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GABAergic Organization of the Cat Medial Geniculate Body

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ABSTRACT

A study of neurons and processes (puncta) immunolabeled by antibodies to γ-aminobutyric acid (GABA) or glutamic acid decarboxylase was undertaken in the medial geniculate body of the adult cat. The proportion and types of GABAergic cells were determined with high resolution methods, including postembbedding immunocytochemistry on semithin plastic sections. A second goal was to draw parallels and differences between the auditory thalamus and other thalamic nuclei. Finally, the types of GABAergic puncta and their concentration in the three major subdivisions of the medial geniculate body were analyzed. The results were that (1) each division had many GABAergic neurons, averaging approximately 26% of the neuronal population; (2) the ventral division had the highest proportion of these cells (33%), the medial division the fewest (18%), and the dorsal division was intermediate (26%); (3) there was a gradient in the proportion of GABAergic neurons, i.e., the ventral and medial division values increased caudorostrally, whereas the value in the dorsal division declined; (4) the predominant GABAergic cell type in each division was a small neuron with a soma approximately 10–12 μm in diameter; (5) a small population of much larger GABAergic neurons was present mainly in the dorsal division; (6) in addition to the fine, granular puncta in each division, a type of giant GABAergic puncta was found only in the dorsal division nuclei. The results obtained with the two antibodies were essentially identical. These findings suggest a structural basis for qualitative differences in the distribution of GABAergic processing within the medial geniculate complex. The GABAergic arrangement in the ventral division was stereotyped, with only one type of putative GABAergic interneuron, and the puncta were correspondingly homogeneous. In contrast, the dorsal division had two types of GABAergic neurons, and the giant GABAergic puncta represent a new substrate for inhibitory interactions. The medial division also had more than one type of GABAergic neuron and a slightly lower concentration of puncta. These qualitative and quantitative distinctions suggest a morphologic basis for possible differences in inhibitory processing among medial geniculate body subdivisions. J. Comp. Neurol. 415:368–392, 1999.

Indexing terms: thalamus; inhibition; interneuron; local circuit; Golgi type II cell

Interneurons play an essential role throughout the neuraxis in the processing and transmission of sensory and motor information (Horridge, 1968). Their contribution in a few special instances to inhibitory and disinhibitory operations is comparatively well understood at both anatomic and physiological levels. Neurons such as Renshaw cells in the spinal cord (Windhorst, 1990), basket cells in the cerebral cortex (DeFelipe et al., 1986), Golgi type II cells in the lateral geniculate body (Friedlander et al., 1980; Cox et al., 1998), and Purkinje cells in the cerebellar cortex (Palay and Chan-Palay, 1974) and their associated circuits are among the best characterized interneuronal populations in the nervous system. However, in other parts of the thalamus, the physiological evidence available from intracellular recording and labeling studies is too limited to permit definitive conclusions about the specific role of intrinsic cells (Jahnsen and Llinás, 1984a,b [guinea pig]). Moreover, there are comparatively few studies available on the medial geniculate body, a structure critical for normal hearing (Winer, 1991) and about whose local circuit interneurons (Golgi type II cells) only a limited

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amount of neurochemical information is available (Rinvik et al., 1987; Rouiller et al., 1990). A more complete analysis of medial geniculate body interneuronal organization is the main object of the present study, with special reference to parallels and differences among the three principal divisions that comprise the auditory thalamus.

One proposed function for thalamic Golgi type II cells is to provide recurrent inhibition onto principal, type I, thalamocortical neurons. Such circuitry might play a role in temporal coding (Morest, 1971) or as a source of disinhibition for nearby type II cells (Winer, 1992). In other primary sensory thalamic nuclei, such as the lateral geniculate body and the ventrobasal complex, γ-aminobutyric acid–accumulating (GABAergic) neurons mediate many aspects of neuronal discharge, including receptive field dynamics and global changes in neuronal excitability, to name just two (Lee et al., 1994; Kim et al., 1997; Ulrich and Huguenard, 1997). A more refined picture of the distribution of such cells in the main sensory thalamic nuclei in each modality is essential to understanding their role. Classic studies of Golgi impregnated material reveal that many neurons have local axons that contribute to the thalamic neuropil and could participate in these processes (Morest, 1964; Guillery, 1966; Scheibel and Scheibel, 1966).

The present study addresses the issues of the proportions and types of GABAergic neurons in the cat medial geniculate body. These questions are pertinent to understanding auditory thalamic function because each of the different parts has a particular pattern of ascending projections from the midbrain (Calford and Aitkin, 1983), a specific set of corticothalamic inputs (Diamond et al., 1969), and a unique physiological arrangement (Clarey et al., 1992). Prior work found that GABAergic neurons and axon terminals and immunoreactive dendrites are a general feature of thalamic organization in many species (Mugnaini and Oertel, 1985; Winer et al., 1995; Arcelli et al., 1997) and that they are plentiful in the auditory thalamus (Rouiller et al., 1990). However, there has been no systematic analysis of possible differences in the intrinsic organization of medial geniculate body subdivisions, nor any quantitative treatment of the GABAergic processes (puncta) in the neuropil. Such differences could have a significant effect on how lemniscal and nonlemniscal streams of information are processed by these nuclei. The purpose of the present study is to contrast and compare the GABAergic arrangements in the subdivisions of the medial geniculate body. This will be accomplished with a qualitative and quantitative high-resolution, light microscopic approach based on several antisera to GABA and glutamic acid decarboxylase (GAD).

A second rationale is to determine the different types of GABAergic puncta within the medial geniculate body. At least two of the sources of GABA are extrinsic, whereas another is intrinsic. One projection arises from the thalamic reticular nucleus (Crabtree, 1998), all of whose neurons are GABAergic (Houser et al., 1980). Reticular nucleus axons have a wide range of terminal architectures within the thalamus (Cox et al., 1996); these could play a role in the diverse inhibitory effects seen in nuclei other than the medial geniculate body (Cox et al., 1997), and it is likely that analogous processes exist within the auditory thalamus. A second GABAergic projection originates in the inferior colliculus, where immunoreactive neurons with a wide range of sizes and shapes exist (Oliver et al., 1994). A correspondingly diverse population of these—most notably the largest cells in the inferior colliculus—project to each subdivision of the auditory thalamus (Winer et al., 1996), where they exert monosynaptic effects on their neuronal targets (Peruzzi et al., 1997). It is unknown whether specific types of GABAergic puncta could represent these intrinsic and extrinsic projection systems. A third origin for GABAergic influence is the several Golgi type II cell populations in the ventral (Morest, 1975), dorsal (Winer and Morest, 1983b), and medial (Winer and Morest, 1983a) divisions of the medial geniculate body. These vary in size, shape, and number and might contribute to the specific patterns of neuropil organization found in each nucleus. The architectonic parcellation chosen follows that used in previous studies (Morest, 1964, 1965; Winer and Morest, 1984; Winer, 1992).

Although experimental identification of the origin of each presumptive type of puncta is beyond the scope of the

Abbreviations

BIC	brachium of the inferior colliculus
BSC	brachium of the superior colliculus
CG	central gray
CM	centre médian nucleus
CN	central nucleus of the inferior colliculus
CP	cerebral peduncle
CSC	commissure of the superior colliculus
D	dorsal nucleus of the medial geniculate body
DC	dorsal cortex of the inferior colliculus
DCa	caudal 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
FF	fields of Forel
Ha	habenula
LD	lateral dorsal nucleus
LGB	lateral geniculate body
LL	lateral lemniscus
LMN	lateral mesencephalic nucleus
LN	lateral nucleus of the inferior colliculus
LP	lateral posterior nucleus
M	medial division of the medial geniculate body
Mes V	mesencephalic trigeminal nucleus and tract
Md	medial dorsal nucleus
ML	medial lemniscus
MRF	mesencephalic reticular formation
MZ	marginal zone of the medial geniculate body
OR	optic radiation
OT	optic tract
Ov	*pars ovoidea* of the ventral division of the medial geniculate body
PHy	posterior hypothalamus
Pol	posterior area of the thalamus, lateral region
Pom	posterior area of the thalamus, medial region
Pt	pretectal area
Pul	pulvinar
Py	pyramidal tract
Re	thalamic reticular nucleus
RN	red nucleus
SGI	intermediate gray layer of the superior colliculus
Sgl	suprageniculate nucleus, lateral part
Sgm	suprageniculate nucleus, medial part
SGI	intermediate gray layer of the superior colliculus
SGP	deep gray layer of the superior colliculus
SGS	superficial gray layer of the superior colliculus
SNC	substantia nigra, *pars compacta*
SNR	substantia nigra, *pars reticulata*
Spf	subparafascicular nucleus
SpN	suprapeduncular nucleus
St	subthalamic nucleus
V	ventral division of the medial geniculate body
Vb	ventrobasal complex
Vl	ventrolateral nucleus of the medial geniculate body
VTA	ventral tegmental area
ZI	zona incerta
III	oculomotor nucleus

present study, an essential step to that end is first to define the differences among subdivisions that could contribute to inhibitory circuits in the medial geniculate body. Because these different sources of inhibitory control may not exert the same effect on postsynaptic neurons, a more precise analysis of their organization could predict patterns of inhibition within the thalamus and clarify modality specific differences in these arrangements.

MATERIALS AND METHODS

General procedures

A complete series of medial geniculate bodies from 15 healthy adult male cats, each weighing 2.5–4.0 kg, were available. Experimental procedures followed the guidelines of the institutional animal care and use committee and standard veterinary protocols (Society for Neuroscience, 1991). Animals were anesthetized with sodium pentobarbital (Abbott Laboratories, North Chicago, IL; 40 mg/kg, i.p.); the perfusion began when the animal was areflexic to nociceptive stimuli. After perfusion and partial dissection, the brain was blocked in situ to approximate the stereotaxic transverse or horizontal plane in a standard atlas (Berman and Jones, 1982). To include the entire medial geniculate body, the block extended approximately 2 mm beyond the caudal and rostral poles of the auditory thalamus; this detail was important because the rostral extremity reaches the level of the ventrobasal complex (Fig. 1G: Vb) in the transverse plane (Winer, 1985). Several experiments were also available in which adult cats received cortical injections of horseradish peroxidase (Winer, 1984), after which the brain was prepared for postembedding immunocytochemistry for the simultaneous demonstration of retrograde labeling and immunostaining (Larue and Winer, 1996). This material was useful as an independent estimate of the proportion of local circuit neurons and for comparative purposes in contrasting GABAergic and thalamocortical neurons (Fig. 9B,C).

Immunocytochemistry

GABA immunocytochemistry for thick sections. Six cats were perfused intracardially with a brief (<1 minute) washout (100–200 ml of phosphate buffered saline [PBS], pH 7.4, 25°C) followed by fixative (2,000 ml of 3–4% paraformaldehyde/0.25–3% glutaraldehyde/in phosphate buffer [PB], pH 7.4, 4–10°C). After 1 hour, cryoprotectant (10% sucrose/0.1 M PB, 500 ml) was perfused and, after dissection, the brain was immersed overnight in a second cryoprotectant (30% sucrose at 4°C). Sections 50-μm-thick were cut on a Vibratome™ (Oxford Laboratories, Foster City, CA) and collected in buffer (cold 0.1 M PB). Sections were placed in blocking serum (5% normal goat serum/PBS; 60 minutes), then incubated in rabbit anti-GABA (DiaSorin, Clearwater, MN; 1:5,000 with 2% normal goat serum; overnight at 4°C). A 1:2 Nissl-stained series was prepared for cytoarchitectonic analysis.

GABA immunocytochemistry for postembedded semithin sections. Three animals were perfused intracardially with a brief washout (<1 minute; 100–200 ml of PBS, 25°C) and then fixed (2,000 ml of 2% paraformaldehyde/3% glutaraldehyde; 4°C). Blocks 200 μm thick and 6 × 8 mm wide were cut on a Vibratome, and the slabs were osmicated, dehydrated in graded alcohols, flat-embedded in a recipe for soft Araldite epoxy suitable for semithin sectioning, then polymerized (15 hours, 60°C). Semithin sections, 1–1.5-μm-thick, were cut with 8-mm-wide glass knives from blocks that contained an entire hemithalamus. For postembedding, sections were heat mounted on clean slides, etched in ethanolic sodium hydroxide (approximately 10% NaOH/100% EtOH), deosmicated, rehydrated, and immunostained on the slide (streptavidin-biotin kit [Histomark, Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD], or with avidin/biotin at twice the recommended dilution ([Vector Laboratories, Inc., Burlingame, CA]; Larue and Winer, 1996). A 1:5 or a 1:3 series counterstained with toluidine blue was used to determine cytoarchitectonic borders.

GAD immunocytochemistry. Six cats were perfused with washout (200–300 ml of normal saline) and then fixative (2,000 ml of 0.5% zinc salicylate/10% unbuffered formalin, 20°C for both solutions; Mugnaini and Dahl, 1983). One hour later, cryoprotectant was perfused (10% sucrose/normal saline, 500 ml) and the brain was dissected, blocked, and stored overnight in a cryoprotectant solution (30% sucrose/saline solution, 4°C). Frozen sections 25 μm thick were collected (0.5 M Tris, pH 7.6) and placed in blocking serum (10% normal rabbit serum/0.1 M DL-lysine in 0.5 M Tris, pH 7.6, 60 minutes; Sigma Chemical Co., St. Louis, MO). They were incubated overnight with sheep anti–GAD-1440 (1:2,000 dilution with 2% normal rabbit serum, at 4°C) following standard protocols (Oertel et al., 1981; Mugnaini and Dahl, 1983; Larue and Winer, 1996). Antigen was localized with the immunoperoxidase technique (ABC Vectastain; Vector Laboratories). A 1:2 Nissl series was also prepared (Winer and Larue, 1988).

Data analysis

Architectonic boundaries. Subdivisions were determined independently from Nissl- or toluidine blue–stained sections that were adjacent to the immunostained sections. The architectonic scheme was derived from Golgi studies (Morest, 1965) and connectional experiments related to auditory midbrain (Calford and Aitkin, 1983) and cortical (Niimi and Matsuoka, 1979) projections. As demonstrated below, the immunostaining patterns also distinguished the medial geniculate body subdivisions from one another. The boundaries derived from the Nissl or the toluidine blue–stained sections were superimposed on the immunostained sections (Winer, 1992); precise alignment was achieved by a careful comparison of major vessels in each set of preparations.

Neurons. GAD-positive neurons from each medial geniculate body division were drawn with a drawing tube with an oil immersion objective (Figs. 3–6). Only darkly immunostained cells with pale nuclei were considered immunopositive. These neurons were readily compared in size, shape, and dendritic origins to specimens recognized in Golgi preparations (Morest, 1975; Winer and Morest, 1983a,b).

Puncta. Many different types of immunopositive profiles, including puncta, preterminal processes, and dendrites were drawn (Figs. 7, 8). For quantitative analysis (Table 1), however, only puncta were counted. Representative examples of such processes appear in Figure 9D.

Quantitative analysis of neurons. The proportion of GABAergic neurons in each medial geniculate body division was determined by using 150 sample areas of various sizes; the total area sampled in one cat was approximately

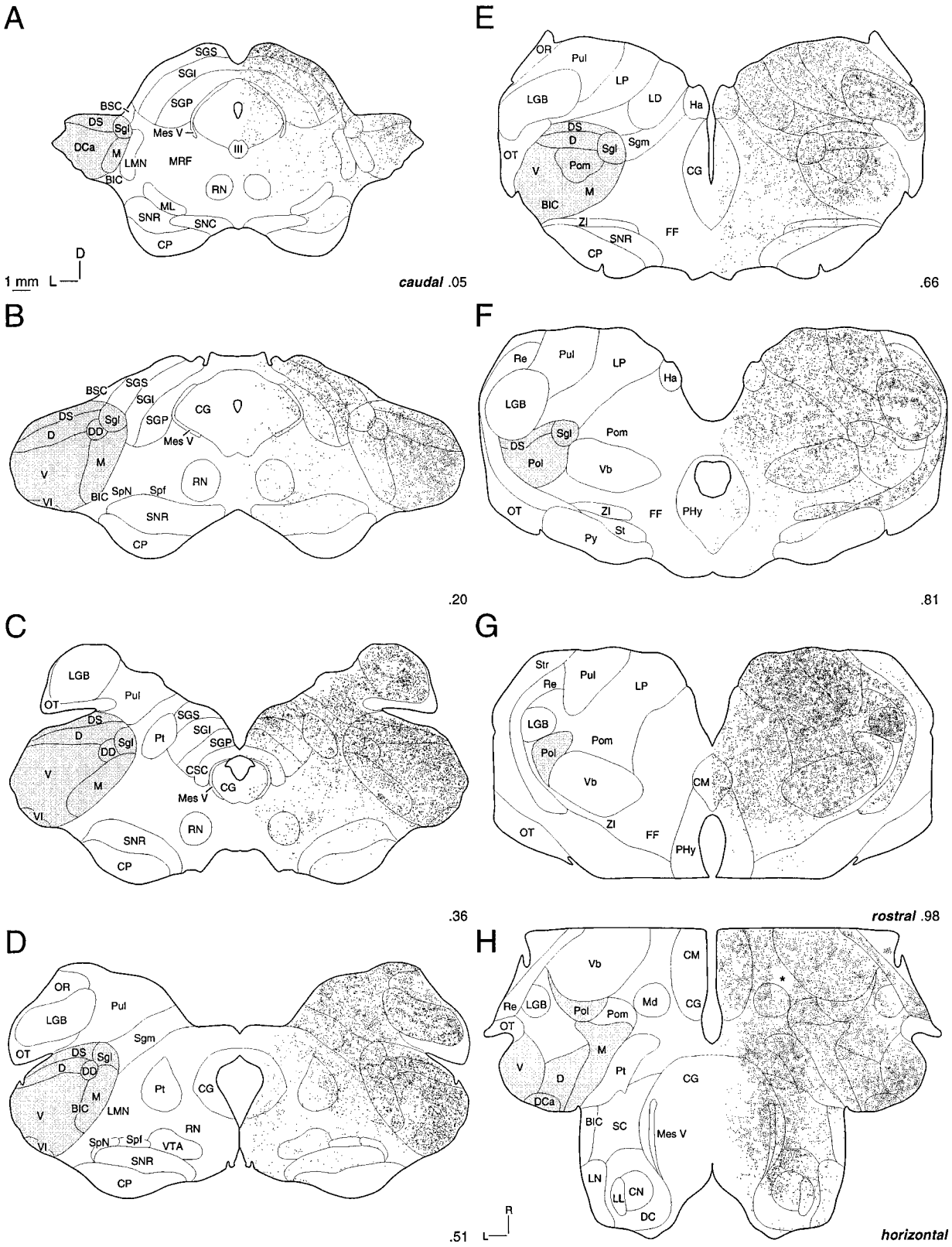


Fig. 1. Architectonic subdivisions of the medial geniculate body (gray stipple, left side) and plots of γ-aminobutyric acid–positive (GABAergic) neurons (dots, right side) in a caudorostral (A–G) transverse sequence and in a representative horizontal section (H). Numbers, percentage of the length of the medial geniculate body. Planachromat, N.A. 0.30, ×125. **A:** The caudal tip had a lower concentration of GABAergic cells than more rostral sections due to the many brachial fibers. **B:** Nuclear differences in the proportion of GABAergic cells were present in the dorsal division. **C:** The dorsal superficial nucleus (DS) had far fewer GABAergic cells than the other parts of the medial geniculate body. **D:** The brachium of the inferior colliculus (BIC) was virtually defined by the absence of immunopositive neurons. **E:** The concentration of GABAergic neurons was similar in the medial geniculate body and in the lateral geniculate body (LGB) and slightly lower in the adjoining pulvinar (Pul) and lateral posterior (LP) nuclei. **F:** Near the rostral pole of the medial geniculate complex (Pol), the thalamofugal and corticofugal axons marked an abrupt decline in the density of GABAergic neurons. **G:** Even the rostral pole had many immunopositive neurons. **H:** Caudal-to-rostral gradients in the proportion of GABAergic neurons in the dorsal division nuclei and in the medial division were present (see Fig. 2F, dorsal, medial). Asterisk, fiducial mark. For abbreviations, see list.

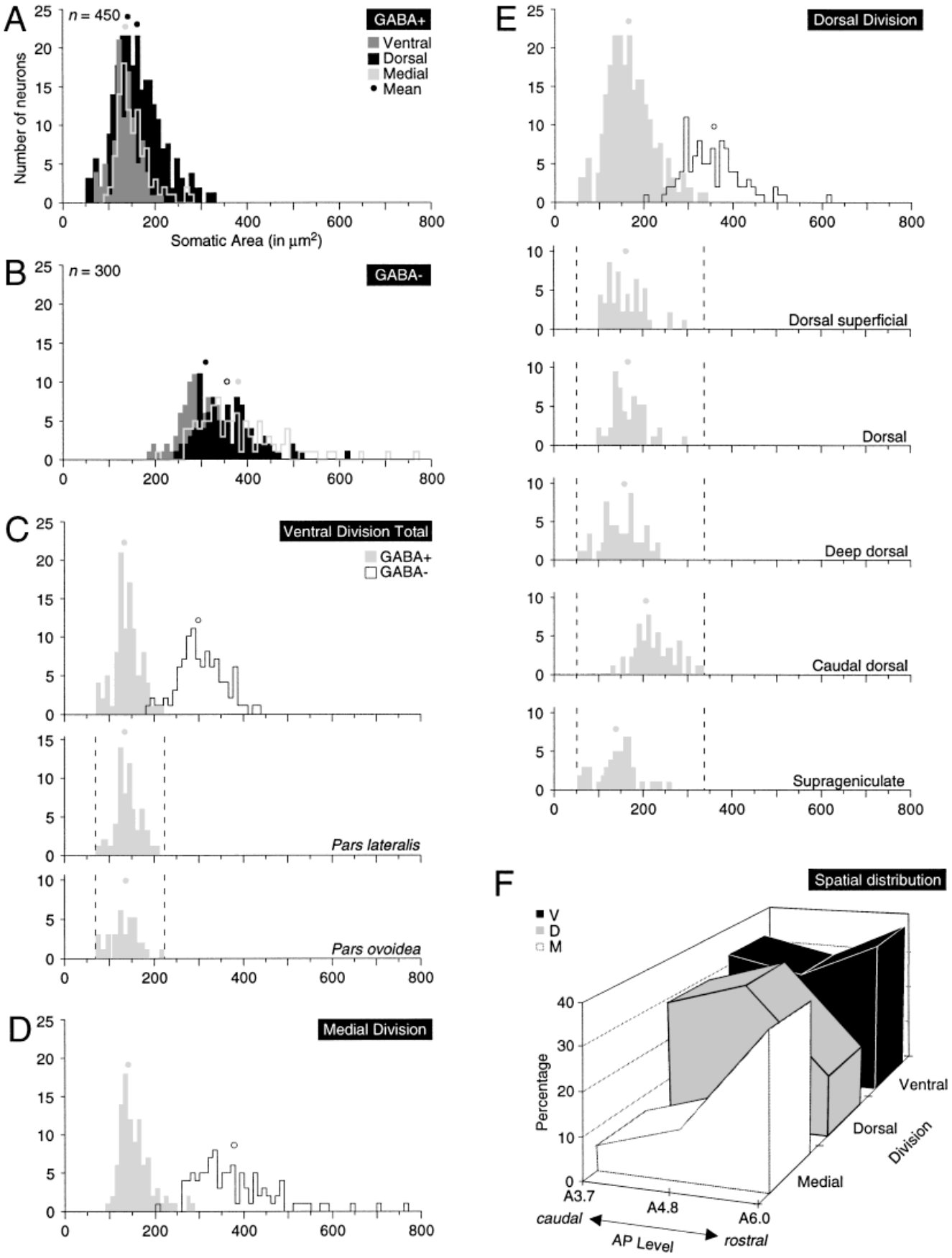

A
n = 450
GABA+
Ventral
Dorsal
Medial
Mean
Number of neurons
Somatic Area (in μm²)
B
n = 300
GABA-
C
Ventral Division Total
GABA+
GABA-
Pars lateralis
Pars ovoidea
D
Medial Division
E
Dorsal Division
Dorsal superficial
Dorsal
Deep dorsal
Caudal dorsal
Suprageniculate
F
Spatial distribution
V
D
M
Percentage
Ventral
Dorsal
Medial
Division
A3.7
A4.8
A6.0
caudal
AP Level
rostral

Figure 2

210 mm^2. The quantitative results from this specimen were compared with those from other animals; only if they were indistinguishable statistically were the results included as representative. Sample fields avoided architectonic boundaries and excluded large blood vessels. GABAergic neurons were plotted from 1-μm-thick, plastic-embedded sections on an X-Y recorder coupled to a microscope stage (Omnigraphics, Austin, TX; Figs. 1, 2F, Table 1). All the neurons from a corresponding toluidine blue–stained section were also plotted in a similar manner (Figs. 1, 2F, Table 1). Only perikarya with a nucleus were selected. The Abercrombie-Floderus correction was applied to compensate for counting errors caused by differences in the mean caliper (nuclear) diameter between the smaller, GABA-positive (Fig. 2A,F) and larger, GABA-negative (Fig. 2B,F) neurons (Smolen et al., 1983). Proportions were determined by averaging the ratios of GABAergic cells to all neurons/area at each anteroposterior level.

Quantitative analysis of perikarya. Representative GABAergic and immunonegative perikarya were drawn from semithin, plastic-embedded GABA sections from each division under oil immersion. A sample of 100 neurons/division was collected. The criteria for inclusion were that neuronal somata were complete, midnucleolar, and remote from architectonic borders. These profiles were scanned and digitized with an image processing system (NIH Image, version 1.61; National Institutes of Health, Bethesda, MD). The perikaryal diameter was fitted with the largest possible oval to measure its area. Two-tailed t-tests of equal variances with an α level = 0.001 were used to make multiple comparisons of the perikarya between nuclear subdivisions. The Bonferroni correction was then applied to minimize the probability of a type I error by using multiple, repeated measures (Winer, 1971).

Quantitative analysis of puncta. Ten representative samples (each 25 × 25 μm) of immunopositive puncta were drawn and counted from 1-μm-thick, deplasticized GABA sections. Sample zones were selected from a numbered grid overlaid on the section; a random number sequence was used to select a particular grid square from the sample space. Each grid was distant from architectonic borders, and regions with blood vessels were excluded. Immunopositive profiles that could not be classified with certainty were omitted from analysis.

For Figure 9, conventional photomicrographs were taken with high resolution, black and white film. Negatives were scanned into Photoshop 5.0 (Adobe Systems, San Jose, CA) on a Super Coolscan LS-1000 film scanner (Nikon Instruments Division, Garden City, NY). Images were imported and composed in Canvas 5.0 (Deneba Software, Miami, FL). For Figures 10 and 11, images were photographed digitally on a Nikon Microphot-FXA equipped with a Spot™ cooled CCD camera (Diagnosticx Instruments, Inc., Sterling Heights, MI), and processed as above in Photoshop and Canvas. No digital editing of the image content was performed.

RESULTS

The results in tissue immunostained for GAD (Fig. 7) or GABA (Fig. 8) were indistinguishable: each antiserum revealed the same types of neurons and comparable varieties of puncta. The term, GABAergic, therefore, refers to the results obtained with both methods unless specific reference is made to one procedure. The superior immunopenetration in the thick GAD sections often revealed immunopositive axons and dendrites, whereas the semithin GABA material gave a high resolution view of puncta and somata that was essential for quantitative studies and for determining their areal and regional distribution.

Regional distribution of GABAergic neurons

Surveys of the rostral midbrain and caudal diencephalon showed that the transition between them was marked by an abrupt increase in the proportion of GABAergic neurons (Fig. 1H). From its caudal pole (Fig. 1A: DCa) to its rostral tip (Fig. 1G: Pol), the medial geniculate body had many more GABAergic cells than the adjoining mesencephalic reticular formation (Fig. 1A: MRF), pretectum (Fig. 1D: Pt) or the fields of Forel (Fig. 1G: FF). The auditory thalamic GABAergic cells were comparable numerically to those in the most superficial one-third of the superior colliculus (Fig. 1A: SGS), ventrobasal complex (Fig. 1G: Vb), and lateral geniculate body (Fig. 1E: LGB). The number of thalamic neurons was far larger than that in the tegmental sector of the midbrain or in other parts of the diencephalon, such as, for example, in the posterior hypothalamus (Fig. 1F: PHy).

Proportion of GABAergic neurons

About 26% of the medial geniculate body neurons were GABAergic; their proportion ranged from 33% in the ventral division to 18% in the medial division, with the dorsal division value intermediate (26%; Table 1). We next considered whether the number of such cells was constant along the caudorostral axis by sampling at levels approximating transverse stereotaxic values of 3.7, 4.8, and 6 mm, respectively, rostral to interaural zero, or the central half of the medial geniculate body. Two of the three divisions, the dorsal and the medial, showed significant changes but in opposite directions: the dorsal division value rose slightly and then declined precipitously, whereas the medial division increased slightly at first and, later, more dramatically (Fig. 2F; Table 1). The three sampling intervals represent a total of less than half the length of the medial

Fig. 2. Somatic size distribution of γ-aminobutyric acid–positive (GABAergic) and non-GABAergic cells (A–E) and spatial gradients of GABAergic neurons (F) in the three major parts of the medial geniculate body. **A:** The distributions of GABAergic neurons overlapped except for the smallest dorsal division cells and a few larger cells in the dorsal and medial divisions. **B:** There was more dispersion in the size range among the non-GABAergic cells, mainly from the magnocellular neurons in the medial division and the large thalamocortical neurons in the suprageniculate nucleus. **C:** In the ventral division, there was little overlap between the GABAergic (stippled) and the non-GABAergic (outline) cell populations. The somatic size of GABAergic neurons did not differ in the lateral (*pars lateralis*) and medial (*pars ovoidea*) subnuclei of the ventral division (Table 2). **D:** Medial division GABAergic neurons were bimodally distributed, as were those in the dorsal division (D). The largest non-GABAergic neurons, the magnocellular neurons and other subtypes, were prominent. **E:** In the dorsal division, the proportion of GABAergic cells was significantly lower than that in the ventral division (Table 1), with a bimodal distribution. Three of the five dorsal division nuclei had significantly smaller or larger GABAergic neurons (Table 2); the deep dorsal nucleus had the smallest such neurons, the caudal dorsal nucleus had the largest. **F:** Spatial gradients of GABAergic neurons in caudal-to-rostral traverses through the central 40% of the auditory thalamus showed a rapid increase in the medial division, slower growth in the ventral division, and a precipitous decline in the dorsal division. The latter decrease coincided with the emergence of the anterior dorsal nuclei (Winer and Morest, 1983b).

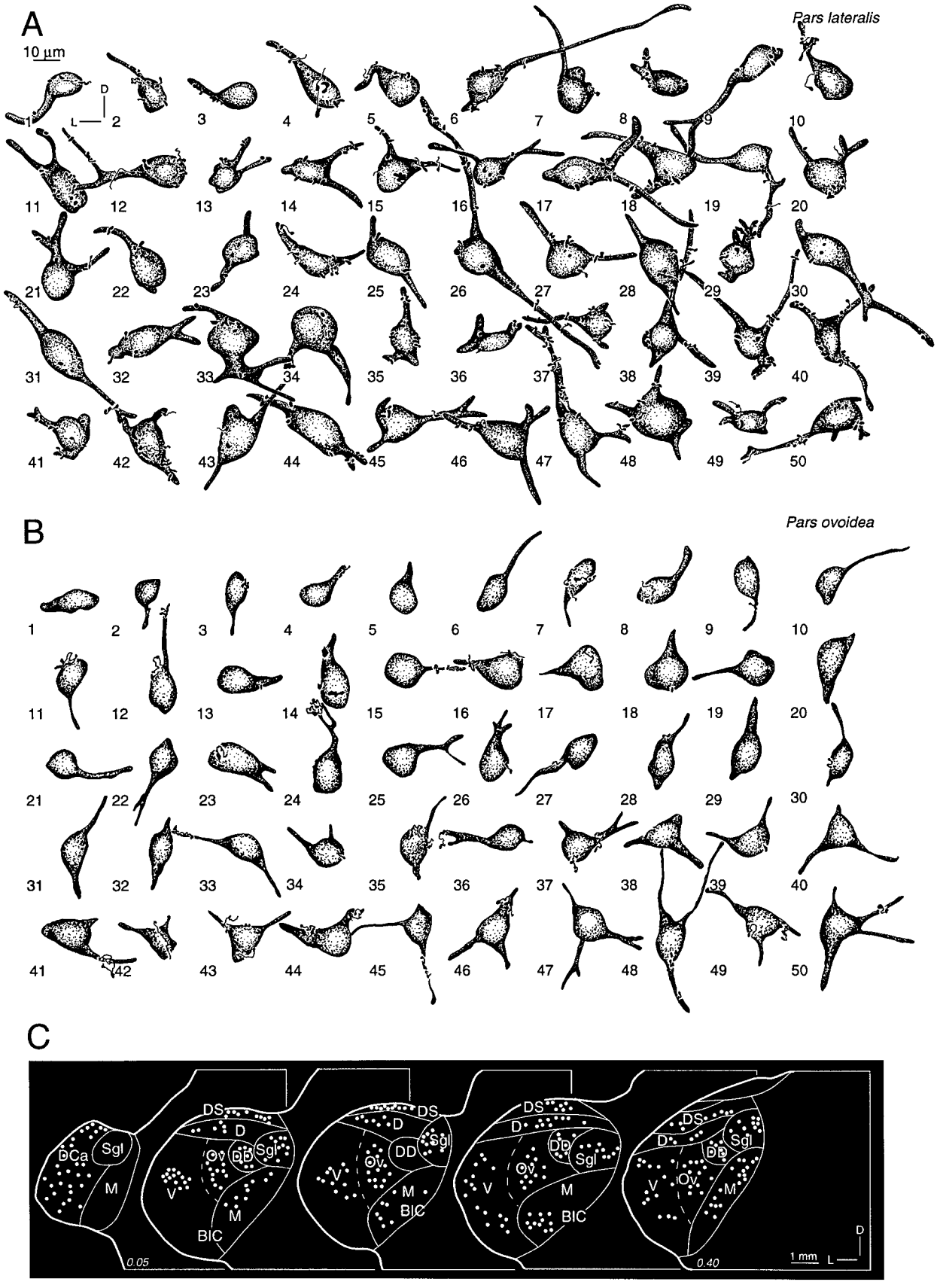


Fig. 3. Representative examples of ventral division glutamic acid decarboxylase (GAD) –immunostained neurons. **A:** In the *pars lateralis*, the neurons were slightly more variable than in the *pars ovoidea* (B). A few cells (4,27) had immunopositive puncta near their somata. Many dendrites were oriented along the mediolateral axis. Protocol for A,B and Figures 4, 5: Planapochromat, N.A. 1.32, ×1,250. **B:** *Pars ovoidea* neurons were more homogeneous than *pars lateralis* cells. Many of the former had processes oriented vertically, parallel to the dendrites of principal neurons. There were even fewer puncta near these neurons than near *pars lateralis* cells. **C:** Plots of the distribution of GAD-immunoreactive neurons in Figures 3–5. Each dot represents one neuron.

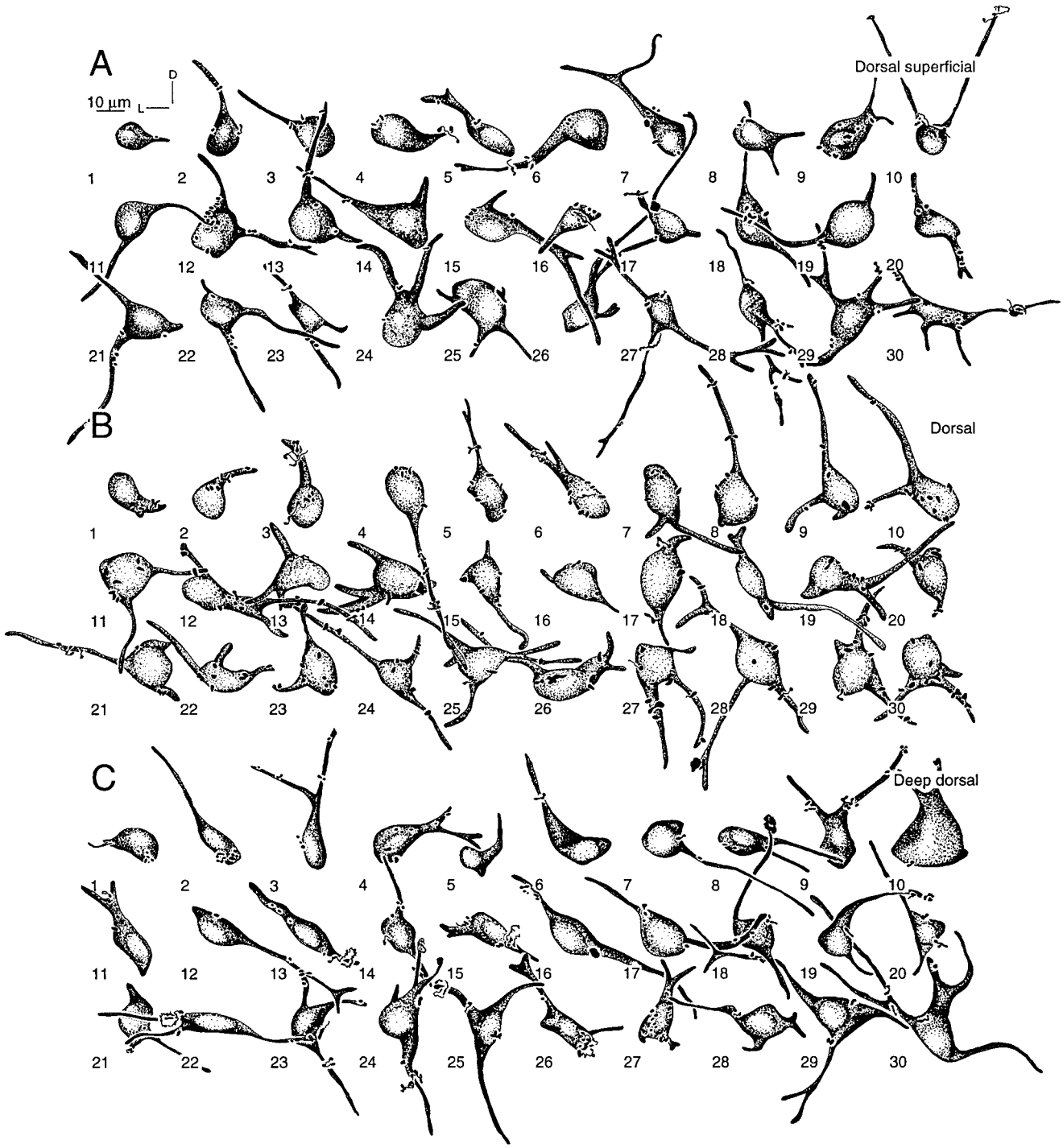


Fig. 4. Characteristic dorsal division glutamic acid decarboxylase (GAD) –immunoreactive neurons (see also Figs. 5A,B and Table 2). **A:** In the dorsal superficial nucleus, dendritic immunoreactivity often reached the first branch point (7,20,28). Many processes followed the long, mediolateral nuclear axis. Protocol as in Figure 3A. **B:** Dorsal nucleus neurons were similar in size to the dorsal superficial nucleus cells, and the puncta near their dendrites and somata ranged from small and delicate (23) to medium in size and coarse (26). **C:** Deep dorsal nucleus neurons were the smallest in the dorsal division (Table 2), with drumstick-shaped somata (7,12,28), or more complex varieties with long, immunostained dendrites (3,29,30).

geniculate body. Measurements were limited to this region because the myeloarchitectonic volume and complexity of the neuropil at the caudal and rostral faces of the auditory thalamus, as seen in fiber stained preparations, made such measurements in those regions difficult to interpret. The present measures excluded the brachium and its associated tracts, and represent a substantial part of the medial geniculate body.

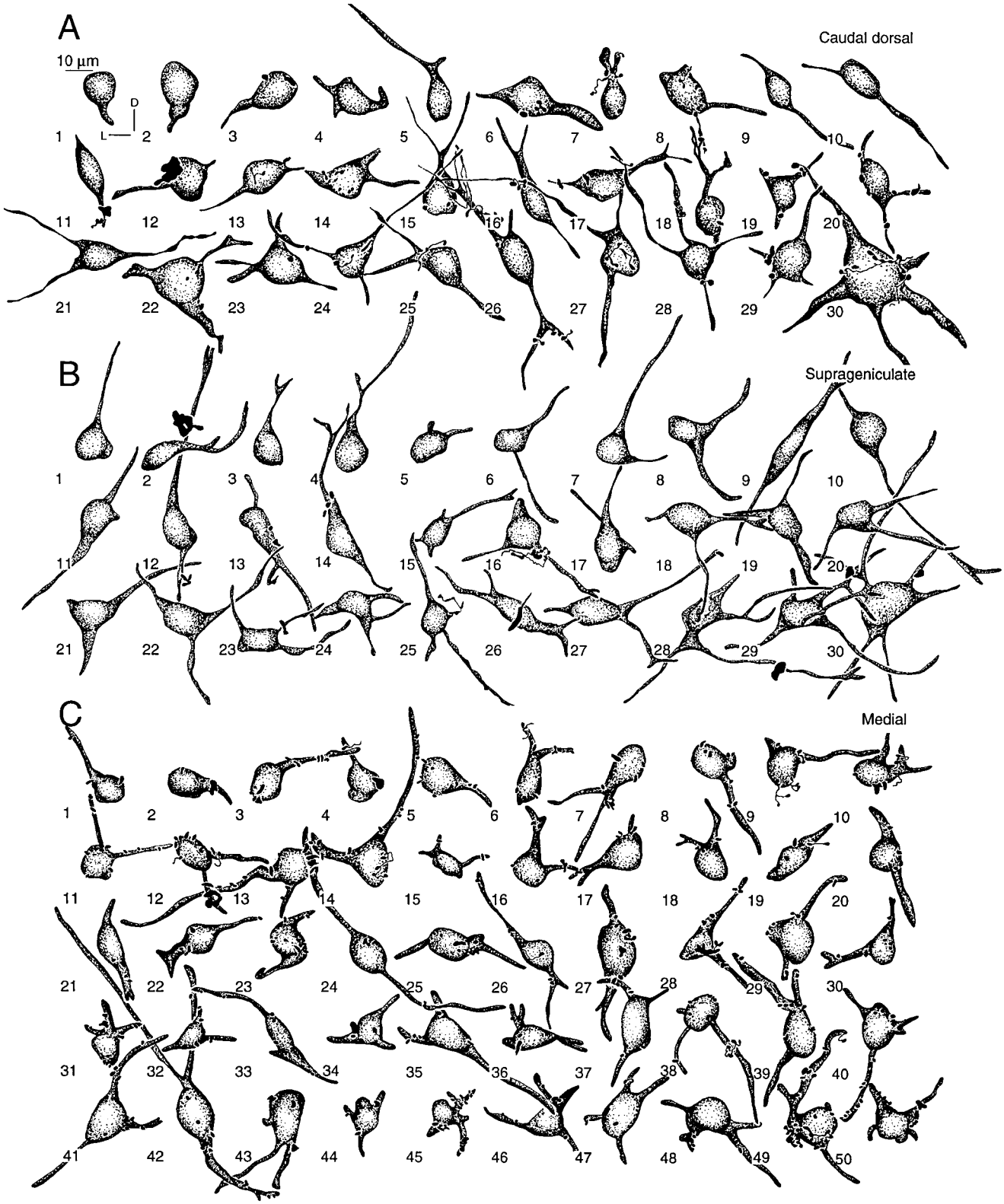


Fig. 5. Glutamic acid decarboxylase–immunopositive dorsal (A,B), and medial division cells (C). **A:** Caudal dorsal nucleus neurons were approximately 30% larger in average somatic diameter than those in other dorsal division nuclei (Table 2). Many had the drumstick-shaped perikaryon typical elsewhere in the dorsal division. Others were more complex, with immunopositive dendrites extending up to 50 µm (24,26,27), and some larger cells had fine perisomatic puncta nearby (6,8,22,30). **B:** Supragenicular nucleus neurons were the second-smallest of the five dorsal division nuclei (Table 2; Fig. 2D), and the neurons were heterogeneous in shape and size. The smallest (6–8) resembled those in the dorsal superficial (Fig. 4A: 1,2,5), dorsal (Fig. 4B: 2,9,19), and deep dorsal (Fig. 4C: 5,12,13) nuclei, whereas the largest (30) were second in size only to some caudal dorsal nucleus cells (Fig. 5A: 30). **C:** In the medial division, some large neurons were present (27,29,42), and cells with either a vertical (20,21,29) or a lateral (22,25,50) dendritic orientation occurred. The smallest neurons had few puncta nearby (15,30,44), and the larger cells (27,49) had a wider range (50). See also Figures 3, 4.

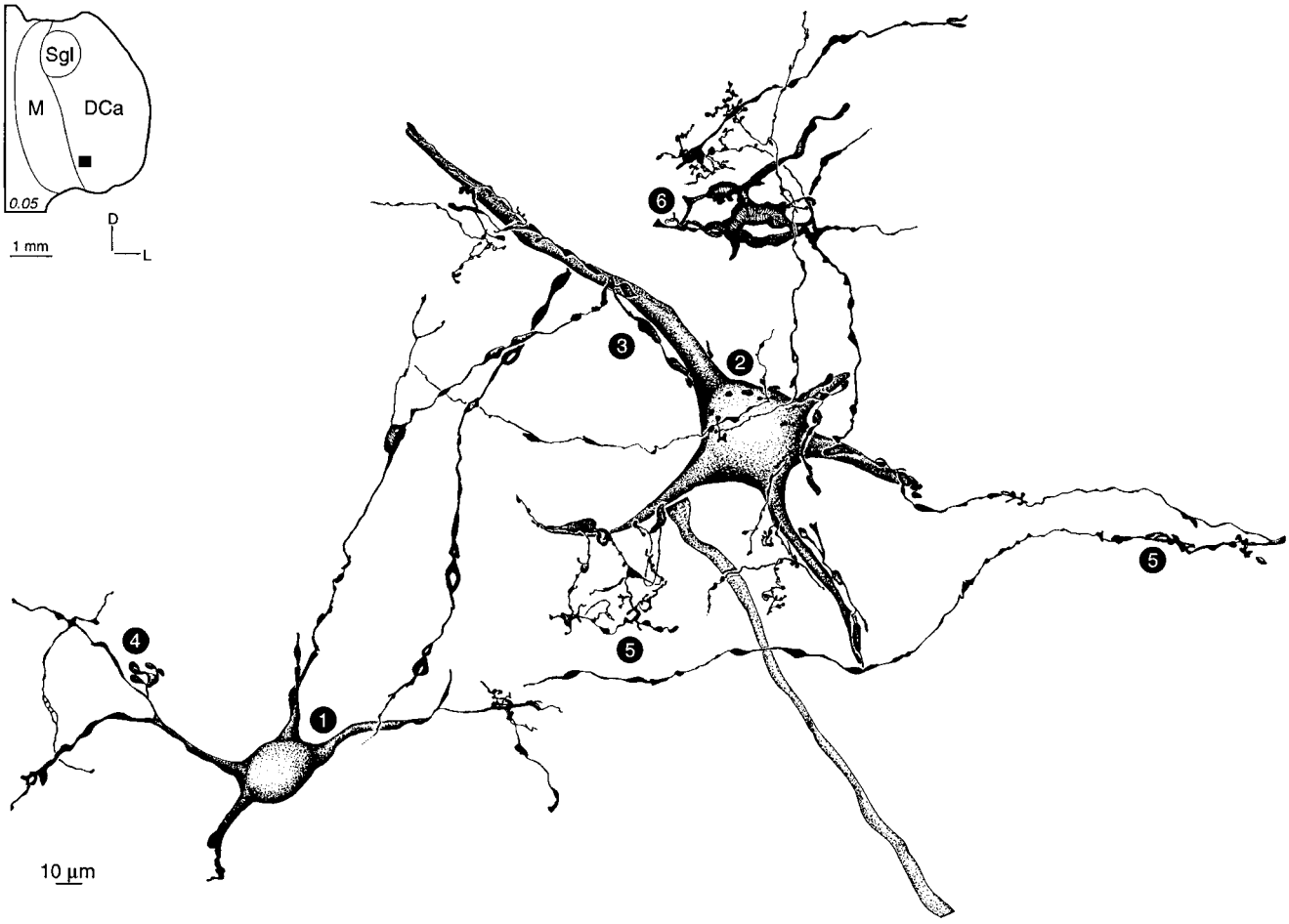


Fig. 6. Apposition of medium (1) and large (2) subvarieties of γ-aminobutyric acid–positive (GABAergic) neurons in the caudal dorsal nucleus (inset, DCa) showing some possible interrelationships in a glutamic acid decarboxylase preparation. The axon of the small type II cell projects among the proximal dendrites of the larger neuron, with some endings up to 4 × 10 µm. Finer grape-like clusters of puncta arose from the putative dendrites (4) of the small GABAergic cell (1). The finest immunopositive afferents formed lacy baskets along with some coarser puncta (5) near the larger GABAergic neuron (2). The largest profiles may have fenestrated processes that form complex arrays (6). Planapochromat, N.A. 1.32, ×1,250.

Neuronal size

Somatic measurements were made from semithin sections in plastic-embedded material. In each division, a sample of midnucleolar GABAergic populations (ventral and medial divisions, n = 100; dorsal division, n = 250) and 100 GABA-negative neurons from each division was drawn and their dendrites excluded. In each division, there was a significant somatic size difference ($P < 0.001$) between the two neuronal populations (t-test; Table 1). The distributions of GABAergic and non-GABAergic populations were largely non-overlapping (Fig. 2A,B), with GABAergic cells on average less than half the size of the GABA-negative neurons (see Figs. 10D: 2, 11B,C: 1 for exceptions).

Comparisons of the size of GABAergic neurons revealed statistically significant differences in two of the three divisions. Thus, dorsal division GABAergic neurons were approximately 20% larger than those in the ventral division, and medial division cells were approximately 15% larger than their ventral division counterparts (Table 1; Fig. 2C–E). However, dorsal division cells (Table 1; Fig. 2E) were not significantly larger than those in the medial division (Table 1; Fig. 2D). Among GABA-negative neurons, ventral division cells were the smallest and those in the medial division were the largest (Table 1).

A subset of GABAergic neurons in the dorsal division (approximately 20%; see Fig. 2E) and in the medial division (approximately 5%; see Fig. 2D) were unusually large and contributed to a bimodal, although not statistically significant, distribution of somatic size. This finding suggests that there might be more than one type of GABAergic neuron (Fig. 2A).

Varieties of GABAergic neurons

From the GAD preparations, 30–50 neurons were drawn from each division to show representative examples (Figs. 3–5). There were three reasons for including these data: (1) they provide independent confirmation of the neuronal types recognized in GABA material; (2) they revealed more of the dendritic configuration of the cells; (3) the superior immunopenetration showed the distribution of GAD-positive puncta in some detail.

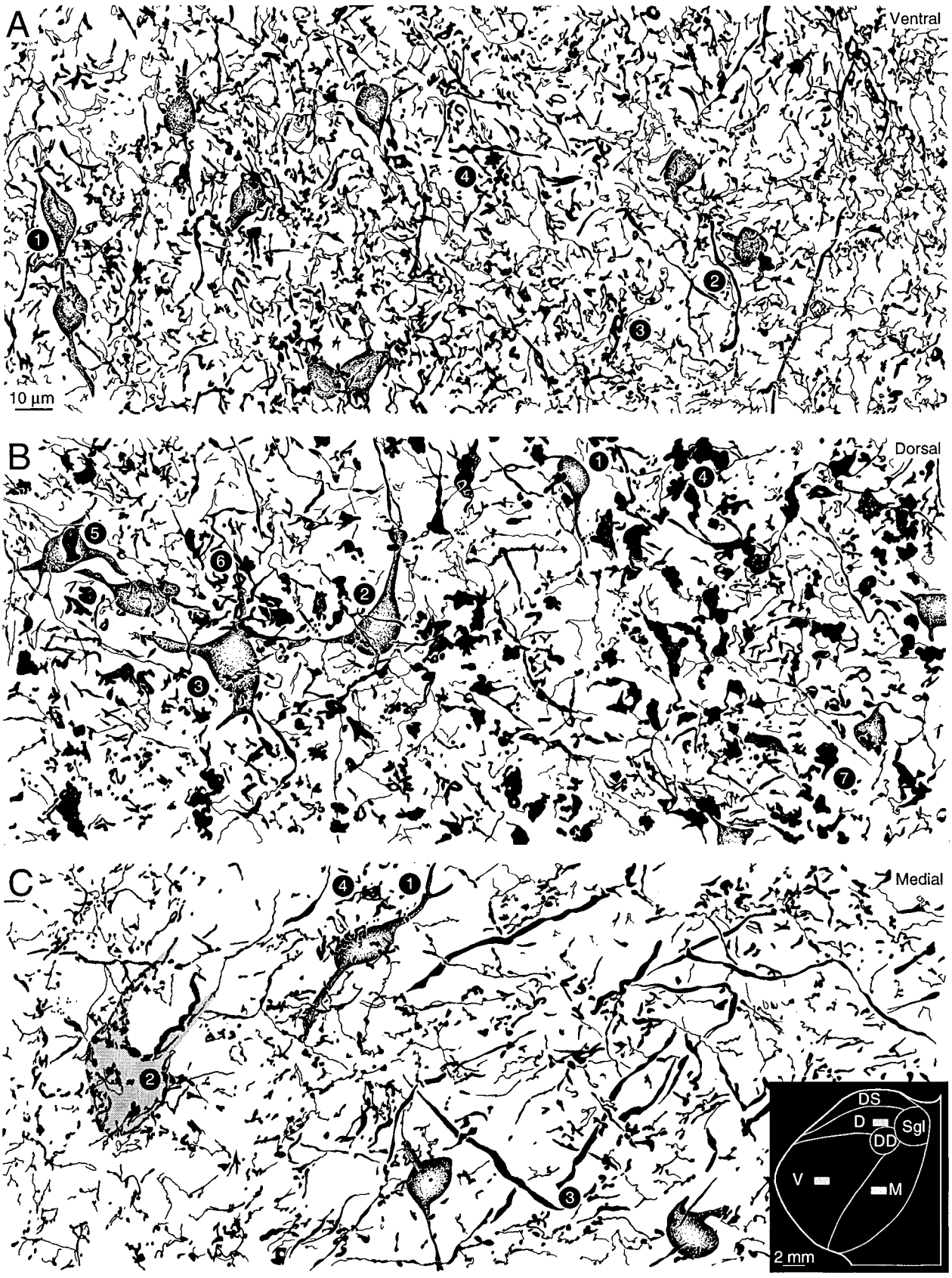


Figure 7

Ventral division. These neurons were the least variable in both the GABA (Table 1) and the GAD preparations (Fig. 3). The typical soma was oval and bipolar (Fig. 3A: 22; Fig. 3B: 18; Fig. 10A,B: 1), approximately 11 × 13 μm on its main axes (Fig. 7A: 1), with either a vertical (Fig. 3A: 7; Fig. 3B: 6) or a horizontal (Fig. 3A: 50; Fig. 3B: 19) orientation. Because of the thickness of the section and the criterion that the perikaryon was confined to it, these views probably included most of the principal (and proximal) dendritic trunks of these neurons, unless the immunoreactivity among these processes was variable. Most cells had two dendritic trunks that emerged from any part of the perikaryon (Fig. 3A: 16,26; Fig. 3B: 31,34) and were variable in thickness, ranging from approximately 2 μm (Fig. 3A: 15; Fig. 3B: 30) to 4 μm (Fig. 3A: 18; Fig. 3B: 50). A summary of the chief types of GABAergic neurons appears in Figure 12D.

Although the puncta (see below for details) were numerous in the ventral division (Figs. 7A, 8A), they were found less often near the perikarya of GAD-positive neurons (Fig. 9C: 1). Some of the largest neurons had no puncta in their immediate vicinity (Fig. 3A: 34,46; Fig. 3B: 17,48), others had a few (Fig. 3A: 29,30; Fig. 3B: 3,43), and a rare neuron had up to five (Fig. 3A: 24,42; Fig. 3B: 14,37). Small clusters of puncta near dendrites were sometimes noted (Fig. 3A: 10,38,47; Fig. 3B: 12,33,50).

Dorsal division. The mean somatic diameter of these GABAergic neurons was the largest in the medial geniculate body (Table 1). They were the most variable in size, and had a wider range in shape and a more heterogeneous dendritic architecture. Because there were regional differences among dorsal division nuclei, the results from five subdivisions were compared (Figs. 4, 5A,B).

GABAergic neurons in the dorsal superficial nucleus were often oriented mediolaterally, with their dendrites parallel to those of the tufted principal cells that are one of the primary cell types in Golgi material (Winer and Morest, 1984). The smallest immunopositive somata were approximately 100 μm^2 in area and had either a horizontal orientation (Fig. 4A: 1) or, more often, a multipolar, radiate configuration with 2–3 primary dendrites (Fig. 4A: 24) that may divide (Fig. 4A: 27). Some neurons had only one process apparent (Fig. 4A: 5,6) and others had up to five (Fig. 4A: 30). Many dendrites were slender (<2 μm in diameter), sparsely branched, bifurcated simply rather than forming tufts, and radiated without an obvious preference. The largest immunopositive perikarya were stellate shaped (Fig. 4A: 24,25,29), and their processes could cross the long axes of immunonegative tufted neuron dendrites. The cell size range in the dorsal superficial nucleus was smaller than that elsewhere in the dorsal division (Figs. 4B,C, 5A,B). These neurons had less dendritic immunoreactivity than ventral division neurons (compare Fig. 4A: 10,13,21–24 and Fig. 3A: 26,39,40).

A few dorsal superficial nucleus neurons had more puncta near their somata (Fig. 4A: 7,9,12) than others elsewhere in the dorsal division. These included some of the smallest cells (Fig. 4A: 16); the largest neurons had few such puncta near them (Fig. 4A: 24,25). Clusters of puncta near their dendrites as seen in the ventral division were uncommon.

The other dorsal division nuclei (dorsal: Fig 4B; deep dorsal: Fig. 4C; caudal dorsal: Fig. 5A; and suprageniculate: Fig. 5B) shared most of these features, including mainly small (approximately 150 μm^2) immunopositive somata (Fig. 4B: 5,16; Fig. 4C: 12,28; Fig. 5A: 13,19; Fig. 5B: 3,17), and about 2–3 slender primary dendrites (Fig. 4B: 20,28; Fig. 4C: 23,25; Fig. 5A: 21,27; Fig. 5B: 8,23); a few neurons had puncta near their somata (Fig. 4B: 6,10; Fig. 4C: 26,27; Fig. 5A: 6,14; Fig. 5B: 16,26). The chief differences among the dorsal division nuclei were the few unusually large neurons in the deep dorsal (Fig. 4C: 10), caudal dorsal (Fig. 5A: 30), and suprageniculate (Fig. 5B: 30; see also Fig. 5C) nuclei; the large endings in some subdivisions (Fig. 5A: 12); and the occasional aggregation of puncta near dendrites (Fig. 5B: 12,28).

Medial division. These neurons were slightly, but not significantly, smaller than dorsal division GAD-positive cells (Table 1: Medial = 158.6 μm^2, Dorsal = 167.9 μm^2). A cardinal feature was the heterogeneity in their dendritic structure and orientation. There were small neurons with radiating dendritic origins (Fig. 5C: 45), medium-sized cells oriented dorsoventrally (Fig 5C: 20) or horizontally (Fig. 5C: 25), or with both vertical and lateral processes (Fig. 5C: 17), neurons with intermediate orientations (Fig. 5C: 41), and larger cells with a corresponding range of diversity. The horizontal subtype was the rarest, whereas the vertical and multipolar neurons were the most common. Even the longest immunostained processes had few branches (Fig. 5C: 39,42).

The density of puncta/625 μm^2 was not statistically different from the value in the ventral division (Table 1), although it was the lowest of the three divisions. Many medial division neurons had at least some puncta near their somata, including small (Fig. 5C: 12), medium-sized (Fig. 5C: 7), and large (Fig. 5C: 27,50) cells. Immunonega-

Fig. 7. Glutamic acid decarboxylase (GAD) –positive puncta in the three principal divisions of the medial geniculate body (locus in C, inset). **A:** Ventral division immunopositive neurons (1) were small (approximately 10 × 12 μm in diameter), with a preferred vertical orientation (Fig. 3A: 16; 3B: 31). There were many GAD-positive puncta near their primary dendrites (2). Some puncta were approximately 1 μm in diameter and formed clusters in the neuropil. The puncta rarely were associated with GAD-positive somata. A second, larger and more globular profile with clusters, possibly of puncta, was also present (4). The neuropil was strikingly regular (compare with B,C), with a vertical orientation of the immunopositive neurons and the processes ascending toward them. Protocol for A–C and for Figure 8: Planapochromat, N.A. 1.32, ×1,250. **B:** Despite the broad somatic size range among dorsal division neurons (Table 2), most had drumstick-shaped somata approximately 10–12 μm in diameter (1); the full range contained slightly larger cells (2) and other neurons (3) with a stellate appearance. The puncta also distinguished the dorsal from the ventral (A) and medial (C) divisions because giant processes from approximately 4–10 μm on their long axes were numerous in the neuropil. These puncta had a complex substructure, with vacuolated regions that, in semithin material, might contain immunonegative dendrites (Fig. 12C: arrow). The profiles were elongated, formed short rows or clusters, and appeared to be interconnected serially (4). Giant puncta near somata were unusual (5), and a peridendritic locus was more common (6). Very fine preterminal processes, some with delicate boutons (7), made a lacy plexus in the dorsal division. **C:** Medial division GAD-immunoreactive puncta were larger and sparser than those of the ventral division (A), and smaller than those in the dorsal division (B). As in the other divisions, the small immunopositive soma was the most common neuronal profile (1). The medial division had some processes up to 4 μm thick that traversed the neuropil at oblique or acute angles (3; but see Figure 11A: arrow). The largest medial division puncta (4) were much smaller than the giant dorsal division profiles, and perhaps rarer than similar endings in the ventral division (A). An immunonegative principal cell soma had many puncta nearby (2).

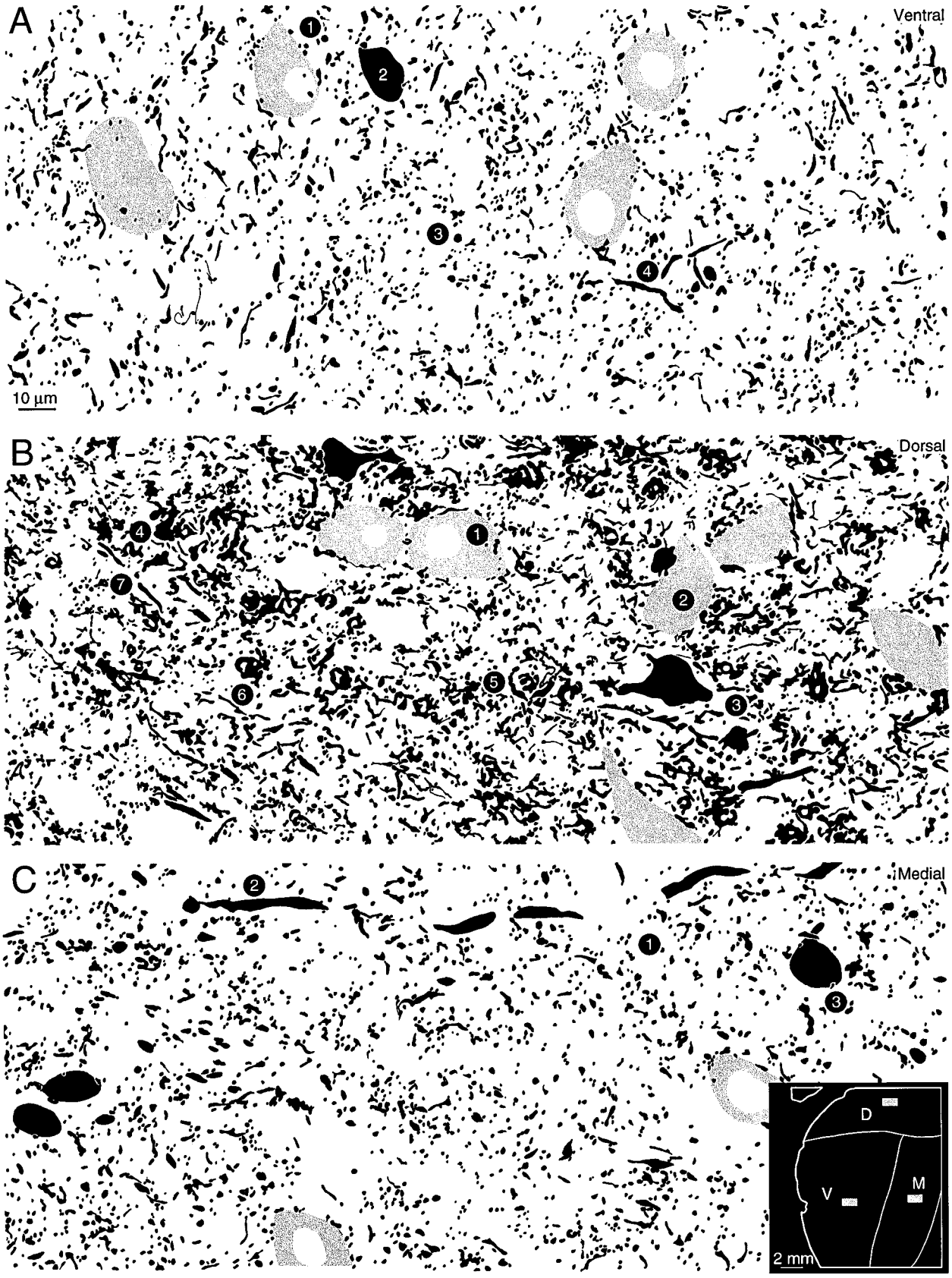


Figure 8

TABLE 1. Quantitative Summary of GABAergic Neurons in the Cat Medial Geniculate Body[1]

	Proportions[2] (in %)				Somatic area[3] (in μm²)					Puncta density[4]		
		Spatial distribution										
Division	Mean ± SD	A3.7[5]	A4.8	A6.0	Mean ± SD	Range	Overlap (in %)	n	GABA statistical comparison[6]	Mean ± SD	Range	Statistical comparison
Ventral												
GABA+	33.3 ± 4.5	33	29	38	140.9 ± 31.7	71–213	7	100	V = M?	41.4 ± 7.0	35–53	V = M?
GABA−					305.2 ± 53.4	181–434			$P = 0.00000000000018$[7]	—	—	$P = 0.34$
Dorsal												
GABA+	26.3 ± 10.3	29	35	15	167.9 ± 52.9	51–331	22[8]	250	D = V?	44.4 ± 12.4	32–72	D = V?
GABA−					352.9 ± 66.0	208–630			$P = 0.00000000039$[7]	—	—	$P = 0.60$
Medial												
GABA+	18 ± 15.9	6	12	36	158.6 ± 38.0	93–282	10	100	M = D?	37.8 ± 8.7	26–49	M = D?
GABA−					382.6 ± 97.3	205–762			$P = 0.015$	—	—	$P = 0.19$

[1]GABAergic, γ-aminobutyric acid positive. For other abbreviations, see list.
[2]Only neurons with nucleated profiles were counted. The Abercrombie-Floderus correction was applied to compensate for sampling from neuronal populations with different perikaryal sizes (Smolen et al., 1983). Two samples (n = 14,936, n = 2,590) had the same proportions.
[3]The soma from 100 GABA-positive and GABA-negative cells with midnucleolar profiles were collected from each division.
[4]Puncta density was collected from 10 25 × 25 μm random samples in each division and evaluated with two-tailed t-tests, which were used for all comparisons.
[5]Anteroposterior values were derived from atlas coordinates (Berman and Jones, 1982) and correspond approximately to the 15%, 35%, and 57% caudorostral intervals, respectively, through the medial geniculate body.
[6]Significance tests compared the somatic area of the GABAergic populations between the three divisions using multiple, two-tailed *t*-tests of equal variances to which the Bonferroni correction has been applied; the nominal α probability was set to 0.001 (Winer, 1971).
[7]Statistically significant.
[8]About 14% of the large neurons were found in DCa.

tive neurons had many more such puncta nearby (compare Fig. 7C: 1,2).

Puncta

These dot-like profiles had a characteristic structure and distribution in each of the primary parts of the medial geniculate body. When there were no quantitative differences between divisions, as in the comparison between the dorsal and the medial division (Table 1), qualitative features nevertheless distinguished them (compare Fig. 8B with C). Only oval or oblate profiles 0.5–3 μm in diameter from semithin sections prepared for GABA were used for quantitative purposes (Fig. 9D). The GAD material was the major source of qualitative observations on puncta size and shape.

Ventral division. In GAD-immunostained thick sections the ventral division neuropil had many immunopositive elements whose delicate texture set it apart from other divisions. Some elongated profiles, which may be either axonal or dendritic, were fine and slender, never more than 1 μm in diameter, and had a predominantly dorsoventral arrangement with occasional lateral branches emerging at right angles to the primary trunk (Fig. 7A: 2). Other much thinner profiles were the prominent in the neuropil, forming a delicate filigree that, once again, had both vertical and lateral components (Fig. 7A: 3).

The puncta were seen best in the 1-μm-thick semithin sections immunostained with GABA (Fig. 8A). Long, uninterrupted processes were rare, and the puncta had a granular quality. Most were approximately 0.5 μm in diameter; some formed clusters in the neuropil (Fig. 8A: 3), whereas others were scattered. A second, much less common, type was approximately 1 μm in diameter, coarser and more globular, and also clustered.

In addition to the puncta in the neuropil, both types of profile were associated with immunonegative (Fig. 8A: 1), but not GABAergic (Fig. 8A: 2), perikarya. This disjunction was not as pronounced in the GAD preparations (Fig. 7A), because immunonegative perikarya had less contrast and were virtually transparent, whereas the toluidine blue counterstaining in the semithin GABA sections revealed neural somata and enhanced the resolution of individual puncta. In contrast, immunopositive neurons rarely had more than 2–3 puncta near their perimeter. This observation was confirmed in other divisions (Figs. 7B,C, 8B,C).

Dorsal division. An unusual arrangement of puncta occurred in the nuclei of the dorsal division, where giant globular profiles were present both in GAD (Fig. 7B: 4) and GABA material (Fig. 8B: 4). Single profiles were up to 8 × 10 μm along their main axes and may be larger because they often extended through even thick sections. These puncta were numerous, e.g., there were more than 75 in the small and representative sample field (Fig. 7B) shown

Fig. 8. Semithin plastic-embedded section immunostained with γ-aminobutyric acid (GABA) (black profiles) and counterstained with toluidine blue to reveal immunonegative neurons (fine stippled profiles). In this material, puncta were revealed at the expense of other, longer processes (see Fig. 7A). **A:** Immunonegative neurons (1) were approximately 20 × 15 μm on their long axes, more than twice the area of GABAergic neurons (2; Table 1); the cells were aligned in dorsoventral rows that may correspond to fibrodendritic laminae (Morest, 1965). The puncta were primarily fine and granular (<2 μm in diameter; Table 3: Puncta) and similar in density to those in the dorsal (B) or medial (C) divisions (Table 1: Puncta density). Some immunonegative neurons had many puncta near their somata, whereas GABAergic perikarya usually had far fewer puncta nearby. Immunopositive processes longer than a few micrometers were rare (4). Protocol as in Figure 7A. **B:** As in the thicker sections immunostained for GAD (Fig. 7B), the most striking feature of the dorsal division neuropil was the giant puncta (4–6). GABA-negative neurons were more than twice the size of GABAergic cells (Table 1) and had either mediolateral (1) or dorsoventral (2) orientations. GABAergic neurons were also aligned on these axes (3; Fig. 4B: 5,15,17). Giant globular puncta were approximately 4–8 × 10–12 μm on their long axes (4) and, in favorable planes of section, they contained many finer elements (5). Sometimes they were fenestrated, with a hollow profile at their core whose size and immunonegativity suggest that they are dendritic. These puncta were rarely found near principal cell perikarya (2; Fig. 7B: 5). **C:** The medial division was distinct: it had only smaller puncta (1), these were sparser than those in the ventral division (A; Table 1), it contained the largest immunopositive and immunonegative neurons, and it had the lowest neuronal density. Some processes >4 μm in diameter passed mediolaterally, consistent with an extrinsic origin. The large, vacuolated regions in the neuropil contain immunonegative brachial axons.

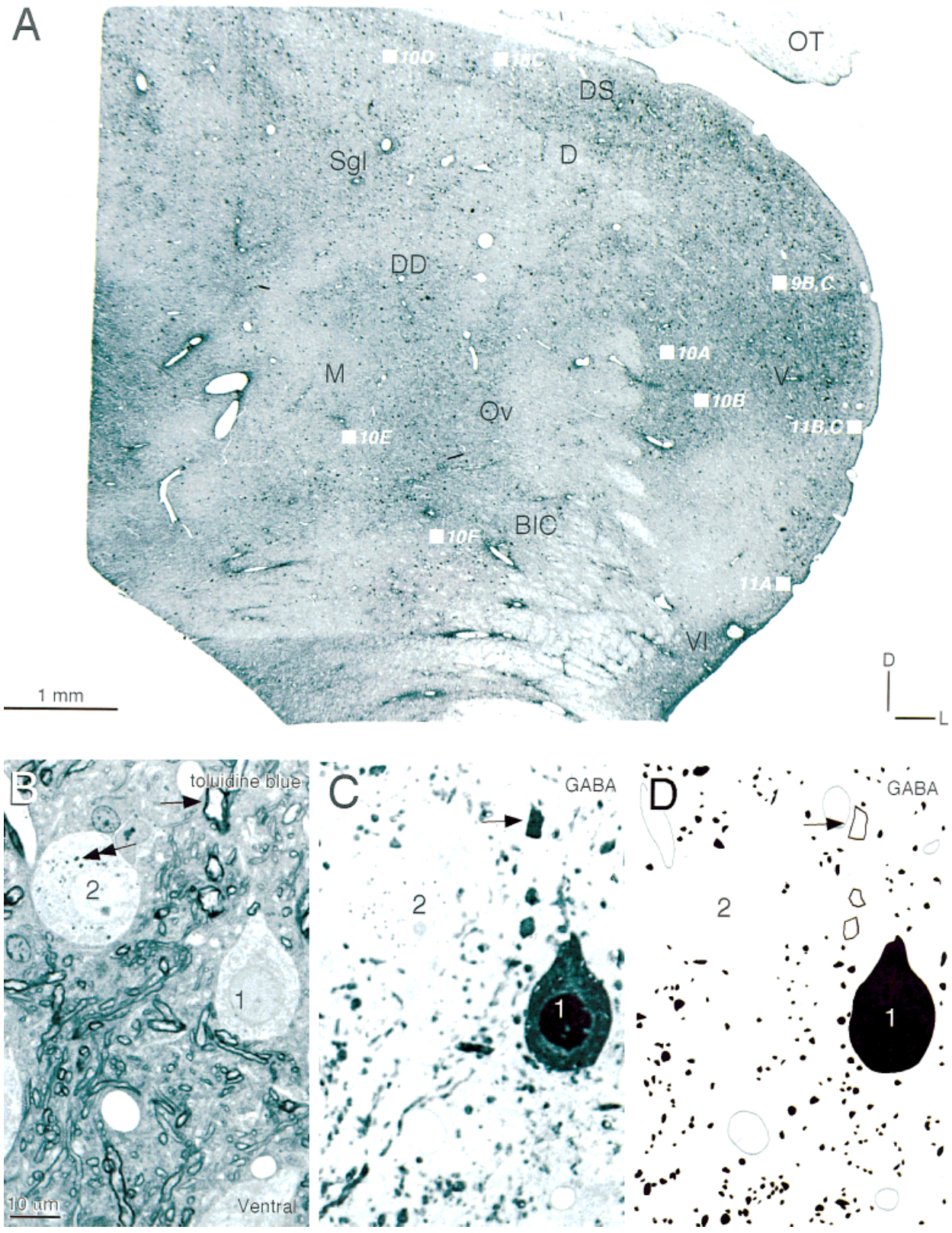


Fig. 9. Patterns of immunostaining. **A:** Photomicrograph of a typical 1 μm thick, γ-aminobutyric acid (GABA) –immunostained section from which quantitative estimates were made (Tables 1, 2). At this low magnification, the GABA positive (GABAergic) neurons were barely visible. The cell-poor brachium of the inferior colliculus (BIC) separated the *pars lateralis* (V) from the *pars ovoidea* (Ov). Architectonic boundaries were determined in adjoining, toluidine blue-stained sections (Figs. 10B,D,F, 11C). Boxes, loci of photomicrographs in Figures 9–11. Planachromat, N.A. 0.04, ×16. **B,C:** Pair of matched, serial semithin sections stained with toluidine blue (B) or GABA (C,D) after a large injection of horseradish peroxidase in the primary auditory cortex (AI), which labeled some ventral division neurons (2) and not others (1). Small intracytoplasmic granules of reaction product were seen in principal cells (B: double headed arrow), and many of the unlabeled cells were GABAergic (C: 1). A thick myelinated axonal profile in (B: arrow) was also GABAergic (C,D: arrow). **D:** Interpretation of C to show the structures classified as puncta (solid black). Profiles omitted were not included as puncta (outlines). There is approximately 5% discrepancy in the concordance between observers with regard to the identification of puncta. Protocol for B,C: Planapochromat, N.A. 1.25, ×788.

TABLE 2. Quantitative Summary of Ventral and Dorsal Division GABAergic Neurons[1]

				Somatic area (in μm²)		
Division	Subdivision	Mean ± SD	Range	n	Statistical comparison[2]	
Ventral	*Pars lateralis*	140.6 ± 26.4	78–204	64	V = Ov?	$P = 0.18$
	Pars ovoidea	132.1 ± 32.7	71–213	36		
Dorsal	Dorsal superficial	165.3 ± 44.7	104–299	50	DS = D?	$P = 0.91$
					DS = DD?	$P = 0.092$
					DS = DCa?	$P = 0.000000000012$[3]
					DS = Sgl?	$P = 0.023$
	Dorsal	167.0 ± 39.2	93–294	50	D = DD?	$P = 0.059$
					D = DCa?	$P = 0.00000000000024$[3]
					D = Sgl?	$P = 0.012$
	Deep dorsal	111.0 ± 38.7	51–230	50	DD = DCa?	$P = 0.00000000000000044$[3]
	Caudal dorsal	216.8 ± 45.0	136–331	50	DCa = Sgl?	$P = 0.000000000000000037$[3]
	Suprageniculate	133.3 ± 58.4	52–255	50	Sgl = DD?	$P = 0.53$

[1]GABAergic, γ-aminobutyric acid positive. For other abbreviations, see list.
[2]See footnote 6 on Table 1.
[3]Statistically significant.

here, and the dorsal division in its entirety, therefore, must contain thousands of them (Winer et al., 1999).

The processes that give rise to the giant profiles were only approximately 1–2 μm in diameter in both GAD (Fig. 7B: 7) and GABA (Fig. 8B: 3) material. The giant puncta themselves were elaborate and had a substructure filled with thorns, globules, and swellings that could represent clusters of smaller puncta. The central part of the profile often had a hollow space. In the plastic-embedded semithin sections counterstained with toluidine blue, this region contained a pale, unmyelinated process that was immunonegative and with a caliber suggesting that it may be dendritic (Fig. 10C, arrow). Such puncta were less common near the perikarya of either immunonegative (Fig. 8B: 2) or immunopositive (Fig. 7B: 5) neurons.

The giant puncta seemed to be less numerous in semithin sections (Fig. 8B) than in the GAD preparations (Fig. 7B), perhaps because of differences in section thickness. A second reason for their apparent enhancement in the GAD material was that far more of the processes associated with them were revealed, whereas in the GABA, the fine, puncta-sparse segments were rarely immunostained. Perhaps the abundant and apparently diffuse clusters of profiles in GABA material (Fig. 8B: 5) would be in continuity if the intervening processes were visible, as they are in the thicker GAD preparations.

In addition to the giant puncta, smaller profiles were also present in the neuropil and near the somata of immunonegative neurons (Figs. 7B, 8B). These puncta were less obvious in the GAD preparations, suggesting that some of the many fine puncta in the GABA material aggregate in the thicker sections by virtue of immunostaining of their intervening processes.

Medial division. The GABAergic organization in the medial division (Figs. 7C, 8C) was unique and featured a population of puncta larger than those in the ventral division (Figs. 7A, 8A) and smaller than those in the dorsal division (Figs. 7B, 8B). It alone of the three divisions had a significant number of thick GABAergic processes (Figs. 7C: 3, 8C: 2), some of which were up to 5 μm in diameter (Saint Marie et al., 1997), and these coursed mediolaterally, a feature consistent with an extrinsic origin (Winer et al., 1996). Other, finer processes, usually devoid of puncta, traversed the medial division neuropil (Fig. 7C).

The dominant medial division puncta were medium in size and globular, and they clustered in the neuropil (Figs. 7C, 8C: 1). These puncta were much smaller than even the fragments of the giant dorsal division puncta, and rarely as granular as those in the ventral division. As with most puncta in the medial geniculate body, they were associated, in descending order of density, with the neuropil, or near the somata of immunonegative neurons (Fig. 7C: 2) and, in rare instances, near the somata of immunopositive cells (Fig. 8C: 3). Their absolute number was indistinguishable among the three division (Table 1).

DISCUSSION

We address six interrelated themes. The first issue is the concordance of the present results with those of previous morphological and immunocytochemical studies of the cat medial geniculate body. This is followed by a consideration of possible physiological implications. Next, we assess the significance of species specific patterns of thalamic GABAergic organization. We then contrast the different patterns of organization seen in specific thalamic sensory and motor nuclei with one another. The features common to thalamic Golgi type II cells and the evidence for more than one variety of Golgi type II cell are assessed. We conclude by evaluating the consequences of these results for parcellations of the auditory thalamus.

Relation to prior morphologic and immunocytochemical results

Golgi studies. A key issue is whether the types of neurons identified in Golgi preparations can be related to the present, immunocytochemically identified profiles. In the ventral division, Golgi type II cells have a drumstick-shaped soma approximately 10–12 μm in diameter from which approximately three to four thin primary dendrites arise without preferential orientation (Morest, 1964). This class represents approximately 35% of ventral division neurons and, with the bushy tufted Golgi type I cells, constitutes the entire neuronal population in the ventral division (Morest, 1975). In semithin, plastic-embedded, GABA-immunostained material, the neuronal profile corresponding to this description was immunopositive and had a small (10–12 μm in diameter) soma with a highly invaginated nuclear envelope, scant cytoplasm, and fine primary dendrites. In contrast, virtually all of the immunonegative neurons were significantly (approximately 25–30%) larger, had smooth somatic contours, and thicker principal dendrites with fewer processes, and these often

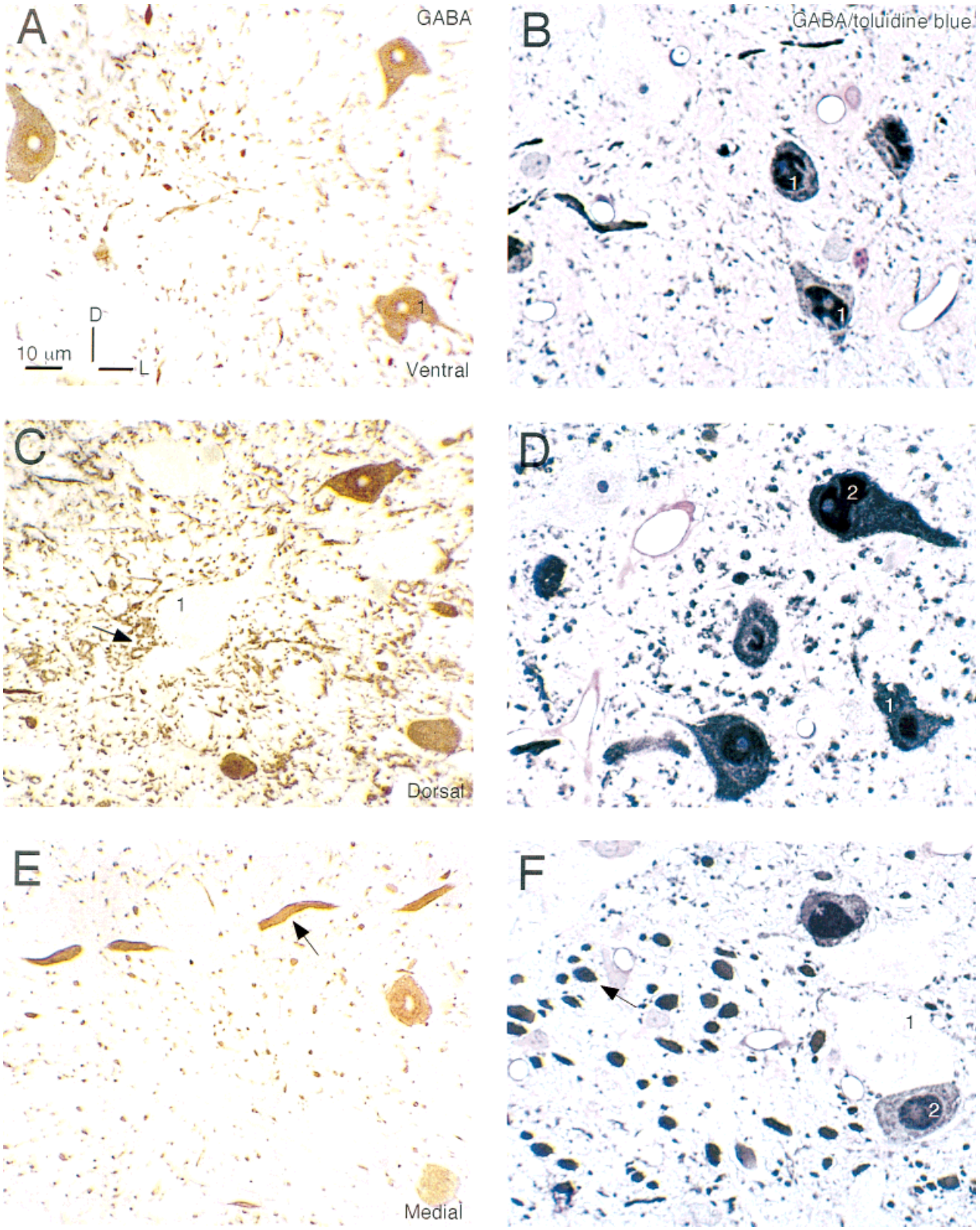


Fig. 10. Figures from sections prepared for γ-aminobutyric acid (GABA) alone (left) or for GABA and toluidine blue (right) from the three main medial geniculate body divisions. **A:** γ-Aminobutyric acid–positive (GABAergic) ventral division neurons were dispersed among immunonegative principal cells (see Fig. 9). In midnucleolar sections, the GABAergic cells had drumstick-shaped somata (1) from which one or two thin primary processes arose; many processes were parallel to the fibrodendritic laminar axis. Protocol for Figures 10, 11: Planapochromat, N.A. 1.25, ×750. **B:** In toluidine blue preparations the nuclear envelope of GABAergic cells (1) was irregular and invaginated, sometimes splitting the cytoplasm into lobules. GABAergic processes crisscrossed the neuropil and were varied in thickness. **C:** In the dorsal division, immunonegative somata (1) had many puncta nearby and clusters of puncta were often near the origins (arrow) or branch points of what may be dendrites. **D:** Both small (1) and large (2) varieties of dorsal division GABAergic neurons were present. The larger subtype lacked the highly invaginated nucleus found in ventral division GABAergic cells (B: 1). **E:** In the medial division, the plexus of GABAergic puncta was simpler than that in the ventral (A,B) and dorsal (C,D) divisions. Fibers up to 4 μm thick passed through the neuropil (arrow). **F:** Medial division GABAergic neurons (2) were much smaller than principal cells (1). Fascicles of large processes, which may be axons sectioned en face, were present (arrow).

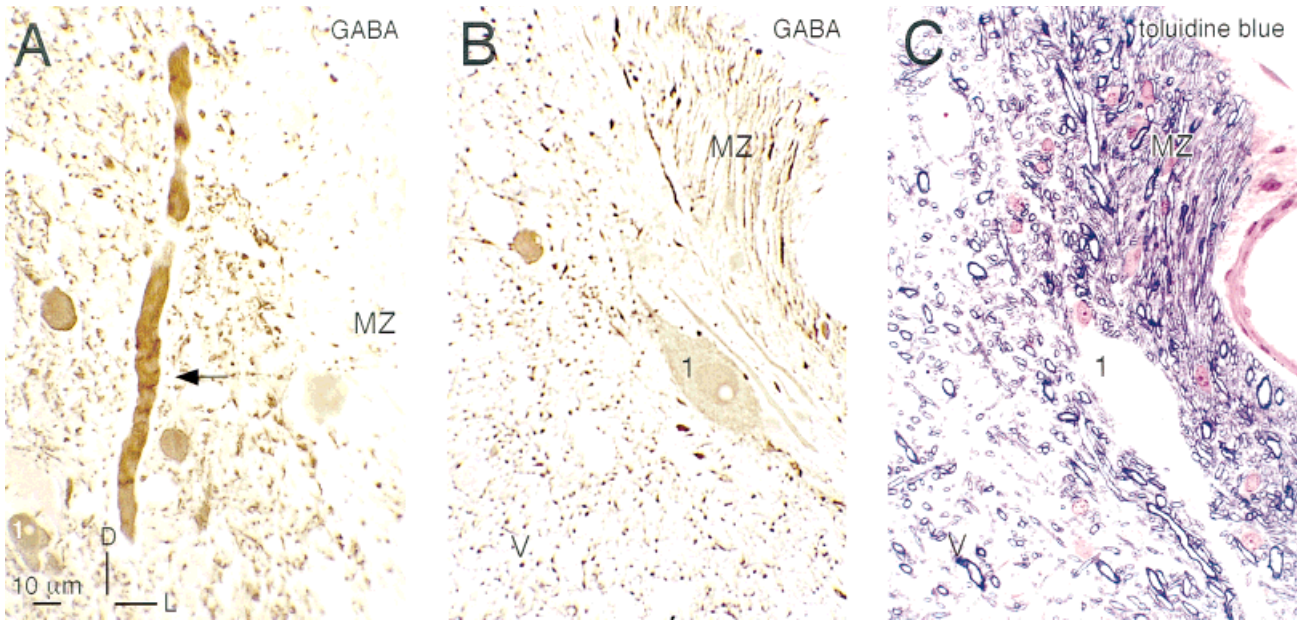


Fig. 11. Some unusual features of medial geniculate body γ-aminobutyric acid–positive (GABAergic) organization. **A:** Possible GABAergic axon approximately 10 μm in diameter (arrow) from the capsule of the ventral division in a semithin section. Changes in caliber may represent nodes of Ranvier. The source and target of these fibers is unknown (Fig. 13). Protocol for all panels appears in legend to Figure 10A. **B,C:** A GABAergic neuron (1) with a soma approximately 35 × 25 μm at the border of the marginal zone (MZ) and the *pars lateralis* (V) in immunostained (B) and toluidine blue–stained (C) sections. Only a few such cells were seen.

arose at the somatic poles. Given the many parallels between the profile of the Golgi type II cell and that of the immunostained ventral division neurons, we conclude that the correspondence between them is substantial.

In the cat dorsal division, two varieties of Golgi type II cell were recognized in rapid Golgi preparations (Winer and Morest, 1983b,1984). One was much smaller (again approximately 10–12 μm in diameter) and far more numerous; it had a thin (approximately 1 μm in diameter), unmyelinated and profusely branched local axon and slender and radiating dendrites with sparse, complex appendages. A second, much rarer Golgi type II cell had a larger soma (approximately 14–18 μm in diameter), thicker dendrites, longer and more variable spines, and a much thicker axon (approximately 2–3 μm in diameter) with fewer branches than the smaller variety (Fig. 12B). As in the ventral division, the correspondence between the Golgi-impregnated neuronal profiles and their immunostained counterparts was substantial. Both the large and small subvarieties were present in the GABA material and, as expected, the larger neurons were rarer (Fig. 4C: 10; see also Fig. 6: 2).

In the medial division, only a small Golgi type II neuron has been characterized definitively in rapid Golgi material (Morest, 1964; Winer and Morest, 1983a), and it resembled those described above in the ventral and dorsal divisions (in Winer, 1992, Table 6.1). The present, immunocytochemical results have identified this neuron readily, and, in addition, a second larger and rarer variety (Fig. 7C: 4) for which the corresponding, Golgi-impregnated neuron remains uncertain. There are several types of medial division neuron that might correspond to the large Golgi type II neuron (for example, tufted or elongated cells). However, too little is known about the structure and local distribution of their axon in the rapid Golgi preparations, and of their dendritic configuration in immunostained material, to permit more secure conclusions about their identity.

The correlations suggested above should be regarded as provisional rather than definitive for two reasons. First, no intracellular recordings in the medial geniculate body from Golgi type II/GABAergic neurons are available that could confirm independently their identity. Second, the results from studies of the lateral geniculate body, in which cells have been characterized physiologically and morphologically, suggest that certain neurons that might appear to be Golgi type II/class III/GABAergic on structural grounds are actually excitatory principal cells with an atypical morphology and that project to the cerebral cortex (Friedlander et al., 1980).

Immunocytochemical investigations. It is more difficult to relate the present, neurochemical results directly to previous studies (Table 3) for several reasons. First, few prior investigations of the medial geniculate body made an effort to relate the profiles of immunopositive neurons with those of Golgi-impregnated cells. Second, our quantitative results are based on semithin postembedded material and use the Abercrombie-Floderus correction, which was not applied in other investigations; when numerical estimates of the proportion of GABAergic neurons are not adjusted for somatic size differences, quantitative differences among studies cannot be compared directly. Third, in studies that used thick sections (>3 μm) for quantitative purposes, technical problems in assessing immunopenetration in these frozen- or Vibratome-sectioned preparations can complicate quantitative estimates of immunonegative and immunopositive neurons. Fourth, earlier work did not always specify the nuclear locus of the observations within the medial geniculate body. We have treated nuclear differences in neuronal subtypes and in puncta size, shape, and density as a primary datum. Fifth, we have validated

our results with different antibodies, whereas prior studies have often used one only.

The present results are largely in accord with those in an earlier investigation of medial geniculate body GABAergic organization (Rinvik et al., 1987). The salient points in both studies were that >20% of the neurons were immunopositive in each subdivision of the auditory thalamus, that there were qualitative and quantitative differences between divisions, and that there was a strong gradient of increasing immunoreactivity along the caudorostral axis of the medial division. An unusually large variety of type II cell also was recognized which may correspond to the class we have identified. Furthermore, in the dorsal division, "ring-like profiles" were described that seem closely to match the giant peridendritic GABAergic puncta in the present account in shape, although not in size.

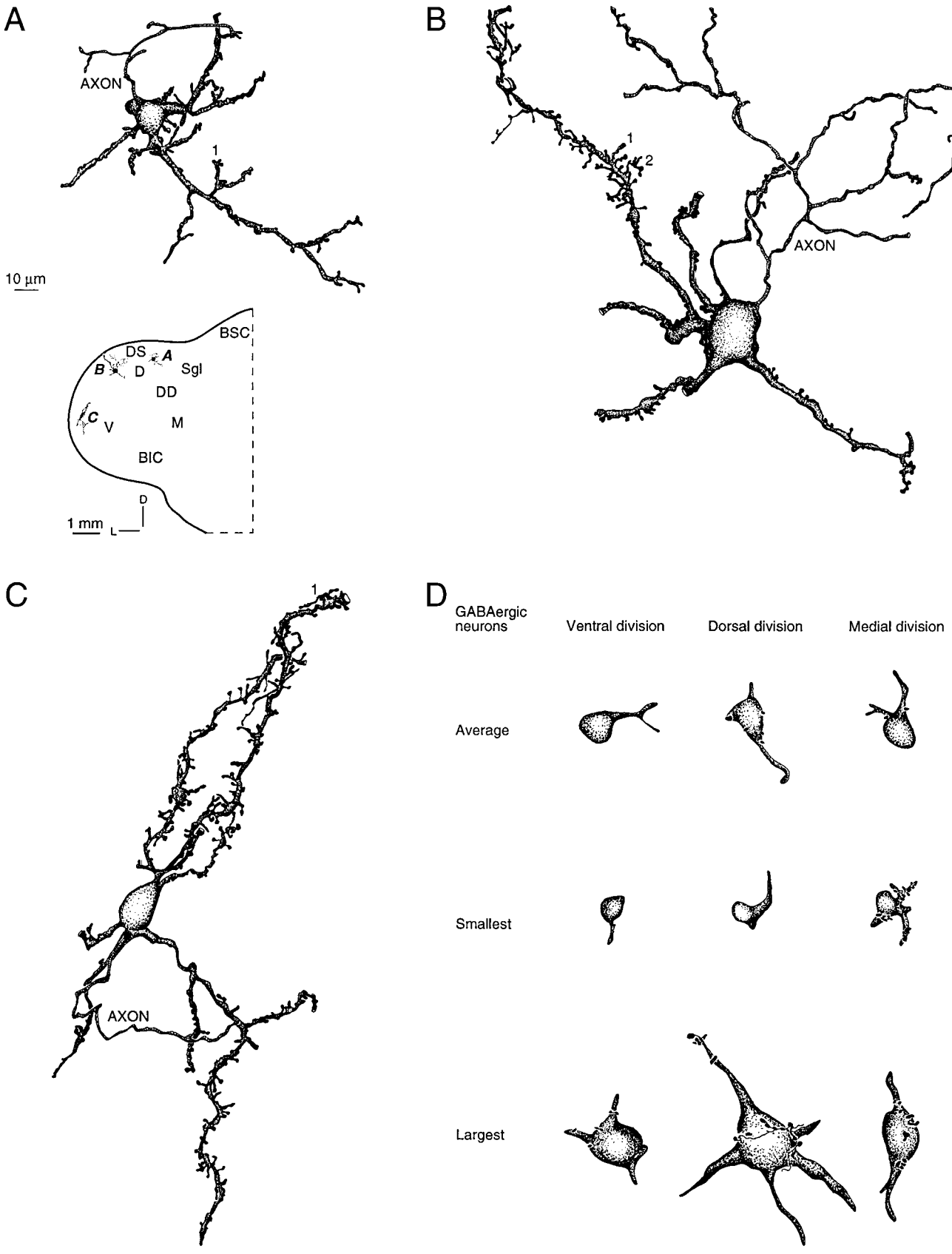
There are fewer points of concordance between the present results and those of another immunocytochemical study (Rouiller et al., 1990). Their maximum proportion of GABAergic neurons was much lower than the value in the present investigation (27 vs. 38%, respectively; see Table 3). They found only a modest range of change in this proportion, i.e., from approximately 18 to approximately 28%, along the caudorostral axis in the medial division at stereotaxic levels corresponding to those in the present study, whereas we found a far larger difference (Fig. 2F: Medial). Moreover, the giant, and possibly peridendritic, puncta in the dorsal division were not recognized. Finally, they reported a caudorostral gradient of GABAergic immunoreactivity for the ventral division that is the opposite of the present finding; the reason for the latter discrepancy is unknown. Some numerical differences may be attributable to methodological variables. We counted neurons from semithin sections only, and all estimates of proportions were adjusted with the Abercrombie-Floderus correction. In contrast, their numerical estimates were based on a comparison of adjacent, immunoreacted or Nissl-stained sections >5 μm thick, with no post hoc compensation for sampling bias due to mean caliper diameter. Differences in tissue penetration between semithin immunostained sections (present results) and the thicker, free-floating immunostained preparations and Nissl material in their study could account for all or part of the disparity.

Physiological implications

The proportion of medial geniculate body GABAergic neurons averaged 33% in this study, a value substantially larger than the 20% reported in the central nucleus of the inferior colliculus (Oliver et al., 1994), and the 25% seen in the auditory cortex (Prieto et al., 1994). With the exception of the dorsal nucleus of the lateral lemniscus, nearly all of whose neurons are GABAergic (Adams and Mugnaini, 1984), the medial geniculate body, at least in carnivores and primates (Winer and Larue, 1996a), may have the largest proportion of GABAergic cells in the auditory system (Aitkin, 1989) and among the highest number in the brain (Emson, 1983). Thus, the degree of local processing and the proportion of interneurons may differ across auditory synaptic stations, assuming that some approximate parity in the ratio of interneurons to their respective synaptic terminations is preserved from nucleus to nucleus. This finding suggests that GABAergic neurons must have a prominent role in medial geniculate body function. Intracellular recordings and iontophoretic studies that would clarify the role of interneurons are not yet available. Without these data, we can only endorse ideas already propounded as to how processing might be influenced by interneurons. These include the temporal modulation of spike activity of ventral division principal cells by means of axodendritic synapses, whereas the dendrodendritic endings might allow for extended regimens of recurring intraglomerular processing (Morest, 1971, 1975). In any event, the presence of only one variety of Golgi type II GABAergic neuron suggests that the local circuit organization within *pars lateralis* and *pars ovoidea* is likely to be similar, and that this pattern may differ from that in the dorsal and medial division, in which significantly different proportions and types of Golgi type II/GABAergic neurons occur (Table 1).

Physiological investigations of the dorsal division have revealed that its neurons respond sluggishly to tonal stimuli and more strongly to complex signals, that their tuning curves are broad and have lower Q_{10} dB values than those of ventral division neurons, and that slow changes in global excitability are a cardinal feature (Aitkin and Dunlop, 1969; Altman et al., 1970; Aitkin and Prain, 1974; Aitkin et al., 1981). Some of these attributes may be conferred by the input from the nucleus sagulum (Calford and Aitkin, 1983; Beneyto et al., 1998), and there is a direct GABAergic projection from the inferior colliculus (Peruzzi et al., 1997) that could play an inhibitory role once delegated exclusively to Golgi type II cells (Winer et al., 1996). Indeed, the totality of extrinsic GABAergic input from the inferior colliculus and the thalamic reticular nucleus (Montero, 1983) could approximate that from the GABAergic Golgi type II cells, suggesting that the collective impact of inhibition on thalamic processing must be enormous. In view of the coarse temporal coding and wide frequency tuning of dorsal division neurons, it can only be surmised that such processes either cannot depend on Golgi type II cells, or that the behavior of dorsal division

Fig. 12. Summary of results and comparison with Golgi studies. **A:** Typical Golgi type II cell from the dorsal division of the cat medial geniculate body; it had a small, approximately 10–12 μm in diameter drumstick-shaped soma, 3–4 main dendrites projecting in a stellate configuration, and a local AXON whose distal processes extended beyond the section. The somatodendritic profile of this cell resembled that of many immunostained dorsal division specimens (D: Average). 1, the dendritic appendages were sparse and longer than those of principal cells. Protocol for panels A–C: rapid Golgi impregnation. For A–D: Planapochromat, N.A. 1.32, ×1,250. Redrawn from Winer and Morest (1983b). **B:** A large Golgi type II (IIb; cf. Fig. 13) from the dorsal division. This cell was more than twice the somatic size of the small Golgi type II (IIa; cf. Fig. 13) dorsal division neuron, had a much larger and more complex AXON, and longer and more elaborate dendritic appendages than those of classic type IIa cells (A). Dorsal division stellate principal cells (not shown) were still larger, with many short dendritic appendages, and an unbranched axon. 1, long pedunculated dendritic spine. 2, complex multilobulated appendage. These irregular and contorted dendrites may be too small to be the source of the giant puncta (Figs. 7B: 4; 8B: 6). Redrawn from Winer and Morest (1984). **C:** A small stellate Golgi type II cell from the ventral division, with a characteristic drumstick-shaped soma, 3–4 main trunks parallel to the long axis of the isofrequency laminae, and with various dendritic appendages (1). The AXON formed only local branches and was thinner than that of the type IIb neuron (B). 1, the intermediate and distal dendrites have long, stringy processes. Redrawn from Winer (1992). **D:** Representative GABAergic neurons from the primary medial geniculate body divisions. The largest such cells were in the dorsal division, and the medial division had the widest absolute size range. Glutamic acid decarboxylase preparation, frozen sections. Scale bar in A applies to A–D.


A
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1
2
AXON
C
1
AXON
D
GABAergic neurons
Ventral division
Dorsal division
Medial division
Average
Smallest
Largest

Figure 12

TABLE 3. Comparison With Other Studies of Thalamic GABAergic Organization[1]

Species	Study	Nucleus	Antisera[2]			Amount (in %)	Percent/ Division			GABAergic neurons[5] (diameter or area)	Puncta[6]
			GAD[3]	GABA[4] Thick	GABA[4] Semithin		V	D	M		
Cat	Rinvik et al. (1987)	MGB	●	●		24–27	23	32	30	12–14, few 35 μm	Thicker and with more irregular outlines in (D)
	Rouiller et al. (1990)		●	●			7–28	—	0	9–19 μm	—
	Arcelli et al. (1997)		●	●	●		—	—	—	—	—
	Present study[3]		●	●	●	6–38	33	26	18	~12, rare 18 (D)* μm	1–3
	Fitzpatrick et al. (1984)	LGB	●			≈25	—	—	—	9–14 μm	—
	Rinvik et al. (1987)		●	●		12–27	—	—	—	12–15, few 35 μm	Light to dark, some large ring-like profiles
	Arcelli et al. (1997)		●	●	●	24–27	—	—	—	—	—
	Penny et al. (1983)	Vb	●	●		27–32	—	—	—	10–12 μm	—
	Spreafico et al. (1983)		●			19–21	—	—	—	≈10–14 μm	Darkly stained
	Rinvik et al. (1987)		●	●		20–30	—	—	—	12–15, few 22–26 μm	—
	Arcelli et al. (1997)		●	●	●	24–27	—	—	—	—	—
Rat	Muganini and Oertel (1985)	MGB	●			<5	—	—	—	—	Very few (D)–few (V, M)
	Winer and Larue (1988)		●			<1	—	—	—	V: 69 μm² D: 64 μm² M: 81 μm²	1–2
	Arcelli et al. (1997)		●	●	●	<1	—	—	—	—	—
Guinea pig	Asanuma (1991)		●	●		>10	—	—	—	Vb: 10–15 μm	Small
	Arcelli et al. (1997)		●	●	●	<1	—	—	—	—	—
Rabbit	Arcelli et al. (1997)		●	●	●	<1	—	—	—	—	—
Bat	Vater et al. (1992)		●	●		—	—	—	—	M: 7–11 μm	Variable
	Winer et al. (1992)		●	●		<1	—	—	—	V: 38 μm² D: 33 μm²	1–2
Monkey	Smith et al. (1987)			●		—	—	—	—		—
	Arcelli et al. (1997)		●	●	●	27–33	—	—	—	—	—

[1]GABAergic, γ-aminobutyric acid positive; MGB, medial geniculate body; approximate symbol; extrapolated/estimated data; —, not described; asterisk, from semithin sections. For other abbreviations, see list.
[2]GAD preparations were sectioned between 15 and 50 μm; GABA preparations were sectioned between 30 and 60 μm for thick sections or prepared from postembedded tissue sectioned at 1–1.5 μm (semithin sections).
[3]GAD-1440 (Oertel et al., 1981).
[4]GABA (DiaSorin; Stillwater, MN).
[5]In μm or μm².
[6]Puncta types: 1, <0.5 μm in diameter; 2, 0.5–2.0 μm; 3, >2 μm.

interneurons differs fundamentally from that of corresponding cells in the ventral division. The presence of the large Golgi type II cells, albeit in small numbers, could represent yet other forms of local circuit processing presumably unique to the dorsal division. The giant GABAergic puncta, many of which are 5 μm in diameter, are a significant departure from the far finer puncta predominating elsewhere in the medial geniculate body. Each of the six dorsal division nuclei has many of these profiles, and they are not present elsewhere in the medial geniculate body (Winer et al., 1998). They are often seen near large caliber, immunonegative dendrites and, thereby, may influence the behavior of principal cells, although ultrastructural confirmation is not yet available.

Medial division neurons have even broader tuning than cells in the dorsal or ventral divisions, they prefer complex or polymodal stimuli, and they show a degree of physiological plasticity not evident in other auditory thalamic nuclei (Aitkin, 1973; Calford, 1983; Gerren and Weinberger, 1983). These attributes certainly seem incompatible with the cycle-by-cycle fine temporal control of signal processing by GABAergic Golgi type II cells that has been hypothesized for the ventral division (Morest, 1974), and they leave open what role the larger GABAergic Golgi type II neuron might serve.

Comparative GABAergic arrangements in the medial geniculate body

Some anatomic features are common to all thalamic nuclei. These include projections to the cerebral cortex (Jones, 1985) which have varying degrees of topography (Jones, 1984), a substantial thalamocortical-corticothalamic reciprocity (Winer and Larue, 1987), and the prevalence of bushy tufted Golgi type I neurons as the main thalamocortical (Winer, 1984) and thalamosubcortical (Shinonaga et al., 1994) projection neuron. Other common denominators are that many of these neurons are glutamatergic (LeDoux and Farb, 1991 [rat]) and that their termination is specific to certain cortical layers (Jones, 1981), and, in the case of the medial geniculate body, they have a projection to the amygdala that targets specific nuclear subdivisions (LeDoux et al., 1985 [rat]). A further feature, i.e., the presence of a population of GABAergic Golgi type II cells, has a more variable and nucleus-specific expression. Because these numerical differences have been addressed by several studies (Ohara and Lieberman, 1993 [rat]; Arcelli et al., 1997 [rat, guinea pig, cat, monkey]), only the salient points are summarized below.

The proportion of GABAergic neurons in the medial geniculate body is species specific (Table 3), ranging from none (Ottersen and Storm-Mathisen, 1984 [mice]) to <1% (Winer and Larue, 1988 [rat], Winer et al., 1992 [mustached bat, *Pteronotus p. parnellii*]) to approximately 5% (J.A. Winer and D.T. Larue, unpublished observations [pallid bat, *Antrozous pallidus*]), to approximately 30% (present results), and to a substantial number in primates (Smith et al., 1987 [squirrel monkey]; Winer and Larue, 1996a [macaque monkey]). In avians, the few species studied suggest that the range is narrower, from none in chicken (Müller, 1988) to <1% in the barn owl (Winer and Larue, 1996b) nucleus ovoidalis, the thalamic homologue for the medial geniculate body. In the few reptiles so far investigated, GABAergic thalamic cells are rare or nonexistent in the nucleus reuniens, which is believed to be homologous to the medial geniculate body (Pritz and Stritzel, 1994; Pritz, 1995). In some instances, the paucity of GABAergic neurons is limited to the auditory thalamus:

in the case of the mustached bat and rat, for example, the inferior colliculus has many such cells, as does the cerebral cortex (Winer and Larue, 1996a). Perhaps numerical variability across species with regard to GABAergic auditory thalamic neurons is either a departure from some unknown ancestral pattern, or the comparative differences reflect regressive developmental events (Cowan et al., 1984) in which immature Golgi type II cells do not survive in some species. Although the present results cannot evaluate which of these scenarios is most plausible, they do predict that, in the absence or severe reduction in the number of Golgi type II cells, there should be a similar reduction in the concomitant ultrastructural features that accompany such cells, including dendrodendritic interactions (Morest, 1971), synaptic glomeruli (Ralston, 1971), or nests (Morest, 1975), and their associated intrinsic thalamic circuitry (Jones, 1985). This is in fact the case (Ohara and Lieberman, 1993; Arcelli et al., 1997). How these might affect thalamic processing is unknown.

We have speculated elsewhere that synaptic glomeruli could act as dynamic frequency-specific or aurally specific temporal filters adapted to the analysis of the fine structure of the stimulus, such as onset or offset behavior, or frequency or amplitude modulation (Winer and Morest, 1983b, 1984; Winer, 1985). Whatever their role proves ultimately to be, that they are largely absent in the auditory thalamus of many rodents, microchiropterans, and avians—and comparatively abundant in carnivores and primates—suggests a difference in processing regimens in the large brains of advanced mammals, although the nature of the difference is unclear. It would be interesting to know which pattern prevails in the auditory thalamus of Cetacea (Kruger, 1959) and whether it entails specific neural and/or psychophysical correlates. Because cetacean sensory neocortex appears to have some features considered as regressive such as a poorly differentiated layer IV, a substantial layer II, and unexpected overall thinness, perhaps the number of thalamic GABAergic Golgi type II cells would resemble the proportion in the rat more closely than the value in the cat or monkey (Morgane et al., 1985; Krubitzer, 1995).

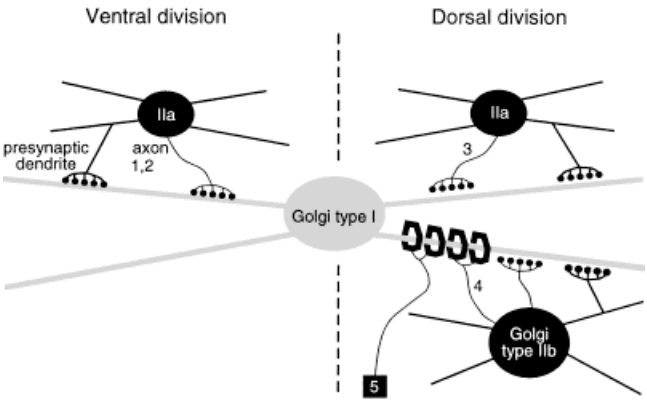

Fig. 13. Schematic diagram of documented and putative circuitry in the subdivisions of the cat medial geniculate body based on Golgi-impregnated material (Winer, 1992 [cat, bat, opossum, human]), electron microscopic investigations of synaptology (Morest, 1975), and the present, immunocytochemical, results. The ventral division (left side) contains primarily (1; Morest, 1975) small stellate Golgi type II (IIa) cells with axons presynaptic to principal neuron dendrites, and presynaptic processes that make dendrodendritic contacts (2; Morest, 1971). Predicted but unconfirmed synaptic relationships are indicated by a small gap. Similar arrangements may be present in the dorsal division: small type IIa cell make axodendritic and dendrodendritic contacts onto principal cells (3; Winer, unpublished observations), whereas the second, much larger Golgi type IIb neuron could form peridendritic rings near principal cell dendritic trunks (4; present results). Alternate sources (5) for the latter circuit include γ-aminobutyric acid–positive inferior colliculus neurons (Oliver et al., 1994) that project to the medial geniculate body (Winer et al., 1996) monosynaptically (Peruzzi et al., 1997), or axons from the thalamic reticular nucleus (Crabtree, 1998).

GABAergic organization in other thalamic nuclei

There is now a sufficient body of data available on the GABAergic organization of the mammalian thalamus to propose some basic principles in carnivores and primates (Table 3). First, the main sensory nuclei each contain at least approximately 20% GABAergic neurons (Spreafico et al., 1983; Winer, 1992). This finding suggests that there may be a core of GABAergic operations common to each modality, although their nature remains a matter of conjecture. Second, the proportion of GABAergic neurons in the lateral geniculate body seems somewhat more conserved across species (Hendrickson et al., 1983; Ohara et al., 1983; Fitzpatrick et al., 1984) than does the number for either the ventrobasal complex (Arcelli et al., 1997) or the medial geniculate body (Winer and Larue, 1996a). This conservation suggests differences among thalamic nuclei that might reflect evolutionary, functional, and modality specific constraints. Third, the vast majority of GABAergic neurons match closely the description of the classic type II cell in the lateral geniculate body (Guillery, 1966; Montero and Zempel, 1985), medial geniculate body (Morest, 1975; present results: Fig. 13A), and ventrobasal complex (Scheibel and Scheibel, 1966; Spreafico et al., 1983). This finding is consistent with the view that Golgi type II and GABAergic neurons represent the same neuronal population. Fourth, the sole exception to the latter generalization is the class of large Golgi type II cells in the dorsal division (Winer and Morest, 1983b), which is presumptively represented by the large GABAergic neurons in immunostained material (Rinvik et al., 1987; present results). This finding suggests that some nuclei may have unique GABAergic processes that the large type II cells subserve. Fifth, the thalamic reticular nucleus is a common source of GABAergic input to the main sensory nuclei in each modality (Jones, 1985). This is in accord with the prediction that principal nuclei receive some common types of corticoreticulothalamic modulation (Jones, 1975). Sixth, the various thalamic nuclei each have different patterns of extrinsic GABAergic input. Thus, there are substantial extrinsic GABAergic inputs to the medial geniculate body from the inferior colliculus (Winer et al., 1996), and from the pretectum to the lateral geniculate body (Cucchiaro et al., 1993). In contrast, the ventrobasal complex appears to lack such input entirely (De Biasi and Rustioni, 1990). This finding is congruent with the principle established in the auditory brainstem, where GABAergic and glycinergic neurons are as likely to be extrinsic projection cells as are glutamatergic or aspartatergic neurons (Winer et al., 1995). Seventh, the few descriptions of the thalamic puncta available suggest that they are subdivision specific in shape and perhaps in density (Winer et al., 1992). Perhaps synaptic density, and the proportion of GABAergic neurons, differs among nuclei. Eighth, none of the thalamic GABAergic neuronal populations appears to project outside the thala-

mus (LeVay and Ferster, 1979; Jones, 1985). This finding suggests that the thalamus represents a significant departure from the auditory brainstem in that its GABAergic neurons are entirely intrinsic, and this principle includes all modalities. Ninth, intrinsic GABAergic cells appear to have fewer GABAergic puncta near their somata than GABA-negative neurons (Winer et al., 1993). This observation may reflect a differential locus for inhibitory control of postsynaptic neurons. This finding is in contrast to the auditory brainstem, where GABAergic projection cells receive abundant axosomatic puncta (Winer et al., 1995).

Evidence for multiple types of thalamic interneuron

The results from Golgi studies (Winer and Morest, 1983b, 1984) and the present immunocytochemical investigations suggests that at least two populations of medial geniculate body interneurons exist: the small (type IIa) and the large (type IIb) varieties. The latter subtype (Fig. 12D) is found in the dorsal division (Fig. 2E) only, and its presence raises fundamental questions about how many subvarieties of type II neurons might exist. An immunocytochemical study of neurons in the cat ventrobasal complex found that a subpopulation of neurons with large somata (18.67 μm ± 2.45) and some smaller cells were GABAergic and nitric oxide synthase positive (Meng et al., 1996). It was estimated that approximately 3–4% of all neurons were nitric oxide synthase positive, a value that would seem consistent with the comparative rarity of the large Golgi type IIb neurons in our material. A similar argument for chemically specific subclasses of intrinsic neuron has been made in the rat lateral geniculate body (Gabbott and Bacon, 1994). If this numerical value can be extrapolated to the medial geniculate body, it could explain the comparative rarity of the Golgi type IIb neurons (Winer and Morest, 1984) and enhance a distinction between classes of such cells that, until now, has rested principally on morphological criteria (Tömböl, 1969). A clue to possible differences is the subsequent finding that nitric oxide synthase–immunoreactive material and GABA are present throughout ventrobasal complex local circuit neurons; if the diffuse nature postulated for nitric oxide–mediated effects and the more restricted, focal actions of type II–mediated presynaptic GABA release can be extrapolated to the present data, then such interneurons may have both modulatory and specific effects (Meng et al., 1997). It remains to be seen whether analogous patterns occur in the medial geniculate body.

Implications for subdividing the auditory thalamus

The present results would have limited relevance for thalamic organization if they could not be used as tools to confirm independently the identity of subdivisions revealed by architectonic and connectional methods (Winer, 1992). Thus, in some early studies, the dorsal division was considered not as a separate entity but as part of the larger principal division of the medial geniculate body (Rose and Woolsey, 1949, 1958). This scheme of classification or close variants of it have persisted until rather recently (Imig and Morel, 1985, their Fig. 1; Rinvik et al., 1987). In the present study, the dorsal division could be defined virtually by the presence of the giant GABAergic axon profiles alone (Winer et al., 1999). By the same token, many of the smaller nuclei within the dorsal division such as the deep dorsal and caudal dorsal nuclei have distinctive patterns of GABAergic organization (Fig. 2E). Other subnuclei, such as the *pars ovoidea* and the *pars lateralis*, are regarded as territorial subdivisions of a single nucleus, the ventral division, that contains a homogeneous neuronal population (Morest, 1964) and a continuous, although complex, arrangement of characteristic frequency (Imig and Morel, 1985). The latter nuclei are not distinguished from one another, either on the basis of neuronal size or the proportion of GABAergic neurons (Fig. 2C), or by their thalamocortical connections (Winer, 1992), but solely on their laminar organization. Moreover, in species and nuclei in which such architectonic features as the giant GABAergic puncta are absent, as in the rat dorsal division (Winer and Larue, 1988) and in the bat medial geniculate body (Winer et al., 1992), this absence suggests a species-specific patterns in auditory thalamic circuitry (Winer and Larue, 1996a) whose significance awaits physiological exploration.

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