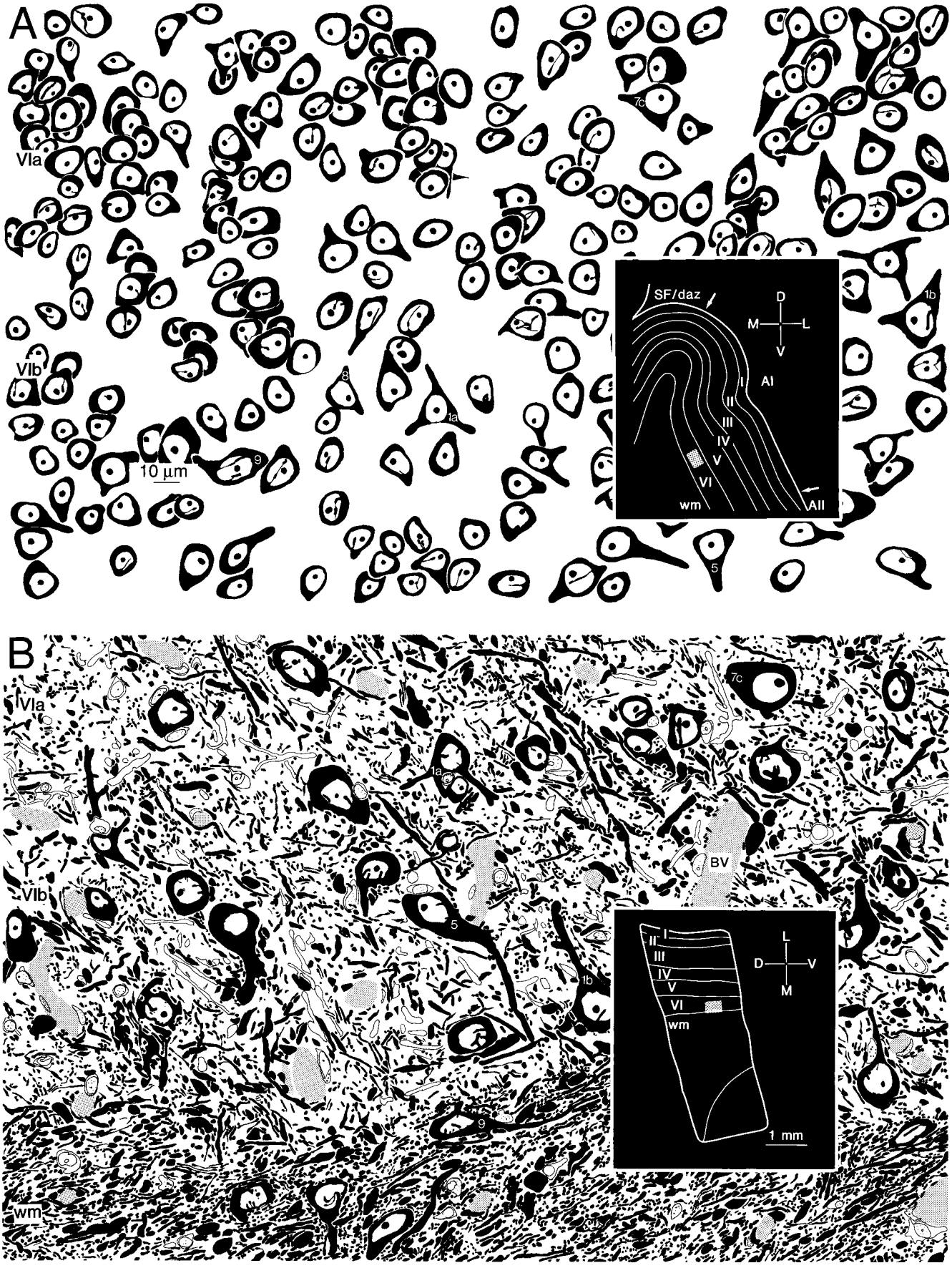
A
VIa
VIb
7c
8
1a
9
10 µm
1b
5
SF/daz
D
M
L
V
I
II
III
IV
V
VI
AI
AII
wm
B
VIa
VIb
7c
1a
BV
5
1b
9
wm
I
II
III
IV
V
VI
wm
L
D
V
M
1 mm

Figure 2

entered the white matter. Such lateral branches were not found on other pyramidal cells.

Medium-sized pyramidal cells (Figs. 4:1b, 5, 7:1b) were common, and they dominated layer VIa. They resembled pyramidal cells in layers III (Winer, 1984a) and V (unpublished observations). Common features included 1) prominent basal dendritic tufts, 2) apical arbors crossing one or two layers, 3) modest lateral dendrites, 4) an axon with local branches that overlapped the dendritic domain, and 5) a collateral that entered the white matter (Fig. 5A).

The oval or triangular perikaryon was smooth and elongated vertically (Figs. 4:1b, 5, 7:1b). Dendrites arose at the poles, and the thick apical trunk formed tufts of short and oblique branches. The trunk ascended radially or curved, with few branches. The terminal tuft arose in layer V and projected for 300–400 μm (Fig. 4:1b). Some processes reached layer V or layer IVb. Basal arbors were short, sinuous, tapered rarely, and ended within 250 μm. Distal dendritic segments were the most spinous in this survey and resembled those of pyramidal neurons in other layers. Eight types of appendages were identified: a thin, filiform variety (Fig 5B:1); hill-like swellings (Fig. 5B:2); finger-like processes (Fig. 5B:3); mushroom-shaped spikes, which were the most common and included large (Fig. 5B:4) and small (Fig. 5B:5) subtypes; complex, convoluted appendages (Fig. 5B:6); elaborate spines with short processes at the stem (Fig. 5B:7); and sessile profiles (Fig. 5B:8).

The axon arose beneath the perikaryon and had an initial segment that was 25–35 μm long and swellings suggestive of a myelin sheath. About four to six local branches ascended parallel to the dendrites and overlapped them. These fibers sometimes acquired myelin, then emitted thinner, unmyelinated segments that ended locally or extended past the apical dendrites for 500–800 μm or more, branching often (Fig. 5A). Both terminal and en passant boutons were seen near the soma and apical dendrite (Fig. 5B). The axon projected toward the apical dendrites near the layer IV/V border.

Smaller pyramidal cells (Fig. 4:1a) had finer, more delicate dendrites and sparser basal arbors. These neurons were neither as numerous as the medium-sized pyramidal cells nor as rare as the largest type, and they were predominantly in layer VIa. Primary dendrites arose at the somatic poles, and the thin apical trunk was smooth, branched sparsely, extended up to 500 μm, and did not enter layer IV. It had no terminal arbor and only a few processes. The four to six basolateral dendrites were very slender and had many spines, like other types of pyramidal cells.

The axon arose beneath the perikaryon and became myelinated, then it emitted horizontal processes that curved and ascended toward the pia as they divided. Sometimes, only horizontal branches were seen (Fig. 4:1a).

Star pyramidal cells. The star pyramidal cell (Fig. 6:2) and the five other neuronal types described below each had 1) spinous dendrites, 2) a prominent apical dendrite, and 3) a corticofugal axon. Star pyramidal cells were rarer than the other pyramidal cells. They resembled small or medium-sized pyramidal cells, save for the unusual basal dendritic organization. A thick apical dendrite arose from the upper or lateral somatic surface. The two or three secondary dendrites were shorter and poorly ramified; like the small pyramidal cells (Fig. 4:1A), these had no terminal tuft. The apical dendrite did not reach layer IV. Primary dendrites arose along the somatic perimeter. Basolateral processes divided richly near the perikaryon, like those of the medium-sized pyramidal cells.

A myelinated axon arose under the soma. It had oblique, ascending local branches, and a collateral entered the white matter. The lateral axonal plexus was more elaborate than that of medium-sized pyramidal cells (Fig. 5), and it extended beyond the dendritic domain.

Fusiform pyramidal cells. These neurons (Fig. 6:3a,b) differed from pyramidal cells (Fig. 4), in having two primary dendrites, and they differed from bipolar cells (Fig. 12) because of their longer ascending and descending dendrites, their many dendritic spines, and a corticofugal axon. One subtype had a vertically elongated soma and was located chiefly in layer VIa (Fig. 6:3a). Other Golgi studies have classified them as spindle-shaped cells (Tömböl, 1984), because the soma was elongated vertically. They were smaller than their layer V counterparts (unpublished observations). Dendrites had tufts with two to four processes. A radial dendrite often was thicker and projected farther. The upper process reached layer IV, the lower one entered the white matter, some 800 μm distant. Primary dendrites divided sparsely, with short, lateral branches like those of small and star pyramidal cells, and they lacked the terminal tuft found on the large and medium-sized pyramidal neuron apical dendrites. The long axis of the dendritic field was at the center. Appendages resembled those of other pyramidal cells.

A myelinated axon arose from a primary, descending trunk and, like that of the medium-sized pyramidal neurons, formed ascending collaterals and corticofugal branches. Local segments spanned layers VIa and V.

Horizontal fusiform neurons (Fig. 6:3b) have been considered as nonpyramidal cells with a corticofugal axon (Tömböl, 1984). Those in AI were oriented horizontally but, otherwise, resembled fusiform vertical pyramidal cells. These neurons were rare and were confined to layer VIb or the white matter. Their two primary dendritic trunks formed an arbor 700–900 μm wide and only 100–125 μm tall. The processes were shorter, thicker, had more spines, and followed a more acute trajectory than the processes of typical pyramidal neurons.

A myelinated axon came from the soma or from a primary dendrite and reached the white matter. Some collaterals reentered the cortex after a short horizontal projection. These remained within layer VI.

Fig. 2. Cytoarchitecture and axonal profiles in layer VI. **A**: Neuronal somata in a Nissl preparation. Several types of neurons were present (the key to the numbered neurons appears in Table 1), including small (1a) and medium-sized (1b) pyramidal cells, inverted pyramidal cells (5) or Martinotti cells (6), large multipolar cells (7c), bipolar cells (8), and horizontal cells (9). Layer VIa cells were more clustered than those in layer VIb. Planapochromat, N.A. 1.32; ×2,000. **Insets**: Locus for the cytoarchitectonic observations (stippled areas). **B**: Neurons and axons in layer VI in a 1-μm-thick, plastic-embedded, toluidine blue-counterstained section. Many cell types resembling those in the Nissl material were discerned, including small (1a) and medium-sized (1b) pyramidal cells, inverted pyramidal cells (5) or Martinotti cells (6), large multipolar cells (7c), and, near the border with the white matter (wm), a horizontal cell (9). Radial arrays of myelinated axons of various calibers ascended between short columns of neuronal somata; secondary branches contributed to the horizontal plexus in layer VIb (above inset). Protocol as in A. Neurons, black areas; axons, thin black profiles; dendrites, open profiles; glia, open oval profiles; blood vessels, stippled areas. SF/daz, suprasylvian fringe/dorsal auditory zone.

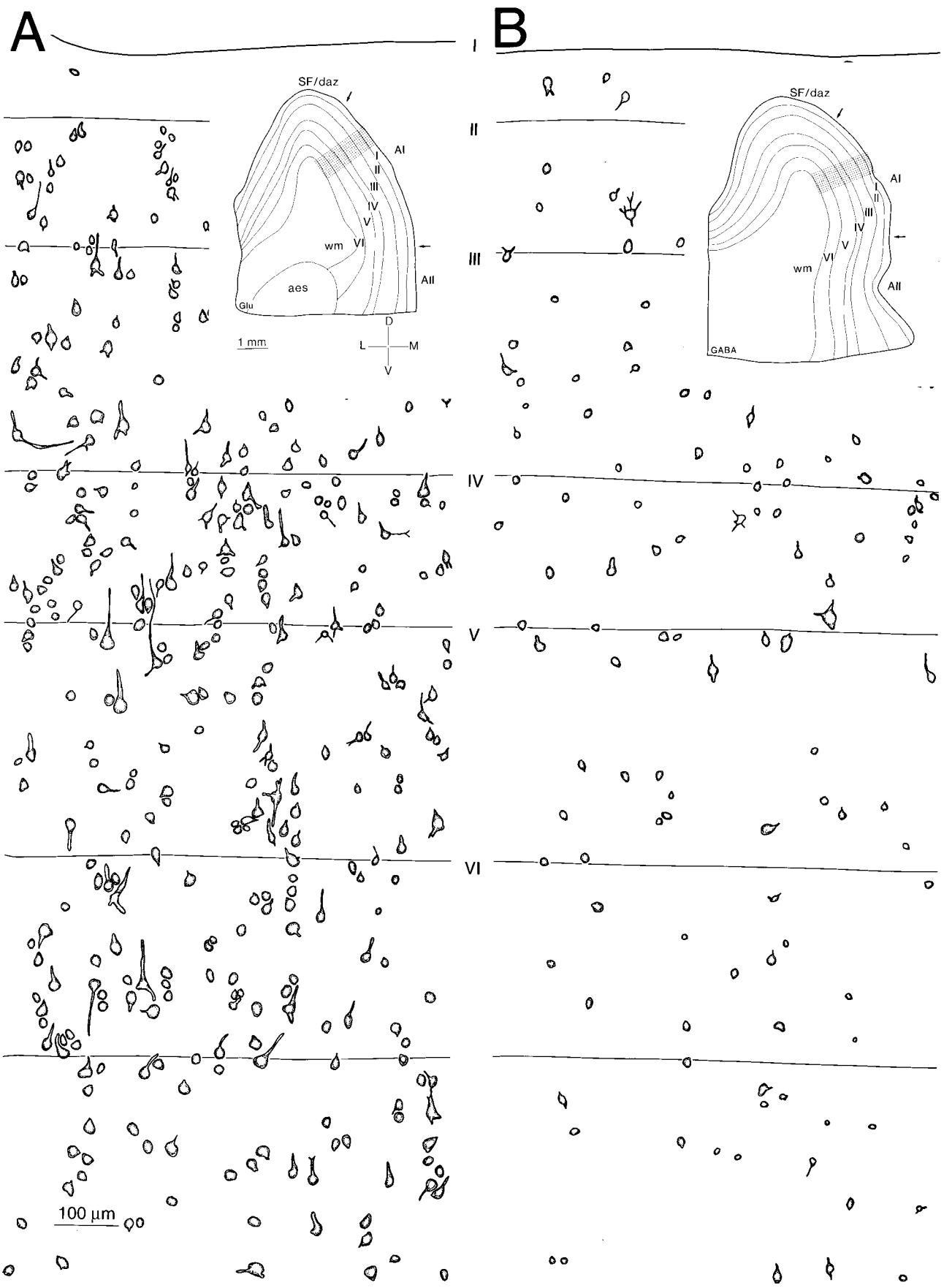

Fig. 3. Neurochemical organization of AI. **A**: Glutamatergic (Glu) neurons. Pyramidal cells were immunostained as well as some bipolar-shaped cells; few horizontal cells were seen. Some layer V cells were the largest in AI. **B**: γ-Aminobutyric acidergic (GABAergic) neurons. Layer VI had far fewer such neurons than layers II–IV. The range in size and shape suggests that several neuronal classes were immunostained (for details and methods, see Prieto et al., 1994a,b). Planapochromat, N.A. 0.65; ×500. **Insets**: Locus for the immunocytochemical observations (stippled area). For abbreviations, see list.

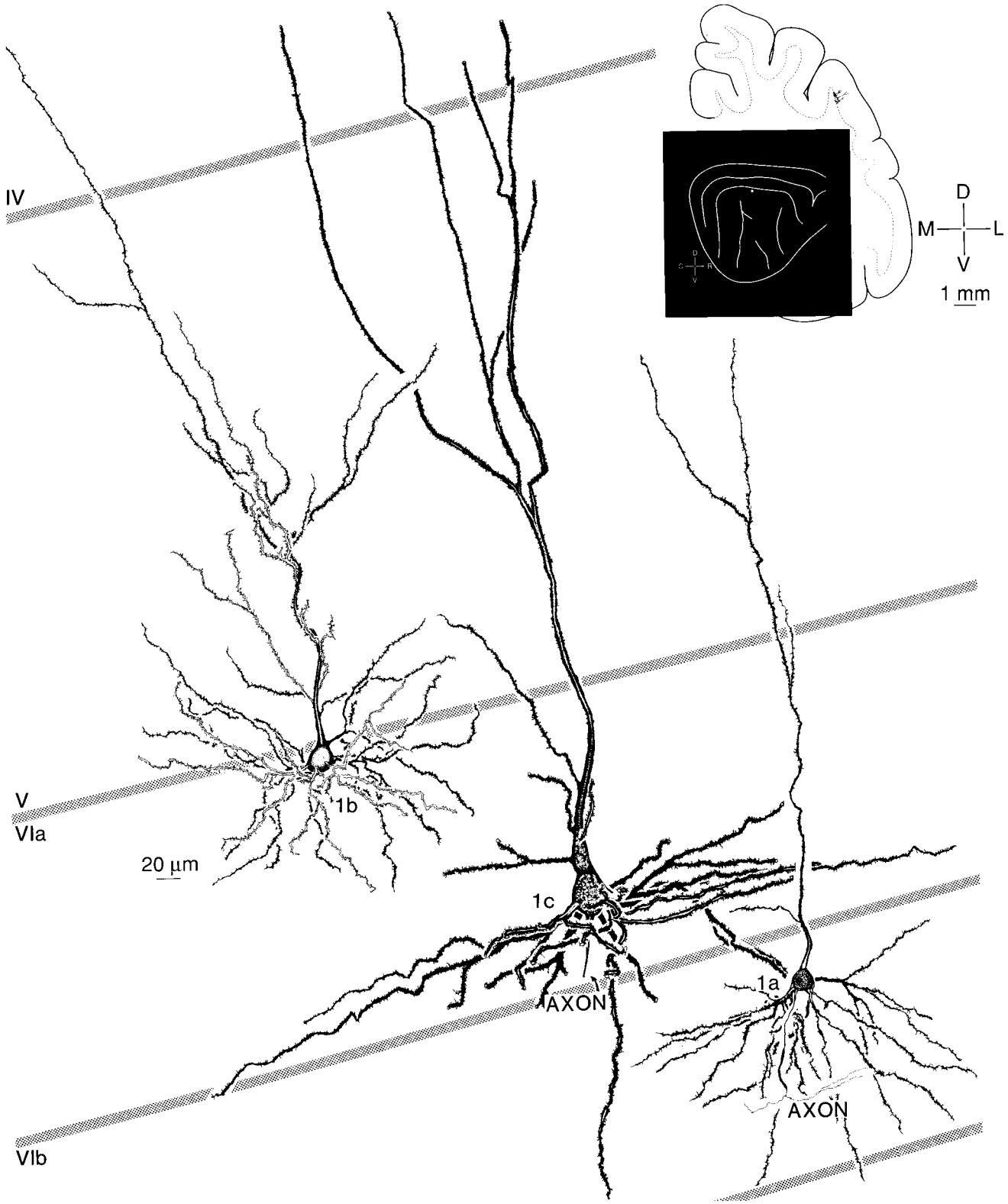

Fig. 4. Layer VI pyramidal cells were varied in size, dendritic architecture, laminar position, and in relative frequency. 1a: Small pyramidal cells had delicate basal dendrites that extended laterally and ventrally without appreciable overlap, in contrast to the processes of the medium-sized pyramidal cells (1b); both types had many dendritic appendages. The myelinated axon (AXON) had thin horizontal collaterals. 1b: Medium-sized pyramidal cells were the principal type in layer VI. Their basal dendrites had elaborate arbors and more complex apical processes than their layer II (Winer, 1985) and layer III (Winer, 1984b) counterparts. Dendritic appendages began after the first division and extended to their tips. 1c: Large pyramidal cells were smaller than the corresponding layer V neurons (see Fig. 1:V); only these cells (and those of some medium-sized layer VI cells; see Fig. 5A) had dendrites long enough to span a layer. These cells had long, lateral, basal dendrites that could equal or exceed the span of those of horizontal cells (see Fig. 13, Table 1); these processes had many spines and contributed to the sublaminar organization by filling strata 100–200 µm thick. Protocol for this figure as well as Figs. 5–13: Golgi-Cox method; 140-µm-thick section; planachromat, N.A. 0.95; ×663. **Insets**: Transverse views of the areal (see Fig. 1, top inset) and laminar (see Fig. 1, bottom inset) position of the neurons; lateral view (white on black) indicates the locus of the observation on the reconstructed hemisphere. An open profile or a short series of interrupted lines at the tip denotes incomplete processes.

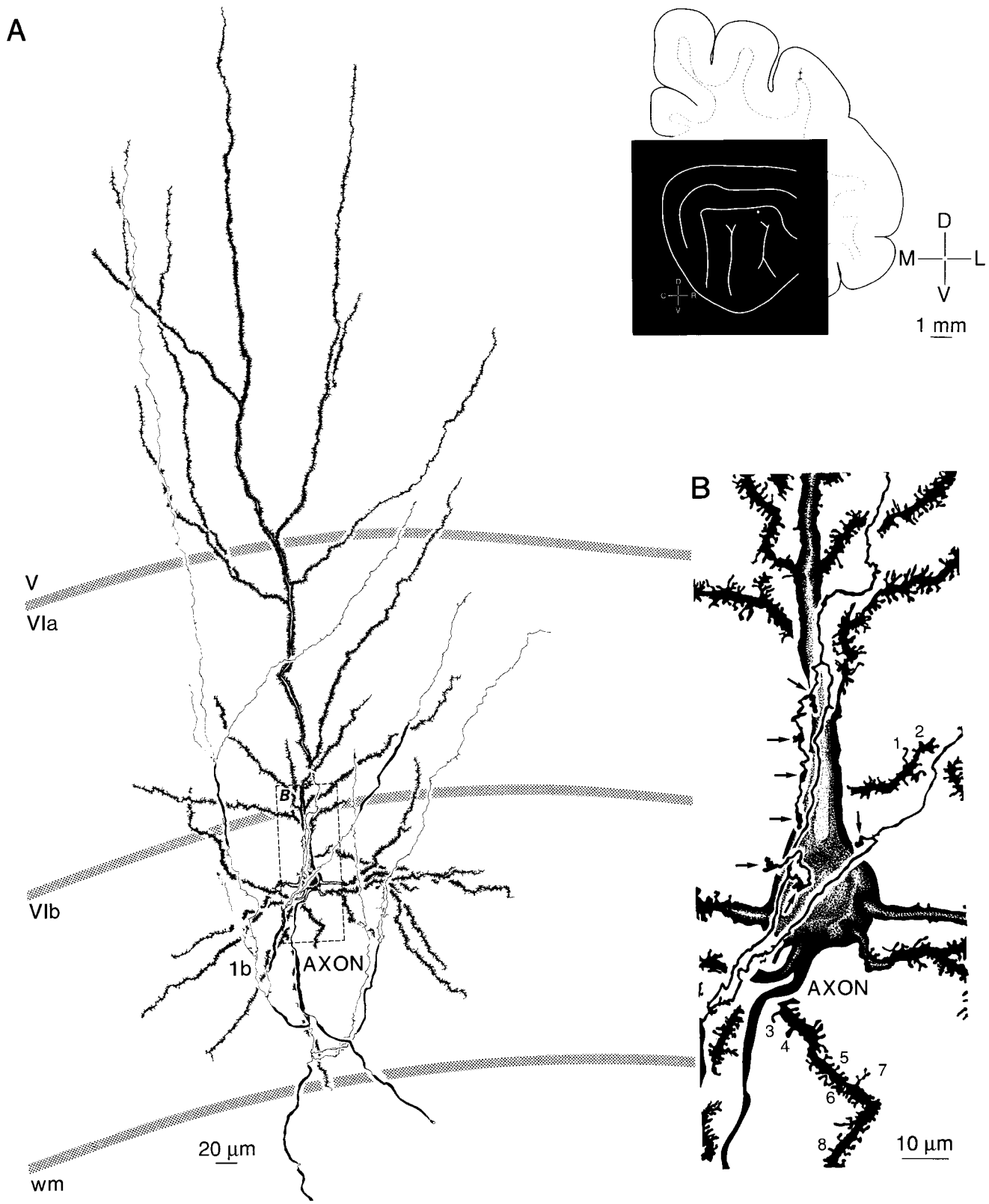

Fig. 5. Medium-sized pyramidal cell. **A**: The basal dendritic field was more spherical than that of the large pyramidal cell (see Fig. 4:1c). Recurrent axonal branches (AXON) overlapped much of the dendritic field and entered the white matter (wm). Dashed box indicates the area shown in B. **B**: Characteristic appendages (1–8) on proximal dendrites of each type of pyramidal cell (see text), and a recurrent axonal collateral system. The numbers 1—8 represent the varieties of dendritic appendages: 1) filiform, 2) hill-like swellings, 3) finger-like processes, 4) large and 5) small mushroom-shaped spikes, 6) convoluted spines, 7) elaborate excrescences with short extensions at the stem, and 8) sessile profiles. A local, unmyelinated axon ≈0.5 µm in diameter with recurrent perisomatic branches and peridendritic boutons en passant (arrows) was present. The collateral entered layer VIa (A) and made a few more boutons of uniform size. For the protocol and an explanation of the inset in this and Figures 6–13, see Figure 4.

Tangential pyramidal cells. These neurons (Fig. 8:4) were sufficiently numerous and distinctive to comprise a separate group. Their unusually long and well-developed apical dendritic system had lateral arbors almost as long as those of horizontal cells (Fig. 13). These dendrites had many spines with diverse shapes. Basal dendrites divided richly in layer VI. They were shorter than their lateral counterparts, only a little less spinous, and they ended after projecting a short distance toward the white matter. These branches were acute and overlapping, whereas the apical processes divided more simply.

The axon was 2–3 μm thick. It descended toward the white matter while emitting a few thin, perhaps unmyelinated, local branches.

Inverted pyramidal cells. These neurons (Figs. 7:5, 8:5/i,ii) resembled medium-sized pyramidal cells, except that their principal dendrite projected toward the white matter, whereas smaller dendritic tufts arose at the top of the soma. Their spinous dendrites distinguished them from those of Martinotti cells, which are smooth (Tömböl, 1984) and are considered here as a separate class (Table 2). Inverted pyramidal cells occurred in layer V (unpublished observations) and were even more common in layer VIa. A primary dendrite arose beneath the triangular soma and extended 400–600 μm, often reaching the white matter. The principal dendrite rarely had a terminal tuft as robust as that of classical pyramidal cells. Thinner, shorter lateral dendrites formed tufts near the soma, like other pyramidal cells. These processes branched once and were 50–200 μm long.

The axonal origin was unusual; it came from the main descending dendrite or from the soma and was myelinated after the initial segment (35–70 μm). The primary axon entered the white matter, and local collaterals ascended obliquely, descended, or ran laterally for 300–500 μm (Fig. 8:5/i).

Nonpyramidal cells

Nonpyramidal neurons had smooth dendrites of uniform caliber and an intrinsic axon. Four main classes were recognized, and all were GABAergic (Prieto et al., 1994a).

Martinotti cells. This class of neuron (Fig. 9:6/i,ii) had smooth dendrites and an inverted configuration (Tömböl, 1984), and they were classified as nonpyramidal cells (see above). They were found both in GAD-immunostained material (Prieto et al., 1994a) and in Golgi preparations, and they were confined to layers V (unpublished observations) and VIa. They resembled spinous inverted pyramidal cells, with a descending principal dendrite arising from the perikaryon, and other dendrites with polar origins. They differed in having shorter, thinner dendrites with smooth contours, a more delicate appearance, and a less regular branching pattern. The main dendrite was 300–400 μm long and sometimes reached the white matter. Superficial dendrites branched sparsely, with irregular swellings and constrictions (Fig. 9:6/i), or had a rough surface and few appendages (Fig. 9:6/ii).

Martinotti cells were also distinguished from spinous inverted pyramidal cells by an axon that ascended vertically, and which did not appear to enter the white matter. It arose from the soma or an upper primary dendrite, had several horizontal collaterals near the soma, and projected towards layer IIIa and beyond.

Multipolar cells. This class included neurons which shared a stellate dendritic configuration and smooth dendrites (Figs. 10, 11). The small, medium-sized, and large varieties differed in somatic size, dendritic form, and sublaminar distribution (Table 1). The small and medium-sized types, like the Martinotti cells, were identified readily in the glutamic acid decarboxylase (GAD) material (Prieto et al., 1994a). Their dendritic diversity suggested that this class may contain additional subtypes.

Small multipolar cells (Fig. 10:7a) had a spherical or oval dendritic field less than 400 μm in diameter. Their cardinal feature was a round soma, usually located in layer VIa, from which five to eight primary dendrites radiated in a stellate configuration. The dendritic arbor was intricate, because processes branched often and overlapped. Dendritic appendages were rare except for an occasional spine with a bulbous tip.

A thin, unmyelinated axon (not illustrated) projected locally and had fine terminal boutons near the dendrites. In contrast, neurogliaform cells had finer and more complex dendrites and an axon that was restricted to the dendritic field (Prieto et al., 1994a).

Medium-sized multipolar cells (Fig. 10:7b/i,ii) also were concentrated in layer VIa (see Prieto et al., 1994a). They differed in the shape of their dendritic field, with five to eight primary dendrites that had a stellate pattern and a spherical configuration (Fig. 10:7b/i). The arbor was 300–400 μm in diameter and was confined to layer VI. Dendrites branched sparsely, with some higher order segments and a rare undivided process; the few spines were clustered on the most distal dendrites.

The axon (not shown) arose from either the soma or a primary dendrite and divided to form a plexus of varicose branches. Some collaterals reached layer Vb.

Large multipolar cells (Fig. 11:7c/i,ii) had thick, radiating dendrites and a stellate dendritic field up to 800 μm wide; they were rarer in layer VI than in layers III–V (Prieto et al., 1994a). Their oval somata were found only in layer VIa and were 20–30 μm in diameter, larger than all but the largest AI pyramidal cells in layer V (unpublished observations). Some six to eight thick primary dendrites extended up to 700–800 μm; a few ventral dendrites reached the white matter, whereas the most dorsal dendrites entered layer IV. The lateral processes were shorter and sparser. The longest dendrites branched two or three times, whereas the lateral dendrites branched only once, and there were few spines. Dendrites were up to 10 μm thick and tapered to a fine tip. They had more branched and complex arbors than those of multipolar cells in other layers (Prieto et al., 1994a).

The axon arose at the somatic apex. It had a thick initial segment; more complete impregnations were uncommon, suggesting that the distal parts may be myelinated.

Bipolar cells. These neurons (Fig. 12:8/i,ii) were rarer in layer VI than in layers III–V (Prieto et al., 1994a). They were found only in layer VIa, near the layer V border, with fusiform (Fig. 12:8/i) or oval (Fig. 12:8/ii) somata 15–20 μm wide and 35–50 μm tall. Hallmarks were the two or three primary trunks that arose at the upper and lower somatic poles and which tapered to a terminal tuft of three or four branches. The dendritic field was sinuous, hour-glass shaped, and ≈500–600 μm tall, with apical processes reaching layer Vb and basal processes entering the white matter. Spines were sparse and irregular on the distal branches (Fig. 12:8/ii).

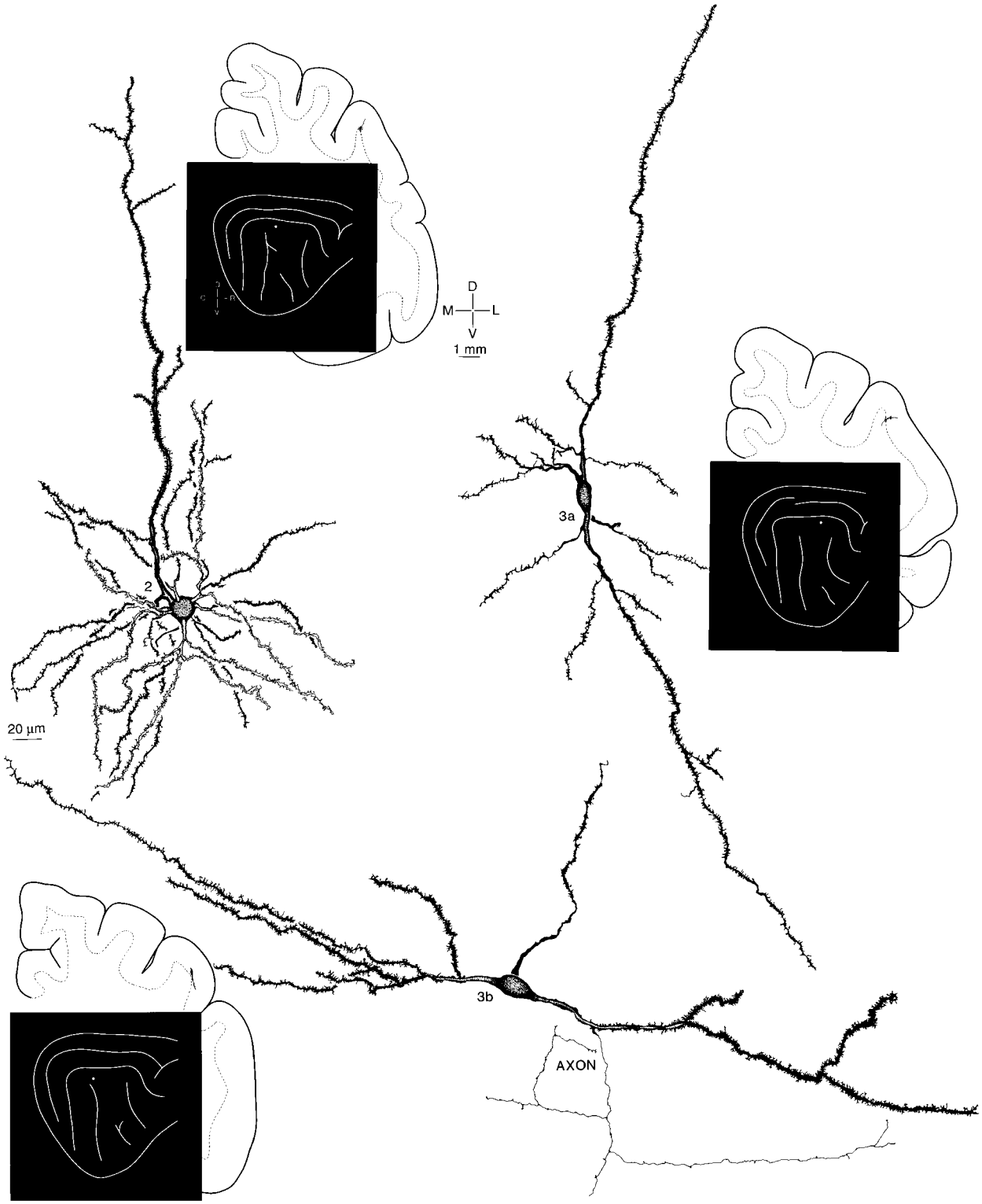

Fig. 6. Other types of pyramidal neuron. 2: Star pyramidal cells were more stellate than medium-sized pyramidal cells (see Fig. 4:1b; Fig. 5) except for the apical dendrite, which branched less than that of the medium-sized pyramidal cell. The robust dendritic branching and the thick processes aligned it with medium-sized pyramidal cells. 3: Fusiform pyramidal cells had a long apical dendrite and characteristic appendages. 3a: Vertical fusiform pyramidal cells had long apical and basal and short lateral processes (see Table 1). The few proximal dendrites had many appendages, especially the distal and basal processes. The shorter lateral dendrites were as thin as those of the small pyramidal neuron (see Fig. 4:1a). 3b: The fusiform horizontal pyramidal cell had spinous lateral dendrites and a few thinner processes at right angles to these; other horizontal cells (see Fig. 13:9a,b) were virtually smooth. The AXON ran parallel to the dendrites, with sparse local branches and only a few tiny boutons en passant.

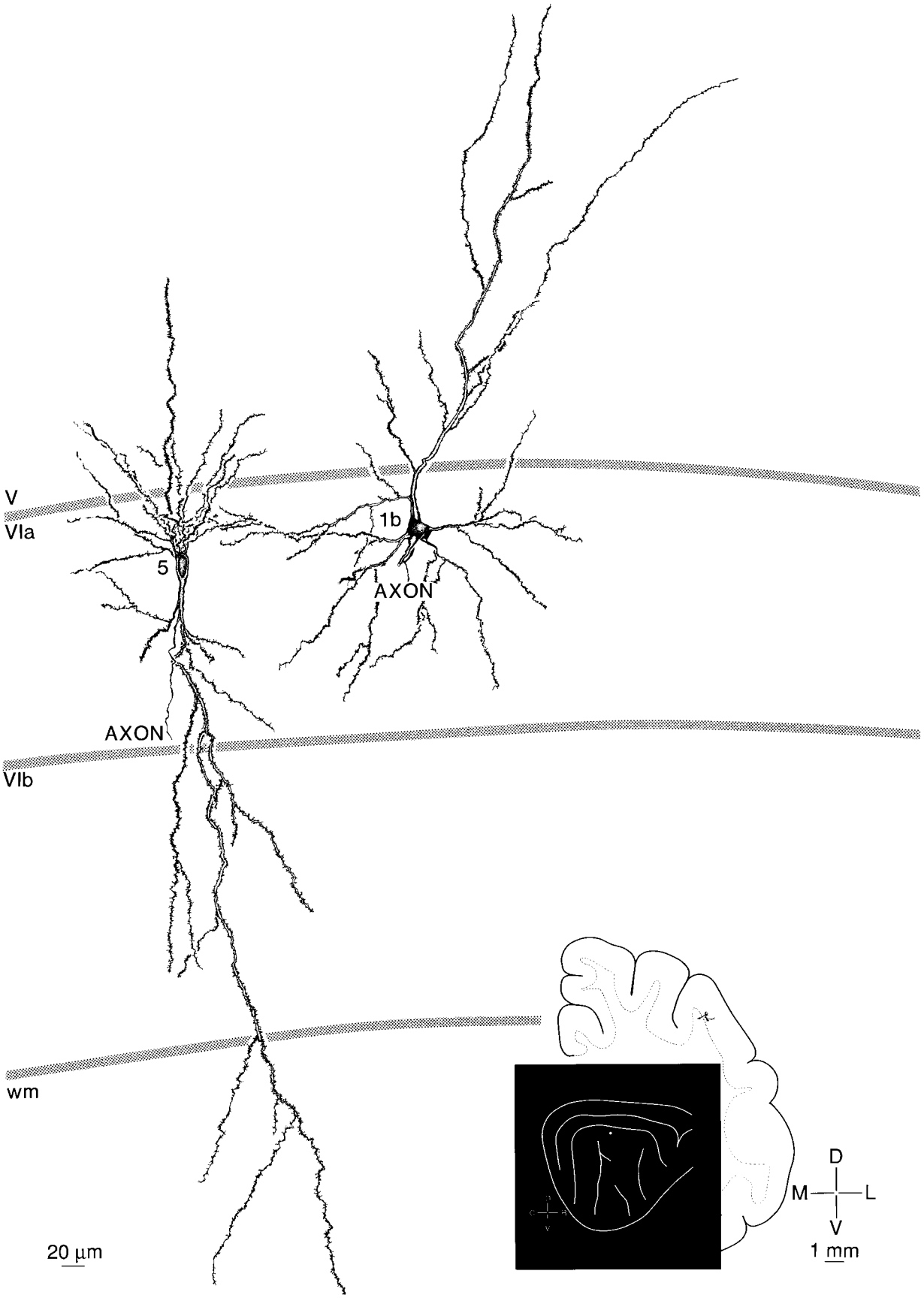

Fig. 7. Spinous inverted pyramidal cell (5) near a medium-sized pyramidal cell (1b). The ventral (apical) dendrite branched often and extended farther than that of the medium-sized neuron, and they were equally spinous. Descending branches had more appendages than the intermediate segments in layer VIb, and they entered the white matter (wm).

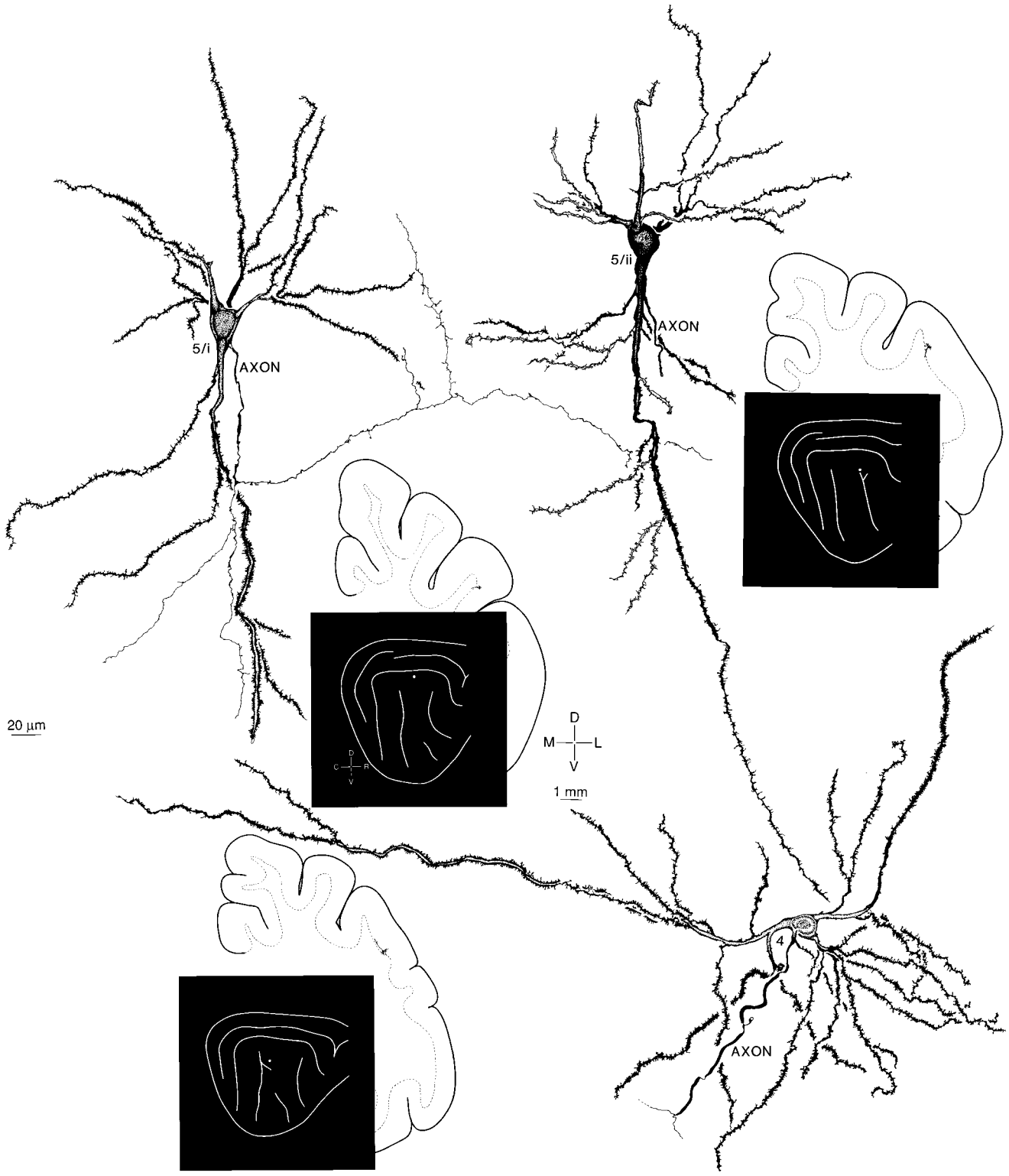

Fig. 8. Inverted pyramidal cells (5/i, 5/ii) and a tangential pyramidal neuron (4). 5: The AXON (5/i) had recurrent and remote branches. Local processes paralleled the long dendritic axis with short, thin side branches; longer processes had few such spurs. Vertical axonal branches ramified among apical dendrites (see Table 1:1a–c,2,3a,5,8). 4: Tangential pyramidal cells were unusual because of their dendritic asymmetry and otherwise resembled medium-sized pyramidal cells (see Figs. 4, 5, 7:1b). The dorsolateral dendrites were unusually thick (top right).

The axon (not shown) originated from an apical dendrite and projected vertically while branching in layers VIa and Vb. It usually was not impregnated.

Horizontal cells. These neurons were confined to layer VIb (Fig. 13), like horizontal fusiform pyramidal cells and tangential pyramidal cells (Table 1). Bitufted or bipolar subtypes occurred. The former had an oval soma (Fig. 13:9a) 25–35 μm wide and 15–20 μm tall. Some four to six thick primary dendrites arose laterally, branched nearby, then projected farther or divided. Dendrites in the plane of section extended up to 800 μm and flared distally; near their origins, they were more constricted. They had a smooth surface with few spines.

A myelinated axon arose from a primary dendrite. It was 2–3 μm thick and projected laterally within layer VI, up to 400 μm away.

Some horizontal cells (Fig. 13:9b) had fusiform or oval somata and a more bipolar shape. Their thick main dendrites arose as two or three isolated trunks that formed a tuft of distal branches; a finer side branch either ascended or descended. The dendritic field was about half as wide (≈400 μm) as that of the horizontal bitufted cells. Higher order dendrites also were sparser, smoother, and straighter than those of bitufted neurons.

The axon arose from the soma and ascended. Usually, only the initial segment was impregnated. The axon contributed preterminal plexuses above the cell and projected laterally, outside the dendritic domain.

Sublaminar distribution of projection neurons

The retrograde tract-tracing experiments showed that layer VI cells project to contralateral AI, ipsilateral AII, and the ventral division of the medial geniculate body. Their somatic and dendritic filling with granular WGA-HRP reaction product allowed their somatodendritic profiles to be classified and related provisionally to the neuronal types defined in the Golgi material (see Code and Winer, 1985).

Commissural neurons. Commissural neurons labeled by AI injections were concentrated in layer VIa (87.6%), with far fewer in layer VIb (12.4%; Fig. 14A); Student's t test showed that this was a statistically significant difference ($P < 0.005$). These neurons were diverse in size and shape. All pyramidal cell classes were labeled except the horizontal fusiform pyramidal cells. Inverted (Fig. 15A:5) or fusiform vertical pyramidal cells (Fig. 15A:3a) were numerous, whereas small (Fig. 15A:1a), medium-sized (Fig. 15A:1b), and large (Fig. 15A:1c) pyramidal cells were labeled less often. Star pyramidal cells (Fig. 15A:2) and tangential pyramidal cells (Fig. 15A:4) were sparse.

Corticocortical cells. In contrast to the commissural neurons, corticocortical projection cells were concentrated in layer VIb. Only 30.1% of the HRP-filled neurons were found in layer VIa, with 69.9% in layer VIb (Fig. 14B; $P < 0.005$). However, the sublaminar distribution of layer VI corticocortical neurons was area-specific. Neurons projecting to ipsilateral AII were concentrated in layer VIb, and the few layer VI neurons projecting to the ipsilateral anterior auditory field, the posterior auditory field, or the temporal cortex were found more often in layer VIa (data not shown). Layer VI ipsilateral corticocortical neurons had diverse structures, like the commissural cells. Each type of pyramidal cell, with the exception of the star pyramidal neurons, was labeled by AII injections, including inverted (Fig. 15B:5) and vertical fusiform (Fig. 15B:3a) pyramidal cells. Classical pyramidal cells were rarer, although small (Fig. 15B:1a), medium-sized (Fig. 15B:1b), and large (Fig. 15B:1c) examples occurred. Fusiform horizontal pyramidal cells were labeled by the AII injection (Fig. 15B:3b), but they did not project to the contralateral hemisphere or the thalamus. The cell types labeled by injections in other ipsilateral auditory cortical areas (not illustrated) were similar to those projecting to AII.

Corticothalamic cells. Corticothalamic cells were distributed evenly within layer VI, with 52.8% in layer VIa and 47.2% in VIb (Fig. 14C). The neurons labeled after an injection in the ventral division of the medial geniculate body differed strikingly from the cases described above: nearly all were classical pyramidal cells, of which the medium-sized cells were dominant (Fig. 15C:1b). Among the modified layer VI pyramidal cells, only a few examples of vertical fusiform pyramidal cells were labeled (Fig. 15C:3a).

DISCUSSION

We address five interrelated themes pertinent to layer VI organization. The first issue is the validity of subdividing layer VI into upper and lower halves and some prospective implications. The nature of the parallels and differences between layer VI and other laminae is then considered. A third topic is possible physiological correlates of layer VI organization. Then, we compare and contrast the roles of layer VI pyramidal and nonpyramidal neurons in cortical processing. We close with a brief, speculative treatment of layer VI function.

Sublaminar organization

Each layer in AI can be divided into sublaminae on the basis of local differences in neuronal form, neuropil organization, and extrinsic connectivity. In layer VI, the upper half is dominated by pyramidal cells, whereas horizontal cells are more prevalent in the lower half. In the visual cortex, where many of the same types of neurons (for summaries, see Table 2, and Fig. 16) occur, the analogous bilaminar organization has been recognized. This has been confirmed both in cytoarchitectonic preparations (Lund et al., 1979) and by connectional results, in which neurons in the upper and lower halves of layer VI project to different thalamic nuclei (Fitzpatrick et al., 1994 [macaque monkey]; McCourt et al., 1986; Conley and Raczkowski, 1990 [prosimian primate]). Thus, the distinction between the pyramidal cell-rich layer VIa and the horizontal cell-dominated layer VIb is equally clear in AI on both cytoarchitectonic and connectional grounds.

We found commissural AI cells of origin concentrated in layer VIa, in agreement with prior results (Kelly and Wong, 1981), whereas corticocortical neurons projecting to ipsilateral AII were found mainly in layer VIb. However, neurons projecting to other ipsilateral areas were more numerous in layer VIa (Rouiller et al., 1991). Our findings suggest that the sublaminar segregation of cells of origin in the commissural and corticocortical systems, although it is not absolute, is significant. In contrast, large injections of retrograde tracer limited to the medial geniculate body that include more than one division, label neurons throughout the depth of layer VI, with no apparent sublaminar preference. Whether smaller or more focal injections

TABLE 2. Layer VI Neurons in Auditory Cortex and in Other Areas and Species

		Concordance with of Golgi studies or with intracellularly injected cortical neurons				
Neuronal type	Subtypes	Study	Species	Area[1]	Denomination	Figure(s)
1. Pyramidal cell	a. Small	Tunturi (1971)	Dog	MES, PES	Small pyramidal	Table 1
		Tömböl (1984)	Cat	—	Small pyramidal	4a; 10c; 27:1
		Meyer (1987)	Human	Motor	Small pyramidal	6:5
		Fitzpatrick and Henson (1994)	Bat	Auditory	Multiform	2S
					True pyramid	26B
	b. Medium sized	Ramón y Cajal (1900)	Human	Auditory	Triangular	14A
					Pyramid	14B
					Ovoid pyramid	14F
		Globus and Scheibel (1967)	Rabbit	—	Class I-pyramid	1:7
		Tunturi (1971)	Dog	MES, PES	Medium pyramidal	Table 1
		Jones (1975)	Monkey	Somatosensory	Type 8	29
		Feldman and Peters (1978)	Rat	17	Pyramidal	12P
		Tömböl (1984)	Cat	—	Medium-sized pyramidal	4b; 10a; 27:2; 28:2
		Mitani et al. (1985)	Cat	AI	Pyramid like	14a, b
		Valverde et al. (1989)	Rat	Somatosensory	Pyramidal	11a
	c. Large	Tömböl (1984)	Cat	—	Triangular pyramidal	29:1
		Ferrer et al. (1986b)	Cat	LG, MSSG	Pyramidal	7B:P
2. Star pyramidal cell	—	Ferrer et al. (1986a)	Mouse	Sensorimotor	Multiapical pyramidal	6 (third from right)
		Miller (1988)	Rat	Visual	Atypically oriented	6Q, R
3. Fusiform pyramidal cell	a. Vertical	Ramón y Cajal (1900)	Cat	Auditory	Fusiform	18D, E
		Tunturi (1971)	Dog	MES, PES	Fusiform	Table 1
		Feldman and Peters (1978)	Rat	17	Sparsely spinous nonpyramidal	12 (*)
		McMullen and Glaser (1982)	Rabbit	Auditory	Fusiform	14B (right)
		Tömböl (1984)	Cat	—	Vertically arranged pyramidal	28:1
		Mitani et al. (1985)	Cat	AI	Bipolar	14C, D
					Fusiform	15B
	b. Horizontal	Feldman and Peters (1978)	Rat	17	Spinous multipolar	7e
		Tömböl (1984)	Cat	—	Fusiform	9, 27:6
		Ferrer et al. (1986b)	Cat	LG, MSSG	Fusiform	7B:F
			Sheep	Parietal, frontal	Fusiform	9F
			Human	Temporal, frontal	Fusiform	10B:F
4. Tangential pyramidal cell	—	Tömböl (1984)	Cat	—	Tangential pyramidal	7a–c; 8a, 20c; 27:5; 29:2
		Ferrer et al. (1986b)	Cat	LG, MSSG	Horizontal pyramid	7B (*)
		Valverde et al. (1989)	Rat	Somatosensory	Inverted pyramidal	12 h, 14B:a
5. Inverted pyramidal cell	—	Ramón y Cajal (1899)	Human	Motor	Triangular	25B
		Ramón y Cajal (1900)	Cat	Auditory	Cell devoid of radial shafts	18A, G
		Globus and Scheibel (1967)	Rabbit	—	Class I-inverted pyramid	2:2, 4, 6, 7, 9
		Tunturi (1971)	Dog	MES, PES	Special auditory	Table 1
		McMullen and Glaser (1982)	Rabbit	Auditory	Inverted pyramidal	15
		Mitani et al. (1985)	Cat	AI	Inverted pyramidal	14F
					Stellate	14E
		Ferrer et al. (1986a)	Rat	Sensorimotor	Inverted pyramidal	11P, 51P
		Miller (1988)	Rat	Visual	Atypically oriented	4J, 7M, 8A, 10W
		Mrzljak et al. (1988)	Human	Prefrontal	Inverted pyramidal	19C
		Valverde et al. (1989)	Rat	Somatosensory	Inverted pyramidal	11b, d; 12a, c–g; 14A:a, b; 14B:b
6. Martinotti cell	—	Ramón y Cajal (1900)	Cat	Auditory	Upside-down pyramidal	18F
		Tömböl (1984)	Cat	—	Martinotti	11a, 28:3
					Tangential pyramid	20c
		Ferrer et al. (1986a)	Rat	Sensorimotor	Martinotti	4A, 5M
7. Multipolar cell	a. Small	Tunturi (1971)	Dog	MES, PES	Star	Table 1
		Peters and Regidor (1981)	Cat	17	Sparsely-spinous multipolar	12U
		Tömböl (1984)	Cat	—	Small round	15a, b; 20d; 29:6
		Ferrer et al. (1986a)	Bat	Sensorimotor	Multipolar	10:7
		Ferrer et al. (1986b)	Human	Temporal, frontal	Local circuit neuron	10B:LC
	b. Medium sized	Globus and Scheibel (1967)	Rabbit	—	Class II-stellate	5:11
		Tunturi (1971)	Dog	MES, PES	Star	Table 1
		Feldman and Peters (1978)	Rat	17	Sparsely spinous multipolar	10g
		Peters and Proskauer (1980)	Rat	Visual	Multipolar	1s, t, v
		Tömböl (1984)	Cat	—	Medium-sized local circuit	10d
					Round	14d, 23b
					Ovoid small	23d
					Local circuit	23a
					Tangential pyramid	29:5
		Ferrer et al. (1986b)	Cat	LG, MSSG	Local circuit	7B:LC
		Fitzpatrick and Henson (1994)	Bat	Auditory	Multipolar	5O, P
	c. Large	Ramón y Cajal (1900)	Human	Auditory	Giant	14E
		Peters and Regidor (1981)	Cat	17	Basket	12T
		McMullen and Glaser (1982)	Rabbit	Auditory	Spine-free multipolar	14B (left)
		Tömböl (1984)	Cat	—	Large basket	12, 28
		Ferrer et al. (1986a)	Rat	Sensorimotor	Multipolar	4A, 5M
		Ferrer et al. (1986b)	Dog	LG, MSSG	Basket	5E
		Fitzpatrick and Henson (1994)	Bat	Auditory	Multipolar	5Q
8. Bipolar cell	—	Meyer (1983)	Cat	17, 18, 19	Bitufted	18b
		Tömböl (1984)	Cat	—	Vertically arranged ovoid	17
		Ferrer et al. (1986b)	Cat	LG, MSSG	Bipolar	7A:B
			Sheep	Parietal, frontal	Bipolar	8B
9. Horizontal cell	a. Bitufted	Tömböl (1984)	Cat	—	Medium-sized ovoid multipolar	6c
					Medium-sized ovoid bipolar	22, 28:7
					Ovoid bipolar bitufted	16a
					Large fusiform local circuit	18
		Rabbit	Sensorimotor	Horizontal	8H	
		Ferrer et al. (1986b)	Cat	LG, MSSG	Bipolar	7B:B
	b. Bipolar	Globus and Scheibel (1967)	Rabbit	—	Class II-stellate	5:10
		Peters and Regidor (1981)	Cat	17	Horizontal bipolar	12V
		Tömböl (1984)	Cat	—	Small ovoid bipolar	14a, 20a, b: 29:3
					Ovoid bipolar bitufted	16b

[1]AI, auditory cortex; LG, lateral gyrus; MES, middle ectosylvian sulcus; MSSG, middle suprasylvian gyrus; PES, posterior ectosylvian sulcus; —, area not specified.
*Cells referred to in present comparison.

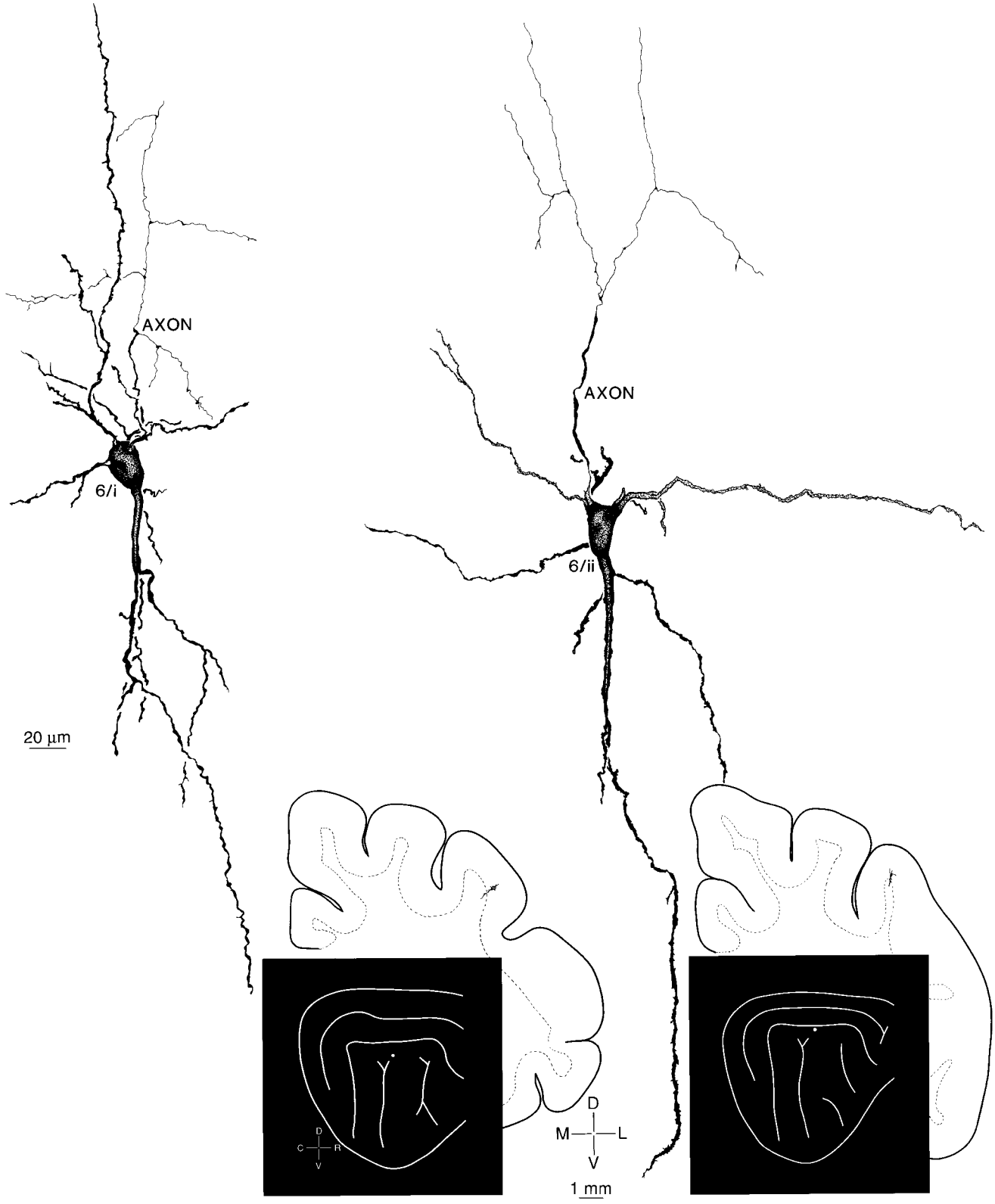

Fig. 9. Martinotti cells (6/i, 6/ii) had smoother, less branched, and simpler dendrites than inverted pyramidal cells (see Fig. 8:5/i,5/ii). The quality of axonal impregnation was comparable to that of pyramidal (see Fig. 5) and inverted pyramidal (see Fig. 8:5i) cells. The thin axon became myelinated in the first 60–100 μm; vertical and lateral branches had tiny boutons at approximately 50-μm intervals.

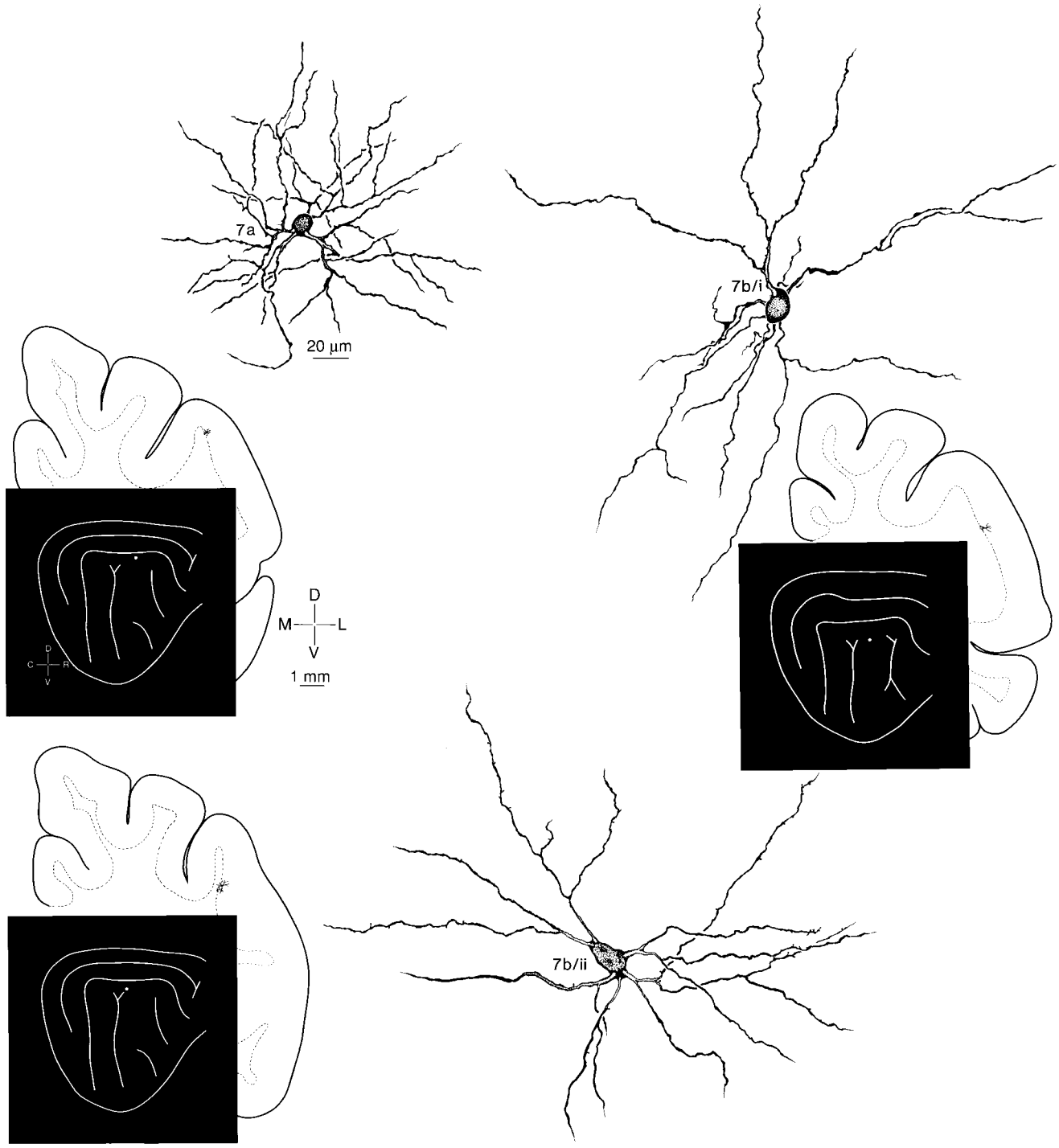

Fig. 10. Small and medium-sized multipolar cells were the major nonpyramidal cells in layer VI. They had spherical dendritic fields and simple, smooth processes. 7a: Small multipolar cell with a limited dendritic domain and sparse appendages (beneath 7a); the arbors filled the dendritic field incompletely. 7b/i: The medium-sized subtype had longer, less branched primary dendrites and an irregularly shaped dendritic domain (7b/ii); their intermediate dendrites were smooth.

into the medial geniculate body would produce more discrete or sublaminar-specific patterns in AI remains unknown. There are reasons to believe that this must be the case, because there is a sublaminar segregation of layer VI cells projecting to different thalamic nuclei from the visual cortex in many species (Bourassa and Deschênes, 1995 [rat]; Conley and Raczkowski, 1990 [prosimian primate]) and from somatosensory cortex (Bourassa et al., 1995; Lévesque et al., 1996; Zhang and Deschênes, 1997 [all in rat]). The laminar origin of commissural, corticocor-

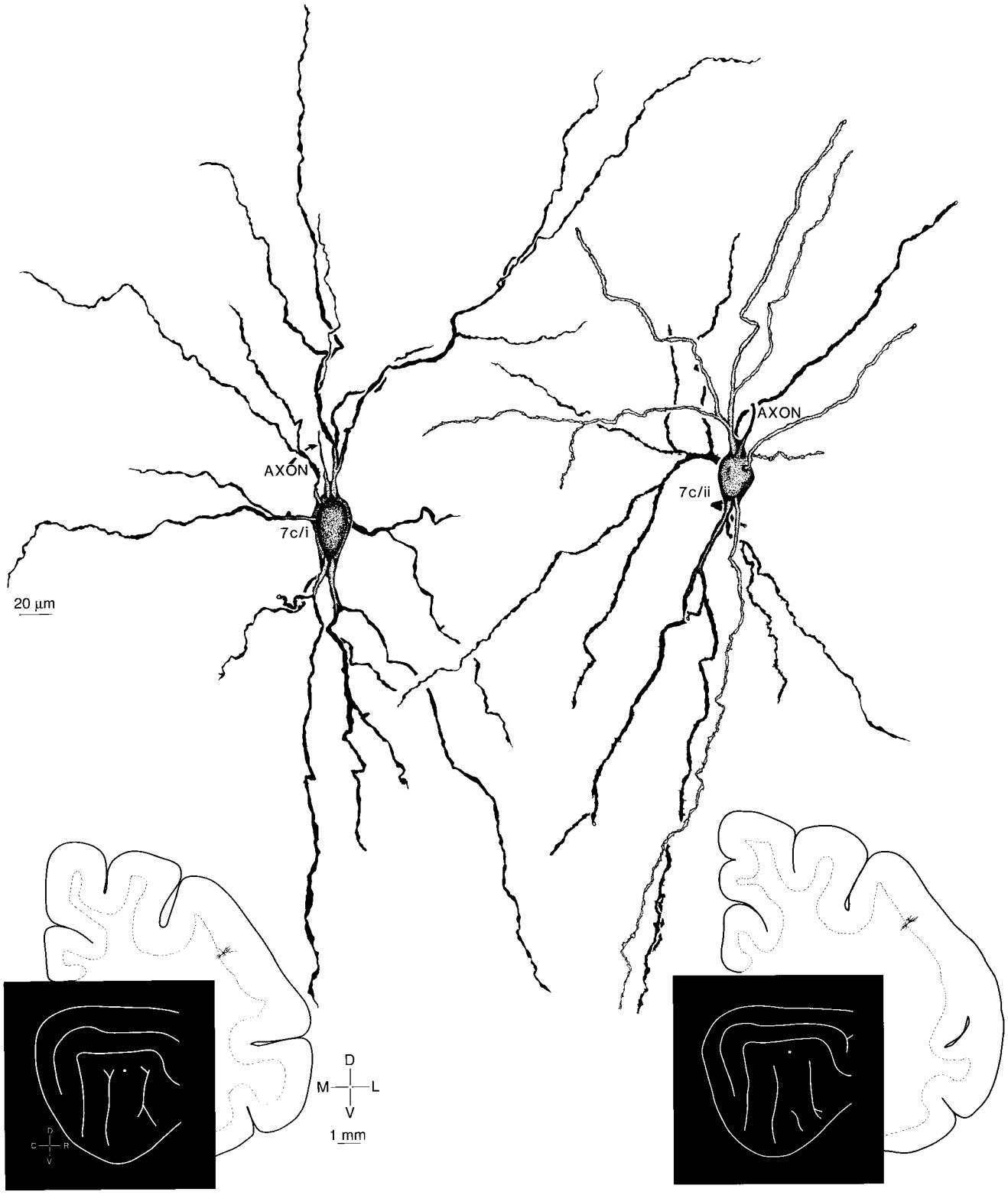

Fig. 11. Large multipolar cell dendritic fields extended into layer V (see Table 1:7c). They branched like the medium-sized multipolar cell (see Fig. 10:7b) but had a more vertical orientation (7c/i), with tufts arising at the apex and base of the soma (7c/ii). Dendrites had a few spines and an irregular surface; they filled a large volume incompletely.

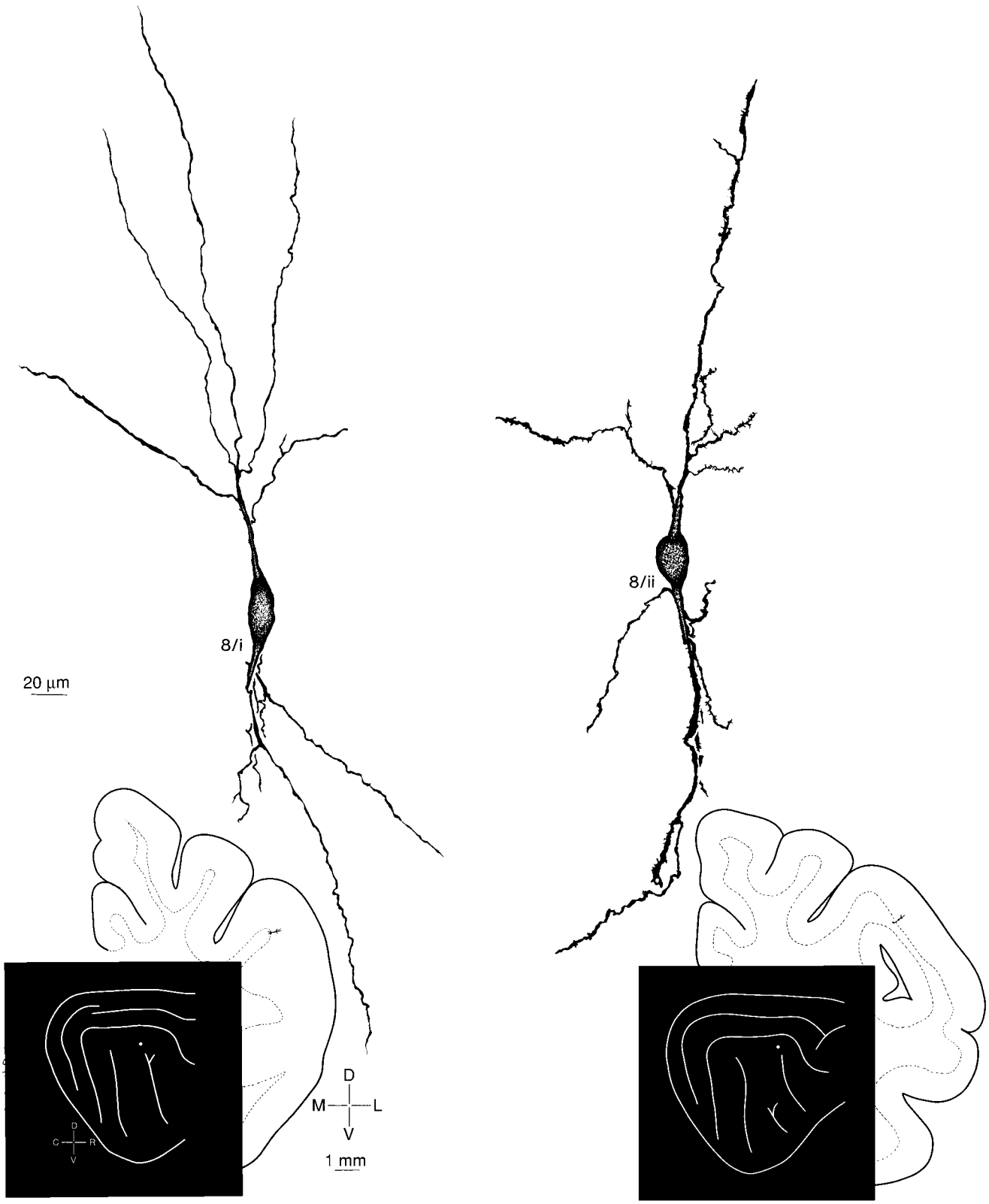

Fig. 12. Bipolar neurons were the vertical counterpart of horizontal cells (see Fig. 13). These two subtypes were smooth (8/i) and moderately spinous (8/ii). The dendritic domain was cylindrical; they had the fewest dendrites in layer VI (see Figs. 6:3a,b; 9:6/i,6/ii; 13:9b).

tical, and corticothalamic layer VI cells in cat striate cortex is like that described here (McCourt et al., 1986), notwithstanding species differences in the sublaminar location of layer VI cells projecting intracortically (Divac et al., 1987 [rat, marmoset, and hedgehog]) and their laminar targets (Burkhalter, 1989 [rat]).

Comparison with other layers

Layer VI (present results) and layer I (Marín-Padilla, 1984) are the only neocortical laminae with horizontal cells (Cobas et al., 1987 [mouse]). Many species and cortical fields have such neurons in layer VI (Table 2:9). Parallels in the organization of layers I and VIb have been pointed out before, and they may reflect the fact that these layers originate from the primordial plexiform neuropil. In contrast, layers II–VIa are derivatives of the cortical plate (Marín-Padilla and Marín-Padilla, 1982; Marín-Padilla, 1983 [human], 1992, 1998 [human]).

A further common denominator in these layers is input from nonprimary thalamic sensory nuclei (Killackey and Ebner, 1972 [opossum and hedgehog]; see also Ojima et al., 1992, citing unpublished observations by Hashikawa and Ojima). The importance of this input for the lateral organization of layers VI and I is that they receive a much weaker projection than layer IV, and the afferents have a more pronounced horizontal distribution (Mitani et al., 1984; Huang and Winer, 1997). In contrast, the input from the thalamus to layer IV is focal, clustered, and vertically oriented in the visual (Ferster and LeVay, 1978), somatic sensory (Landry and Deschênes, 1981), and auditory (Hashikawa et al., 1995 [monkey]; Huang and Winer, 1997) cortex. If the layer VI input terminates on horizontal cell lateral dendrites, then their receptive fields might differ fundamentally from those of pyramidal or bipolar cells in layer VI, neurons whose dendritic domain is much more vertical. Layer VI horizontal neurons might thereby integrate input within or across broad domains in AI (Schreiner, 1995). Perhaps horizontally oriented dendritic fields receive binaural input that is limited to one functional band, whereas cells with dendritic domains that extend rostrocaudally may cross several isofrequency contours. Neurons in the first case could act as interaural disparity detectors, like cells in the lateral superior olive, whereas the second type might subserve combination sensitivity or integrate signals across frequency modulated sweeps (Swarbrick and Whitfield, 1972; see also Evans, 1992).

Only in layers I and VI in AI is the sublaminar segregation of two kinds of neuron as pronounced as that of the many vertically oriented layer VIa cells and the concentration of horizontally arranged layer VIb neurons. The case for such a distinction in layer I is less compelling, because the neuronal density is so low that any vertical, columnar, or lateral arrangement is obscure (Winer and Larue, 1989 [rat]). In layers II–V, the various neuronal populations usually are intermingled, with only rare exceptions, such as the extraverted multipolar cells concentrated in layer IIa (Winer, 1985). The connectional and cytological partition of layer VI, thus, differs from other layers, suggesting that its sublayers may process different types of information, despite the fact that both halves contribute to the corticothalamic system.

Physiological parallels with other cortical areas

A conspicuous omission in the substantial literature on auditory cortex neurophysiology (for summary, see Clarey et al., 1992) is the virtual absence of data on layer VI neurons. A study combining intracellular recording and filling in AI revealed that a layer VI pyramidal cell showed short latency antidromic activation both from the ipsilateral medial geniculate body and from cortical area AII, which suggests a branched axon. Some stellate and bipolar cells (which we would consider as inverted and vertical fusiform pyramidal cells, respectively) responded to medial geniculate stimulation with excitatory postsynaptic potentials and a latency <1.4 millisecond; this suggests either monosynaptic activation or a very rapid polysynaptic pathway (Mitani et al., 1985). Because the data available for layer VI in auditory cortex are limited, we will consider the results from other fields to establish a framework for thinking about possible parallels in AI.

In primary visual cortex, far more is known about the specific inter- and intralaminar projections of single neurons than in AI. A combined electrophysiological and anatomical study proposed the following stages of information processing in the cortex: thalamocortical input ends mainly in layer IV, with much weaker terminations in the upper half of layer VI. Information then flows from layer IV to the supragranular layers, which, in turn, project to layer V and, finally, to layer VI (Gilbert and Wiesel, 1979). This view of cortical processing resembles that proposed for the cat AI (Mitani et al., 1985).

Corticothalamic cells in the rodent primary somatosensory cortex reside mainly in layer VI and have intracortical collaterals that synapse onto nonspiny multipolar cells and onto pyramidal cell dendritic spines (White and Keller, 1987 [mouse]). Such pyramidal cells may have a significant intracortical role, providing both feed-forward excitation and, through GABAergic interneurons, transsynaptic inhibition. The main intracortical target for layer VI cells is layer IV in visual cortex (McGuire et al., 1984 [cat]; see also Yoshioka et al., 1994 [macaque monkey]), and this is thought to provide either feed-forward facilitation or (less often) inhibition to cells in layers II–IV (Grieve and Sillito, 1991, 1995). Layer VI also projects to layer V, possibly providing corticotectal neurons with layer VI influence (Kenan-Vaknin et al., 1992 [rat]). In some species, the layer VI–IV projection preserves the ON and OFF channels from each eye through parallel systems (Fitzpatrick, 1996 [tree shrew]). Although an apparently corresponding projection has been described in AI (Ojima et al., 1992), its role is obscure. Perhaps the lateral dendrites of horizontal cells and the basal dendrites of pyramidal cells in layer VI receive input from broadly tuned neurons in the medial division of the medial geniculate body (Aitkin, 1973). This could propagate nontopographic information widely for cross-modal integration or interareal analysis. In awake, unanesthetized cats, the tuning or bandwidth of single AI neurons in layers III–IV is broader (Evans and Whitfield, 1964; Evans et al., 1965) than in barbiturate-anesthetized preparations (Merzenich et al., 1975), suggesting that the input(s) that broadens tuning in conscious animals is suppressed by barbiturates. These observations can be used to predict that 1) layer VIb horizontal cells may be tuned broadly; 2) such broad-band influences might converge on layer III–IV cells to represent a parallel

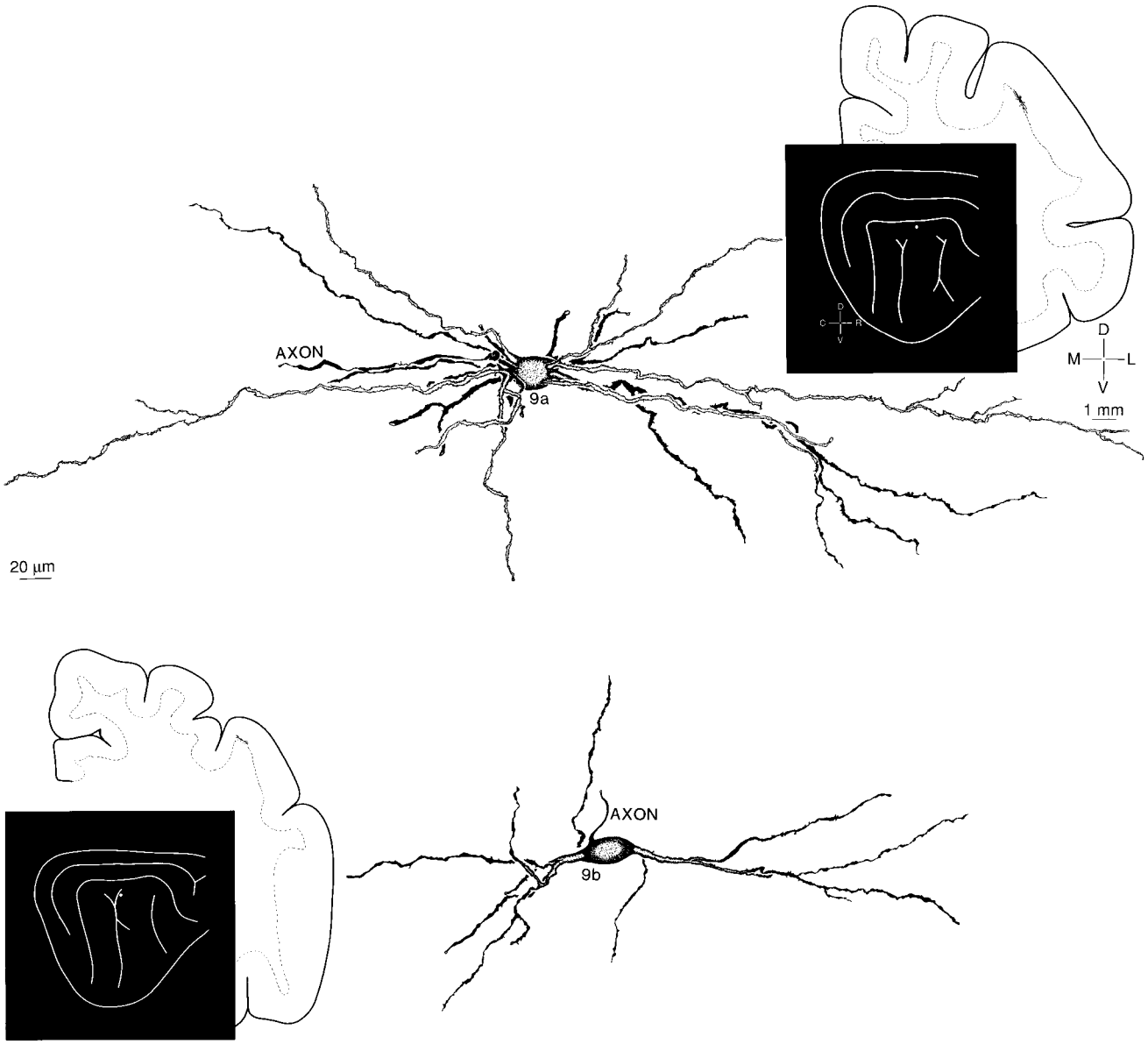

Fig. 13. Horizontal cells (see Table 1:9) were either bitufted, with long, richly tufted lateral processes (9a), or had short branches that divided simply (9b). They shared a lateral dendritic orientation with horizontal fusiform cells (see Fig. 6:3b) and tangential pyramidal cells (see Fig. 8:4). Horizontal cells dominated layer VIb (see also Fig. 1:VI). Both types of horizontal cells had few spines.

stream for purposes internal to layer IV, such as the generation of end-inhibition (Bolz and Gilbert, 1986); or 3) they may affect corticofugal projection cells (Reblet et al., 1992 [rabbit]; and finally 4) some layer VI neurons could encode more global acoustic properties (for example, frequency-modulated cues or responsiveness to formants) rather than specific features (such as high Q_{10} dB values or the preservation of temporal fine structure in interstimulus interval spike statistics) of the stimulus (Evans, 1992). Each proposition can be tested experimentally.

Role of layer VI pyramidal cells in cortical processing

The several subtypes of pyramidal neuron may have different physiological properties, because dendritic patterns in the cortex can correlate with differences in synaptic input and receptive fields (Katz, 1987). Layer VI pyramidal cells form two categories in cat motor cortex (Kang and Kayano, 1994) and in rat motor and somatic sensory cortex (Kaneko et al., 1995). A glutaminase-negative category had large, fast after-hyperpolarizations and no depolarizing after-potentials. In contrast, glutaminase-positive neurons have no or small after-hyperpolarizations and depolarizing after-potentials. These physiological differences have morphological correlates: glutaminase-positive cells have shorter dendrites, more extensive basal dendritic arbors, and axons that project laterally. Glutaminase-negative neurons have the opposite features. This suggests that glutamatergic and nonglutamatergic subtypes must differ in their membrane properties and input-

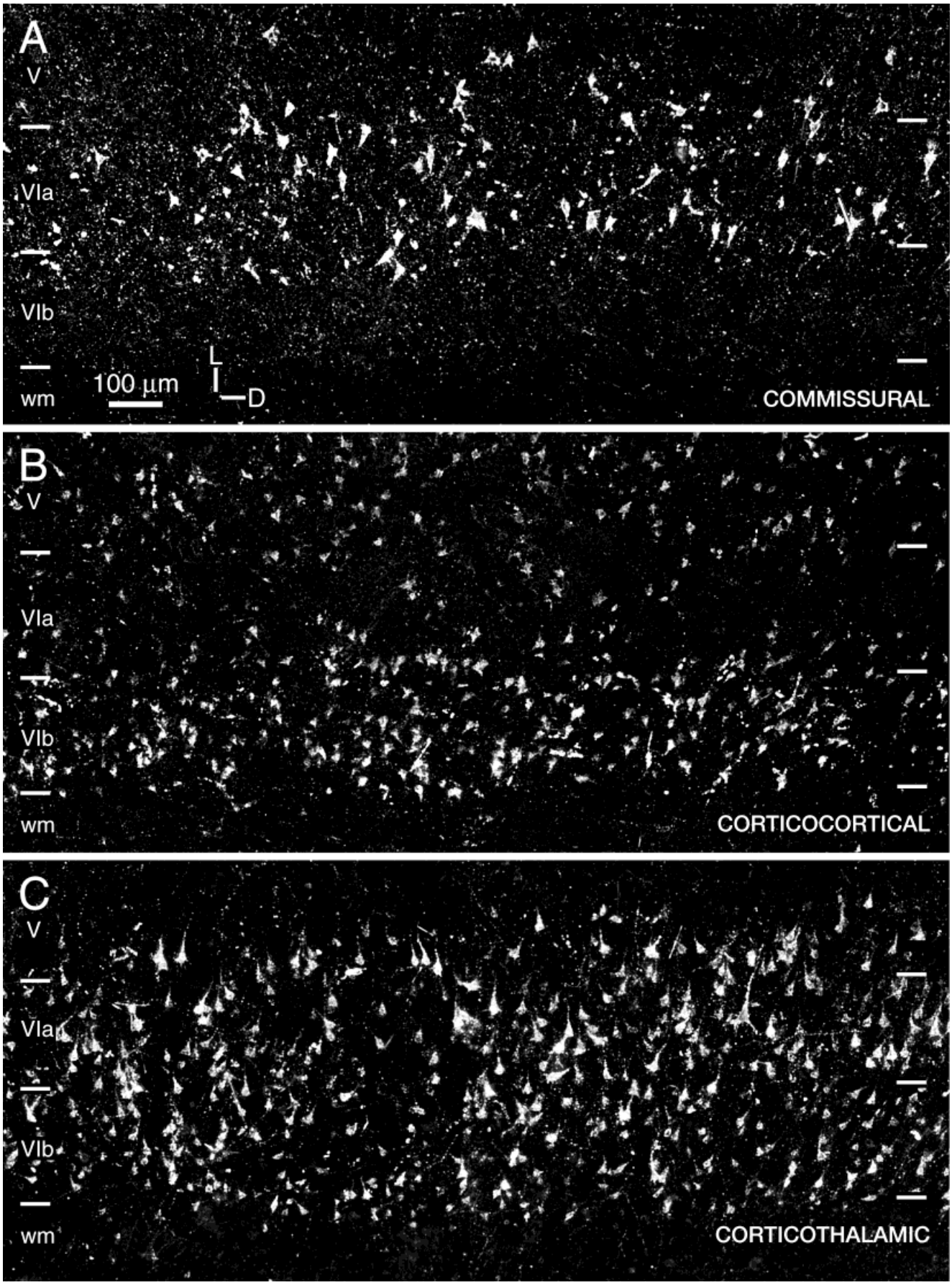

Fig. 14. Layer VI projection neurons after tracer deposits in various targets. **A**: Commissural cells that were labeled retrogradely after a wheat germ agglutinin-horseradish peroxidase (WGA-HRP) injection into the contralateral AI. These cells were mainly in layer VIa (for injection sites and examples of corticofugal neurons, see Fig. 15). **B**: Layer VIb neurons labeled by an injection into the ipsilateral second auditory cortical area (AII). **C**: Corticothalamic cells of origin were distributed more evenly. Protocol: 60-µm-thick section, polarized light; planachromat, N.A. 0.12; ×63.

output organization (Kaneko et al., 1995). This raises intriguing questions about the nature of these properties in the modified layer VI pyramidal cells in AI.

Some neurons in our study (Figs. 4:1b,c; 7:1b) resemble the glutaminase-positive cells noted elsewhere (Kaneko et al., 1995; Fig. 6:3–6), and another of our neurons (Fig. 5) was like the glutaminase-negative neurons described by others (Kaneko et al., 1995, Fig. 11:14,19,21,22). Although the classification of pyramidal cells in Golgi material may be useful, it also can include neurons that, in physiological studies, could correspond to physiologically distinct subgroups. In layer V, pyramidal neurons likewise consist of two classes, similar to those in layer VI (Chagnac-Amitai et al., 1990 [rat]).

In cat AI, thalamocortical axons from the ventral nucleus of the medial geniculate body end in layers IIIb and IV (Sousa-Pinto, 1973), and those from the medial division target layers I and VI (Mitani et al., 1984; Niimi et al., 1984). Layers III and IV receive substantial corticocortical input (Diamond et al., 1968), and commissural axons terminate mainly in layer III and progressively less in layers I, V, and VI (Code and Winer, 1986). Layer VI horizontal cell dendrites would be well placed to receive input from nonprimary thalamocortical afferents, whereas the vertically oriented neurons are candidates for corticocortical, commissural, and/or thalamocortical sources. Pyramidal cell types with specific sublaminar locations participate selectively in corticofugal projection systems (Einstein and Fitzpatrick, 1991; Ojima et al., 1992; present results; Zhang and Deschênes, 1997 [rat]).

Because large pyramidal cells are the only layer VI neurons with apical dendrites that reach layer III, they alone could receive monosynaptic commissural (in layer III) and thalamic input (in layer IV). Both medium-sized and vertical fusiform pyramidal cells cross layer IV and would receive synaptic input from neurons in the ventral division of the auditory thalamus, much as layer IV cells in other areas (Davis and Sterling, 1979). Because these three types of neurons were labeled retrogradely only by ventral division injections, the primary thalamocortical-corticothalamic circuit might involve relatively few cell types. An electrophysiological-morphological study found that nearly all layer VI neurons activated antidromically by medial geniculate body stimulation were classical pyramidal cells, with the exception of an occasional vertical fusiform neuron (Mitani et al., 1985). This finding is consistent with the present results and with observations in somatic sensory cortex (Zhang and Deschênes, 1997 [rat]).

Speculation on layer VI

Although each layer in AI has a unique cytoarchitecture and pattern of connectivity (Winer, 1992), layer VI appears to have the most diverse neurons and the most widespread connections in AI. The reason for these laminar differences may reflect the dual ontogenetic origins postulated for layers VIa and VIb. Layer VIb, as noted above, arises from the primordial plexiform neuropil, a structure composed of afferent and efferent fibers and scattered neurons, the appearance of which precedes that of the cortical plate. On the other hand, layer VIa is a derivative of the cortical plate, an origin that it shares with layers II–V (Marín-Padilla and Marín-Padilla, 1982; Marín-Padilla, 1983, 1984, 1992, 1998). Marín-Padilla has proposed that all cortical pyramidal cells belong to the cortical plate and that, at least initially, their apical dendrites contact layer I, a feature that constrains their morphology and defines their orientation, as recognized in classical descriptions of their dendritic form in many species (Ramón y Cajal, 1911) and in more recent work on their intracortical axonal projections (Winer, 1984b). Nearly all of the pyramidal cells in layer VI conform to these principles. The apparent absence of apical dendrites among layer VIb inverted pyramidal cells is consistent with the notion that their primordial plexiform origins differ from those of layer VIa neurons and are aligned more closely with those of cells in layer I.

In neocortical ontogeny, the neuroblasts that form the deepest layers are generated first in the ventricular zone to construct the cortical subplate; this is followed by the emergence of the cortical plate itself in layer VIb in somatic sensory cortex (Valverde et al., 1989 [rat]). If this pattern occurs in AI, then layer VI could play two interrelated developmental roles. First, it might serve as a spatial and temporal target for migrating, presumptive neocortical neuroblasts that will constitute layer VIa as well as the more superficial laminae. Such layer VI neurons, especially those with a vertical orientation and dendrites that extend into the superficial layers (Coogan and Van Essen, 1996 [macaque monkey]), may serve a role analogous to that of the radial glia, except that the specification of neuronal position (and perhaps identity) is contingent on laminar rather than areal cues (McConnell and Kaznowski, 1991 [ferret]). Because the maturational sequence of neurons in layers V and VI itself is protracted (McConnell et al., 1994 [cat and ferret]), its completion may involve changes in the other layers that could initiate, permit, or sustain corticofugal axonal outgrowth. Perhaps layer VI neurons assist in the temporal control of the development of layer V neurons, the corticofugal projections of which predate those even of layer VI cells (Clascá et al., 1995 [ferret]). A second and even more radical idea is that the early generation of layer VI neurons enables these prospective corticofugal and other infragranular cells to form the most rudimentary extrinsic and intrinsic connections at a time when the granular and supragranular layers barely have begun their much more protracted sequence of differentiation. This is consistent with the view that corticofugal projections appear to emerge before local connections (Callaway and Lieber, 1996 [ferret]).

Such a role is also consistent with the diversity of layer VI neuronal form and the breadth of mature neuronal connections. Therefore, perhaps early in cortical development, layer VI functions as a protocortical template con-

Fig. 15. Layer VI projection neurons. **A**: The inset shows that the injection (black dots) and diffusion (stippled area) were confined to AI. Commissural neurons included small (1a), medium-sized (1b), and large (1c) pyramidal cells and fusiform vertical (3a) and inverted (5) pyramidal cells. Some star (2) and tangential (4) pyramidal cells also were labeled. **B**: All pyramidal cell types with the exception of the star pyramidal cells were labeled after injections into the ipsilateral second auditory area (AII). Some classes of corticocortical neurons projected commissurally, such as inverted (5) and vertical fusiform (3a) pyramidal cells. Fusiform horizontal (3b) and classical pyramidal cells were rarer. **C**: Layer VI neurons labeled by an injection into the ventral division of the medial geniculate body. Almost all were classical pyramidal cells of different sizes; medium-sized neurons predominated (1b) except for a few vertical fusiform pyramidal cells (3a). For abbreviations, see list.

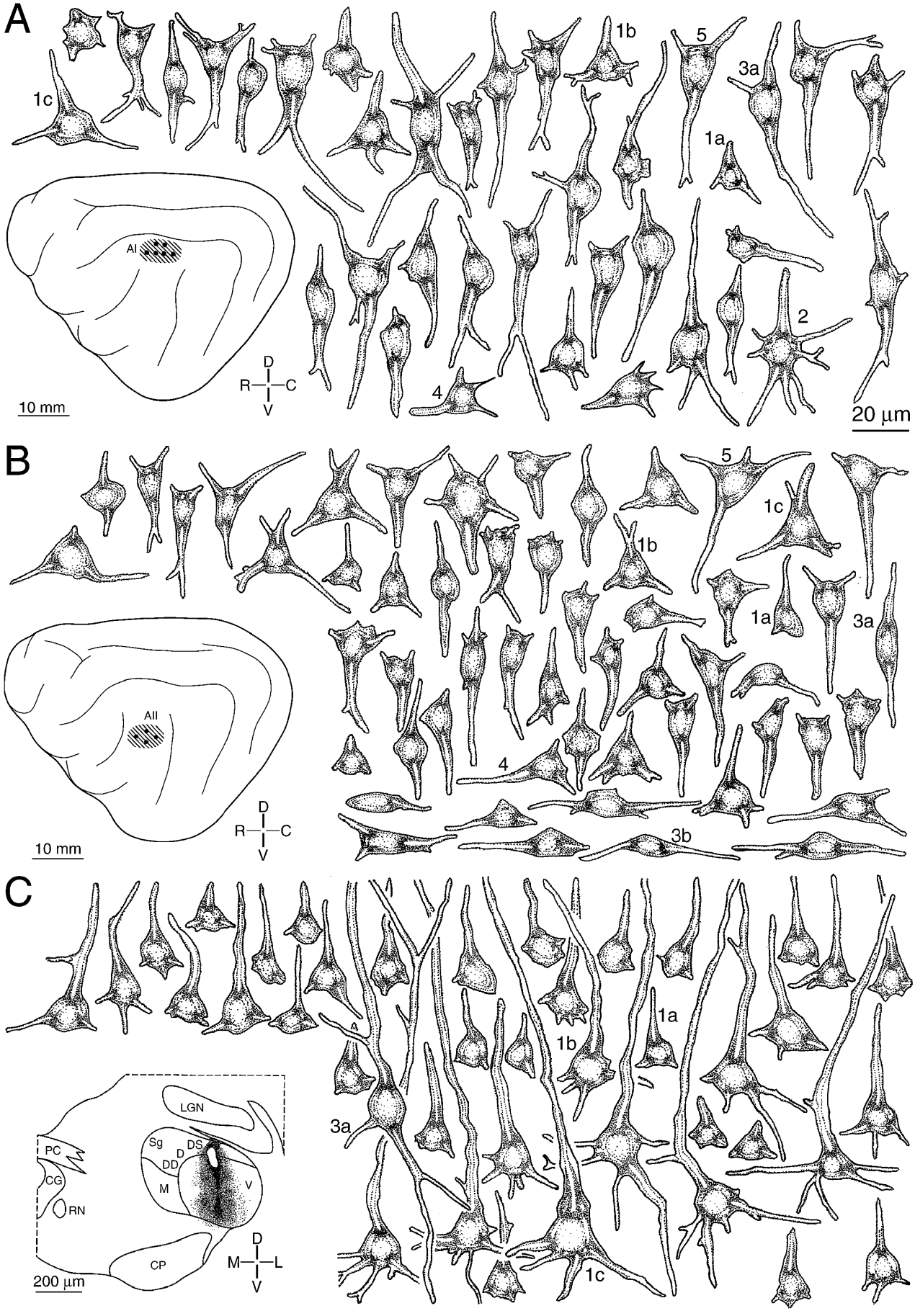
A
1c
1b
5
3a
1a
2
4
AI
D
R
C
V
10 mm
20 µm
B
5
1c
1b
1a
3a
AII
4
3b
D
R
C
V
10 mm
C
1a
1b
3a
1c
LGN
Sg
DS
D
DD
M
V
PC
CG
RN
CP
D
M
L
V
200 µm

Figure 15

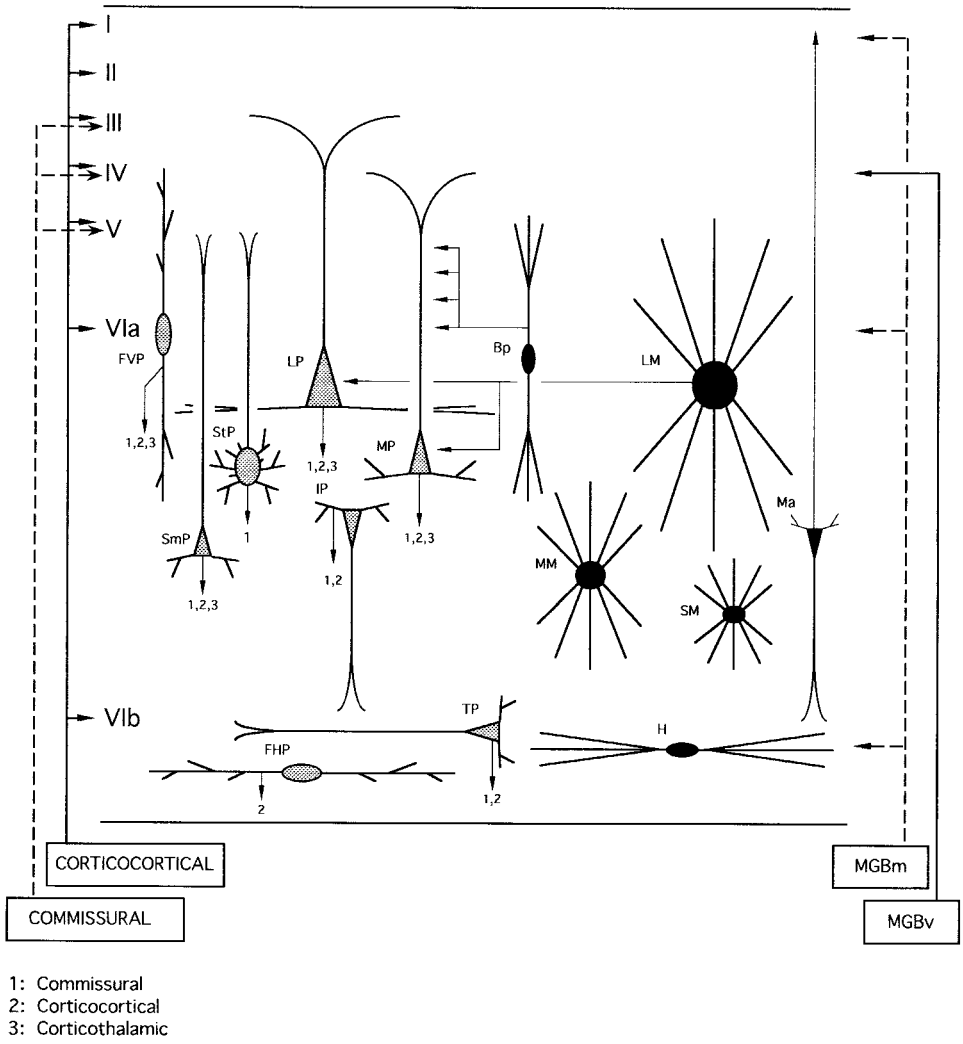

Fig. 16. Summary of neurons, immunocytochemical results (from Prieto et al., 1994a,b), and connections. All eight types of pyramidal neurons are glutamatergic (stippled), and they project to various targets. Some types, such as the fusiform vertical (FVP), small (SmP), medium-sized (MP), and large (LP) pyramidal cells, project in the commissural, corticocortical, and corticothalamic pathways, whereas inverted (IP) and tangential (TP) pyramidal cells are part of two systems (commissural, corticocortical). The two neurons with the most specific projections are the star pyramidal cell (StP), which projects only to contralateral AI, and the fusiform horizontal pyramidal (FHP) cell, which is a member only of the corticocortical system. The six nonpyramidal neuronal types are GABAergic black. Large multipolar (LM) neurons probably correspond to basket cells, and their axons form axosomatic synapses on pyramidal cells and other basket cells (see Prieto et al., 1994a,b). Bipolar cell axons are presynaptic to pyramidal cell apical dendrites. Martinotti (Ma) cells project to layer I and contact the apical tuft of pyramidal cells, none of which are from layer VI. These neurons may receive inputs from the ipsilateral cortical areas (left) and from the medial division of the medial geniculate body (MGBm; right). The ascending dendrites of fusiform vertical, star, small, medium-sized, and large pyramidal cells as well as bipolar and large multipolar neurons cross commissural-recipient layers (far left). Only three layer VI cell types are likely to be subject to lemniscal thalamocortical input (MGBv; right): fusiform vertical, medium-sized, and large pyramidal cells, the apical dendrites of which might reach layer IV. For other abbreviations, see list.

taining most of the principal types of neurons and making many of the chief connections in other layers, although neither the form of the cells nor their function can be considered as mature. This idea entails several predictions, the accuracy of which can be examined. For example, layer VI ought to contain most of the cell types present in the other layers; the results of Golgi studies indicate that this is the case (Winer, 1984a–c, 1985, 1992; present results). By the same token, layer VI should have an array of mature connections that is wider than those in most other layers, which it has; other than its reciprocal connections with the thalamus (Winer, 1992) and the ipsilateral (Winguth and Winer, 1986) and contralateral hemispheres (Code and Winer, 1985), it forms significant interlaminar intrinsic connections in sensory cortex (Ojima et al., 1992; Usrey and Fitzpatrick, 1996 [tree shrew]). Finally, layer VI ought to contain most of the types of GABAergic neuron found in the other layers, which it does (with the exception of the extraverted multipolar cell in layer II; Winer, 1985; Prieto et al., 1994a). This suggests that layer VI will have many of the same structural arrangements for, if not the same proportions of, local inhibition/disinhibition as other layers, a prediction that has been confirmed (Prieto et al., 1994a,b). The principle that informs these speculations is

the idea that cortical structure, function, and development are linked indissolubly with the physiology and projections of each type of neuron.

ACKNOWLEDGMENTS

Ms. Mónica Beneyto generously contributed to the completion of this work and prepared Figures 15 and 16. We thank Mr. Stuart Ingham for help with photographic processing and Ms. Pamela Woronoff and Ms. Esther A. Kim for editorial assistance.

LITERATURE CITED

Aitkin LM. 1973. Medial geniculate body of the cat: responses to tonal stimuli of neurons in medial division. J Neurophysiol 36:275–283.

Bolz J, Gilbert CD. 1986. Generation of end-inhibition in the visual cortex via interlaminar connections. Nature 320:362–365.

Bourassa J, Deschênes M. 1995. Corticothalamic projections from the primary visual cortex in rats: a single fiber study using biocytin as an anterograde tracer. Neuroscience 66:253–263.

Bourassa J, Pinault D, Deschênes M. 1995. Corticothalamic projections from the cortical barrel field to the somatosensory thalamus in rats: a single-fibre study using biocytin as an anterograde tracer. Eur J Neurosci 7:19–30.

Burkhalter A. 1989. Intrinsic connections of rat primary visual cortex: laminar organization of axonal projections. J Comp Neurol 279:171–186.

Callaway EM, Lieber JL. 1996. Development of axonal arbors of layer VI pyramidal neurons in ferret visual cortex. J Comp Neurol 376:295–305.

Chagnac-Amitai Y, Luhmann HJ, Prince DA. 1990. Burst generating and regular spiking layer 5 pyramidal neurons of rat neocortex have different morphological features. J Comp Neurol 296:598–613.

Clarey JC, Barone P, Imig TJ. 1992. Physiology of thalamus and cortex. In: Popper AN, Fay RR, editors. Springer handbook of auditory research, vol 2: The mammalian auditory pathway: Neurophysiology. New York: Springer-Verlag, p 232–334.

Clascá F, Angelucci A, Sur M. 1995. Layer-specific problems of development in neocortical projection neurons. Proc Natl Acad Sci USA 92:11145–11149.

Cobas A, Welker E, Fairén A, Kraftsik R, Van der Loos H. 1987. GABAergic neurons in the barrel cortex of the mouse: an analysis using neuronal archetypes. J Neurocytol 16:843–871.

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

Code RA, Winer JA. 1986. Columnar organization and reciprocity of commissural connections in cat primary auditory cortex (AI). Hearing Res 23:205–222.

Conley M, Raczkowski D. 1990. Sublaminar organization within layer VI of the striate cortex in *Galago*. J Comp Neurol 302:425–436.

Coogan TA, Van Essen DC. 1996. Development of connections within and between areas V1 and V2 of macaque monkeys. J Comp Neurol 372:327–342.

Cox W. 1891. Impregnation des Nervensystems mit Quecksilbersalzen. Arch Mikrosc Anat 37:16–21.

Crick F. 1984. Functions of the thalamic reticular complex: the searchlight hypothesis. Proc Natl Acad Sci USA 81:4586–4590.

Davis TL, Sterling P. 1979. Microcircuitry of cat visual cortex: classification of neurons in layer IV of area 17, and identification of the patterns of lateral geniculate input. J Comp Neurol 188:599–628.

Deschênes M, Hu B. 1990. Electrophysiology and pharmacology of the corticothalamic input to lateral thalamic nuclei: an intracellular study in the cat. Eur J Neurosci 2:140–152.

Diamond IT, Jones EG, Powell TPS. 1968. Interhemispheric fiber connections of the auditory cortex of the cat. Brain Res 11:177–193.

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

Einstein G, Fitzpatrick D. 1991. Distribution and morphology of area 17 neurons that project to the extrastriate cortex. J Comp Neurol 303:132–149.

Evans EF. 1992. Auditory processing of complex sounds: an overview. Phil Trans R Soc London B 336:295–306.

Evans EF, Whitfield IC. 1964. Classification of unit response in the auditory cortex of the unanesthetized and unrestricted cat. J Physiol (London) 171:476–493.

Evans EF, Ross HF, Whitfield IC. 1965. The spatial distribution of unit characteristic frequency in the primary auditory cortex of the cat. J Physiol (London) 179:238–247.

Feldman ML, Peters A. 1978. The forms of non-pyramidal neurons in the visual cortex of the rat. J Comp Neurol 179:761–794.

Ferrer I, Fabregues I, Condom E. 1986a. A Golgi study of the sixth layer of the cerebral cortex: I. The lissencephalic brain of Rodentia, Lagomorpha, Insectivora and Chiroptera. J Anat (London) 145:217–234.

Ferrer I, Fabregues I, Condom E. 1986b. A Golgi study of the sixth layer of the cerebral cortex: II. The gyrencephalic brain of Carnivora, Artiodactyla and primates. J Anat (London) 146:87–104.

Ferster D, LeVay S. 1978. The axonal arborizations of lateral geniculate neurons in the striate cortex of the cat. J Comp Neurol 182:923–944.

Fitzpatrick D. 1996. The functional organization of local circuits in visual cortex: insights from the study of the tree shrew striate cortex. Cerebral Cortex 6:329–341.

Fitzpatrick DC, Henson OW. 1994. Cell types in the mustached bat auditory cortex. Brain Behav Evol 43:79–91.

Fitzpatrick D, Usrey WM, Schofield BR, Einstein G. 1994. The sublaminar organization of corticogeniculate neurons in layer 6 of macaque striate cortex. Vis Neurosci 11:307–315.

Gilbert CD, Wiesel TN. 1979. Morphology and intracortical projections of functionally characterised neurones in the cat visual cortex. Nature 280:120–125.

Globus A, Scheibel AB. 1967. Pattern and field in cortical structure: the rabbit. J Comp Neurol 131:155–172.

Grieve KL, Sillito AM. 1991. A re-appraisal of the role of layer VI of the visual cortex in the generation of cortical end inhibition. Exp Brain. Res 87:521–529.

Grieve KL, Sillito AM. 1995. Non-length-tuned cells in layers II/III and IV of the visual cortex: the effect of the blockade of layer VI on responses to stimuli of different lengths. Exp Brain Res 104:12–20.

Hashikawa T, Molinari M, Rausell E, Jones EG. 1995. Patchy and laminar terminations of medial geniculate axons in monkey auditory cortex. J Comp Neurol 362:195–208.

Huang CL, Winer JA. 1997. Areal and laminar distribution of cat auditory thalamocortical projections. Proc. Soc Neurosci 24:185.

Jones EG. 1975. Varieties and distribution of non-pyramidal cells in the somatic sensory cortex of the squirrel monkey. J Comp Neurol 160:205–268.

Kaneko T, Kang Y, Mizuno N. 1995. Glutaminase-positive and glutaminase-negative cells in layer VI of the primary motor and somatosensory cortices: a combined analysis by intracellular staining and immunocytochemistry in the rat. J Neurosci 15:8362–8377.

Kang Y, Kayano F. 1994. Electrophysiological and morphological characteristics of layer VI pyramidal cells in cat motor cortex. J Neurophysiol 72:578–591.

Katz LC. 1987. Local circuitry of identified projection neurons in cat visual cortex brain slices. J Neurosci 7:1223–1249.

Kelly JP, Wong D. 1981. Laminar connections of the cat's auditory cortex. Brain Res 212:1–15.

Kenan-Vaknin G, Malach R, Segal M. 1992. Excitatory inputs to layer V pyramidal cells of rat primary visual cortex revealed by acetylcholine activation. Brain Res 574:147–156.

Killackey HP, Ebner FF. 1972. Two different types of thalamocortical projections to a single cortical area in mammals. Brain Behav Evol 6:141–169.

Landry P, Deschênes M. 1981. Intracortical arborizations and receptive fields of identified ventrobasal thalamocortical afferents to the primary somatic sensory cortex in the cat. J Comp Neurol 199:345–372.

Lévesque M, Gagnon S, Parent A, Deschênes M. 1996. Axonal arborizations of corticostriatal and corticothalamic fibers arising from the second somatosensory area in the rat. Cerebral Cortex 6:759–770.

Lund JS, Henry GH, MacQueen CL, Harvey AR. 1979. Anatomical organization of the primary visual cortex (area 17) of the cat: A comparison with area 17 of the monkey. J Comp Neurol 184:599–618.

Lund JS, Hendrickson AE, Ogren MP, Tobin EA. 1981. Anatomical organization of primate visual cortex VII. J Comp Neurol 202:19–45.

Marín-Padilla M. 1983. Structural organization of the human cerebral cortex prior to the appearance of the cortical plate. Anat Embryol (Berlin) 168:21–40.

Marín-Padilla M. 1984. Neurons of layer I. Developmental analysis. In: Peters A, Jones EG, editors. Cerebral cortex, vol 1, cellular components of the cerebral cortex. New York: Plenum Press, p 447–478.

Marín-Padilla M. 1992. Ontogenesis of the pyramidal cell of the mammalian neocortex and developmental cytoarchitectonics: A unifying theory. J Comp Neurol 321:223–240.

Marín-Padilla M. 1998. Cajal-Retzius cells and the development of the neocortex. Trends Neurosci 21:64–71.

Marín-Padilla M, Marín-Padilla T. 1982. Origin, prenatal development and structural organization of layer I of the human cerebral (motor) cortex: A Golgi study. Anat Embryol (Berlin) 164:161–206.

McConnell SK, Kaznowski CE. 1991. Cell cycle dependence of laminar determination in developing cerebral cortex. Science 254:282–285.

McConnell SK, Ghosh A, Shatz CJ. 1994. Subplate pioneers and the formation of descending connections from cerebral cortex. J Neurosci 14:1892–1907.

McCourt ME, Boyapati J, Henry GH. 1986. Layering in lamina 6 of cat striate cortex. Brain Res 364:181–185.

McGuire BA, Hornung J-P, Gilbert CD, Wiesel TN. 1984. Patterns of synaptic input to layer 4 of cat striate cortex. J Neurosci 4:3021–3033.

McMullen NT, Glaser EM. 1982. Morphology and laminar distribution of nonpyramidal neurons in the auditory cortex of the rabbit. J Comp Neurol 208:85–106.

Merzenich MM, Knight PL, Roth GL. 1975. Representation of cochlea within primary auditory cortex in the cat. J Neurophysiol 38:231–249.

Meyer G. 1983. Axonal patterns and topography of short-axon neurons in visual areas 17, 18, and 19 of the cat. J Comp Neurol 220:405–438.

Meyer G. 1987. Forms and spatial arrangement of neurons in the primary motor cortex of man. J Comp Neurol 262:402–428.

Meyer G, Albus R. 1981. Spiny stellates as cells of origin of association fibres from area 17 to area 18, in the cat's neocortex. Brain Res 210:335–341.

Miller MW. 1988. Maturation of rat visual cortex: IV. The generation, migration, morphogenesis, and connectivity of atypically oriented pyramidal neurons. J Comp Neurol 274:387–405.

Mitani A, Itoh K, Nomura S, Kudo M, Kaneko T, Mizuno N. 1984. Thalamocortical projections to layer I of the primary auditory cortex of the cat: A horseradish peroxidase study. Brain Res 310:347–350.

Mitani A, Shimokouchi M, Itoh K, Nomura S, Kudo M, Mizuno N. 1985. Morphology and laminar organization of electrophysiologically identified neurons in the primary auditory cortex in the cat. J Comp Neurol 235:430–447.

Mrzljak L, Uylings HBM, Kostovic I, Van Eden CG. 1988. Prenatal development of neurons in the human prefrontal cortex: I. A qualitative Golgi study. J Comp Neurol 271:355–386.

Niimi K, Ono K, Kusunose M. 1984. Projections of the medial geniculate nucleus to layer 1 of the auditory cortex in the cat traced with horseradish peroxidase. Neurosci Lett 45:223–228.

Ojima H, Honda CN, Jones EG. 1992. Characteristics of intracellularly injected infragranular pyramidal neurons in cat primary auditory cortex. Cerebral Cortex 2:197–216.

Peters A, Proskauer CC. 1980. Smooth or sparsely spined cells with myelinated axons in rat visual cortex. Neuroscience 5:2079–2092.

Peters A, Regidor J. 1981. A reassessment of the forms of nonpyramidal neurons in area 17 of cat visual cortex. J Comp Neurol 203:685–716.

Prieto JJ, Peterson BA, Winer JA. 1994a. Morphology and spatial distribution of GABAergic neurons in cat primary auditory cortex (AI). J Comp Neurol 344:349–382.

Prieto JJ, Peterson BA, Winer JA. 1994b. Laminar distribution and neuronal targets of GABAergic axon terminals in cat primary auditory cortex (AI). J Comp Neurol 344:383–402.

Ramón y Cajal S. 1899. Estudios sobre la corteza cerebral humana II: Estructura de la corteza motriz del hombre y mamíferos superiores. Rev Trim Micrográf Madrid 4:117–200.

Ramón y Cajal S. 1900. Estudios sobre la corteza cerebral humana III: Corteza acústica. Rev Trim Micrográf Madrid 5:129–183.

Ramón y Cajal S. 1911. Histologie du Système Nerveux de l'Homme et des Vértébres. Azoulay L, translator. Paris: Maloine [original French edition]. Madrid: Consejo Superior de Investigaciones Científicas [1972 reprint].

Ramón-Moliner E. 1970. The Golgi-Cox technique. In: Nauta WJH, Ebbesson SOE, editors. Contemporary research methods in neuroanatomy. Berlin: Springer-Verlag, p 32–55.

Reblet C, López-Medina A, Gomez-Urquijo M, Bueno-López JL. 1992. Widespread horizontal connections arising from layer 5/6 border inverted cells in rabbit visual cortex. Eur J Neurosci 4:221–234.

Rouiller EM, Simm GM, Villa AEP, de Ribaupierre Y, de Ribaupierre F. 1991. Auditory corticocortical interconnections in the cat: Evidence for parallel and hierarchical arrangement of the auditory cortical areas. Exp Brain Res 86:483–505.

Schreiner CE. 1995. Order and disorder in auditory cortical maps. Curr Opin Neurobiol 5:489–496.

Sillito AM, Cudeiro J, Murphy PC. 1993. Orientation sensitive elements in the corticofugal influence on centre-surround interactions in the dorsal lateral geniculate nucleus. Exp Brain Res 93:6–16.

Sillito AM, Jones HE, Gerstein GL, West DC. 1994. Feature-linked synchronization of thalamic relay cell firing induced by feedback from the visual cortex. Nature 369:479–482.

Sousa-Pinto A. 1973. Cortical projections of the medial geniculate body in the cat. Adv Anat Embryol Cell Biol 48:1–42.

Swarbrick L, Whitfield IC. 1972. Auditory cortical units selectively responsive to stimulus "shape". J Physiol (London) 224:68–69.

Tömböl T. 1984. Layer VI cells. In: Peters A, Jones EG, editors. Cerebral cortex, vol 1: Cellular components of the cerebral cortex. New York: Plenum Press, p 479–519.

Tunturi AR. 1971. Classification of neurons in the ectosylvian auditory cortex of the dog. J Comp Neurol 142:153–166.

Usrey WM, Fitzpatrick D. 1996. Specificity in the axonal connections of layer VI neurons in tree shrew striate cortex: Evidence for distinct granular and supragranular systems. J Neurosci 16:1203–1218.

Valverde F, Facal-Valverde MV. 1988. Postnatal development of interstitial (subplate) cells in the white matter of the temporal cortex of kittens: A correlated Golgi and electron microscopic study. J Comp Neurol 269:168–192.

Valverde F, Facal-Valverde MV, Santacana M, Heredia M. 1989. Development and differentiation of early generated cells of sublayer VIb in the somatosensory cortex of the rat: a correlated Golgi and autoradiographic study. J Comp Neurol 290:118–140.

White EL., Keller A. 1987. Intrinsic circuitry involving the local axon collaterals of corticothalamic projection cells in mouse SmI cortex. J Comp Neurol 262:13–26.

Winer JA. 1984a.Anatomy of layer IV in cat primary auditory cortex (AI). J Comp Neurol 224:535–567.

Winer JA. 1984b. The pyramidal neurons in layer III of cat primary auditory cortex (AI). J Comp Neurol 229:476–496.

Winer JA. 1984c. The non-pyramidal cells in layer III of cat primary auditory cortex (AI). J Comp Neurol 229:512–530.

Winer JA. 1985. Structure of layer II in cat primary auditory cortex (AI). J Comp Neurol 238:10–37.

Winer JA. 1992. The functional architecture of the medial geniculate body and the primary auditory cortex. In: Webster DB, Popper AN, Fay RR, editors. Springer handbook of auditory research, vol 1: The mammalian auditory pathway: Neuroanatomy. New York: Springer-Verlag, p 222–409.

Winer JA, Larue DT. 1989. Populations of GABAergic neurons and axons in layer I of rat auditory cortex. Neuroscience 33:499–515.

Winguth SD, Winer JA. 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.

Yoshioka T, Levitt JB, Lund JS. 1994. Independence and merger of thalamocortical channels within macaque primary visual cortex: Anatomy of interlaminar projections. Vis Neurosci 11:467–489.

Zhang Z-W, Deschênes M. 1997. Intracortical axonal projections of lamina VI cells of the primary somatosensory cortex in the rat: A single-cell labeling study. J Neurosci 17:6365–6379.