THE JOURNAL OF COMPARATIVE NEUROLOGY 319:172–190 (1992)

Patterns of GABAergic Immunoreactivity Define Subdivisions of the Mustached Bat's Medial Geniculate Body

JEFFERY A. WINER, JEFFREY J. WENSTRUP, AND DAVID T. LARUE
Division of Neurobiology, Department of Molecular and Cell Biology, University of California, Berkeley, California 94720-2097

ABSTRACT

The anatomy and the spatial distribution of neurons and axonal endings (puncta) immunoreactive for glutamic acid decarboxylase (GAD) or gamma-aminobutyric acid (GABA) were studied in the medial geniculate body of the mustached bat (*Pteronotus parnellii*). The principal findings are that: 1) most GABAergic neurons are present in the dorsal and ventral divisions with few, if any, in the medial division; 2) only a small fraction, about 1% or less, of auditory thalamic neurons are immunopositive; 3) the density of immunoreactive puncta is independent of the number of GABAergic neurons in the thalamic divisions, with the ventral division having the largest number/unit area, the medial division about 75% of this value, and the dorsal division only about 50%; and 4) the form of the puncta was unique to each division, those in the ventral division being medium-sized and comparatively simple, those in the medial division predominantly large, coarse, and complex, while dorsal division endings were finer and more delicate. These patterns recapitulate, with some significant exceptions, those found in the rat and cat.

The puncta could originate from several sources; while many may arise from intrinsic GABAergic Golgi type II local circuit neurons, these cells may not be the only or even the principal source. Thus, the dorsal division contains comparatively many immunopositive cells though fewer puncta than might be expected if the bulk of these were to arise from auditory thalamic interneurons. This suggests that other, extrinsic sources, such as the thalamic reticular nucleus, may be the source of such endings. A second point is that the form and density of the puncta is regionally specific within the medial geniculate complex. These local patterns might have a significant and regionally specific role in controlling the differential excitability of auditory thalamic neurons. The distribution of presumptive synaptic endings also has implications for the number and arrangement of glomeruli or synaptic nests. Thus, these circuit elements, which are common to the thalamic nuclei in other species, might play an important role in local synaptic circuits between different types of cells. If so, then the structural variations embodied in these patterns could subserve functional arrangements that differ among species. Such patterns might reflect concomitant physiological differences in the organization of local circuits within the microchiropteran medial geniculate body.

Key words: GAD, comparative neuroanatomy, neuropil, interneurons, *Pteronotus parnellii*, bat

Gamma-aminobutyric acid (GABA) is one of the principal inhibitory neurotransmitters at virtually every synaptic station in the central auditory pathway, in neurons and in synaptic endings ranging from the cochlear nucleus to the cerebral cortex (Aitkin, '89). As such, it is localized both in neuronal perikarya and in axon terminals (puncta) whose distribution does not always covary (Winer and Larue, '88, '89). Iontophoretic studies of single neurons have shown that GABA agonists or antagonists can profoundly alter receptive field structure in rodent auditory brain stem neurons and may thus modulate local circuitry (Caspary et al., '85; Faingold et al., '91). Whether analogous morphological substrates for such effects exist in the auditory thalamus of a microchiropteran species is the primary question addressed by this study.

The mustached bat is an aerial predator that relies on echolocation to acquire its prey, accomplishing this task

Accepted February 1, 1992.

Jeffrey J. Wenstrup's present address is Department of Neurobiology, Northeastern Ohio Universities College of Medicine, 4209 State Route 44, P.O. Box 95, Rootstown, OH 44272.

with an auditory system enormously expanded and highly differentiated relative to other sensory pathways. While there is a considerable body of anatomical and physiological data on brain stem and cortical neurons and circuitry (reviewed in Suga, '84; Pollak and Casseday, '89), the same cannot be said for the medial geniculate body, for which the literature is much more limited and has concentrated largely on single unit studies (Olsen, '86; Olsen and Suga, '91a,b) or on combined physiological-connectional approaches (Frisina et al., '89; J.J. Wenstrup, D.T. Larue, and J.A. Winer, in preparation). This work has demonstrated a rich variety in auditory thalamic neuronal responses to acoustic input and a pattern of tectothalamic connections that in its main outlines resembles that found in other mammals, with certain significant exceptions. However, there are only sparse data on the intrinsic organization of the highly developed auditory thalamus.

The function(s) of GABAergic neurons and axon terminals in the auditory thalamus is unknown. The plentiful number of such elements in most other thalamic sensory and motor nuclei (Jones, '85) suggests that they play some fundamental role. The present study is the first of an interrelated series, and it is devoted to the arrangement of GABAergic neurons and puncta in various auditory thalamic subdivisions. Such data would be helpful in discerning whether GABA is a universal constituent in the mammalian auditory thalamus (Winer, '91, '92). It bears also on the question of how this distribution matches or differs from that in other species, and whether the mustached bat embodies homologous neuronal arrangements or reflects a significant departure from such a pattern (Morest and Winer, '86; Winer and Larue, '87). A second goal is to compare and reconcile the subdivisions derived on the basis of neuronal form (Winer and Wenstrup, '92a) and cytoarchitectonic grounds (Winer and Wenstrup, '92b) with those suggested by patterns of midbrain connectivity (Olsen, '86; Kobler et al., '87; Frisina et al., '89).

MATERIALS AND METHODS

Tissue preparation and immunocytochemistry

Adult mustached bats (*Pteronotus p. parnellii*) of either sex and weighing 10–15 g were captured at Windsor Cave, Jamaica. After acclimatization, they were anesthetized with sodium pentobarbital (60 mg/kg) and perfused through the heart preparatory to either glutamic acid decarboxylase (GAD) or GABA immunocytochemistry. For GAD, a peristaltic pump delivered the wash of about 25 ml of normal saline that preceded fixation, which used up to 125 ml of unbuffered 10% formalin with 0.5% zinc salicylate, pH 6.5, and at 20°C. The details of the procedure were similar to those in prior work (Mugnaini and Dahl, '83; Winer and Larue, '88). Briefly, pairs of 25-μm-thick frozen sections were collected, from which alternate GAD and Nissl series were prepared. Primary antiserum, sheep anti-GAD 1440 (developed by

Abbreviations

AD	anterodorsal thalamic nucleus
ALD	anterolateral division of the central nucleus of the inferior colliculus
AM	anteromedial thalamic nucleus
Am	amygdala
APt	anterior pretectum
BIC	brachium of the inferior colliculus
Cb	cerebellum
CIC	commissure of the inferior colliculus
CN	cochlear nucleus
CP	cerebral peduncle
CPu	caudoputamen
D	dorsal nucleus *or* dorsal division of the medial geniculate body
DC	dorsal cortex of the inferior colliculus
DMN	deep mesencephalic nucleus
DNLL	dorsal nucleus of the lateral lemniscus
DPD	dorsoposterior division of the central nucleus of the inferior colliculus
DS	dorsal superficial nucleus of the dorsal division of the medial geniculate body
EpN	entopeduncular nucleus
Ex	external nucleus of the inferior colliculus
Fim	fimbria
GP	globus pallidus
Ha	habenula
Hip	hippocampus
Hyp	hypothalamus
IC	inferior colliculus
INLL	intermediate nucleus of the lateral lemniscus
Int	intralaminar thalamic nucleus
Ip	interpeduncular nucleus
L	limitans nucleus
LD	lateral dorsal thalamic nucleus
LGBv	ventral nucleus of the lateral geniculate body
LL	lateral lemniscus
LMN	lateral mesencephalic nucleus
LP	lateral posterior thalamic nucleus
LV	lateral ventricle
M	medial division of the medial geniculate body
MB	mamillary body
MCP	middle cerebellar peduncle
MD	medial division of the central nucleus of the inferior colliculus
MRF	mesencephalic reticular formation
MZ	marginal zone of the medial geniculate body
PIN	posterior intralaminar nucleus
PL	posterior limitans nucleus
Pt	pretectum
Py	pyramidal tract
Ra	raphe
Ret	thalamic reticular nucleus
RN	red nucleus
RP	rostral pole subdivision of the medial geniculate body
Sag	sagulum
SC	superior colliculus
Sg	suprageniculate nucleus of the dorsal division of the medial geniculate body
SN	substantia nigra
Spf	subparafascicular nucleus
SpN	suprapeduncular nucleus
V	ventral nucleus *or* ventral division of the medial geniculate body
Vl	lateral subdivision of the ventral division of the medial geniculate body
Vm	medial subdivision of the ventral division of the medial geniculate body
Vpl	ventral posterolateral thalamic nucleus
Vpm	ventral posteromedial thalamic nucleus
VTA	ventral tegmental area
wm	white matter
I, II, III	cortical layers
IIIv	third ventricle
IVv	fourth ventricle
Planes of section:	
C	caudal
D	dorsal
L	lateral
M	medial
R	rostral
V	ventral

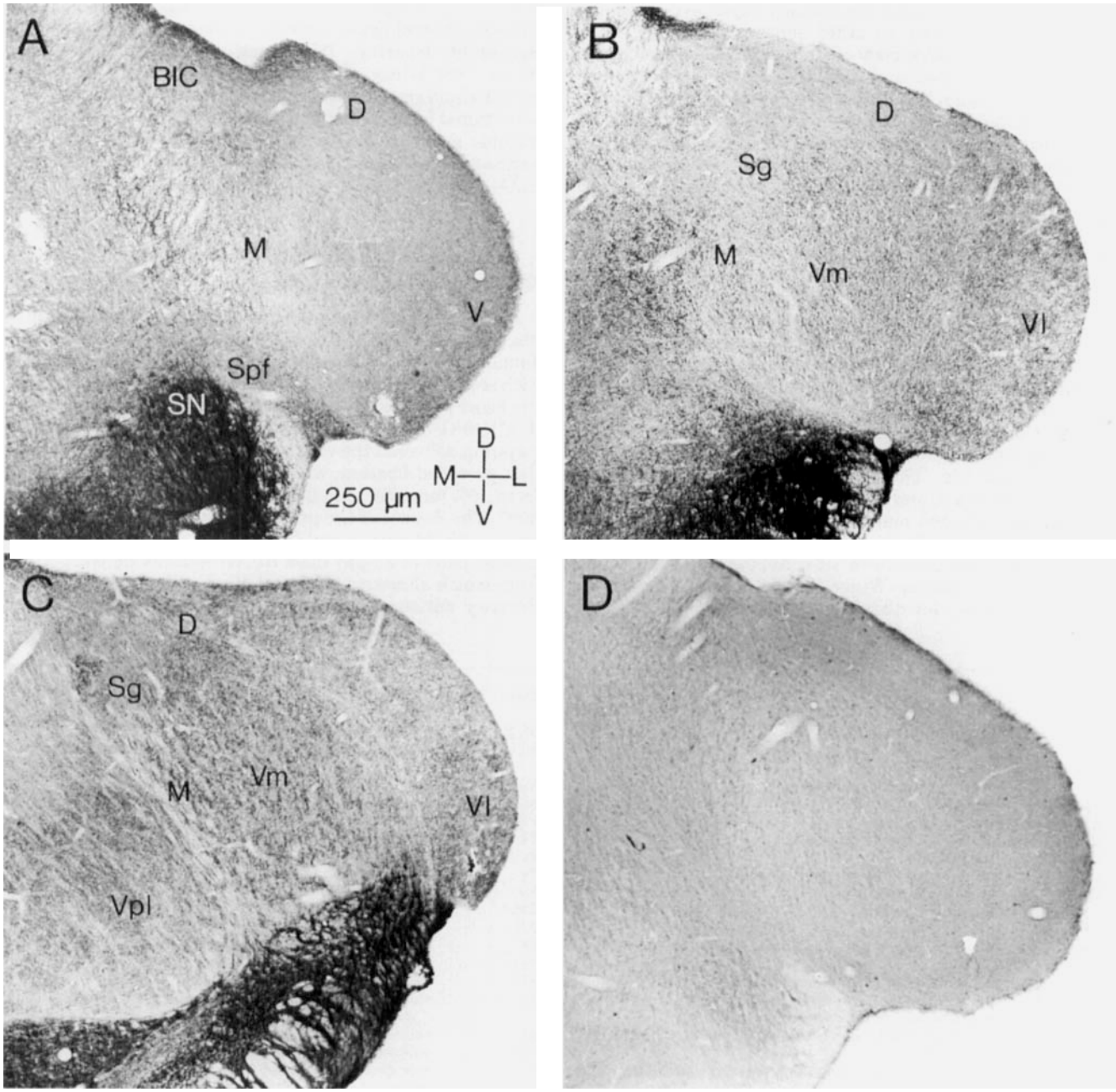

Fig. 1. Patterns of GAD immunoreactivity in the mustached bat midbrain and medial geniculate complex and in a control section. **A–C:** GAD immunoreactivity. **A:** At the caudal third of the auditory thalamus, showing moderately dense ventral division puncta (*V*), fine and sparse dorsal division puncta (*D*), and light immunostaining in the medial division (*M*). At this magnification, the few immunopositive medial geniculate neurons (see Fig. 3A–F) are not visible. Long segments of strongly immunoreactive dendrites are visible near the substantia nigra (*SN*). Protocol for panels A–D: planapochromat, N.A. 0.14, ×50. **B:** GAD immunoreactivity midway through the medial geniculate body, showing a lateral-to-medial gradient of decreasing ventral division immunostaining, the pale dorsal division pattern, and a concentration of prominent medial division puncta among immunonegative fiber fascicles. **C:** Near the rostral one-third of the auditory thalamus, showing distinct lateral (*Vl*) and medial (*Vm*) territories within the ventral division, continuation of the characteristic dorsal division pattern with an obvious regional specialization within the suprageniculate nucleus (*Sg*), and conspicuous, coarse medial division immunoreactivity. **D:** Control incubated in pre-immune serum and free of immunostaining.

Oertel et al., '81, '83) was used at 1:2,000 dilution. Controls were incubated either in preimmune serum or in buffer omitting primary antiserum. Immunoperoxidase labeling was accomplished with the peroxidase-antiperoxidase method (Sternberger and Joseph, '79) or the avidin-biotin technique (Hsu et al., '81); the latter proved most effective, and used the Vectastain avidin-biotin immunoperoxidase (ABC) kit ([Vector Laboratories, Burlingame, CA]; see also Roberts et al., '85). To enhance the contrast of the diaminobenzidine staining, the osmium-thiocarbohydrazide-

osmium method was used (Willingham and Rutherford, '84; see also Winer and Larue, '88 for details).

For the GABA immunocytochemistry, the animals were perfused with .01 M phosphate-buffered saline (PBS) for about 2 minutes followed by 4% paraformaldehyde and 0.1–0.25% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, at room temperature for 15 minutes. After immersion in 10% buffered sucrose overnight at 4°C, serial transverse or horizontal sections were cut on a Vibratome at 50 μm. From each set of three, two were incubated in rabbit-anti-GABA (1:5,000; Incstar, Clearwater, MN) and processed by the ABC technique, followed by the osmium intensification described above, while the third was stained for Nissl substance.

Data analysis

Neurons. Only cells that were indisputably GAD-immunoreactive (*GAD+*) or GABA-positive (*GABA+*) were included. Assignment of neurons to a particular architectonic subdivision was made independently from the Nissl preparations. These data were related to the disposition of neuronal populations identified in Golgi studies, and correlated with the results of tract-tracing studies of patterns of terminal input from physiologically identified inferior colliculus subdivisions upon the medial geniculate complex.

Puncta. Axon terminals were studied with oil-immersion, planapochromatic objectives. To be classified as puncta, the profiles must be oval or round; processes resembling dendrites were excluded. Size, shape, and concentration were noted, and also when possible, location, that is, perisomatic or in the neuropil. All observations were made in zones remote from architectonic borders. Densities were determined by using a grid with 25 × 25 μm squares on representative areas whose architectonic borders were verified in the adjoining Nissl preparation. Each average included four such 625 μm^2 samples; the standard deviation within a set was ±5%.

Quantitative analysis. Perikaryal areas were measured by using SigmaScan on a digitizing tablet (Jandel Scientific; Numonics) whose output was used to generate histograms and as the basis for statistical comparisons on an IBM PC AT computer.

RESULTS

The pattern of immunostaining was readily comparable in the GAD (Figs. 1A–C, 2A–F, 3A,C,E, 4–6, 10) and GABA (Figs. 2G–L, 3B,D,F,G–I) material. In both preparations, comparatively few neurons were immunoreactive (Figs. 1, 2) and their locus within the medial geniculate complex was virtually identical. A second, independent index of the comparability between the antisera was the form and locus of immunoreactive terminals (puncta) in the neuropil, where distinct patterns were readily distinguished in the ventral (Figs. 3G, 4), dorsal (Figs. 3H, 5), and medial (Figs. 3I, 6) divisions, and where regional variations were evident [for example, the high concentration of *GAD+* axon terminals in the rostral pole of the suprageniculate nucleus (Fig. 1C:*Sg*)]. Control material had no specific immunostaining (Fig. 1D).

Neurons

Surveys of the medial geniculate complex revealed that single sections had only a few immunostained neurons (Fig. 2), even when many immunopositive extrathalamic neurons were present. Immunostained medial geniculate neurons were found in declining numbers in the dorsal, ventral, and medial divisions (Fig. 2B,J), respectively. The borders of thalamic subdivisions were defined from the adjoining Nissl preparations (Figs. 2A,C, 7A,B; cf. Winer and Wenstrup, '92b).

Single sections rarely had more than 15 *GAD+* or *GABA+* medial geniculate neurons. Most sections had at least one to two neurons; in some sections with otherwise excellent neuronal immunostaining in nonauditory centers, there were no immunopositive auditory thalamic neurons.

The morphology of *GAD+* or *GABA+* neurons within each medial geniculate subdivision in different experiments was comparable (Fig. 3A–F). In the ventral division, these neurons had a spindle- or drumstick-shaped perikaryon whose width was about half of their length, and from whose somatic pole(s) sparse, slender dendrites 1–3 μm thick issued. There was little dendritic immunoreactivity past the initial 30–40 μm. While their branches could sometimes be followed farther in favorable preparations, they were never immunostained to the extent routinely achieved in the rat (Winer and Larue, '88) and cat (Winer, '91). Some immunopositive dendrites had beaded profiles (Fig. 3C: *left side*), while others were much smoother (Fig. 3F). The modest degree of dendritic immunoreactivity does not permit any conclusion about cellular orientation of the *GABA+* cells relative to the arbors of the tufted, immunonegative neurons. However, the long somatic axis of *GABA+* neurons often followed a dorsomedial-to-ventrolateral arc, parallel to the main dendritic arbors of the larger, non-GABAergic, presumptive thalamocortical relay neurons (Winer and Wenstrup, '92a).

Three features—number, orientation, and size—distinguished immunopositive dorsal division neurons from their counterparts in the ventral division. Thus, such neurons were comparatively common in various nuclei of the dorsal division, and sometimes formed small clusters of two to four cells. Their relative number was accentuated by the modest size of mustached bat dorsal division nuclei (Winer and Wenstrup, '92b) compared to those in other species (Winer, '84, '85, '91, '92; Winer and Larue, '87, '88). Few immunopositive cells were noted in the suprageniculate

Fig. 2. Distribution of GAD- or GABA-immunoreactive somata in and near the medial geniculate body. **A–F:** In transverse sections, many *GAD+* somata occur in the dorsal division (*D*), fewer are evident in the ventral division (*V*), and the medial division *M* is virtually devoid of such neurons. While the rostral pole nucleus (*RP;* **E,F**) is included here within the territory of the ventral division, it may actually belong to the dorsal division on the basis of its connections with the inferior colliculus (Wenstrup and Winer, '87) and cytoarchitecture (Winer and Wenstrup, '92b). If so, then there is a relative paucity of GAD-positive (E,F) or GABAergic neurons except in its most superficial aspects (**H–L**), which contrasts with their comparatively higher density in the dorsal nuclei proper. The **inset** (*black figurine*) shows the approximate anteroposterior locus of the six sections imposed on a horizontal silhouette of the auditory thalamus. Slight inconsistencies between the plane of section and the absolute position of a nuclear border reflect individual variations in the thalamic nuclear configuration or plane of section in the two bats from which this and the other figurine were derived. **G–L:** In horizontal sections, the number of *GAD+* neurons is greatest in the dorsal division, smallest in the medial division, and intermediate in the ventral division. More ventral sections contain progressively fewer immunopositive neurons. Compared to the thalamic intralaminar nuclei (*J*: Int) or the thalamic reticular nucleus (*K*: Ret), few medial geniculate neurons are immunopositive. Planapochromat, N.A. 0.32, ×85. The **inset** shows the approximate dorsoventral locus of the six sections projected on a transverse silhouette.

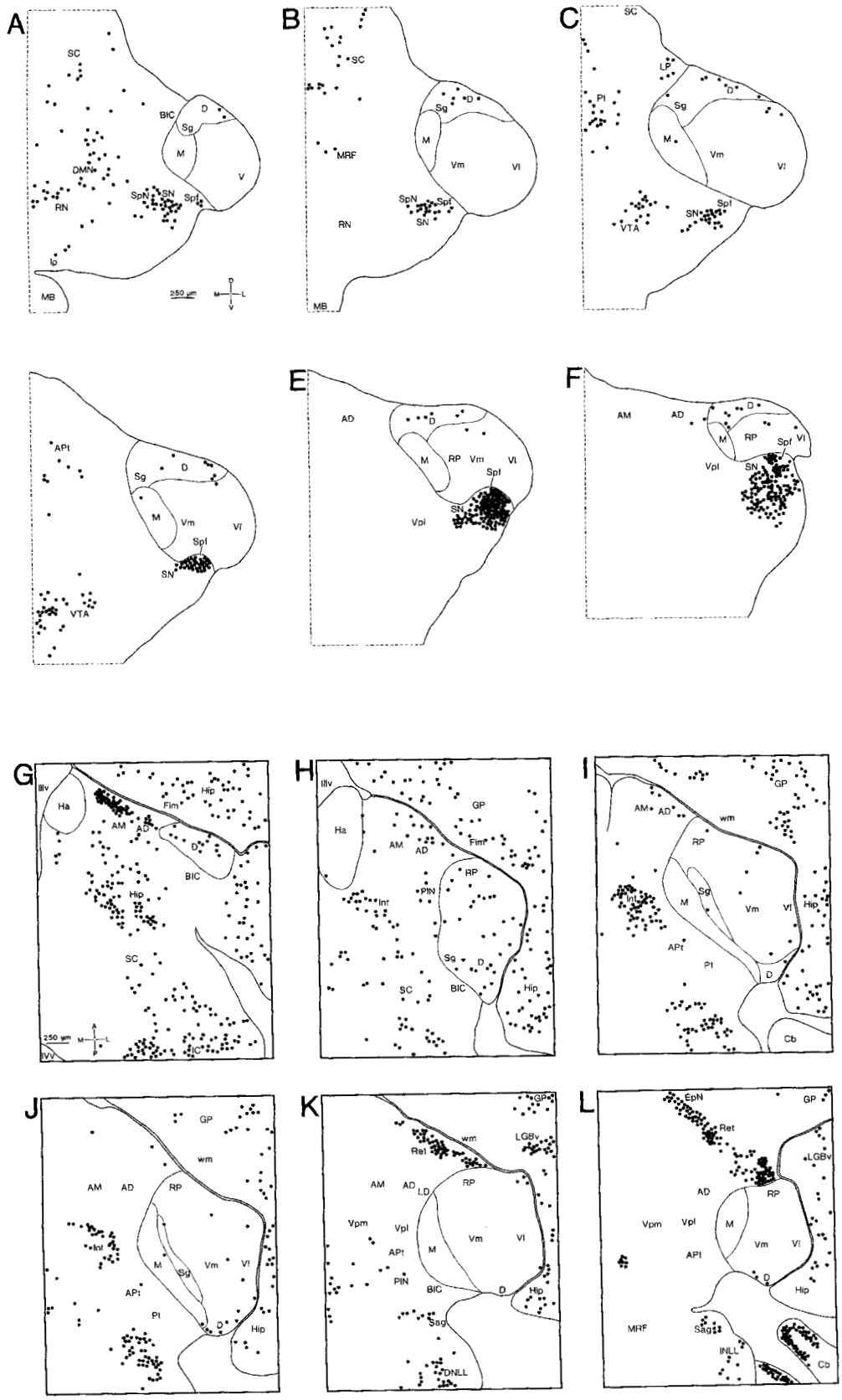
A
SC
BIC
D
Sg
M
V
DMN
SpN
SN
Spl
RN
lp
MB
250 μm
B
SC
MRF
Sg
D
M
Vm
Vl
SpN
Spl
SN
RN
MB
C
SC
LP
Pl
D
Sg
M
Vm
Vl
SN
Spl
VTA
APt
Sg
D
M
Vm
Vl
Spl
SN
VTA
E
AD
D
M
RP
Vm
Vl
Spl
SN
Vpl
F
AM
AD
D
M
RP
Spl
Vl
Vpl
SN
G
IIIv
Ha
Fim
Hip
AM
AD
D
BIC
Hip
SC
250 μm
IVV
H
IIIv
GP
Ha
AM
AD
Fimf
RP
PIN
Int
Sg
D
SC
BIC
Hip
I
GP
AM
AD
wm
RP
Sg
M
Vm
Vl
Hip
Int
APt
Pl
D
Cb
J
GP
wm
AM
AD
RP
Int
M
Sg
Vm
Vl
APt
Pl
D
Hip
K
GP
wm
Ret
LGBv
AM
AD
LD
RP
Vpm
Vpl
M
Vm
Vl
APt
PIN
BIC
D
Hip
Sag
DNLL
L
EpN
GP
Ret
LGBv
AD
RP
Vpm
Vpl
M
Vm
Vl
APt
D
Hip
MRF
Sag
INLL
Cb

Figure 2

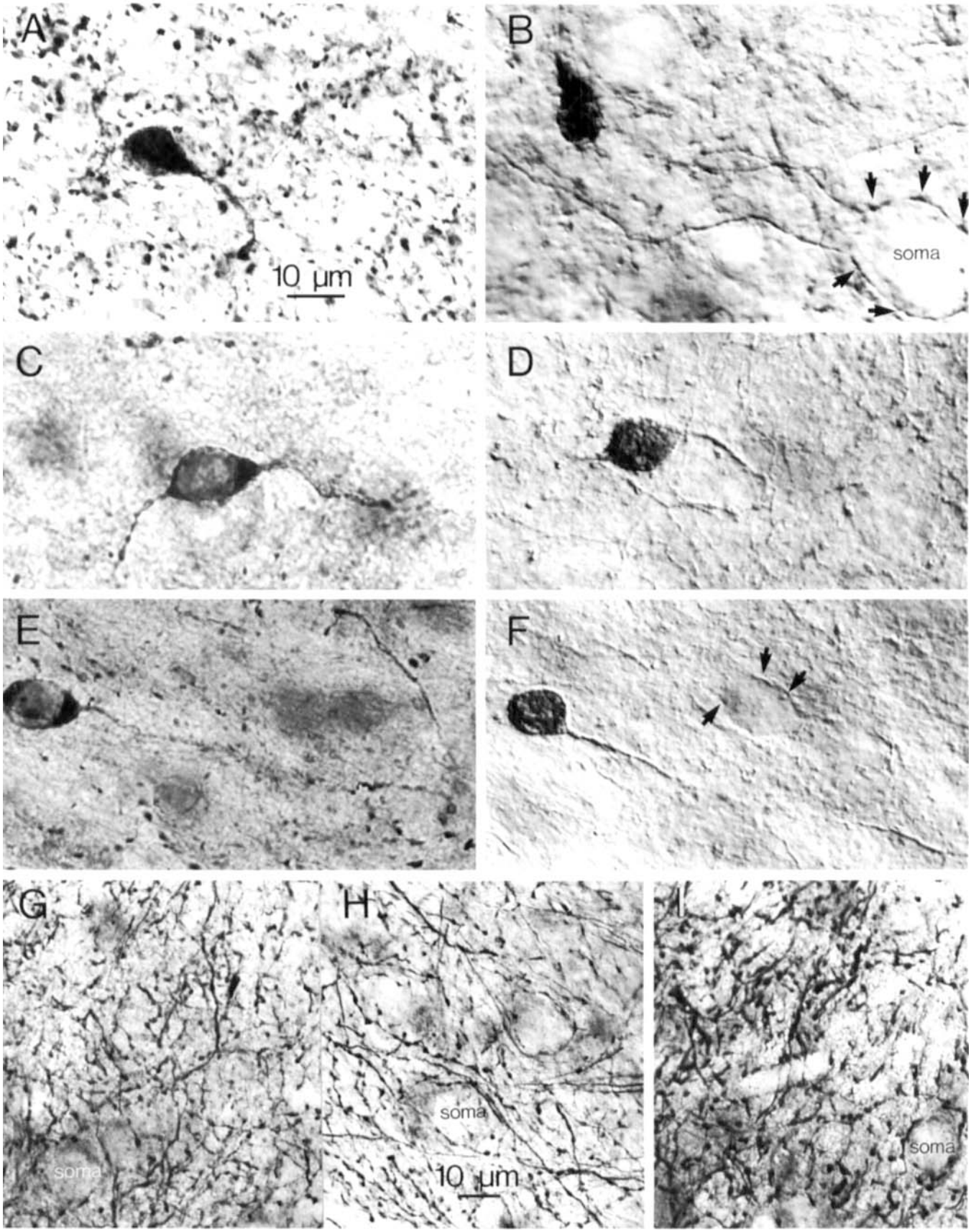

Figure 3

nucleus (Fig. 2B,C,I,J), with most in the superficial dorsal and dorsal nuclei proper (Fig. 2D,G). The prevailing somatodendritic orientation was medial to lateral (Fig. 3C–F), which aligned them with the long dendritic axis of immunonegative tufted (but not radiate) cell dendrites (Winer and Wenstrup, '92a). Finally, in GAD preparations, perikarya in the dorsal nuclei were significantly smaller in area (about 12%) than immunostained ventral division neurons (Table 1), save for rare suprageniculate immunopositive neurons with somatic areas exceeding 100 μm^2, which is as large as that of principal cells.

Few or no immunopositive medial division neuronal perikarya were seen (Fig. 2). Such cells were often located near the borders of adjoining nuclei, so that their type(s) and disposition could not be determined with confidence.

Puncta: general pattern

The relatively few immunopositive neurons (Fig. 1A–C) and the fine caliber of immunostained puncta (Fig. 3G–I) could contribute to a misleading impression that the medial geniculate complex as a whole was only weakly GABAergic, especially in contrast to other, more conspicuously immunopositive areas such as the substantia nigra (Fig. 1A:*SN*) or the hippocampal formation (Fig. 10A:*Hip*). While many immunoreactive cell bodies and far more pronounced dendritic immunostaining occurred in the latter areas, the density and distinctive pattern of immunopositive puncta imparted a characteristic texture that differentiated each of the major auditory thalamic subdivisions (Figs. 4–6), along with the pattern of neuronal immunoreactivity.

Ventral division. The number, distribution, and size of puncta distinguished the ventral division from adjoining nuclei. For example, the marginal zone, which forms the free surface of the medial geniculate complex, was virtually free of immunolabeled profiles (Fig. 4:*MZ*). This was not a consequence of inconsistent immunostaining since the neuropil in layer I of auditory cortex from nearby sections in the same experiments had many puncta in even the most superficial parts (Fig. 9D).

Besides the qualitative differences in the form of the puncta, there were important quantitative differences as well. Thus, puncta in the lateral part of the ventral division were almost twice as numerous as those in the dorsal division, and one-third more common than those in the medial division (Table 2). A broad range of sizes was evident, including small, fine ones (less than 0.5 μm in diameter; Fig. 4:*1*), medium-sized terminals (about 0.5–2 μm; Fig. 4:*2*), and large, coarse profiles (exceeding 2 μm; Fig. 4:*3*). Their numbers were comparable to those in the rat (with one exception; see Table 2) and they were in general uniformly distributed, although some immunonegative neurons clearly received more such terminals than did others (Fig. 4: *stippled outlines*). Occasional, much longer and thicker immunoreactive endings, some relatively smooth (Fig. 4:*4*), others coarser and beaded (Fig. 4:*5*), occurred. Some of these may represent preterminal axonal fragments, while others resembled immunopositive dendrites (Fig. 3A) and were consequently excluded from any quantitative analysis.

Within the ventral division, there were regional differences in the density of puncta. Thus, they decreased (from 119.3 to 81.1/625 μm^2) and then increased slightly (to 84.7) along sampling tracks running laterally-to-medially (Figs. 7, 8). While thalamoperforating vessels (Fig. 7A,C: *open spaces*) sometimes confounded attempts to construct perfectly straight tracks through the sampling space, the relatively modest standard deviations among these samples (range: 4.9–24.1; median: 12.5) suggested that puncta density was homogeneous along a line but differed among tracks as well as between divisions (Table 2). No consistent departure from these numerical values was observed along the caudorostral axis (Fig. 8).

Dorsal division puncta. The density of immunoreactive dorsal division puncta was clearly lower both in GAD- (Fig. 3C,E) and GABA-immunostained (Fig. 3D,F) preparations relative to the ventral division, despite the fact that the dorsal division contained the bulk of immunolabeled medial geniculate neurons (Fig. 2). The comparatively small size and fine, granular appearance of dorsal division puncta contributed to the impression of modest immunoreactivity compared to the ventral and medial divisions (Figs. 1A, 3H). Quantitatively, there were about half as many puncta per sample as in the ventral division (Table 2; Fig. 7D). Along a posterior-to-anterior axis, the number of dorsal nucleus puncta varied while gradually increasing (Fig. 8), although the present sample is too limited to reveal other, finer local patterns. While this quantitative analysis was limited to the dorsal nucleus proper, other subdivisions, notably the suprageniculate nucleus, had a much different arrangement, with aggregates of many coarse and strongly immu-

Fig. 3. *GAD+* (**A,C,E**) or *GABA+* cells and puncta (**B,D,F,G–I**) in the mustached bat. A,B: Ventral division, showing typical flask-shaped immunopositive neurons among clusters of puncta or near immunonegative somata, some of the latter receiving *GABA+* endings (*arrows* on *soma* in *B*). The immunopositive neurons are similar in each auditory thalamic subdivision, with their small oval or drumstick-shaped somata, sparse perikaryal cytoplasm (*E*), and a few thin, sometimes beaded, primary dendrites. Protocol for all panels: semi-apochromat, N.A. 1.25, ×787. C–F: Dorsal nucleus GABAergic cells, with a characteristic medial-to-lateral orientation, and generally fewer puncta than in the ventral division (compare A,C). G–I: *GABA+* puncta in, respectively, the ventral (G), dorsal (H), and medial (I) divisions. In each, perisomatic baskets ring immunonegative cells, and the plexus of *GABA+* preterminal branches has a preferred orientation, with a superior-to-inferior arrangement in the ventral and medial divisions, and a medial-to-lateral dorsal division configuration. Single puncta are more obvious in the dorsal division, and less so in the ventral and medial divisions, where the heavy immunostaining of preterminal elements obscures them.

Fig. 4. Ventral division GAD immunoreactivity. All of the neurons (*stippled outlines*) are immunonegative and receive varying numbers of immunopositive axon terminals, and the distribution of puncta includes fine, small elements (*1*), medium-sized ones (*2*), and large profiles (*3*); moderately thick, smooth preterminal axonal fragments (*4*) as well as much larger, beaded axonal segments (*5*) are also present. Some zones of rarefaction are attributable to thalamoperforating vessels (Fig. 1), while other areas (for example, beneath *Vl*) have above average local concentrations whose significance is unknown. The marginal zone (*MZ*) has little immunoreactivity. Planapochromat, N.A. 1.32, ×2,000.

Fig. 5. Dorsal division immunoreactivity, spanning the superficial dorsal (*DS*) and dorsal (*D*) nuclei (see also Fig. 7D). The number of immunopositive neurons is greater than in the ventral division (see Fig. 2), while the puncta are far fewer (Table 2). Small, delicate puncta (*1*) predominate, and these are often finer than their ventral (Fig. 4:*1*) or medial (Fig. 6:*1*) division counterparts. Many rather long (50–100 μm) preterminal segments are visible (beneath *DS*), and the thinnest of these (*4*) are thread-like, approximately 0.3 μm in diameter and with tiny beads, while thicker segments (*5*) have coarser swellings. Some immunonegative neurons have few axosomatic endings, while others have many more, though their number rarely approaches that of ventral or medial division cells. Planapochromat, N.A. 1.32, ×2,000.

Figure 4

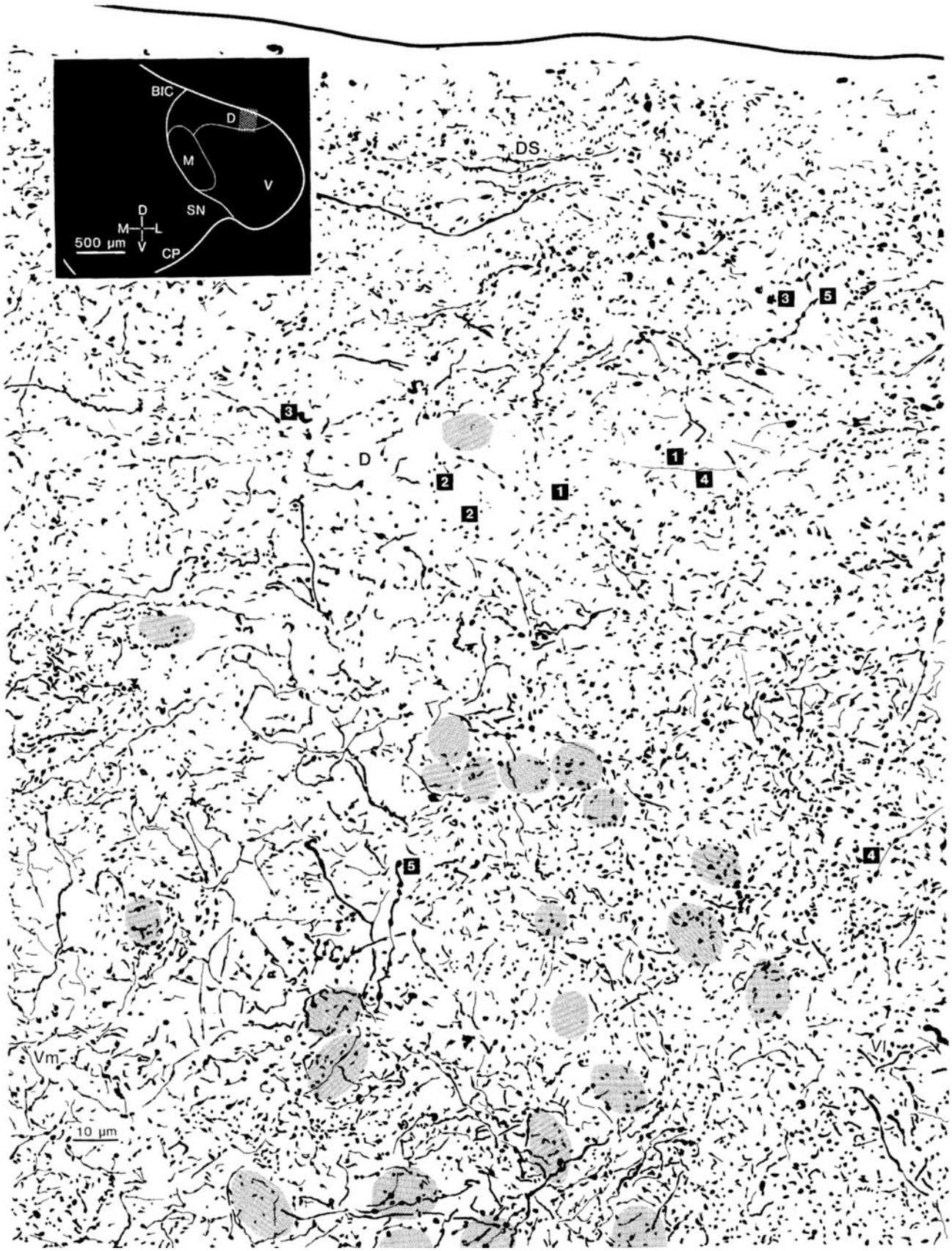

Figure 5

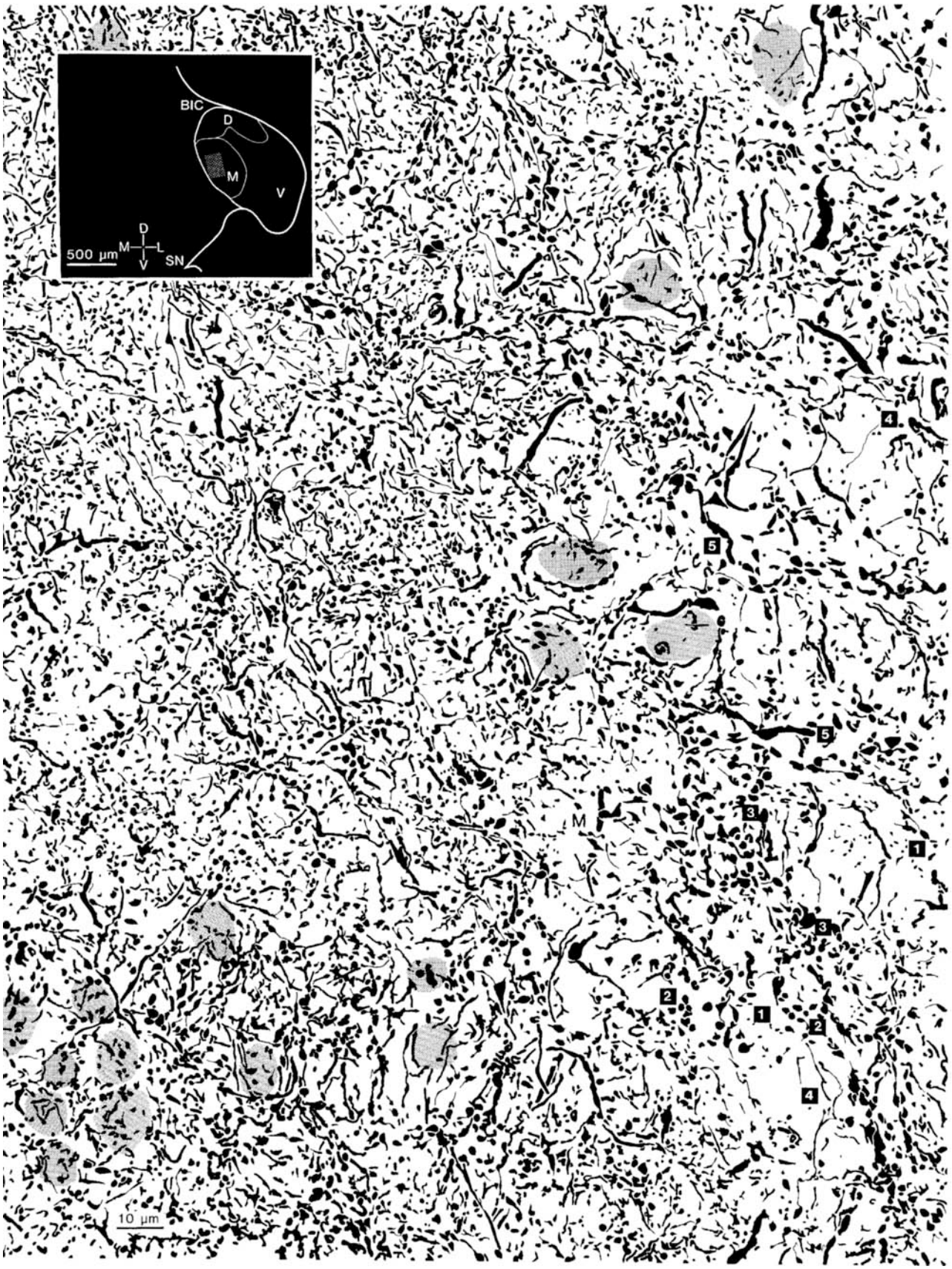

Figure 6

TABLE 1. Somatic Size Comparisons of GAD Immunoreactive Neurons[1] in the Ventral and Dorsal Divisions of the Mustached Bat Medial Geniculate Body

Division	Mean area[2,*]	SD	Range	No.
Ventral division	38.3	6.9	28.7–62.7	46
Dorsal division*	33.4	7.1	18.7–56.6	65

[1]Too few medial division neurons were immunostained to permit their inclusion; see the text for details.
[2]All measurements in μm^2.
*$P < 0.001$, t-test; df = 110.

TABLE 2. Number of Puncta/625 μm^2 in Different Subdivisions of the Mustached Bat's Medial Geniculate Complex, and Comparison With the Rat

Division	Mustached bat[1,2]	Rat[3]	Ratio (%)
Ventral division (lateral part)	119.3 ± 17.4 (97–151)	113.1 ± 36.2 (31–200)	105
Dorsal division	65.0 ± 14.8 (39–96)	28.7 ± 18.7 (0–87)[4]	226
Medial division	88.9 ± 18.1 (43–141)	114 ± 31.9 (31–206)	78

[1]Mean ± SD (range).
[2]Total = 10,538.
[3]Modified from Winer and Larue ('88).
[4]Since these values were interpolated from the 100 μm^2 sampling grid used in an earlier study (Winer and Larue, '88), the zero value is certainly an underestimate given the present, larger sampling zone.

noreactive puncta (Fig. 1C:*Sg*). Puncta density also increased along the dorsoventral axis (Figs. 5, 7D).

GAD-immunoreactive dorsal division puncta (Fig. 5) appeared on average smaller than those in the ventral division (Fig. 4). Nonetheless, classes of small, medium-sized, and large puncta were readily noted (Fig. 5:*1–3*, respectively). As in the ventral division, where many puncta formed apparent axosomatic endings upon immunonegative neurons (Fig. 4: *stippled outlines*), some dorsal nucleus neurons received a moderate number of terminals (Fig. 3F: *arrows;* Fig. 3H: *soma*; Fig. 5: *stippled outlines*), while others had far fewer. Besides these endings, very fine, thread-like, presumably preterminal profiles were seen (Fig. 5:*4*). Much thicker, beaded elements (Fig. 5:*5*) also occurred, some perhaps of dendritic origin (Fig. 3C) and up to 50–100 μm long (Fig. 5, below *DS*).

Medial division puncta. *GAD+* medial division puncta were three-fourths as numerous as those in the ventral division (Table 2) and, despite considerable variability in their number along dorsoventral tracks running through the long axis of the medial division (Fig. 7C,D), the anteroposterior gradient (Fig. 8) was more uniform.

Although the density of puncta was intermediate to that in the ventral and dorsal divisions (Table 2), the appearance of medial division immunostaining was different, and many puncta were coarser and larger and had complex shapes (Fig. 6). The three primary size classes—small, medium-sized, and large—were seen (Fig. 6:*1–3*, respectively). The small puncta were as fine as the most delicate dorsal nucleus puncta (compare Figs. 5:*1*, 6:*1*), but the largest profiles were much bigger and more complex than their counterparts in the ventral (Fig. 4:*3*) and dorsal (Fig. 5:*3*) divisions. Thin, delicate profiles were present, some with trajectories that recapitulated those of axons of brachial origin (Figs. 3I, 6:*4*; see also Winer and Wenstrup, '92b). Given the relatively few intrinsic immunopositive medial division neurons (Fig. 2), it is probable that such profiles represent extrinsic axons (Fig. 6). Many puncta terminated freely in the neuropil, while others formed axosomatic endings (Figs. 3I, 6).

DISCUSSION

The present results have three main implications. First, they independently validate and extend the plan for subdividing the mustached bat medial geniculate complex derived from studies of neuronal architecture (Winer and Wenstrup, '92a) and cytoarchitecture (Winer and Wenstrup, '92b), and from investigations of midbrain (Wenstrup and Winer, '87; Frisina et al., '89) and cortical (Olsen, '86) connections. Second, they provide a context for considering comparative aspects of sensory thalamic organization, with particular reference to homologous and nonhomologous elements in the evolution of thalamic circuitry. Finally, the patterns of immunostaining are specific to particular nuclei and consistent with the idea that GABAergic elements, either of interneuronal or extrinsic origin, might play an important physiological role whose precise nature remains to be defined. However, before considering these implications, it is appropriate to assess first the reliability of the method since our conclusions depend in part on a negative result, viz., the relative paucity of Golgi

Fig. 6. Medial division GAD immunoreactivity, showing a numerical pattern comparable to the ventral division (Table 2; Fig. 4) but with much coarser and more complex puncta. Small (*1*), medium-sized (*2*), and large (*3*) endings occur among a population chiefly of immunonegative perikarya (*stippled profiles*; see also Fig. 2:*M*), upon which varying numbers of puncta (*lower left*) terminate. The many prominent puncta (*5*) are the largest and most elaborate in the auditory thalamus, and a few scattered, beaded profiles occur (*4*) among the often lengthy preterminal segments. Planapochromat, N.A. 1.32, ×2,000.

Fig. 7. Quantitative analysis of concentrations of *GAD+* puncta. **A:** Nissl preparation showing the track (*1*) along which the puncta counts were made in an adjoining, immunostained section. Tracks were selected to avoid thalamoperforating vessels, and the anteroposterior position of the section is comparable to that of Figure 1B. Planapochromat, N.A. 0.32, ×200. **B:** Cytoarchitectonic subdivisions and schematic view of puncta concentration along the track in A. Many lie in the lateral part of the ventral division (see also D:*1*), and the absolute value is rarely less than 60 puncta/625 μm^2. The values for the medial division are somewhat underestimated due to the many fibers (see also D:*4*). **C:** Puncta concentrations at an architectonic level near that of Figure 1C. **D:** Four dorsal-to-ventral tracks (*1–4*) show that the lateral part of the ventral division has the highest concentration, followed by the medial part of the ventral division, the medial division, and then the dorsal division (*2,3*), respectively; exact values from other counts are shown differently in Figure 8.

Fig. 8. Number of *GAD+* puncta/625 μm^2 within and along a caudal-to-rostral sequence in the auditory thalamus. **A–F:** The number to the right of each black box identifies each of the 30 sampling distributions; every numerical value is the mean of four samples (see also Table 2). Most of the trends confirm the patterns shown in Figure 7, for example, the lateral part of the ventral nucleus (*Vl*) consistently has the highest values, the medial division (*M*) and the medial part of the ventral division (*Vm*) are comparable, and the dorsal division (*D*) generally has the fewest puncta. **G–K:** Puncta counts arranged according to architectonic subdivisions and across posterior-to-anterior sequences through different medial geniculate body subdivisions. The italic numbers in panels A–F identify the locus of the sample. **G:** From the lateral part (*Vl*) of the ventral division. **H:** From the intermediate part of the ventral division. **I:** From the medial part (*Vm*) of the ventral division. **J:** From the dorsal division. **K:** From the medial division (*M*). **L:** Three-dimensional surface view of the spatial distribution of puncta from panels A–F. For purposes of illustration, each point has been represented as two lines to generate a contour map. The orientation for this figure is drawn as if the reader were viewing the left medial geniculate body from a caudomedial perspective.

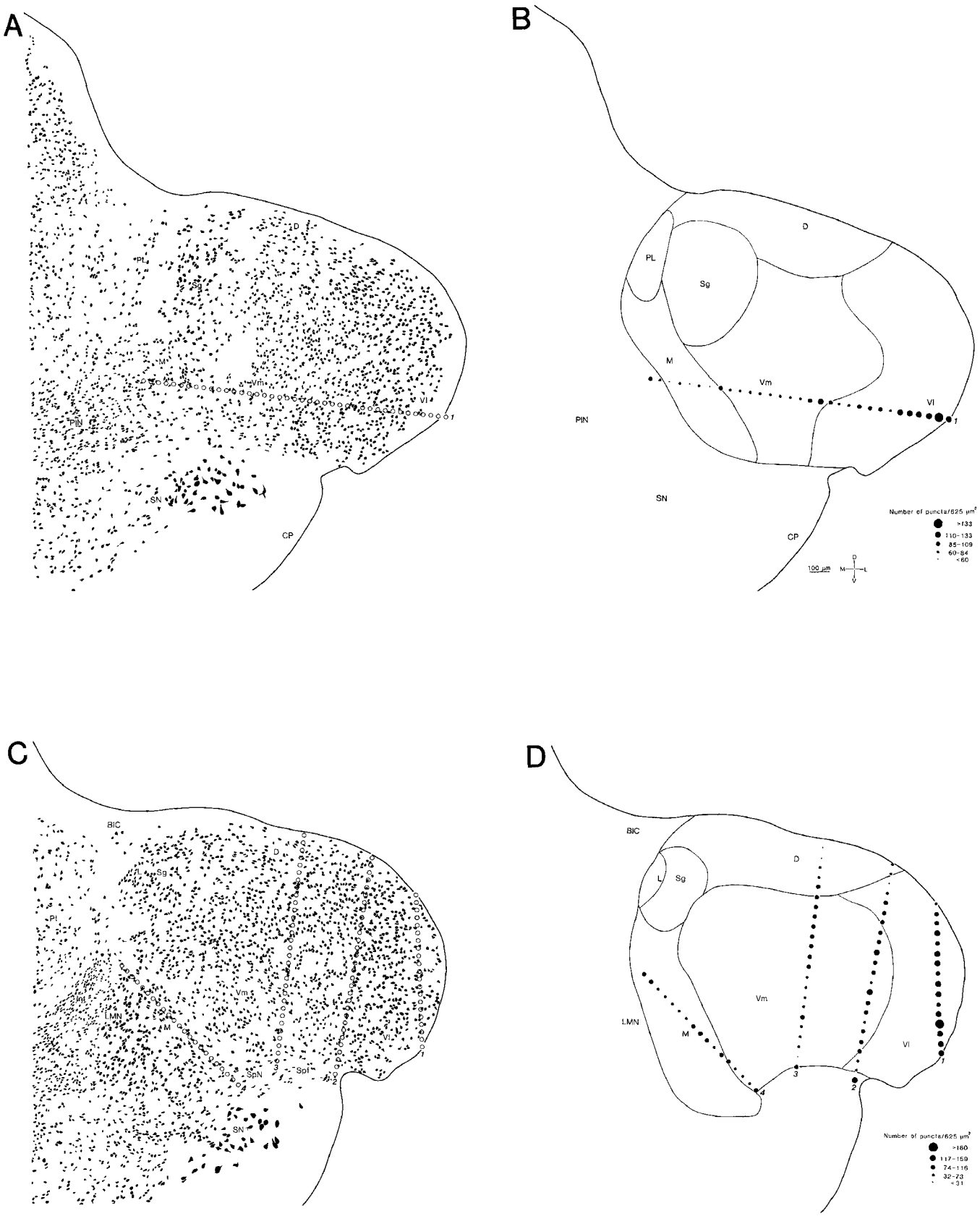
A
D
PL
M
Vm
VI
PIN
SN
CP
B
D
PL
Sg
M
Vm
VI
PIN
SN
CP
Number of puncta/625 μm²
>133
110-133
85-109
60-84
<60
100 μm
C
BIC
D
PL
Sg
Pl
LMN
M
Vm
VI
SpN
Spf
SN
D
BIC
L
Sg
D
Vm
LMN
M
VI
Number of puncta/625 μm²
>160
117-159
74-116
32-73
<31

Figure 7

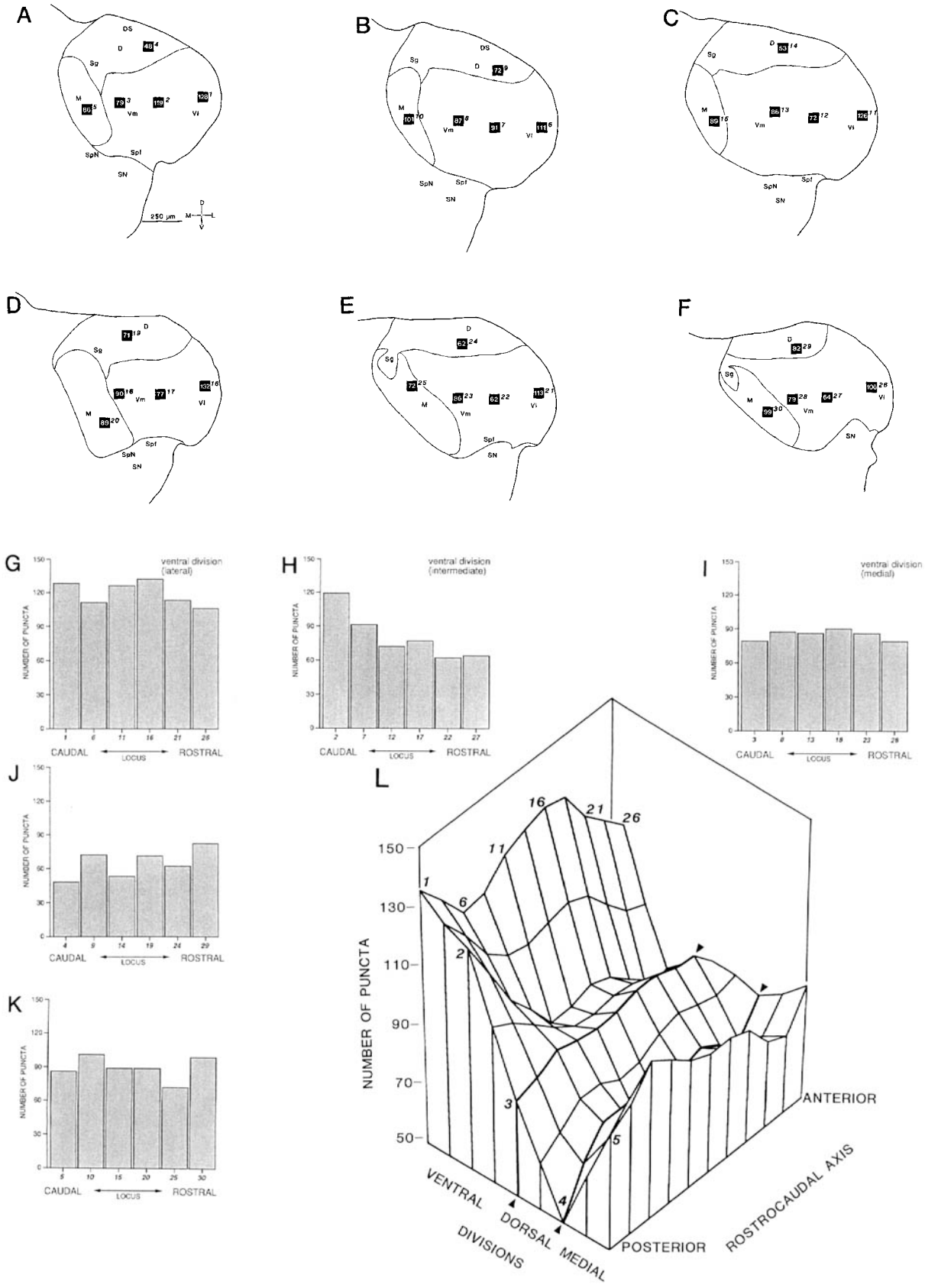
A
B
C
D
E
F
G
H
I
J
K
L
DS
Sg
D
M
Vm
Vl
SpN
Spl
SN
250 μm
ventral division (lateral)
ventral division (intermediate)
ventral division (medial)
NUMBER OF PUNCTA
CAUDAL
LOCUS
ROSTRAL
NUMBER OF PUNCTA
VENTRAL
DORSAL
MEDIAL
DIVISIONS
POSTERIOR
ANTERIOR
ROSTROCAUDAL AXIS

Figure 8

type II neurons, and the reliability of GAD or GABA immunostaining as a probe for such neurons.

Methodological considerations

Many different strategies might be used to identify local circuit neurons within the medial geniculate body. Thus, Golgi or Nissl preparations show small cells with sparse, pale cytoplasm, a locally ramifying axon, and slender dendrites with a few elaborate appendages; each of these features is suggestive, though not diagnostic, of such interneurons. Unfortunately, in the adult specimens available, the extent of axonal impregnation was insufficient in these Golgi-Cox preparations to warrant any conclusion that more than a few such cells are present (Winer and Wenstrup, '92a), nor could these classes of neurons be differentiated reliably on the basis of somatic size (Winer and Wenstrup, '92b).

Another approach to identifying medial geniculate body interneurons would entail the study of retrogradely labeled thalamocortical relay cells in semi-thin plastic sections for post-embedding immunocytochemistry using a variety of antisera to mark neurons through a sequence of semi-serial sections. The latter method has successfully identified GABAergic intrinsic neurons in the primate lateral geniculate body (Montero and Zempel, '85, '86) and glycinergic superior olivary neurons in the cat (Saint Marie et al., '89) and mustached bat (Park et al., '91) whose axons project to the inferior colliculus. Since the present study used immunostaining only, several questions arise. These are: 1) how specific and veridical are the antisera for identifying GABAergic elements? 2) with so few immunopositive neurons, how reliable are quantitative estimates of puncta? 3) could other neuroactive compounds besides GABA fulfill a role in the operation of local thalamic circuits? and 4) what is the concordance between GAD and GABA?

Relatively few, and approximately the same proportion, of medial geniculate neurons were immunostained for GAD or GABA in every experiment. Despite the comparatively sparse auditory thalamic immunoreactivity, many non-auditory structures, either remote from or adjoining the medial geniculate complex, were robustly immunopositive for both in GAD and GABA preparations, including the substantia nigra (Fig. 1A–C), the subparafascicular nucleus (Fig. 2E), certain hippocampal neurons (Figs. 2G, 10A), and cerebellar Purkinje cells (Fig. 2K). Neural populations known to be immunoreactive for GAD or GABA in rodents (see Mugnaini and Oertel, '85) were always positive in the present study, whereas structures known to be GABA-negative were likewise free of immunoreactivity, as were both omission controls (not shown) and sections incubated in pre-immune serum (Fig. 1D). GAD-immunoreactive auditory neurons and puncta were consistently and darkly immunostained in the inferior colliculus (Fig. 9A,B), auditory cortex (Fig. 9C,D), and cochlear nucleus (not illustrated). Their number and locus were always consistent within an experiment and between cases (J.A. Winer, G.D. Pollak, and D.T. Larue, in preparation).

A parallel argument supports the reliability of the numerical observations on puncta. First, the variability between sections within an experiment was low, approximately 10–15%, and between cases it was rarely as much as 30%. In making quantitative estimates (Fig. 8) nuclear boundaries were avoided, further reducing the standard deviation. The concordance between independent observers in the puncta count was greater than 90%.

The consistency and continuity among these independent variables suggest that the present results faithfully reflect the arrangement of GABAergic cells and puncta in the mustached bat auditory thalamus. However, the functional implication of regionally specific GABAergic arrangements remains to be explored physiologically.

In contrast to other studies, in which antisera to GAD or GABA appear to identify somewhat different neuronal populations (see Hurd and Eldred, '89), the same proportion of cells, with a similar morphology, was marked in the present experiments. However, it should be stressed that our entire sample of immunolabeled auditory thalamic neurons (about 110) is too small to permit the careful scrutiny that a larger population of such cells would encourage, and we are therefore unable to make definitive quantitative statements about differences between the antisera. Moreover, since immunopenetration is superior in the GAD preparations, even quantitative comparisons would of necessity be indirect. The only appreciable qualitative difference was related to the puncta: longer preterminal bouton-free axonal segments were marked by GABA, while the punctate character of the boutons and perhaps the intensity of the immunostaining was more prominent in the GAD material.

Possible sources of GABAergic input

If the mustached bat medial geniculate complex contains as few GABAergic neurons as estimated in this study—perhaps 1–2% of such cells in the dorsal nucleus, less than 1% of ventral division cells, and only a rare medial division neuron—then what is the origin of the comparatively rich plexus of GABAergic endings? Two known sources are Golgi type II cells in the medial geniculate body and GABAergic thalamic reticular nucleus neurons. Other potential contributors, which remain to be demonstrated experimentally, are GABAergic cells in or near the ventral nucleus of the lateral lemniscus or the lateral tegmental projection neurons in the midbrain, and/or inferior colliculus cells.

The proportion of local circuit neurons in the bat auditory thalamus is unknown, and few such cells have been impregnated in Golgi preparations (Winer and Wenstrup, '92a). Since these neurons appear to comprise only a minute proportion of the population, they would presumably require relatively enormous local axonal branches to generate the formidable GABAergic terminal plexus common in these experiments. Thus, while they probably contribute to local thalamic circuits, they are unlikely to be the largest or even the principal source. A second possible avenue of influence is that their dendrites might participate in dendro-dendritic glomerular complexes or synaptic nests that are one hallmark of medial geniculate body ultrastructural organization in carnivores (Morest, '71, '75; Ohara et al., '89), though such arrangements are considered to be

Fig. 9. Characteristic patterns of GAD immunostaining in auditory structures outside the medial geniculate complex. **A:** *GAD+* inferior colliculus neurons. For analysis of subdivisions, see Zook et al. ('85). Protocol for panels A,C: planachromat, N.A. 0.50, ×500. **B:** *GAD+* inferior colliculus neuron with many immunopositive axosomatic endings (*solid black, lower left*) among immunonegative neurons (*stippled profiles*). Compare with Figures 4–6. Protocol for panels B,D: planapochromat, N.A. 1.32, ×2,000. **C:** *GAD+* cells in layers I–III of auditory cortex. **D:** View of layers I–II at higher power showing small *GAD+* cells and fine, numerous puncta.


A
DC
CIC
B
DPD
Ex
MD
ALD
LL
100 µm
D
L
M
V
B
10 µm
C
D
I
II
III
IC
DC
DPD
Ex
IVv
LL
Cb
MCP
Ra
MRF
CN
Py
1 mm
LV
Ha
CPu
wm
Am
Hyp

Figure 9

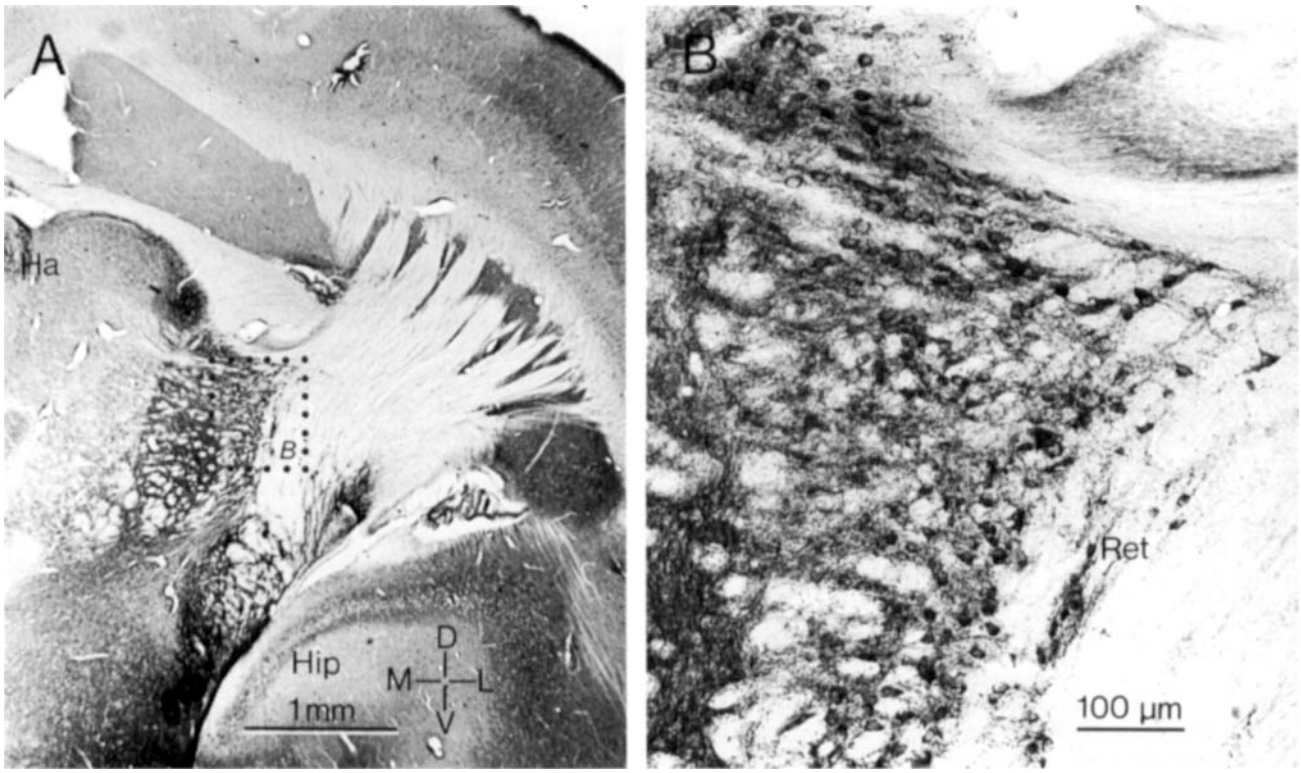

Fig. 10. Identification of the presumptive thalamic reticular nucleus. **A:** Overview of the rostral thalamus. The **inset** (*dotted square*) frames the putative reticular nucleus in this GAD-immunostained section. Planachromat, N.A. 0.04, ×20. **B:** At higher magnification, the *GAD+* neurons and the characteristic lamellar configuration of the reticular nucleus (*Ret*) are evident. See text for further discussion. Planapochromat, N.A. 0.32, ×156.

quite rare in many small mammals (Špaček and Lieberman, '74).

The overwhelmingly GABAergic neuronal population in the thalamic reticular nucleus of many species (Houser et al., '80) is a probable source of much of the GABAergic input to the cat medial geniculate body (Rouiller et al., '85). Many of these neurons, which are heavily GAD- or GABA-immunoreactive in the mustached bat (Fig. 10), are also retrogradely labeled after auditory thalamic injections of horseradish peroxidase (E. Covey, personal communication; J.F. Olsen, personal communication). Presumably, they could represent some of the synaptic endings containing flattened vesicles, profiles thought to arise from extrageniculate sources in other thalamic nuclei (Montero and Scott, '81). This hypothesis has been confirmed in recent studies, which have demonstrated precise patterns of connections between particular sectors of the reticular nucleus and subdivisions of the prosimian medial geniculate complex (Conley et al., '91).

Many neurons in the ventral nucleus of the mustached bat's lateral lemniscus (Pollak and Winer, '89) and others in the lateral tegmental system of the midbrain (J.A. Winer, unpublished observations) are GABAergic. Whether some of these cells project to the medial geniculate body remains to be demonstrated in bats. If the mustached bat auditory thalamus resembles the cat's, this projection should be heaviest in the dorsal division (Morest, '65). Since the proportion of GABAergic cells is greatest in the bat dorsal division (Fig. 2), while the relative number of *GAD+* puncta is lowest among the three primary divisions (Table 2), this suggests 1) that GABAergic thalamic interneurons are unlikely to account for the bulk of the immunopositive puncta; and 2) that multiple sources of extrinsic GABAergic input (besides the thalamic reticular nucleus) may exist for each auditory thalamic subdivision, and that these remain to be identified. Another potential origin for such a projection is the neurons of the nucleus of the central acoustic tract whose axons terminate within the suprageniculate nucleus (Casseday et al., '89), although the identity of their transmitter remains uncertain. In the cat, neurons in the posteromedial region of the ventral nucleus of the lateral lemniscus, as well as cells in every subdivision of the inferior colliculus, in the superior colliculus, and from the vicinity of the brachium conjunctivum, all send axons to the medial geniculate body (Henkel, '83; Hutson, '88). Some of these neurons, too, may be GABAergic.

Once the sources of GABAergic projections and the functional role of intrinsic inhibitory neurons can be established with more precision, it should then be possible to draw parallels and specify differences with other, chemically distinct auditory thalamic components. Thus, in rodents and carnivores, monoamines (Fuxe, '65; Fitzpatrick et al., '89) and enkephalins (Covenas et al., '86) each have a particular regional distribution whose function is unknown but that overlaps spatially with the GABAergic territories defined here, as does the pattern of glutamate immunopositive neurons (Winer, '91). Iontophoretic application of acetylcholine alters the firing pattern of thalamic sensory neurons (McCormick and Prince, '87), and there is a substantial cholinergic axonal plexus in subregions of the

rat (Levey et al., '87) and cat (Fitzpatrick et al., '89) medial geniculate body whose functional role is unknown.

Comparative aspects of GABAergic organization in the auditory thalamus

Are the immunocytochemical patterns described in the present study unique to the mustached bat, or do they embody trends common to other mammals? With some exceptions, the major features of medial geniculate body GABAergic organization are remarkably conserved in rodents, in carnivores, and possibly in primates. As a rule, the numbers and types of immunopositive puncta have a relatively consistent interspecific organization: the ventral division invariably has a dense concentration of small and medium-sized puncta and some large ones, the dorsal division has far fewer puncta, most of which are more delicate, while the density in the medial division is comparable to that in the ventral division, and the puncta are generally larger and coarser in appearance. Species differences include the relative ratios between divisions (see Table 2) as well as certain extremely dense aggregates of puncta in the macaque monkey auditory thalamus (J.A. Winer, unpublished observations).

There are substantial species differences in the number of immunopositive neuronal perikarya. Thus, in rats and mustached bats, only a comparatively few neurons, on the whole, are immunopositive, and in the rat (Winer and Larue, '88), most of these were in the ventral and medial divisions, respectively. In the bat, the dorsal division contained the preponderance of such cells, followed by the ventral division, with rare immunopositive medial division neurons. In the cat (Huchton et al., '91) and monkey (J.A. Winer, unpublished observations), every auditory thalamic subdivision has many GABAergic neurons, including more than one such type in some nuclei. In the opossum and rabbit, the proportion of GAD-immunoreactive medial geniculate neurons appears greater than in the mustached bat (Penny et al., '84), though still much lower than in the cat (Rinvik et al., '87) and squirrel monkey (Smith et al., '87). Of course, the observation that comparable types of neurons are immunostained in different species or that the numbers of immunopositive puncta are comparable (or different) is only indirect evidence of any functional relationship, and, without a physiological frame of reference, it is of limited value. Nevertheless, some inferences are possible. Thus, the relative density of puncta is comparable in species with many synaptic nests or glomeruli, such as the cat (Morest, '71, '75) and perhaps in the monkey (J.A. Winer, unpublished observations), and in species with fewer such local arrangements; however, rigorous quantitative ultrastructural studies are incomplete (see Špaček and Lieberman, '74) and the synaptic dispositions within, not to mention the absolute number of, putative glomeruli in the bat, remain unknown. However, if they exist their targets might well include postsynaptic sites outside the glomeruli, while in the cat and monkey the density of these arrangements is consistent with the hypothesis that both glomerular and extraglomerular synaptic targets are involved. This raises the intriguing comparative question of the physiological role of GABAergic Golgi type II cells in the auditory thalamus of mammals with few or many such neurons, respectively. If a large number of GABAergic neurons in a given nucleus is a mark of advanced function, their overall rarity in the bat medial geniculate body constitutes something of a paradox. Perhaps another transmitter has subsumed the role of GABA in the bat, or the auditory thalamic circuitry represents an important departure from the pattern in carnivores and primates and aligns the mustached bat more closely with rodents and lagomorphs.

The species differences and parallels in auditory thalamic GABAergic organization could support the conclusion that, even among the principal nuclei of the lemniscal acoustic pathway, there may be significant differences in the number and arrangement of local neuronal circuits. Since different patterns of neurochemical architecture distinguish the main sensory thalamic nuclei, perhaps each modality has a unique pattern of intrinsic organization. For example, in rodents, the lateral geniculate body has a comparatively large number of GABAergic neurons and axon terminals (Ottersen and Storm-Mathisen, '84; Mugnaini and Oertel, '85), the medial geniculate complex possesses only a few such cells and distinct local variations of puncta (Winer and Larue, '88), while the ventrobasal complex has no apparent GABAergic neurons and a moderate number of puncta (Barbaresi et al., '86; Harris and Hendrickson, '87; Williams and Faull, '87). The presumably unique functional role of these different patterns of organization remains to be studied and could belie the claim that the various nuclei differ only with respect to their modality-specific input.

Speculation on function

GABAergic inhibition is likely to play multiple roles in auditory thalamic signal processing, much as it does in other brain stem regions (Caspary et al., '85; Faingold et al., '91). Various inhibitory effects have been described in other species that could plausibly reflect GABAergic mechanisms integrated within the medial geniculate body (Aitkin et al., '66; Aitkin and Dunlop, '69; Whitfield and Purser, '72; Aitkin, '73; Aitkin and Prain, '74; Ryugo and Weinberger, '76; Calford and Webster, '81; Rodrigues-Dagaeff et al., '89). In the mustached bat's medial geniculate, many neurons display frequency tuning curves with inhibitory sidebands and upper thresholds (Olsen, '86). While these inhibitory response features could reflect local thalamic GABAergic mechanisms, the inferior colliculus also displays such features (Bodenhamer and Pollak, '83; O'Neill, '85). Nonetheless, thalamic inhibition could contribute to what is thought to be a progressive sharpening of frequency tuning curves from the level of the auditory nerve or cochlear nucleus to the auditory cortex in the mustached bat (Suga and Tsuzuki, '85).

GABAergic mechanisms may contribute to another inhibitory phenomenon related specifically to the mustached bat's analysis of biosonar information. Olsen and Suga ('91b) have recorded delay-tuned, combination-sensitive neurons in the medial geniculate body. These neurons respond preferentially to the fundamental, frequency-modulated (FM) downsweep of the biosonar signal followed by a higher harmonic frequency-modulated sweep presented at a particular delay. Such delay tuning is believed to represent a neuronal analysis of sonar target range. Of particular interest to the present study is that neurons tuned to long best delays (more than 4 ms) appear to depend on inhibitory mechanisms for at least part of their delay tuning. These mechanisms are likely to be integrated in the medial geniculate body, since it is the first station of the ascending auditory system to demonstrate such responses (O'Neill, '85; Olsen, '86).

ACKNOWLEDGMENTS

We are indebted to Drs. D.E. Schmechel and W.H. Oertel for their generous gift of GAD antiserum and pre-immune serum, and we thank J.G. Van de Vere, J. Shin, and D. Lambert for secretarial assistance. Dr. C.E. Schreiner kindly assisted in the preparation of Figure 8L. Preliminary announcements of these results have appeared (Winer and Wenstrup, '87; Wenstrup et al., '88). This research was supported by United States Public Health Service grants R01 NS16832-13 and University of California Faculty Research Awards (J.A.W.) and F32 NS07733-03 (J.J.W.).

LITERATURE CITED

Aitkin, L.M. (1973) Medial geniculate body of the cat: Responses to tonal stimuli of neurons in the medial division. J. Neurophysiol. *36:*275–283.

Aitkin, L.M. (1989) The auditory system. In A. Björklund, T. Hökfelt, and L.W. Swanson (eds): Handbook of Chemical Neuroanatomy, Vol. 7, Integrated Systems of the CNS, Part II. Central Visual, Auditory, Somatosensory, Gustatory. Amsterdam: Elsevier Science Publishers B.V., pp. 165–218.

Aitkin, L.M., and C.W. Dunlop (1969) Inhibition in the medial geniculate body of the cat. Expl. Brain Res. *7:*68–83.

Aitkin, L.M., and S.M. Prain (1974) Medial geniculate body: Unit responses in the awake cat. J. Neurophysiol. *37:*512–521.

Aitkin, L.M., C.W. Dunlop, and W.R. Webster (1966) Click-evoked response patterns of single units in the medial geniculate body of the cat. J. Neurophysiol. *29:*109–123.

Barbaresi, P., R. Spreafico, C. Frassoni, and A. Rustioni (1986) GABAergic neurons are present in the dorsal column nuclei but not in the ventroposterior complex of rats. Brain Res. *382:*305–326.

Bodenhamer, R.D., and G.D. Pollak (1983) Response characteristics of single units in the inferior colliculus of mustache bats to sinusoidally frequency modulated signals. J. Comp. Physiol. *153:*67–79.

Calford, M.B., and W.R. Webster (1981) Auditory representation within principal division of cat medial geniculate body: An electrophysiological study. J. Neurophysiol. *45:*1013–1028.

Caspary, D.M., L.P. Rybak, and C.L. Faingold (1985) The effects of inhibitory and excitatory amino-acid transmitters on the response properties of brainstem auditory neurons. In D.G. Drescher (ed): Auditory Biochemistry. Springfield, IL: Charles C Thomas, pp. 198–226.

Casseday, J.H., J.B. Kobler, S.F. Isbey, and E. Covey (1989) Central acoustic tract in an echolocating bat: An extralemniscal auditory pathway to the thalamus. J. Comp. Neurol. *287:*247–259.

Conley, M., A.C. Kupersmith, and I.T. Diamond (1991) Organisation of the auditory sector of the thalamic reticular nucleus in *Galago:* Comparison of the reciprocal connexions between the reticular nucleus and the ventral and magnocellular divisions of the medial geniculate complex. Eur. J. Neurosci. *3:*1089–1103.

Covenas, R., R. Romo, A. Cheramy, F. Cesselin, and M. Conrath (1986) Immunocytochemical study of enkephalin-like cell bodies in the thalamus of the cat. Brain Res. *377:*355–361.

Faingold, C.L., G. Gehlbach, and D.M. Caspary (1991) Functional pharmacology of inferior colliculus neurons. In R.A. Altschuler, R.P. Bobbin, B.M. Clopton, and D.W. Hoffman (eds): Neurobiology of Hearing, Vol. II, The Central Auditory System. New York: Raven Press, Ltd., pp. 223–251.

Fitzpatrick, D., I.T. Diamond, and D. Raczkowski (1989) Cholinergic and monoaminergic innervation of the cat's thalamus: Comparison of the lateral geniculate nucleus with other principal sensory nuclei. J. Comp. Neurol. *288:*647–675.

Frisina, R.D., W.E. O'Neill, and M.L. Zettel (1989) Functional organization of mustached bat inferior colliculus: II. Connections of the FM_2 region. J. Comp. Neurol. *284:*85–107.

Fuxe, K. (1965) Evidence for the existence of monoamine neurons in the central nervous system. IV. The distribution of monoamine nerve terminals in the central nervous system. Acta Physiol. Scand. *247 (Suppl. 64):*37–85.

Harris, R.M., and A.E. Hendrickson (1987) Local circuit neurons in the rat ventrobasal thalamus—a GABA immunocytochemical study. Neuroscience *21:*229–236.

Henkel, C.K. (1983) Evidence of sub-collicular auditory projections to the medial geniculate nucleus in the cat: An autoradiographic and horseradish peroxidase study. Brain Res. *259:*21–30.

Houser, C.R., J.E. Vaughn, R.P. Barber, and E. Roberts (1980) GABA neurons are the major cell type of the nucleus reticularis thalami. Brain Res. *200:*341–354.

Hsu, S.-M., L. Raine, and H. Fanger (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. J. Histochem. Cytochem. *29:*577–580.

Huchton, D.M., D.T. Larue, J.Y.-M. Sun, and J.A. Winer (1991) The organization of GABAergic neurons in the cat medial geniculate body: A quantitative immunocytochemical study of post-embedded material. Proc. Soc. Neurosci. *17:*300 (abstract).

Hurd II, L.B., and W.D. Eldred (1989) Localization of GABA- and GAD-like immunoreactivity in the turtle retina. Vis. Neurosci. *3:*9–20.

Hutson, K.A. (1988) Connections of the Auditory Midbrain: Efferent Projections of the Dorsal Nucleus of the Lateral Lemniscus, the Nucleus Sagulum, and the Origins of the GABAergic Commissure of Probst. Doctoral dissertation, The Florida State University College of Letters and Sciences, Tallahassee, FL.

Jones, E.G. (1985) The Thalamus. New York: Plenum Press.

Kobler, J.B., S.F. Isbey, and J.H. Casseday (1987) Auditory pathways to the frontal cortex of the mustache bat, *Pteronotus parnellii.* Science *236:*824–826.

Levey, A.I., A.E. Hallanger, and B.H. Wainer (1987) Choline acetyltransferase immunoreactivity in the rat thalamus. J. Comp. Neurol. *257:*317–332.

McCormick, D.A., and D.A. Prince (1987) Actions of acetylcholine in the guinea-pig and cat medial and lateral geniculate nuclei, *in vitro.* J. Physiol. (Lond.) *392:*147–165.

Montero, V.M., and G.L. Scott (1981) Synaptic terminals in the dorsal lateral geniculate nucleus from neurons of the thalamic reticular nucleus: A light and electron microscope autoradiographic study. Neuroscience *6:*2561–2577.

Montero, V.M., and J. Zempel (1985) Evidence for two types of GABA-containing interneurons in the A-laminae of the cat lateral geniculate nucleus: A double-label HRP and GABA-immunocytochemical study. Expl. Brain Res. *60:*603–609.

Montero, V.M., and J. Zempel (1986) The proportion and size of GABA-immunoreactive neurons in the magnocellular and parvocellular layers of the lateral geniculate nucleus of the rhesus monkey. Expl. Brain Res. *62:*215–223.

Morest, D.K. (1965) The lateral tegemental system of the midbrain and the medial geniculate body: Study with Golgi and Nauta methods in cat. J. Anat. (Lond.) *99:*611–634.

Morest, D.K. (1971) Dendrodendritic synapses of cells that have axons: The fine structure of the Golgi type II cell in the medial geniculate body of the cat. Z. Anat. Entwicklungsgesch. *133:*216–246.

Morest, D.K. (1975) Synaptic relationships of Golgi type II cells in the medial geniculate body of the cat. J. Comp. Neurol. *162:*157–194.

Morest, D.K., and J.A. Winer (1986) The comparative anatomy of neurons: Homologous neurons in the medial geniculate body of the opossum and the cat. Adv. Anat. Embryol. Cell Biol. *97:*1–96.

Mugnaini, E., and A.-L. Dahl (1983) Zinc-aldehyde fixation for light-microscopic immunocytochemistry of nervous tissues. J. Histochem. Cytochem. *31:*1435–1438.

Mugnaini, E., and W.H. Oertel (1985) An atlas of the distribution of GABAergic neurons and terminals in the rat CNS as revealed by GAD immunohistochemistry. In A. Björklund and T. Hökfelt (eds): Handbook of Chemical Neuroanatomy, Vol. 4, GABA and Neuropeptides in the CNS, Part I. Amsterdam: Elsevier Scientific Publishers B.V., pp. 436–608.

Oertel, W.H., D.E. Schmechel, E. Mugnaini, M.L. Tappaz, and I.J. Kopin (1981) Immunocytochemical localization of glutamate decarboxylase in rat cerebellum with a new antiserum. Neuroscience *6:*2715–2736.

Oertel, W.H., D.E. Schmechel, and E. Mugnaini (1983) Glutamic acid decarboxylase (GAD): Purification, antiserum production, immunocytochemistry. In J.L. Barker and J.F. McKelvey (eds): Current Methods in Cellular Biology, Vol. 1, Anatomical Techniques. New York: John Wiley & Sons, pp. 63–110.

Ohara, P.T., G. Chazal, and H.J. Ralston, III (1989) Ultrastructural analysis of GABA-immunoreactive elements in the monkey thalamic ventrobasal complex. J. Comp. Neurol. *283:*541–558.

Olsen, J.F. (1986) Processing of Biosonar Information by the Medial Geniculate Body of the Mustached Bat, *Pteronotus parnellii.* Doctoral dissertation, Washington University, St. Louis, MO.

Olsen, J.F., and N. Suga (1991a) Combination-sensitive neurons in the

medial geniculate body of the mustached bat: Encoding of relative velocity information. J. Neurophysiol. *65:*1254–1274.

Olsen, J.F., and N. Suga (1991b) Combination-sensitive neurons in the medial geniculate body of the mustached bat: Encoding of target range information. J. Neurophysiol. *65:*1275–1296.

O'Neill, W.E. (1985) Responses to pure tones and linear FM components of the CF-FM biosonar signal by single units in the inferior colliculus of the mustached bat. J. Comp. Physiol. A. *157:*797–815.

Ottersen, O.P., and J. Storm-Mathisen (1984) Neurons containing or accumulating transmitter amino acids. In A. Björklund, T. Hökfelt, and M.J. Kuhar (eds): Handbook of Chemical Neuroanatomy, Vol. 3, Classical Transmitters and Transmitter Receptors in the CNS, Part II. Amsterdam: Elsevier Science Publishers B.V., pp. 141–246.

Park, T., G. Pollak, J. Winer, and D. Larue (1991) Colocalization of inhibitory transmitters and HRP in projection cells of mustache bat's auditory brain stem: Projections from the lateral superior olive to the inferior colliculus. Assoc. Res. Otolaryngol. *14:*89 (abstract).

Penny, G.R., M. Conley, D.E. Schmechel, and I.T. Diamond (1984) The distribution of glutamic acid decarboxylase immunoreactivity in the diencephalon of the opossum and the rabbit. J. Comp. Neurol. *228:*38–56.

Pollak, G.D., and J.H. Casseday (1989) The Neural Basis of Echolocation in Bats. Zoophysiology series, Vol. 25. Berlin: Springer-Verlag.

Pollak, G.D., and J.A. Winer (1989) Glycinergic and GABAergic auditory brain stem neurons and axons in the mustache bat. Proc. Soc. Neurosci. *15:*1115 (abstract).

Rinvik, E., O.P. Ottersen, and J. Storm-Mathisen (1987) Gamma-aminobutyrate-like immunoreactivity in the thalamus of the cat. Neuroscience *21:*781–805.

Roberts, R.C., H.L. Kim, and C.E. Ribak (1985) Increased numbers of neurons occur in the inferior colliculus of the young genetically epilepsy-prone rat. Dev. Brain Res. *23:*277–281.

Rodrigues-Dagaeff, C., G. Simm, Y. de Ribaupierre, A. Villa, F. de Ribaupierre, and E.M. Rouiller (1989) Functional organization of the ventral division of the medial geniculate body of the cat: Evidence for a rostro-caudal gradient of response properties and cortical projections. Hearing Res. *39:*103–125.

Rouiller, E.M., E. Colomb, M. Capt, and F. de Ribaupierre (1985) Projections of the reticular complex of the thalamus onto physiologically characterized regions of the medial geniculate body. Neurosci. Lett. *53:*227–232.

Ryugo, D.K., and N.M. Weinberger (1976) Corticofugal modulation of the medial geniculate body. Expl. Neurol. *51:*377–391.

Saint Marie, R.L., E.-M. Ostapoff, D.K. Morest, and R.J. Wenthold (1989) Glycine-immunoreactive projection of the cat lateral superior olive: Possible role in midbrain ear dominance. J. Comp. Neurol. *279:*382–396.

Smith, Y., P. Séguéla, and A. Parent (1987) Distribution of GABA-immunoreactive neurons in the thalamus of the squirrel monkey (*Saimiri sciureus*). Neuroscience *22:*579–591.

Špaček, J., and A.R. Lieberman (1974) Ultrastructure and three-dimensional organization of synaptic glomeruli in rat somatosensory thalamus. J. Anat. (Lond.) *117:*487–516.

Sternberger, L.A., and S.A. Joseph (1979) The unpaired antibody method. Contrasting color staining of paired pituitary hormones without antibody removal. J. Histochem. Cytochem. *27:*1424–1429.

Suga, N. (1984) The extent to which biosonar information is represented in the bat auditory cortex. In G.M. Edelman, W.E. Gall, and W.M. Cowan (eds): Dynamic Aspects of Neocortical Function. New York: John Wiley & Sons, pp. 315–373.

Suga, N., and N. Tsuzuki (1985) Inhibition and level-tolerant frequency tuning in the auditory cortex of the mustached bat. J. Neurophysiol. *53:*1109–1145.

Wenstrup, J.J., and J.A. Winer (1987) Projections to the medial geniculate body from physiologically defined frequency representations of the mustached bat's inferior colliculus. Proc. Soc. Neurosci. *13:*324 (abstract).

Wenstrup, J.J., D.T. Larue, and J.A. Winer (1988) Distribution of GAD-immunoreactivity in the medial geniculate body of the mustached bat. Assoc. Res. Otolaryngol. *11:*163 (abstract).

Whitfield, I.C., and D. Purser (1972) Microelectrode study of the medial geniculate body in unanaesthetized free-moving cats. Brain Behav. Evol. *6:*311–322.

Williams, M.N., and R.L.M. Faull (1987) The distribution and morphology of identified thalamocortical projection neurons and glial cells with reference to the question of interneurons in the ventrolateral nucleus of the rat thalamus. Neuroscience *21:*766–780.

Willingham, M.C., and A.V. Rutherford (1984) The use of osmium-thiocarbohydrazide-osmium (OTO) and ferrocyanide reduced osmium methods to enhance membrane contrast and preservation in cultured cells. J. Histochem. Cytochem. *32:*455–460.

Winer, J.A. (1984) Identification and structure of neurons in the medial geniculate body projecting to primary auditory cortex (AI) in the cat. Neuroscience *13:*395–418.

Winer, J.A. (1985) The medial geniculate body of the cat. Adv. Anat. Embryol. Cell Biol. *86:*1–98.

Winer, J.A. (1991) Anatomy of the medial geniculate body. In R.A. Altschuler, D.W. Hoffman, R.P. Bobbin, and B.M. Clopton (eds): Neurobiology of Hearing, Vol. II, The Central Auditory System. New York: Raven Press, Ltd., pp. 293–333.

Winer, J.A. (1992) The functional architecture of the medial geniculate body and the primary auditory cortex. In A.N. Popper, R.R. Fay, and D.B. Webster (eds): The Mammalian Auditory Pathway: Neuroanatomy, Vol. 1, Springer Handbook of Auditory Research. New York: Springer-Verlag, pp. 222–409.

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

Winer, J.A., and D.T. Larue (1988) Anatomy of glutamic acid decarboxylase (GAD) immunoreactive neurons and axons in the rat medial geniculate body. J. Comp. Neurol. *274:*47–68.

Winer, J.A., and D.T. Larue (1989) Populations of GABAergic neurons and axons in layer I of rat auditory cortex. Neuroscience *33:*499–515.

Winer, J.A., and D.K. Morest (1983) The medial division of the medial geniculate body of the cat: Implications for thalamic organization. J. Neurosci. *3:*2629–2651.

Winer, J.A., and J.J. Wenstrup (1987) Anatomy of the mustached bat's medial geniculate body: Cytoarchitectonics, neuronal architecture, and GAD-immunoreactivity. Proc. Soc. Neurosci. *13:*324 (abstract).

Winer, J.A., and J.J. Wenstrup (1992a) The neurons of the medial geniculate body in the mustached bat (*Pteronotus parnellii*). J. Comp. Neurol. (submitted).

Winer, J.A., and J.J. Wenstrup (1992b) Cytoarchitecture of the medial geniculate body in the mustached bat. J. Comp. Neurol. (submitted).

Zook, J.M., J.A. Winer, G.D. Pollak, and R.D. Bodenhamer (1985) Topology of the central nucleus of the mustache bat's inferior colliculus: Correlation of single unit response properties and neuronal architecture. J. Comp. Neurol. *231:*530–546.