THE JOURNAL OF COMPARATIVE NEUROLOGY 355:317–353 (1995)

GABA and Glycine in the Central Auditory System of the Mustache Bat: Structural Substrates for Inhibitory Neuronal Organization

JEFFERY A. WINER, DAVID T. LARUE, AND GEORGE D. POLLAK
Division of Neurobiology, Department of Molecular and Cell Biology, University of California at Berkeley, Berkeley, California 94720-3200 (J.A.W., D.T.L.), and Department of Zoology, The University of Texas, Austin, Texas 78712-1064 (G.D.P.)

ABSTRACT

The distribution and morphology of neurons and axonal endings (puncta) immunostained with antibodies to gamma-aminobutyric acid (GABA) and glycine (Gly) were analyzed in auditory brainstem, thalamic, and cortical centers in the mustache bat. The goals of the study were (1) to compare and contrast the location of GABAergic and glycinergic neurons and puncta, (2) to determine whether nuclei containing immunoreactive neurons likewise have a similar concentration of puncta, (3) to assess the uniformity of immunostaining within a nucleus and to consider regional differences that were related to or independent of cytoarchitecture, and (4) to compare the patterns recognized in this bat with those in other mammals.

There are nine major conclusions. (1) Glycinergic immunostaining is most pronounced in the hindbrain. (2) In the forebrain, GABA alone is present. (3) Some nuclei have GABAergic or glycinergic neurons exclusively; a few have neither. (4) Although there is sometimes a close relationship between the relative number of immunopositive neurons and the density of the puncta, just as often there is no particular correlation between them; this reflects the fact that many GABAergic and glycinergic neurons project beyond their nucleus of origin. (5) Even nuclei devoid of or with few GABAergic or glycinergic neurons contain relatively abundant numbers of puncta; some neurons receive axosomatic terminals of each type. (6) In a few nuclei there are physiological subregions with specific local patterns of immunostaining. (7) The patterns of immunostaining resemble those in other mammals; the principal exceptions are in nuclei that, in the bat, are hypertrophied (such as those of the lateral lemniscus) and in the medial geniculate body. (8) Cellular colocalization of GABA and Gly is specific to only a few nuclei. (9) GABA and glutamic acid decarboxylase (GAD) immunostaining have virtually identical distributions in each nucleus.

Several implications follow. First, the arrangements of GABA and Gly in the central auditory system represent all possible patterns, ranging from mutually exclusive to overlapping within a nucleus to convergence of both types of synaptic endings on single neurons. Second, although both transmitters are present in the hindbrain, glycine appears to be dominant, and it is often associated with circuitry in which precise temporal control of aspects of neuronal discharge is critical. Third, the auditory system, especially at or below the level of the midbrain, contains significant numbers of GABAergic or glycinergic projection neurons. The latter feature distinguishes it from the central visual and somatic sensory pathways.

Indexing terms: inhibition, axon terminals, local circuits, inhibitory projections, disinhibition

Gamma-aminobutyric acid (GABA) and glycine (Gly) are the principal inhibitory neurotransmitters throughout the mammalian neuraxis (Ottersen and Storm-Mathisen, 1984; Venter, 1984; Alger, 1985; Wenthold and Hunter, 1990). Their manifold actions may contribute to an enormous spectrum of postsynaptic consequences, including lateral

Accepted September 24, 1994.

Address reprint requests to Jeffery A. Winer, Division of Neurobiology, Department of Molecular and Cell Biology, Room 289 Life Sciences Addition, University of California at Berkeley, Berkeley, CA 94720-3200.

inhibition (Boos et al., 1990; Vater et al., 1992b; Yang et al., 1992), recurrent inhibition (Babb et al., 1989), disinhibition (Adams and Mugnaini, 1990; Park and Pollak, 1993a), spontaneous activity (Faingold et al., 1989), and feedforward inhibition (Shneiderman and Oliver, 1989; Bledsoe et al., 1990), as well as modulating other facets of receptive field organization (Alloway et al., 1988; Masland, 1988; Pollak et al., 1992; Yang et al., 1992) and intrinsic inhibition (Saint Marie et al., 1991). With the development of antisera specific for GABA and Gly conjugates, their particular pattern of localization in neuronal perikarya and axon terminals has been investigated in some detail in many parts of the central auditory pathway in several species (reviewed in Aitkin, 1989). However, there has been neither a systematic study nor a detailed comparison of their distribution throughout the entire auditory system of a single species. Such knowledge would be important in understanding why more than one such transmitter might exist, and whether they covary or are largely independent.

We present an overview of the immunocytochemical profile of the mustache bat's auditory system that is based on the patterns of immunoreactivity in both neuronal somata and axonal endings for GABA and glycine. We chose the mustache bat for these studies because the remarkable hypertrophy of its central auditory pathway encourages precise architectonic distinctions within and between even relatively minute nuclear centers, and the small absolute size of the brain renders the processing and evaluation of data more tractable than in larger animals. In its main outlines, the central auditory system is readily compared with that of other mammals (Pollak and Casseday, 1989; Ross et al., 1988; Pollak et al., 1995). Moreover, neurophysiological studies have revealed response features of neurons in almost all auditory nuclei that clearly represent the neural correlates for sound localization, target distance, fine-frequency discriminations, and target recognition (Pollak, 1988; Suga, 1988; Pollak and Casseday, 1989). It seems likely that many of the physiological operations that would represent these attributes depend on inhibitory circuits that remain to be defined with more precision.

In this report we describe the major patterns of immunostaining in auditory nuclei of the hindbrain, midbrain, and forebrain, and compare them with one another. The study presents a somewhat unusual perspective on the organization of the auditory system that is difficult to obtain from reports that address only one or two nuclei in particular or that are limited to one transmitter or that are restricted solely to considerations of neurons or puncta. This account focuses on the relevance of the patterns of immunoreactivity for four main issues. The first issue concerns the spatial distribution of GABAergic and glycinergic neurons and puncta in the principal auditory nuclei, and the functional implications that would follow from these arrangements. A second question concerns the extent to which the distribution of GABAergic and glycinergic neurons in the hindbrain auditory nuclei serve as predictors of and substrates for one functional pattern or another. If one transmitter predominates in nuclei that are part of pathways with established functions, then perhaps each has circuit-specific roles in processing information. However, if GABAergic and glycinergic neurons are more or less completely commingled within each nucleus or pathway, then perhaps the two transmitters are functionally equivalent, or they may be segregated on a neuron-by-neuron basis. A third issue is the differential degree to which each transmitter is represented in the hindbrain, midbrain, and forebrain and the significance of any differential concentrations for information processing. The fourth question addresses the generality of the immunostaining patterns in the auditory systems of different species. We will show that the chemoarchitectonic organization of the mustache bat's brainstem and auditory cortex is comparable in many (but not all) respects to that of other mammals, although there are major differences in the acoustic thalamus. These findings could suggest some

Abbreviations

AlD	anterolateral division of the central nucleus of the inferior colliculus
Am	amygdala
ar	fiducial artifact
AvCNp	anteroventral cochlear nucleus, posterior subdivision
D	dorsal nucleus of the dorsal division of the medial geniculate body or dorsal division
DC	dorsal cortex of the inferior colliculus
DCN	dorsal cochlear nucleus
DNLL	dorsal nucleus of the lateral lemniscus
DmD	dorsomedial division of the central nucleus of the inferior colliculus
DpD	dorsoposterior division of the central nucleus of the inferior colliculus
DPo	dorsomedial periolivary region
DS	superficial dorsal nucleus of the dorsal division of the medial geniculate body
DSCF	Doppler-shifted constant frequency cortical area
Ex	external nucleus of the inferior colliculus
FM_1–FM_3	combination-sensitive cortical area
IC	inferior colliculus
ICC	central nucleus of the inferior colliculus
IcT	intercollicular tegmentum
INLL	intermediate nucleus of the lateral lemniscus
LL	lateral lemniscus
LSO	lateral superior olive
M,MGBm	medial division of the medial geniculate body
MGB	medial geniculate body
MNTB	medial nucleus of the trapezoid body
MSO	medial superior olive
NCAT	nucleus of the central acoustic tract
PvCNa	posteroventral cochlear nucleus, anterior subdivision
PvCNp	posteroventral cochlear nucleus, posterior subdivision
Sg	suprageniculate nucleus of the dorsal division of the medial geniculate body
SN	substantia nigra
TB	trapezoid body
V	ventral division of the medial geniculate body
VIIINr	nucleus of the auditory nerve root
VIIIr	auditory nerve root
Vl	lateral subdivision of the ventral nucleus of the medial geniculate body
Vm	medial subdivision of the ventral nucleus of the medial geniculate body
VmPo	ventromedial periolivary nucleus
VNLL	ventral nucleus of the lateral lemniscus
VNLLd	ventral nucleus of the lateral lemniscus, dorsal subdivision
VNLLv	ventral nucleus of the lateral lemniscus, ventral subdivision
VNTB	ventral nucleus of the trapezoid body
wm	white matter
I–VI	cortical layers

Planes of section

C	caudal
D	dorsal
L	lateral
M	medial
V	ventral

principles of inhibitory organization that are relevant to species differences in mammalian auditory forebrain function.

MATERIALS AND METHODS

Bats were captured at Mt. Plenty or Windsor Cave, Jamaica, West Indies, transported to the United States, and housed in a colony room under daily veterinary supervision. All animal husbandry, anesthesia, and perfusion protocols were reviewed and approved by a University Animal Care and Use Committee and took place in an accredited facility. For glycine and GABA immunostaining, adult mustache bats (*Pteronotus parnellii*) weighing 10–12 g were anesthetized with intraperitoneal sodium pentobarbital to a point sufficient to abolish nociceptive hindlimb withdrawal and corneal reflexes. The bat was perfused transcardially with a brief wash (2–3 ml) of phosphate buffered saline, followed by a mixture of paraformaldehyde (2–4%) and glutaraldehyde (0.25–3%) in 0.1 M phosphate buffer. For GAD, the zinc-salicylate-formalin method (Mugnaini and Dahl, 1983) was used (see Winer and Larue, 1988 for details). A peristaltic pump delivered the perfusate at a uniform, controlled pressure comparable to the rate and volume of the circulation in a living animal.

The brain was blocked stereotaxically in the frontal plane and then postfixed in 30% sucrose/0.9% saline (for GAD), 10% sucrose/0.1 M phosphate buffer (for GABA or glycine), or 2% paraformaldehyde/3% glutaraldehyde (for plastic embedding). For GAD, brains were sectioned on a freezing microtome at 25 μm and collected in 0.5 M Tris at pH 7.6 (Mugnaini and Dahl, 1983). For GABA and glycine, sections were cut on a Vibratome (Oxford) at 50 μm for free-floating immunocytochemistry, or at 100–300 μm for plastic embedding; the details are given in each figure legend. The thinner sections were immunostained using avidin-biotin peroxidase (ABC) kit (Vectastain™; Vector Laboratories, Burlingame, CA). Sections were placed in rabbit-anti-glycine antiserum (courtesy of R.J. Wenthold; typical dilutions: Gly I, 1:400; Gly II, 1:1,500), or in rabbit-anti-GABA antiserum (from R.J. Wenthold; 1:2,000; or INCstar, Clearwater, MN; 1:5,000) overnight at 4°C, following a 60-minute incubation in blocking serum (5% normal goat serum) at room temperature. For postembedding, the 100–300-μm-thick slabs were first block stained in 0.5–2% osmium tetroxide for 1–3 hours, then dehydrated in ascending ethanols to propylene oxide and an epoxy (Araldite 6005) modified with the plasticizer dibutyl phthalate, then embedded flat and polymerized at 60°C for 16 hours. Sections 1–1.5-μm thick were cut from blocks containing either whole or hemibrain stems with 8-mm wide glass knives on an LKB Ultratome III. The sections were heat annealed onto uncoated glass slides, etched in ethanolic sodium hydroxide for 1–2 hours, deosmicated in 0.3% hydrogen peroxide in ethanol, then rehydrated, and incubated on the slide in 5% normal goat serum; this was followed by immersion in the primary antiserum as above and at up to 5 times the concentration used in the free-floating procedure. Immunoperoxidase staining was done with ABC reagents at double their normal concentrations or with the streptavidin-biotin (Histomark™) kit (Kirkegaard & Perry Inc., Gaithersburg, MD) at the ready-to-use dilution. The chromogen was cobalt nickel-intensified diaminobenzidine (Adams, 1981). Controls for GABA or glycine included omission of the primary antiserum or adsorption of the primary with the antigen conjugate and, for GAD, incubation in preimmune serum. In both instances, specific immunostaining was absent.

Material from 20 bats was studied. Most of the tissue was prepared by the free-floating immunoperoxidase method for GAD, GABA, or glycine. Semithin sections from four brains flat-embedded in Araldite epoxy were processed for postembedding immunocytochemistry.

The survey of immunostained structures shown in Table 1 (see also Figs. 1–3) was made at a dual-viewing microscope and represented the consensus of both evaluators; it included all the material available. Each value is an estimate of the comparative level of immunoreactivity along a qualitative, ordinal scale of intensity.

RESULTS

Immunoreacted sections of the mustache bat's brain demarcated many parts of the auditory system with a

Fig. 1. (appears on page 320) Global views of immunoreactivity for gamma-aminobutric acid (GABA; panel A) and of glutamic acid decarboxylase (GAD; panel B) immunoreactivity for neurons and puncta in the nuclei of the medulla and midbrain in the mustache bat. This shows a regionally specific pattern of staining which should be compared with the corresponding distribution of glycine immunoreactivity shown in Figure 2; see also Table 1 and Figures 3 and 4 for summaries. For abbreviations, see the accompanying list. Details of the immunostaining procedure are noted at the end of each figure caption. Planapochromat, N.A. 0.04, × 16. **A:** Among the medullary auditory nuclei, the anteroventral cochlear nucleus (AVCN) had few GABAergic neurons and a comparatively dense concentration of puncta, whereas the lateral superior olive (LSO) had its densest GABA puncta immunostaining in its medial limb and much sparser reactivity in the lateralmost part. Even nuclei with rather modest levels of GABA immunostaining, such as the medial superior olive (MSO), were demarcated clearly by their immunoreactivity. **B:** GAD immunoreactivity in the trapezoid body (MNTB, VNTB) and midbrain also revealed diverse and regionally specific patterns, with the nuclei of the lateral lemniscus (DNLL, INLL, VNLL) being especially noteworthy. Some nuclei (for example, INLL) also had distinct, nonuniform internal concentrations of immunoreactivity. Where the GAD immunostaining was the palest, such as in the lateral lemniscal entry zone (LL), the glycinergic elements were often most prominent (see Fig. 2B:LL). The artifact (ar) is a fiducial mark. A: Vibratome section, 50-μm thick; GABA (INCstar), 1:5,000 dilution, ABC avidin-biotin (Vector Laboratories), free-floating. B: Frozen section, 25-μm thick; GAD 1440 (W. Oertel), 1:2,000 dilution, ABC avidin-biotin, free-floating.

Fig. 2. (appears on page 321) Glycinergic immunoreactivity under darkfield illumination in the brainstem at loci matching those in Figure 1. Each cochlear nucleus subdivision displayed a moderate level of regionally specific immunostaining, and the level of glycine immunoreactivity in the principal olivary nuclei (LSO, MSO) included both resident elements and the many immunopositive fibers traversing them. At the level of the midbrain, many of the auditory nuclei showed conspicuous immunoreactivity, with ascending glycinergic axons especially prominent in the lateral lemniscus (LL). Same scale and protocol as in Figure 1. **A:** In the medulla, the glycinergic elements were concentrated preferentially in auditory nuclei, and both the preterminal axons and the puncta were as prominent as the immunopositive neurons (see Fig. 8). Some immunoreactive axons are visible just beneath the floor of the IVth ventricle. Perhaps these represent commissural glycinergic axons arising from neurons in the ventral cochlear nuclei (Wenthold, 1987). **B:** All nuclei from the trapezoid body to the inferior colliculus showed prominent Gly immunoreactivity, especially the lateral lemniscal entry zone, where GAD-positive elements were rarer (see Fig. 1B:LL). A, B: Vibratome section, 50-μm thick; Gly I (R.J. Wenthold), 1:400 dilution, ABC avidin-biotin, free-floating.

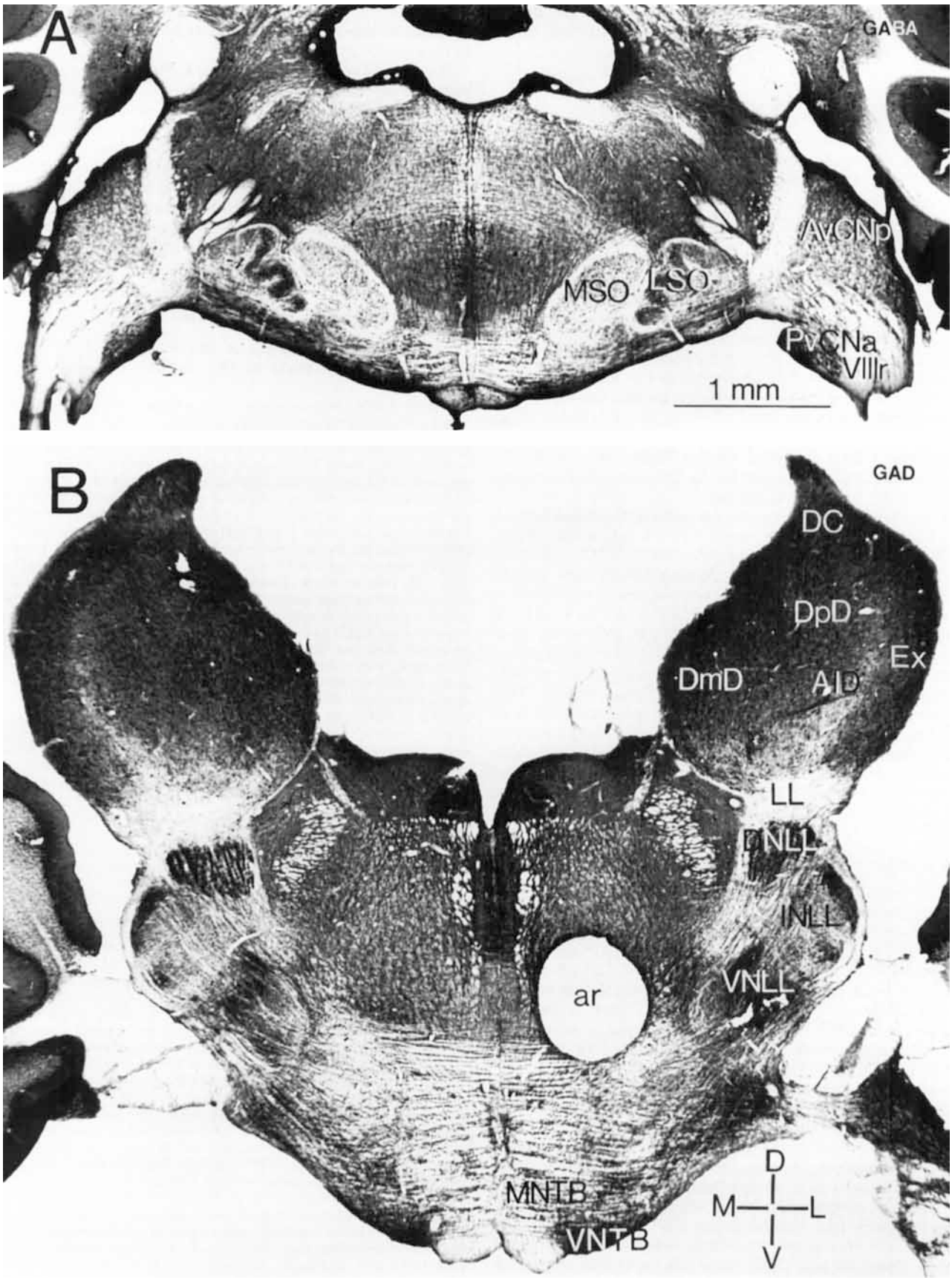


Figure 1. Legend on page 319.

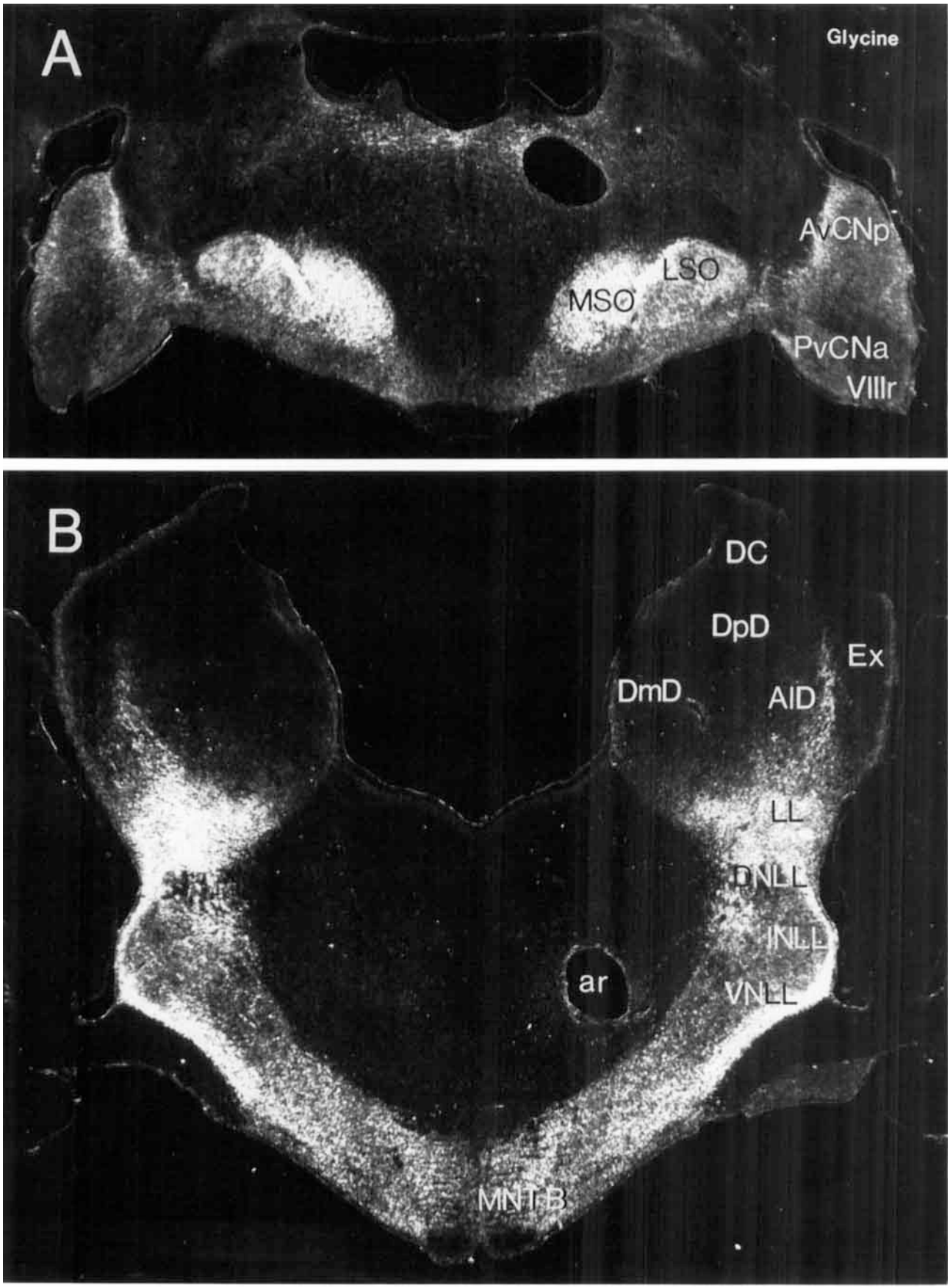


Figure 2. Legend on page 319.

clarity that complemented and confirmed the results from cytoarchitectonic preparations. The immunostaining revealed the prominence of inhibitory components in this pathway (Figs. 1, 2). The many immunopositive axons in the major fiber tracts (Fig. 2B:LL) suggest that some cells may participate in ascending projections rather than only local circuits. We will consider the specific patterns of immunoreactivity for each of the principal nuclei of the auditory pathway, beginning with the cochlear nucleus and extending to the auditory cortex. Schematic summaries of the chief results appear in Figures 3 and 4 and in Table 1. For purposes of economy, we have illustrated only the principal and representative findings in the auditory pathway, while in the interest of completeness the text contains brief descriptions of nearly every nucleus or center whose affiliations are primarily auditory (Aitkin, 1989).

The GABA and GAD material revealed virtually the same distribution of immunostained elements (not illustrated; see Winer et al. [1992] and Prieto et al. [1994a] for a more complete discussion); for purposes of continuity in the exposition, emphasis was placed on the GABA preparations except as noted. Our criteria for assessing neuronal immunostaining are presented in the legend to Figure 6.

Cochlear nucleus

Three cochlear nucleus subdivisions—the dorsal, anteroventral, and posteroventral cochlear nuclei—were recognized on cytoarchitectonic grounds (Zook and Casseday, 1982a). Immunostaining for GABA and Gly defined the borders of each with equal precision (Figs. 1A, 2A, 5). In every subdivision, glycine-positive (Gly+) neurons were more common than GABA-positive (GABA+) cells. The puncta were comparable in density but had characteristic patterns that were specific to each nucleus.

The dorsal cochlear nucleus had more GABA+ neurons than the other subdivisions (compare Figs. 5A,C). Most of these cells were dispersed in small clusters throughout layers I and II, and another group lay deep in the pyramidal cell layer (Fig. 5A). The bulk of the immunoreactive cells were oval or fusiform in shape and 8–10 μm in diameter (Fig. 6A:1–3). Most of these cells were also immunoreactive for glycine (Figs. 5A,B, 6A,B). The posteroventral cochlear nucleus had scattered, small GABA+ cells that also colocalized Gly (Fig. 5B:PvCNp). In the anteroventral cochlear nucleus, only a few very small neurons (less than 8 μm in diameter) were GABA+ (Figs. 5C, 6E), as were some slightly larger cells. Both populations were smaller than the immunonegative spherical bushy cells.

Many more—up to half—of dorsal cochlear nucleus neurons were Gly+ (Fig. 5B). These were heterogeneous in shape and ranged from 8 to 12 μm in diameter (Fig. 6B). Both the posteroventral and anteroventral subdivisions contained far fewer Gly+ cells (Fig. 5D). These represented a broad range of shapes and sizes. The few smaller cells usually colocalized GABA, whereas the more plentiful larger cells, which ranged from 12 to 18 μm in diameter, showed no GABA immunoreactivity (Fig. 6D,F).

The distribution of immunoreactive puncta also distinguished the various subdivisions. These endings differed primarily in density, form, their arrangement in the neuropil, and their pattern of perisomatic terminals. In the exposition that follows, we have used the terms puncta, terminal, and ending interchangeably, always with the proviso that we have not established a direct synaptic relationship between their light- and their electron-microscopic identities (see Prieto et al. [1994b] for a more complete treatment of this issue). We first compare the configuration of GABA+ puncta among the three cochlear nucleus subdivisions and then turn to Gly+ puncta.

The similarity in the pattern of the puncta between cochlear nucleus subdivisions (Figs. 5C,D) should not obscure important differences between them (Fig. 6). In the dorsal cochlear nucleus, GABA+ puncta were especially abundant: they were densest in the superficial layers and decreased markedly in the deepest ones (Fig. 6A). The endings were common on both neuronal perikarya and in the neuropil, and they were granular and fine-to-medium sized (Fig. 6A). In contrast, there were fewer GABA+ puncta in the posteroventral cochlear nucleus and these were coarser and more globular. They formed perisomatic rings about the somata of immunonegative neurons (Fig. 6C:2), much like those in the anteroventral cochlear nucleus (Fig. 6E).

The dorsal cochlear nucleus also had many Gly+ puncta that, like the GABA+ terminals, were especially prominent in the upper two layers, then diminished abruptly in layers III and IV, although they were still present in considerable numbers (Fig. 6B). Occasionally, Gly+ axosomatic endings ringed Gly+ somata at the border between layers II and III; such terminals were remote from the heaviest concentration of Gly+ neurons. Terminals apposing Gly+ somata were often large and coarse, especially in the superficial two layers, whereas puncta on Gly+ cells in layers III and IV were smaller and much less common. This pattern contrasted with that in the posteroventral cochlear nucleus. Here, both Gly+ and Gly− somata and proximal dendrites had many more Gly+ endings, some larger than 1 μm in diameter, and the number of terminals in the neuropil was far less than that of GABA+ endings. The arrangement of Gly+ puncta in the anteroventral cochlear nucleus recalled that of GABA+ terminals, with many perisomatic endings on immunonegative neurons (Fig. 6F). Gly+ neurons received fewer such endings. A few much finer endings were seen in the neuropil.

Olivary nuclei

Both the lateral and medial superior olives were prominent in the mustache bat (Figs. 1, 2; see Zook and Casseday, 1982a). The most notable aspects of their chemical anatomy were (1) regional heterogeneity of the GABA immunostaining (Fig. 1A), and (2) the conspicuous concentration of glycinergic neurons and puncta, as well as the many preterminal axons that passed through the nuclei (Fig. 2A).

Lateral superior olive. The comparatively few GABA+ neurons were concentrated in the lateral one-third of the nucleus and along the perimeter, rather than more centrally (Fig. 7A). The most medial and central regions had only rare GABA+ cells, and these, too, lay along its margin, interspersed among trapezoid body axons (Fig. 7A:TB). Typically, these neurons were 8–10 μm in diameter, with an elongated soma that had little or no dendritic immunoreactivity. A few larger cells were immunopositive (Fig. 8A), and such neurons were sometimes immunopositive for Gly (Fig. 8B).

The Gly+ neurons, in contrast, were far more numerous, and they comprised up to one-third of the cells. These lay along the ventral aspect of the olivary limbs (medially) and were still more numerous and evenly dispersed in the lateral limb (Fig. 7B). These oval cells were among the largest in our sample (15–18 μm in diameter), and they

sometimes had an immunostained dendrite. They resembled the immunonegative olivary neurons (Fig. 8B). Some of the immunopositive neurons also colocalized GABA (Fig. 8A,B:3).

Despite the relative paucity of GABA+ neurons, there was a robust plexus of intensely immunoreactive GABA+ preterminal axons mixed with puncta; this was concentrated in the central and medial limbs and sparser in the more lateral parts (Figs. 1A, 7A). The arrangement of Gly+ puncta presented a rather different pattern. First, these endings had a more homogeneous, predominantly axosomatic, organization throughout the nucleus (Figs. 7B, 8B). Indeed, every lateral superior olivary neuron appeared to receive Gly+ endings. Second, Gly+ endings encircled the somatic membrane completely; these terminals, in turn, were ringed by, or were interdigitated among, a fine-textured plexus of GABA+ puncta (Fig. 8A,B:1,2).

Medial superior olive. The organization of GABA+ and Gly+ cells in the medial superior olive was likewise distinctive. GABA+ neurons were more common in the dorsomedial half (Figs. 1A, 7C). These cells were 15–18 μm in diameter, and their slightly elongated somata were oriented across the long axis of the nucleus. Sometimes, a large dendritic trunk extended ventrolaterally. The distribution of Gly+ neurons recapitulated that of the GABA+ cells, aggregating along the dorsomedial aspect of the nucleus (Fig. 7D). Cells immunopositive for both GABA and Gly were comparatively common.

The density of GABA+ medial superior olivary puncta was substantially lower than in the lateral superior olive (compare Fig. 8A and C). However, their distribution within the nucleus was also nonuniform. There were moderate numbers dorsomedially, both on somata and in the neuropil, and even more ventrolaterally, where most immunonegative somata received fine or medium-sized endings (Fig. 8C). In contrast, the Gly+ puncta were much more evenly distributed. As in the lateral superior olive, both immunopositive and immunonegative neurons were ringed with perisomatic endings (Fig. 8D), some of which interdigitated with GABA+ axosomatic terminals (Fig. 8C,D:1).

Trapezoid body and associated regions

The most salient features of the medial nucleus of the trapezoid body were the entirely glycinergic neuronal population (Fig. 7F) and the striking paucity of Gly+ puncta, especially those associated with neuronal somata, which were almost entirely absent despite the exclusively Gly+ neuronal population. Gly+ endings, if present, were beyond the resolution of the light microscope; all of these profiles represent preterminal axons (Fig. 8F; compare Figs. 6B,D,F, and 8B,D). However, almost every perikaryon received GABA+ boutons of many different sizes and shapes, ranging from tiny granular puncta (Fig. 8E: small arrowheads), to coarse, globular endings (Fig. 8E: medium-sized arrowheads), to long, clasp-like terminals (Fig. 8E: large arrowheads), the latter resembling those immunopositive elsewhere for glycine. Their preterminal portions could be followed for appreciable distances within the sheets of neuropil separating the cells. A similar arrangement of puncta was noted in the GAD+ material, in which the medium-sized, globular endings were most prominent.

The pattern of Gly+ neurons in the medial nucleus of the trapezoid body resembled that so far reported for every mammal: all of the neurons were immunopositive, including those with somata up to 12–18 μm in diameter, among the largest Gly+ neurons we found (Fig. 8F). There was considerable heterogeneity in size and shape among these neurons, suggesting that they include several types (Kuwabara and Zook, 1991). The paucity of Gly+ puncta, in both free-floating sections and in semithin material, was noteworthy (compare Fig. 8B and F). Most Gly+ preterminal axons in the medial nucleus were of comparatively large caliber and were probably in transit through the medial nucleus.

The lateral nucleus of the trapezoid body (not illustrated; see Table 1) had a very different pattern of GABAergic organization. The few immunopositive neurons here were stained lightly, and most such cells were small (about 8 μm in diameter) and were dispersed among immunonegative neurons. The Gly+ cells were far more numerous than the GABA+ neurons. Moreover, they were clustered in small groups between which the fascicles of immunonegative axons passed. In contrast to the GABA+ cells, the Gly+ neurons included small, medium-sized, and large cells; many had their long somatic axis oriented parallel to the plexus of afferent axons. In the neuropil, linear arrays of GABA+ preterminal axons up to 50-μm long ran parallel to the primary dendritic axis, although axosomatic endings were sparse; these puncta were moderate in number. However, there were comparatively few Gly+ puncta in the lateral nucleus; the most conspicuous type was a large globular ending found predominantly in the neuropil and, occasionally, on Gly+ neurons. Many preterminal segments were evident; some were parallel to the long nuclear axis, whereas others crossed it.

The ventral nucleus of the trapezoid body (Fig. 7E) had a moderate number of GABA+ neurons with heterogeneous shapes and sizes. Those in the most ventral, laminated part were more uniform in size and their perikarya were elongated mediolaterally; they were about 10–12 μm in diameter, with a somatic axis up to 20-μm long, and they dominated this part of the nucleus. A different pattern of Gly+ cells was evident in more dorsal parts of the ventral nucleus, where the neurons were dispersed and solitary (Fig. 7F), with their perikarya separated by fascicles of immunonegative trapezoid body axons. The number of such cells was moderate, like that of the GABA+ neurons. Whereas most of these cells had a similar orientation to that of the Gly+ neurons located beneath them, others were arranged orthogonally, and a few were much larger than other ventral nucleus cells.

The disposition of GAD+ puncta was largely independent of the concentration of GAD+ and GABA+ neurons. The prominence of the endings alone would have sufficed to delineate the ventral nucleus (Fig. 1B:VNTB). Their density was highest dorsomedially and laterally, and lowest centrally. Overall, they were moderately heavy in number, and included axosomatic terminals on both immunopositive and immunonegative cells as well as endings in the neuropil. The puncta ranged in size from small, dot-like boutons to medium-sized, granular terminals, to large globular profiles. Whereas the density of Gly+ puncta in the ventral nucleus was comparable to that of their GABA+ counterparts, their distribution was consistent with the concentration of Gly+ neurons. Just as the cells were concentrated in the ventral half, so were the puncta. Many of the preterminal fibers, like the Gly+ neurons, were oriented mediolaterally. Those located more dorsally had a

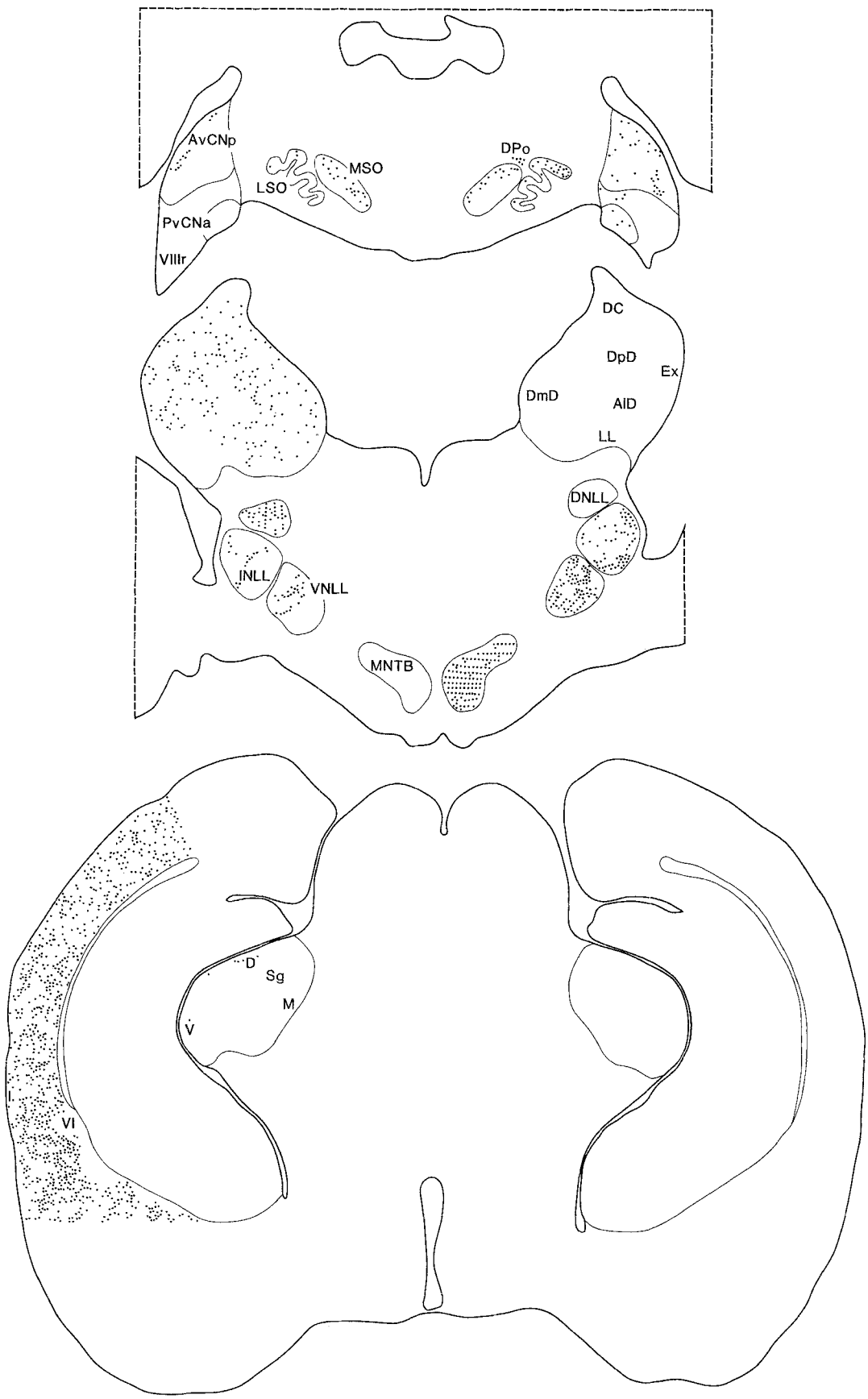


Fig. 3. Summary of immunoreactive neurons in the mustache bat's central auditory pathway. The neuronal density reflects their relative concentration. The principal, lemniscal nuclei are illustrated chiefly, and others are presented in the text or in photomicrographs. See also Figures 1 and 2 and Table 1 for a summary of the primary patterns. Planapochromat, N.A. 0.14, × 80.

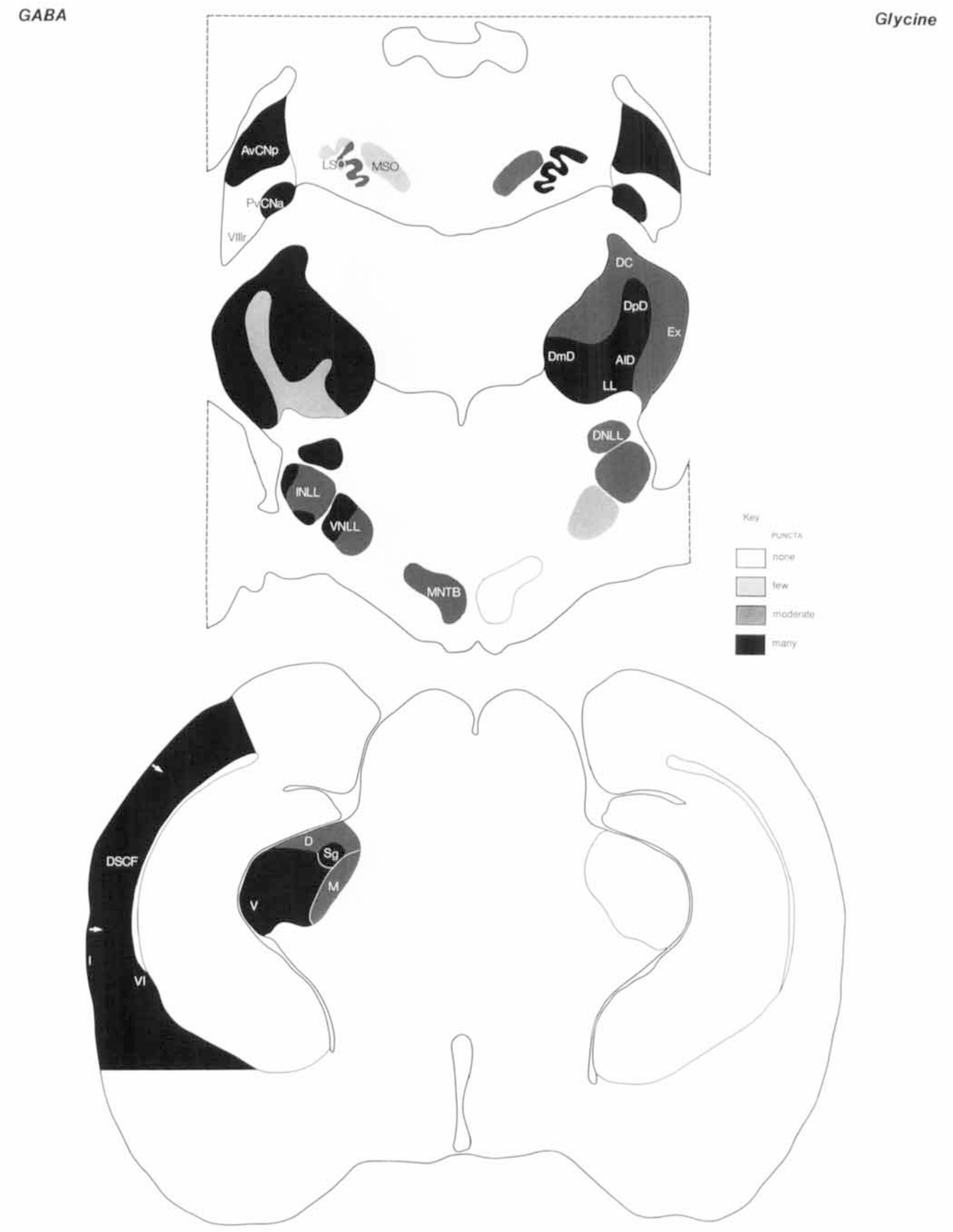
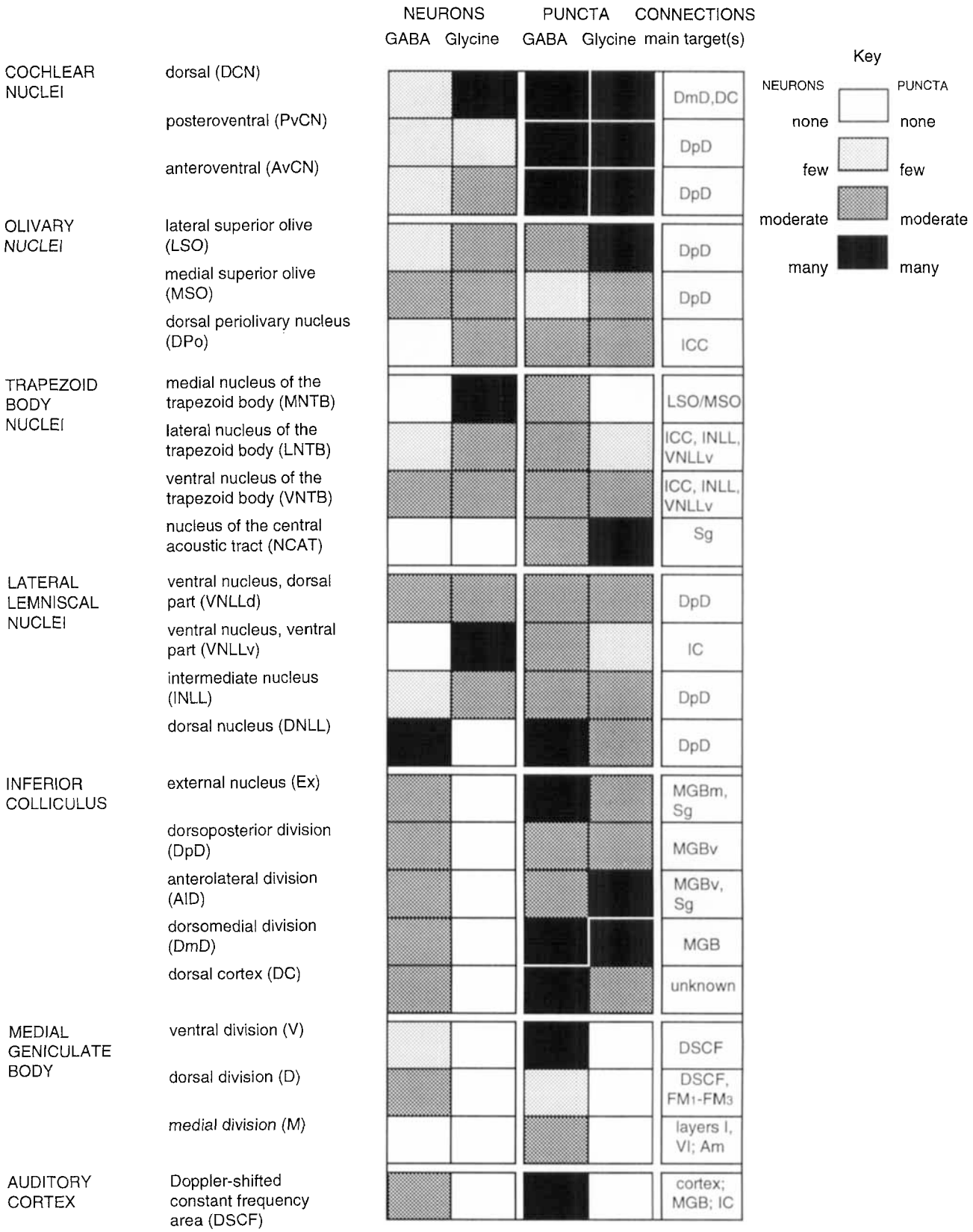


Fig. 4. Schematic summary of immunoreactive axonal endings (puncta) in the mustache bat's central auditory pathway. See also Figures 1–3 and Table 1. Protocol as in Figure 3.

TABLE 1. Summary of Patterns of GABAergic and Glycinergic Immunoreactivity in Auditory Centers in the Mustache Bat[1]



vertical configuration, again recapitulating the somatic arrangement. The ventralmost puncta were more diverse in size and shape and tended to be coarse and globular. Endings apposed to the somata of Gly+ cells were more spherical than clasp-like.

In summary, the patterns of GABA+ and Gly+ elements in the trapezoid body nuclei were diverse, and Gly+ neurons and puncta dominated the picture. Only the ventral and lateral nuclei contained GABA+ neurons, and only in the former were these numerous. The distribution of puncta was more equal or even slightly favored GABA+ endings; indeed, no obvious Gly+ puncta were evident in the medial nucleus, which was the only brainstem center in this survey without them (Table 1).

The nucleus of the central acoustic tract is affiliated with the extralemniscal auditory pathway, and it has prominent connections with the medial geniculate body, through which it influences the frontal lobes (Casseday et al., 1989). It corresponds to the anterolateral periolivary nuclei recognized in other species. It had one of the more unusual neurochemical arrangements in this study, as it was devoid entirely of GABA+ and Gly+ neurons, although it had a comparatively large number of each type of puncta. Only one other structure—the medial division of the medial geniculate body—also lacked both types of neuron (Table 1). Like many other brainstem nuclei, however, almost every neuron received both GABA+ (Fig. 9A) and Gly+ (Fig. 9B) endings. On balance, the GABA+ puncta were finer and more granular, and they seemed to fill the interstices between the much coarser Gly+ axosomatic endings. Although the most conspicuous terminals were axosomatic, others were present in the neuropil as well.

In summary, neurons in the nucleus of the central acoustic tract received both GABA+ and Gly+ extrinsic inputs, and the latter were apparently dominant; the GABA+ endings in the neuropil were somewhat sparser, and the overall pattern of immunoreactivity was quite distinct from the other trapezoid body components, and, indeed, from almost all other nuclei of the central auditory pathway.

[1]Schematic summary of patterns of GABAergic and glycinergic immunoreactivity in auditory centers in the mustache bat. The table was constructed by evaluating the patterns of immunostaining in all the material available for study. Two observers examined each section and assigned to each nucleus an ordinal value. The most extreme cases were given less weight than those in which the immunostaining was more consistent and internally reliable. In practice, the experiments were virtually identical. Where *few* refers to neurons, less than 10% of the cells were immunopositive; *moderate*, 10–40%; and *many*, more than 40%. At one extreme of the distribution (as seen in the medial nucleus of the trapezoid body and the dorsal nucleus of the lateral lemniscus), most or almost all of the neurons were immunopositive, whereas the other extreme, *none*, implies that no immunopositive neurons were observed in any specimen. The same procedure was followed for the puncta; in this instance, the scale refers only to an average density and does not represent qualitative differences. For structures with further subdivisions, such as the cerebral cortex and the intermediate nucleus of the lateral lemniscus, the relative density included every layer and all regions; subdivisions of these or other loci sometimes contained local concentrations or gradients of neurons or puncta, which are averaged across the entire area. The column, CONNECTIONS, refers to one or more principal projection targets that are summarized elsewhere and is not intended to be exhaustive (Zook and Casseday, 1982b, 1987; Olsen, 1986; Frisina et al., 1989; Pollak and Casseday, 1989; Pollak et al., 1992); the chemical identity of these output pathways may include both excitatory and inhibitory components, of which some of the latter are summarized in Figure 16.

The primary conclusions are that (1) each architectonic area has a unique arrangement of immunoreactivity; (2) the relationship between the number of immunoreactive neurons and the concentration of puncta in any nucleus was, at best, inconsistent; (3) almost every possible pattern of immunostaining occurred; (4) no glycinergic neurons were present above the level of the intermediate nucleus of the lateral lemniscus, although such puncta were prominent throughout the inferior colliculus; and (5) the many mismatches between immunoreactive neurons and puncta in a particular nucleus reflect the prominence of ascending (see Fig. 16) or possible descending (not illustrated) inhibitory projection neurons.

Fig. 5. (appears on page 328) GABAergic (left-hand panels) and glycinergic (right-hand panels) neurons and puncta in the subdivisions of the cochlear nuclear complex compared in adjacent, semithin postembedded sections at two caudorostral levels. The noteworthy features are that (1) comparatively few neurons were immunostained except for the substantial number of Gly+ cells in the dorsal cochlear nucleus (B:DCN); (2) each subdivision had a specific arrangement of immunoreactivity, including the nucleus of the cochlear nerve root (D:VIIINr); (3) within a subdivision, there were regional patterns, such as the concentration of GABA+ neurons in the superficial layers of the dorsal cochlear nucleus (panel A) or the population of Gly+ cells in the anteroventral cochlear nucleus, posterior subdivision (D:AvCNp). The small boldface numbers on these panels (and in subsequent illustrations, respectively) refer to the approximate loci from which the photomicrographs shown at higher power in Figure 6 (or in other figures) were taken. The left-hand column represents GABA, the right-hand column, glycine, in all figures except where noted otherwise. **A:** In the caudalmost part of the cochlear nucleus, which was dominated by the dorsal cochlear nucleus, a few GABA+ cells were concentrated chiefly in layer II (see Fig. 6A). Other clusters of immunoreactive neurons lay in the deepest part of the dorsal cochlear nucleus, abutting the nerve root. A few GABA+ cells were scattered in the posterior part of the posteroventral cochlear nucleus (PvCNp). Protocol for A–D: planapochromat, N.A. 0.16, × 83. **B:** In Gly+ material, many more neurons were immunostained, and these were found mainly in layers II–IV (see Fig. 6B). **C:** The posteroventral cochlear nucleus (PvCNa) contained only a few GABA+ cells, and in this section only rare examples were present. The anteroventral cochlear nucleus (AvCNp) also had a few such neurons, and these lay mainly in the ventrolateral quadrant. **D:** Glycinergic anteroventral cochlear nucleus cells were moderate in number; smaller cells were found chiefly in the dorsolateral one-third of the nucleus, and larger neurons were scattered ventromedially. In contrast, those in the posteroventral cochlear nucleus were larger and more evenly distributed. A and C: Plastic embedded section, 1.5-μm thick; GABA (INCstar), 1:2,000 dilution, streptavidin-biotin (Kirkegaard and Perry Inc.). B and D: Plastic embedded section, 1.5-μm thick; Gly II (R.J. Wenthold), 1:300 dilution, streptavidin-biotin.

Fig. 6. (appears on page 329) Higher power views of GABA (panels A, C, and E) and glycine (panels B, D, and F) immunostaining in cochlear nucleus subdivisions in serial semithin sections prepared alternately for each transmitter. The specific loci from which these photomicrographs were taken are shown in Figure 5. Neurons were considered immunopositive if their cytoplasm was immunostained darkly and uniformly (D:1–3), although some others were lighter (D:4); very pale neurons were classified as immunonegative (A, C, E:1,2). See Discussion for further analysis. **A:** In the dorsal cochlear nucleus, the GABA+ neurons were fewer in number and smaller than their Gly+ counterparts (panel B). The fine, granular nature of the puncta was not especially evident at this magnification, although their density imposed a dusty quality upon the neuropil (compare with panel B). 1–3: Neurons colocalizing GABA and Gly; see text for further discussion. Planapochromat, N.A. 0.65, × 390. **B:** The Gly+ neurons were heterogeneous in size and shape, and most densely concentrated in the middle layers of the dorsal cochlear nucleus; note the fine, discrete puncta and their tendency to end in the neuropil and to avoid the perikarya of Gly+ neurons. **C:** The paucity of GABA+ posteroventral cochlear nucleus neurons was evident, although some more lightly stained neurons were seen (1–3; see above). Many cells, some of them Gly+ (D:1–3), received substantial arrays of perisomatic GABA+ endings. **D:** The Gly+ neurons in the posteroventral cochlear nucleus were more abundant than their GABA+ counterparts (panel C; Table 1: PvCN), the puncta were numerous and comparatively coarse, especially in the neuropil, and perisomatic endings were, as in the dorsal cochlear nucleus (B), sparse. **E:** In the anteroventral cochlear nucleus, only a few small cells were GABA+, and the puncta formed rosettes about immunonegative somata (1, 2). **F:** The Gly+ neurons were larger and more varied in staining intensity. The distribution of Gly+ puncta was similar to that of the GABA+ endings (1, 2), indicating that most of the immunonegative neurons of the anteroventral cochlear nucleus received both kinds of axosomatic terminals or that these terminals might colocalize both amino acids. A, C, E: Plastic embedded section, 1.5-μm thick; GABA (INCstar), 1:2,000 dilution, streptavidin-biotin. B, D, F: Plastic embedded section, 1.5-μm thick; Gly II (R.J. Wenthold), 1:300 dilution, streptavidin-biotin.

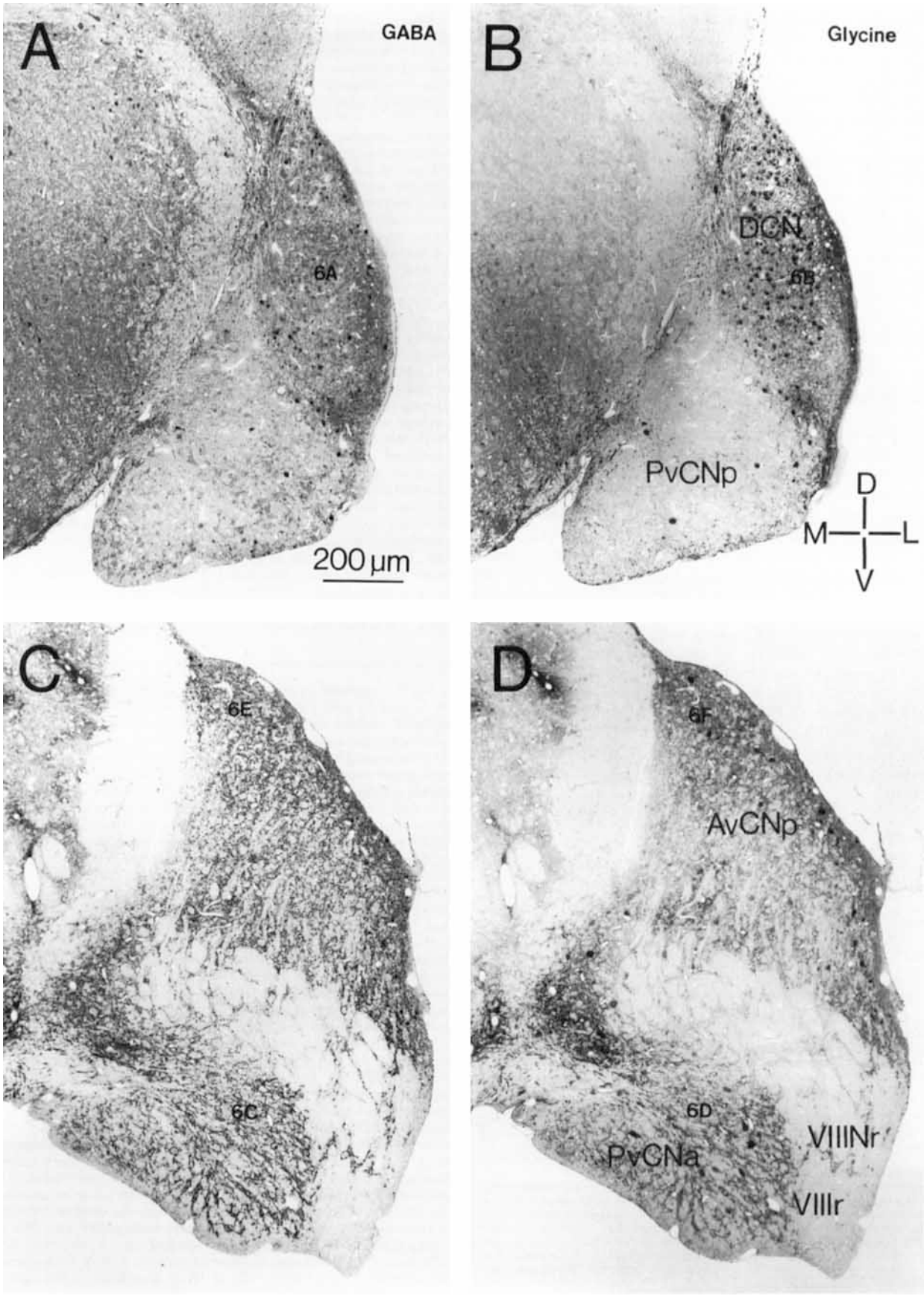


Figure 5. Legend on page 327.

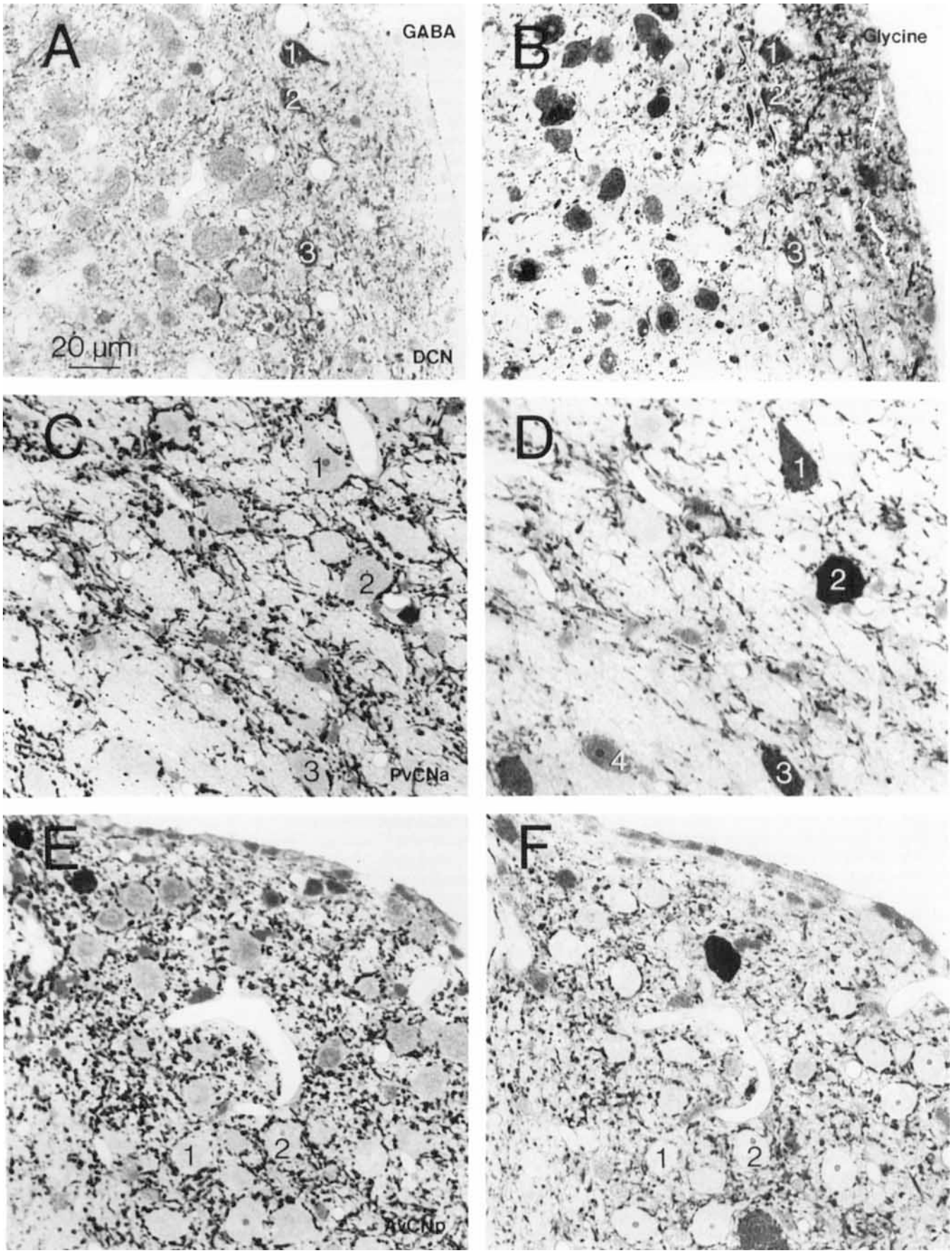


Figure 6. Legend on page 327.

Lateral lemniscal nuclei

The lateral lemniscus contained three parts—the ventral, intermediate, and dorsal nuclei—and each was exceptionally prominent and well developed in the mustache bat (Zook and Casseday, 1982a). The ventral nucleus had two cytoarchitectonic subdivisions, a dorsal and a ventral part, and each had a particular pattern of immunostaining (Fig. 10B). The dorsal part was smaller and lacked the conspicuous columnar neuronal organization that defined the ventral part.

The lateral lemniscal nuclei embodied, collectively, the most diverse patterns of GABA+ and Gly+ immunoreactivity in this series of nuclei (Table 1). The dorsal nucleus had only GABA+ cells, whereas the columnar portion of the ventral nucleus contained exclusively Gly+ neurons. All subdivisions received moderate-to-large numbers of puncta, and both GABA+ and Gly+ endings were almost always present; sometimes (as in the intermediate nucleus) their distribution was essentially identical.

Ventral nucleus. The arrangement of immunoreactive neurons in the two subdivisions of the ventral nucleus was different. Thus, the many Gly+ cells (Fig. 10B) in the columnar (ventral) part were oriented in rows between fascicles of lemniscal axons, which were dispersed throughout the ventral region. The dorsal part had no apparent columnar orientation and contained clusters of GABA+ cells intermingled with Gly+ cells. Some neurons in the dorsal part colocalized both GABA and Gly in alternate semithin sections; such colocalization did not occur in the columnar part of the nucleus (Fig. 11A,B).

The organization of Gly+ and GABA+ puncta further distinguished these subnuclei. The Gly+ puncta were more concentrated in the dorsal part than in the columnar region, and they were coarse and globular; some puncta ended on somata, but most terminated in the neuropil and onto the somata of immunonegative neurons. The plexus of Gly+ puncta in the ventral part was appreciably lighter and included finer and more delicate axosomatic contacts (Figs. 10B, 11B). The GABA+ puncta were also more prominent dorsally (Fig. 10A). In the dorsal part, endings were mainly in the neuropil, with some axosomatic and perisomatic terminals. In the ventral part, GABA+ puncta were much sparser, almost exclusively axosomatic, and, in contrast to the dorsal part, most were small and medium-sized granular endings (Fig. 11A).

Intermediate nucleus. This structure was greatly hypertrophied and formed a prominent lobe on the surface of the brainstem (Fig. 1B). Immunostaining for GAD, GABA, and Gly revealed striking, regionally specific, arrangements both of neurons and puncta (Figs. 1B, 10C,D) that were not immediately evident in normal cytoarchitectonic preparations. There was only a modest population of GABA+ cells and these occurred in small clusters, most often in the central region (Figs. 10C, 11C). Gly+ cells, on the other hand, were more common and widely dispersed, and they had regional concentrations in the dorsolateral and ventrolateral parts (Fig. 10D: asterisk). Serial semithin sections stained alternately for GABA and Gly revealed that most of the GABA+ cells were immunopositive for glycine as well (Fig. 11C,D:1,2).

The GABA+ puncta formed similar regional concentrations in the dorsolateral and ventrolateral regions (Fig. 1B, Fig. 10C: asterisk). They also ended as conspicuous perisomatic rings on both immunonegative and immunopositive neurons, whereas in the puncta-poor central zone few such axosomatic endings occurred. Gly+ puncta (Fig. 2B) had a similar distribution, the dorsolateral and ventrolateral areas receiving far more terminals than the central region (Fig. 10D). Many perisomatic endings were coarse or clasp-like, and among the largest Gly+ puncta in this survey. They encircled both immunopositive and immunonegative perikarya, whereas far finer terminals were plentiful in the neuropil.

Dorsal nucleus. The dorsal nucleus had a prominent columnar organization, and nearly all of the neurons were GABAergic (Fig. 10E). These perikarya were interspersed among fascicles of immunonegative axons, many of which proved to be glycinergic (Fig. 10F; see below), especially those outside the central part of the dorsal nucleus. Two groups of GABA+ cells could be distinguished by the degree of their immunoreactivity: darkly stained and paler neurons. The latter were somewhat larger than the more heavily stained neurons (see also Figs. 10E,F, 11E). Connectional and colocalization studies find that the paler cells project to the contralateral inferior colliculus, whereas the darker staining cells project ipsilaterally. In these experiments a few retrogradely labeled cells were found bilaterally that appeared to be immunonegative, suggesting that not all dorsal nucleus cells are GABA+ (G.D. Pollak, T.J. Park, D.T. Larue, and J.A. Winer, unpublished observations).

The GABA+ puncta formed dense islands between the pale columns of GABA-negative lateral lemniscal axons (Fig. 10E). Delicate strands of preterminal axonal fragments passed between these GABA-rich domains. These aggregations contained a matrix of medium-sized and large globular puncta that ended both upon somata and in the neuropil (Fig. 11E), with the latter arrangement the most common. Some axosomatic endings were quite large and encircled much of the perikaryal membrane.

The organization of Gly+ puncta in the dorsal nucleus was quite different (Figs. 10F, 11F). Their overall contribution to the cells and neuropil was far more delicate than that of their GABA+ counterparts (Figs. 10E, 11E). Some neurons received many Gly+ puncta, and others, very few. Puncta-recipient neurons were found chiefly in the lateral aspect of the nucleus. The puncta could represent endings of collaterals of the large Gly+ axons that ascended in conspicuous fascicles through the nucleus. The thicker, vertically oriented Gly+ preterminal trunks were traced directly into the inferior colliculus (Fig. 2B:LL).

No single picture or simple pattern can capture the complexity and diversity of the GABAergic and glycinergic arrangements in the nuclei of the lateral lemniscus. Like the trapezoid body nuclei, the lateral lemniscus embodied the widest range of patterns seen here. One nucleus was predominantly GABAergic (dorsal nucleus), another was purely glycinergic (ventral nucleus, ventral part), and others contained both types of neurons (ventral nucleus, dorsal part; intermediate nucleus). Neuronal colocalization of GABA and glycine was not seen in the former, more chemically homogeneous nuclei, whereas it was common in the latter. The range of immunopositive endings was, likewise, broad, ranging from large axosomatic terminals (GABA in the ventral nucleus, Gly in the intermediate nucleus) to far finer populations in the neuropil (GABA and Gly in the ventral nucleus). Such richness in form supports the idea of an equally specific regional segregation of function within the lateral lemniscal nuclei.

Inferior colliculus

The chief subdivisions of the inferior colliculus included the dorsal cortex, the external nucleus, and the central nucleus (Zook et al., 1985). Each has a unique set of connections, physiological properties, and neurochemical organization. The exposition that follows concentrates primarily on the central nucleus because it is the principal target of the ascending auditory pathway (Zook and Casseday, 1982b; Aitkin, 1986; Ross et al., 1988; Frisina et al., 1989; Pollak and Casseday, 1989).

The immunostaining patterns here were very different from those in other brainstem auditory nuclei in several significant ways. First, the inferior colliculus had only GABAergic neurons (Fig. 12A,C) and no glycinergic neurons (Fig. 12B,D). Second, there was an abundance of GABAergic (Fig. 13A,C,E) and glycinergic puncta (Fig. 13B,D,F) with close proximity to most, if not all, central nucleus neurons. Third, the pattern of GABA+ and Gly+ puncta innervation was complementary: where GABA+ puncta were most abundant, Gly+ puncta were sparsest, and where Gly+ puncta were most plentiful, GABA+ puncta were fewest (compare Fig. 12A and B:LL).

We estimate that about 20% of central nucleus neurons were GABAergic (Fig. 12C), and there were no glycinergic neurons nor or any somatic colocalization (Fig. 12D). The GABAergic cells were distinguished by their diversity and included small, medium-sized, and large neurons (Fig. 12C); in fact, the smallest and largest neurons in the inferior colliculus were GABAergic. GABAergic cells may therefore include both intrinsic neurons, many of which are small cells, and projection or principal neurons, which presumably are larger cells. Every part of the central nucleus shared these traits.

The puncta were everywhere extremely abundant, and each subdivision had among the heaviest concentration of both GABAergic and glycinergic puncta (especially the former) of any auditory region in this series. The GABAergic puncta presented several patterns. There were prominent axosomatic endings (Fig. 13A:1) as well as many terminals in the neuropil (Fig. 13C), and both populations were variable in size. There was, moreover, a striking internal gradient such that the GABAergic puncta were prevalent dorsally (Fig. 13A) and less pronounced more ventrally, near the lateral lemniscal entry zone (Fig. 13E).

The Gly+ puncta in the central nucleus were equally prominent. Their preterminal trunks were traced as a massive glycinergic projection ascending with the lateral lemniscus toward the inferior colliculus (Figs. 2B, 12B). Like their GABAergic counterparts, the Gly+ puncta had diverse sizes, shapes, and targets, including prominent axosomatic endings in the dorsoposterior (Fig. 13D:DpD) and dorsomedial (Fig. 13F:DmD) subdivisions, and fewer in the dorsal cortex (Figs. 12B, 13B:DC). Delicate terminals were present in the neuropil in each subdivision. The spatial distribution of Gly+ puncta was the inverse of the GABAergic pattern: they were much more numerous in the ventral portion of the central nucleus and sparser dorsally (Fig. 12B). Although still plentiful in the most superficial parts of the dorsal cortex, there were progressively fewer axosomatic endings (compare the upper and lower halves of Fig. 13B).

Fig. 7. (appears on page 332) Distributions of GABA (left side) and glycine (right side) in the olivary complex and trapezoid body nuclei. Planapochromat, N.A. 0.32, × 156. The small numbers refer to higher magnification views shown in Figure 8. **A, B:** Lateral superior olive, with GABA+ preterminal fibers and puncta pronounced in the medial limbs and glycinergic neurons most conspicuous in the lateral one-third. Many peri- and paraolivary neurons were immunoreactive; these cells often abutted the margins of the olive (arrows), and their dendrites followed or crossed the neuropil border. Few olivary neurons were immunostained for GABA, and approximately one-third were glycinergic. **C, D:** In the medial superior olive, where neurons positive for either GABA or glycine were concentrated along the dorsomedial half, a segregation especially marked in the glycine material. Many more preterminal glycinergic fibers were immunostained, which imparted a coarser texture to the neuropil. In other regions, such as in the inferior colliculus, where the preterminal glycine immunoreactivity was not so robust, the granular quality of the terminal plexus was much more evident and readily comparable to the GABAergic puncta (see Fig. 12C, D). A question arises about the correspondence between the structure we have designated as the medial superior olivary nucleus and the corresponding nucleus in other mammals. Although we have so denoted it on the basis of prior, architectonic studies (Zook and Casseday, 1982a), the question of its identity is by no means resolved (see Grothe et al. [1992] for further discussion; see also Irvine [1986] and Schofield and Cant [1991] for comparative considerations). **E, F:** In the medial nucleus of the trapezoid body, there were no GABAergic neurons but many GABAergic puncta terminating upon the immunonegative perikaryal silhouettes, and few or no glycine-positive puncta among the many intensely immunoreactive glycinergic neurons. All neurons received a dense investment of somatic endings. There was a broad distribution in neuronal somatic size. In contrast, the ventral nucleus of the trapezoid body (VNTB) had both GABAergic (E) and glycinergic (F) neurons; some of the GABAergic cells projected to the ipsilateral inferior colliculus (D.T. Larue, T.N. Park, G.D. Pollak, and J.A. Winer, unpublished observations). A, C, E: Vibratome sections, 50-μm thick, GABA (INCstar), 1:5,000 dilution, avidin-biotin immunoperoxidase, free-floating. B, D, F: Glycine (Gly I, R.J. Wenthold), 1:400 dilution, avidin-biotin, free-floating.

Fig. 8. (appears on page 333) Higher magnification views of the patterns of immunoreactivity in the lateral superior olive (A, B), medial superior olive (C, D), and medial nucleus of the trapezoid body (E, F). These photomicrographs were taken from semithin material. The numbers in Figure 7 indicate their comparable locations on matching sections only. Planapochromat, N.A. 1.0, × 825. Adjacent, serial semithin sections prepared for postembedding immunocytochemistry. **A:** GABA immunonegative neurons (1, 2) received more axosomatic endings than immunopositive (3) cells. Many of the puncta were fine and granular. **B:** All lateral superior olivary neurons (1–3) were the target of glycinergic axosomatic endings, and these were comparatively large and globular. Sometimes they ringed the cell almost completely (2), and in other cases they were less concentrated (1). An occasional neuron (3) was immunopositive for both GABA and Gly. **C:** In the medial superior olive, all the GABA-negative neurons received some axosomatic endings, many of which were larger than those terminating on lateral superior olivary cells. Most cells (1, 2) received both GABA+ and Gly+ endings. **D:** Axosomatic glycinergic terminals in the medial superior olive tended to be sparser than their counterparts in the lateral superior olive. Some GABA axonal profiles were glycine immunoreactive (3), and some coarse, presumptively preterminal, axons were also immunostained. **E:** The medial nucleus of the trapezoid body was devoid of GABA+ cells and had a moderate number of immunoreactive axosomatic puncta, including small (small arrowheads), medium-sized (medium-sized arrowheads), and large (large arrowheads) types. Like the GABAergic endings in the lateral (A) and medial (C) superior olives, these formed irregular perisomatic clusters, although their concentration was much lower than that elsewhere in the olivary complex. **F:** No glycinergic puncta were seen in the medial nucleus, and only a few preterminal segments were present. The resolution of the semithin material was not adequate to detect whether any extremely small axosomatic endings might have been present. A, C, E: Plastic embedded section, 1.5-μm thick; GABA (R.J. Wenthold), 1:1,000 dilution, avidin-biotin. B, D, F: Plastic embedded section, 1.5-μm thick; Gly II (R.J. Wenthold), 1:300 dilution, avidin-biotin.

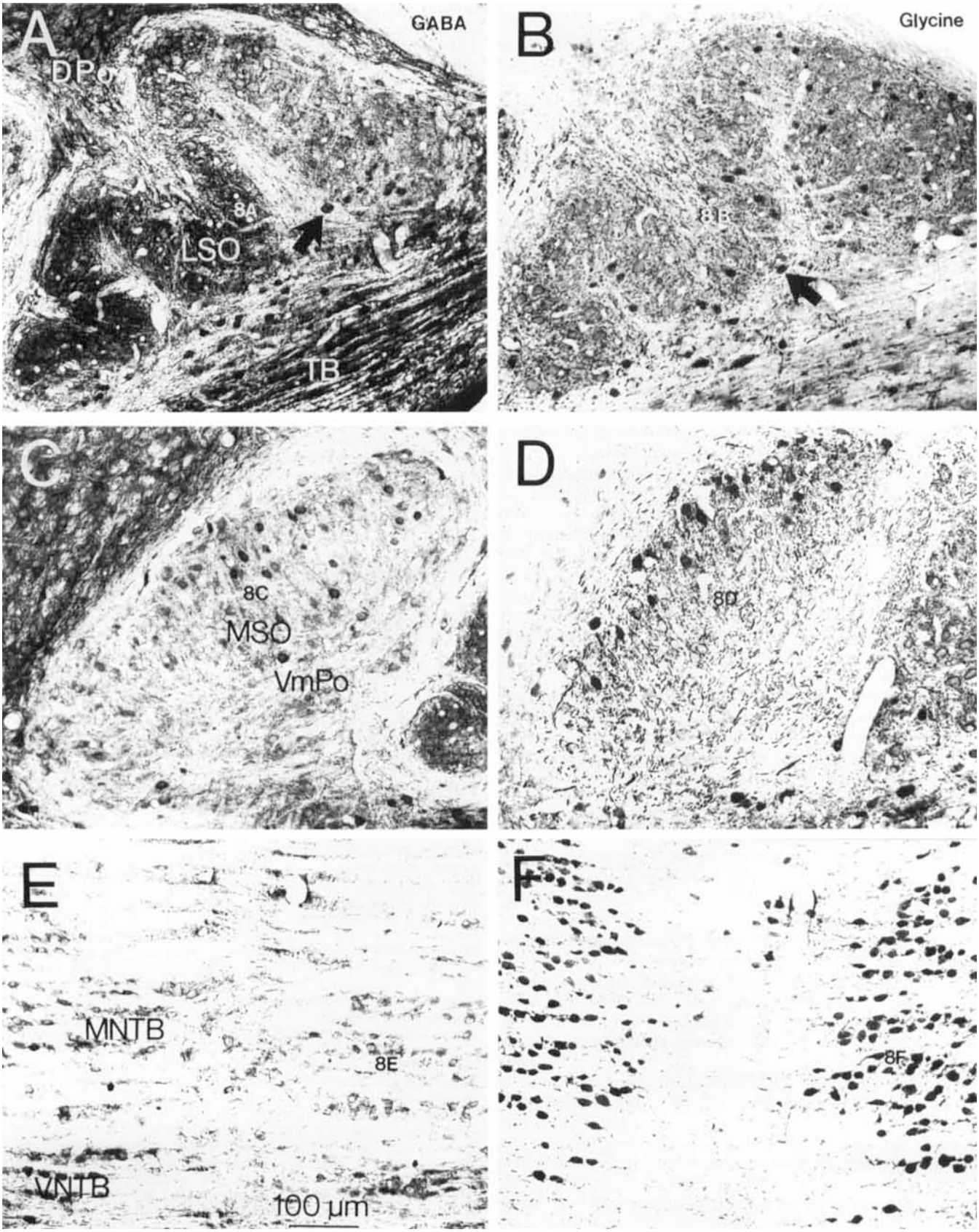


Figure 7. Legend on page 331.

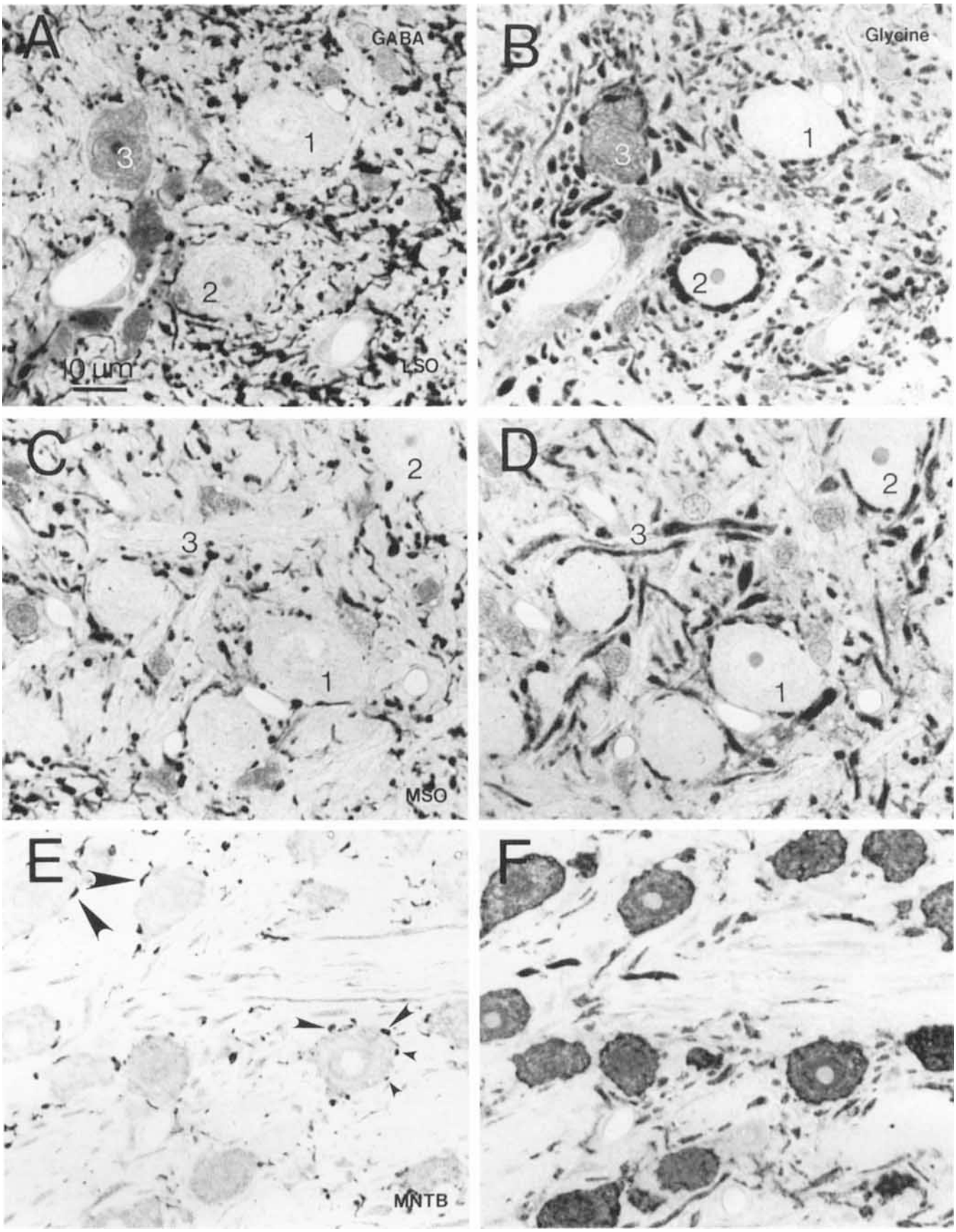


Figure 8. Legend on page 331.

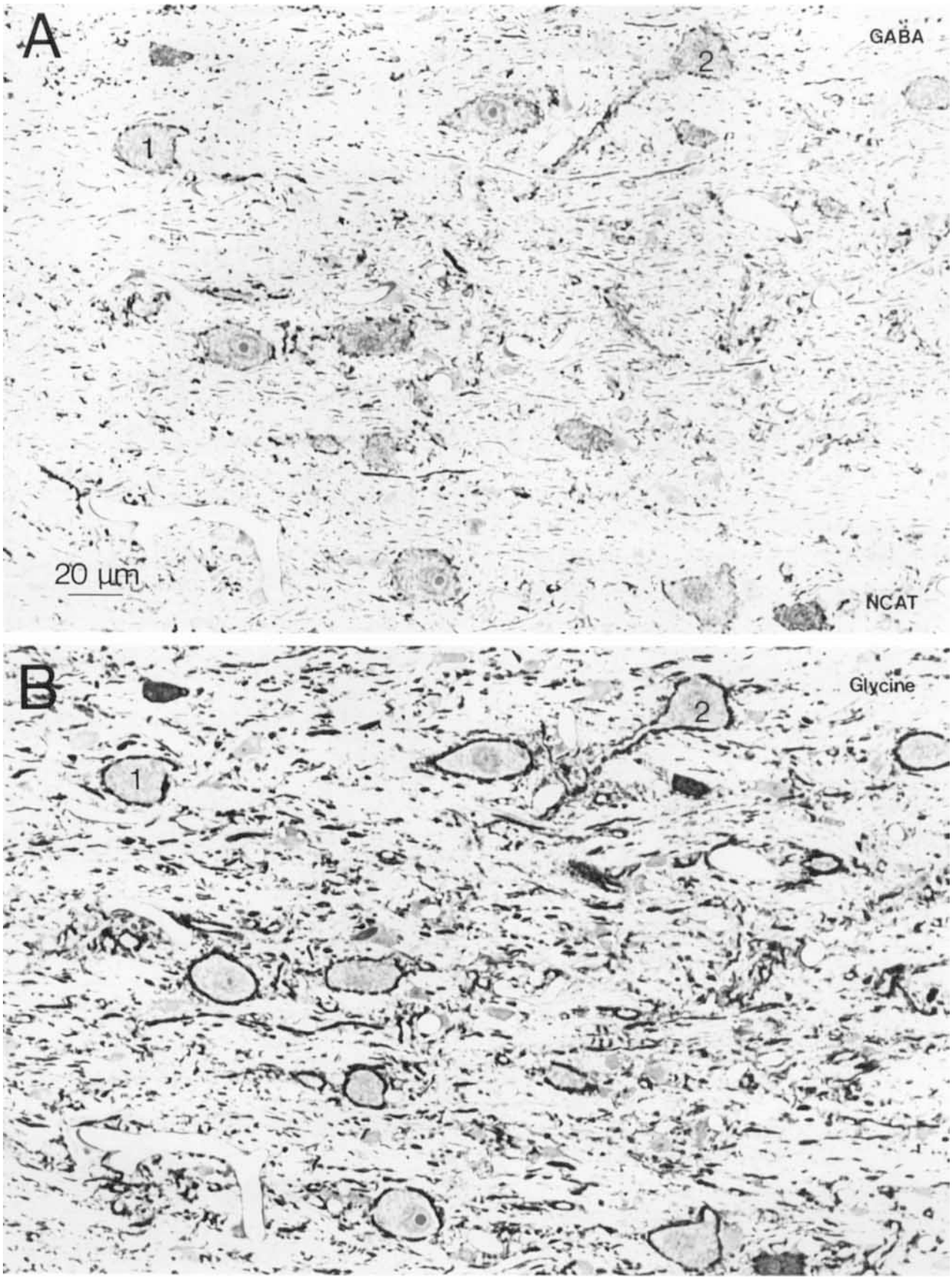


Figure 9

Medial geniculate body

The principal auditory thalamic architectonic subdivisions recognized in other studies (Olsen, 1986; Casseday et al., 1989) also had patterns of GABA immunostaining that were consistent with them. Since a more complete report on GABA/GAD immunoreactivity is available (Winer et al., 1992), only the principal features are summarized here and some new observations are documented. While very few—no more than 1%—of the cells were GABAergic, abundant immunoreactive puncta were found in every subdivision (Fig. 14A). In a global sense, this pattern resembled that in the rat medial geniculate body (Winer and Larue, 1988). No immunoreactivity to glycine was observed.

Almost all the small number of GABAergic cells were found in the dorsal division, which is considered as outside the targets of the lemniscal auditory pathway (Winer and Morest, 1983, 1984). There were none or only a very few immunostained neurons in the medial division, whereas the ventral division had an intermediate number. Such cells were 8–10 μm in diameter with oval or drumstick-shaped somata and little dendritic immunoreactivity.

Despite the overall paucity of auditory thalamic GABAergic neurons, each division had a particular and robust pattern of GABAergic puncta whose form and density were specific and differentiated them on both numerical and qualitative grounds (Fig. 14C–E). Thus, many ventral division puncta (Fig. 14C) were medium-sized (1–2 μm in diameter), with a few finer endings, many of which encircled immunonegative neuronal somata. In contrast, the dorsal division puncta had a different arrangement (Fig. 14D). Numerically, they were about half as dense as those in the ventral division, and they were mostly fine (ranging from less than 0.5 to 1 μm in diameter) and granular (as opposed to globular) in form. They appeared to end predominantly in the neuropil, although some immunonegative perikarya also received a significant number; immunopositive neurons received few terminals, like cells in the ventral division. The medial division puncta differed strikingly from those in the other auditory thalamic regions. They were the largest (2–3 μm in diameter) and coarsest in the medial geniculate body (Fig. 14E), and their number was about three-fourths of the value in the ventral division. Their size and the density of the preterminal segments imparted a highly reticulated texture onto the medial division neuropil and enhanced the impression of its GABAergic nature. Most of the endings terminated freely in the neuropil, and immunonegative cells received some as well. In many respects, this pattern matched that seen in the rat (Winer and Larue, 1988).

In summary, the medial geniculate body had very few GABAergic neurons—about 1% of the total number of cells—and these were concentrated primarily in the dorsal division, with few in the ventral division and none, apparently, in the medial division. In contrast, each division differed with respect to the GABA+ puncta, with the dorsal division having a sparse number of fine endings, the ventral division many medium-sized terminals, and the medial division abundant coarse puncta. Subdivisions with the most conspicuous puncta (the medial and ventral divisions) had the fewest GABA+ cells, whereas the dorsal division,

Fig. 9. Immunostaining in the nucleus of the central acoustic tract in an adjacent pair of semithin sections. These large neurons were immunonegative for either GABA or glycine; the small, stained soma (lower right-hand corner) is a lemniscal neuron outside the nucleus of the central acoustic tract. Planapochromat, N.A. 0.8, × 413. **A:** All neurons received GABA+ axosomatic endings, and these extended, in fortuitously sectioned examples, well onto the proximal dendrites (2); similar observations also applied to the glycinergic puncta (B:2). Although the GABA immunostaining was more punctate and delicate than that for glycine, the number of axosomatic terminals was substantial. **B:** An appreciable number of glycine immunoreactive puncta virtually enveloped the somatic membrane of nonglycinergic neurons. Glial profiles were occasionally immunostained (above 1, beneath 2). A: Plastic embedded section, 1.5-μm thick; GABA (INCstar), 1:3,000 dilution, streptavidin-biotin. B: Plastic embedded section, 1.5-μm thick; Gly II (R.J. Wenthold), 1:300 dilution, streptavidin-biotin.

Fig. 10. (appears on page 336) Immunostaining for GABA (panels A, C, E) and glycine (panels B, D, F) in the lateral lemniscal nuclei. Planapochromat, N.A. 0.65, × 390. **A, B:** Comparison of GABA and glycine immunoreactivity. The dorsal part of the ventral nucleus had many GABAergic neurons and a dense plexus of puncta and a comparable number of glycinergic cells and terminals. In the ventral or columnar part, however, rows of intensely glycinergic neuronal somata (B) were ringed with axosomatic GABAergic terminals (A); see also Figure 11A,B. **C, D:** Immunostaining in the intermediate nucleus was remarkably heterogeneous. Thus, both the GABAergic and the glycinergic puncta were concentrated in the dorsolateral (presumptive 60 kHz) and ventrolateral (presumptive 90 kHz) subregions (asterisks). However, the glycinergic cells were distributed widely and were numerous (Table 1). In contrast, the GABAergic cells were less common and they were scattered singly or formed small groups in the central part, where they were concentrated. **E, F:** The dorsal nucleus contained almost exclusively GABA+ cells and had a columnar organization resembling that in other species (Adams and Mugnaini, 1984; Mugnaini and Oertel, 1985). The glycinergic axons were abundant and coursed among the immunonegative lateral lemniscal fibers entering the inferior colliculus, suggesting that many ascend from sublemniscal sources (see Table 1 and Fig. 16). A, C, E: Vibratome section, 50-μm thick; GABA (INCstar), 1:5,000 dilution, avidin-biotin immunoperoxidase, free-floating. B, D, F: Gly I (R.J. Wenthold), 1:400 dilution, avidin-biotin, free-floating.

Fig. 11. (appears on page 337) Higher magnification views of postembedded, adjacent pairs of semithin sections from the vicinity of the lateral lemniscal nuclei. The approximate locus from which these observations were taken is denoted by the small numbers in Figure 10. Immunoreactivity in these nuclei of the lateral lemniscus encompassed the full range of patterns encountered in this survey and included those with almost entirely GABAergic (E:DNLL) or glycinergic (B:VNLLv) neuronal populations, whereas others (C,D:INLL) contained intermediate concentrations of both types. The puncta, in contrast, were more or less prominent throughout these nuclei (Table 1: LATERAL LEMNISCAL NUCLEI). Planapochromat, N.A. 0.65, × 390. **A:** All neurons in the ventral nucleus received axosomatic GABA+ endings, and there were only sparse terminals in the neuropil. **B:** In contrast, the Gly+ endings were concentrated in the neuropil, with far fewer on the Gly+ perikarya. **C:** A cluster of GABA+ cells in the intermediate nucleus received few axosomatic puncta compared with those ending on neurons in the columnar part of the ventral nucleus, and there was an appreciable number of large, coarse endings in the neuropil. See panel D for the significance of the numbers. **D:** In contrast, Gly+ endings covered much of the somatic surface and were equally abundant in the neuropil. Both GABA+ and GABA− neurons received these endings. Many neurons were immunopositive for both (1, 2), whereas others were immunonegative for glycine (3) yet positive for GABA (C:3). **E:** The dorsal nucleus had almost exclusively GABA+ cells and abundant puncta (Table 1: DNLL) that ended both on neuronal somata and in the neuropil. Note the larger, more lightly stained neurons and the small, darkly stained cells. **F:** The Gly+ puncta also ended on perikarya and in the neuropil, and fascicles of preterminal trunks (left-hand side) ascended toward the inferior colliculus (Fig. 12B). A, C, E: Plastic embedded section, 1.5-μm thick; GABA (INCstar), 1:1,000 dilution in A, 1:2,000 dilution in C and E. B, D, F: Plastic embedded section, 1.5-μm thick; Gly II (R.J. Wenthold), 1:300 dilution, streptavidin-biotin.

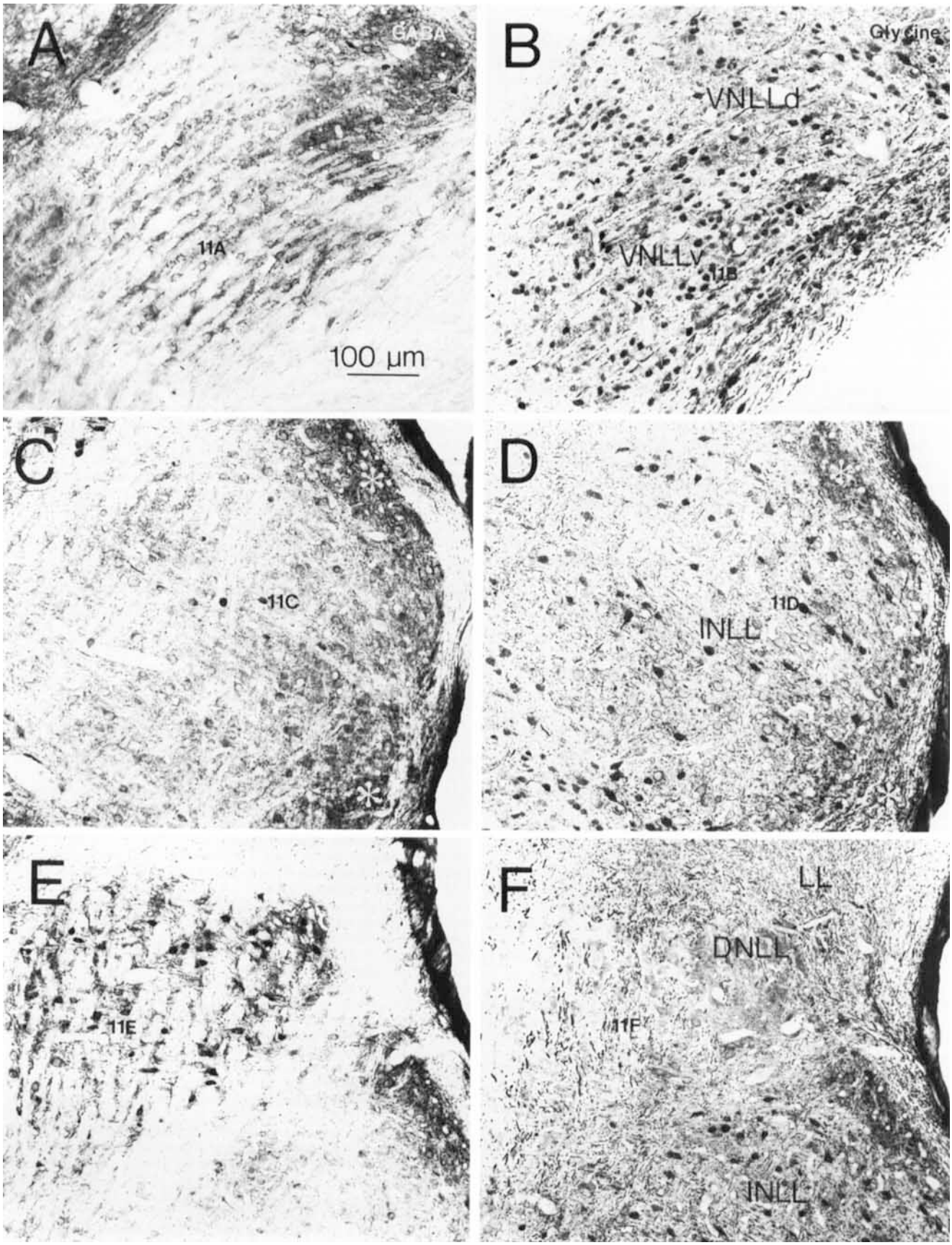


Figure 10. Legend on page 335.

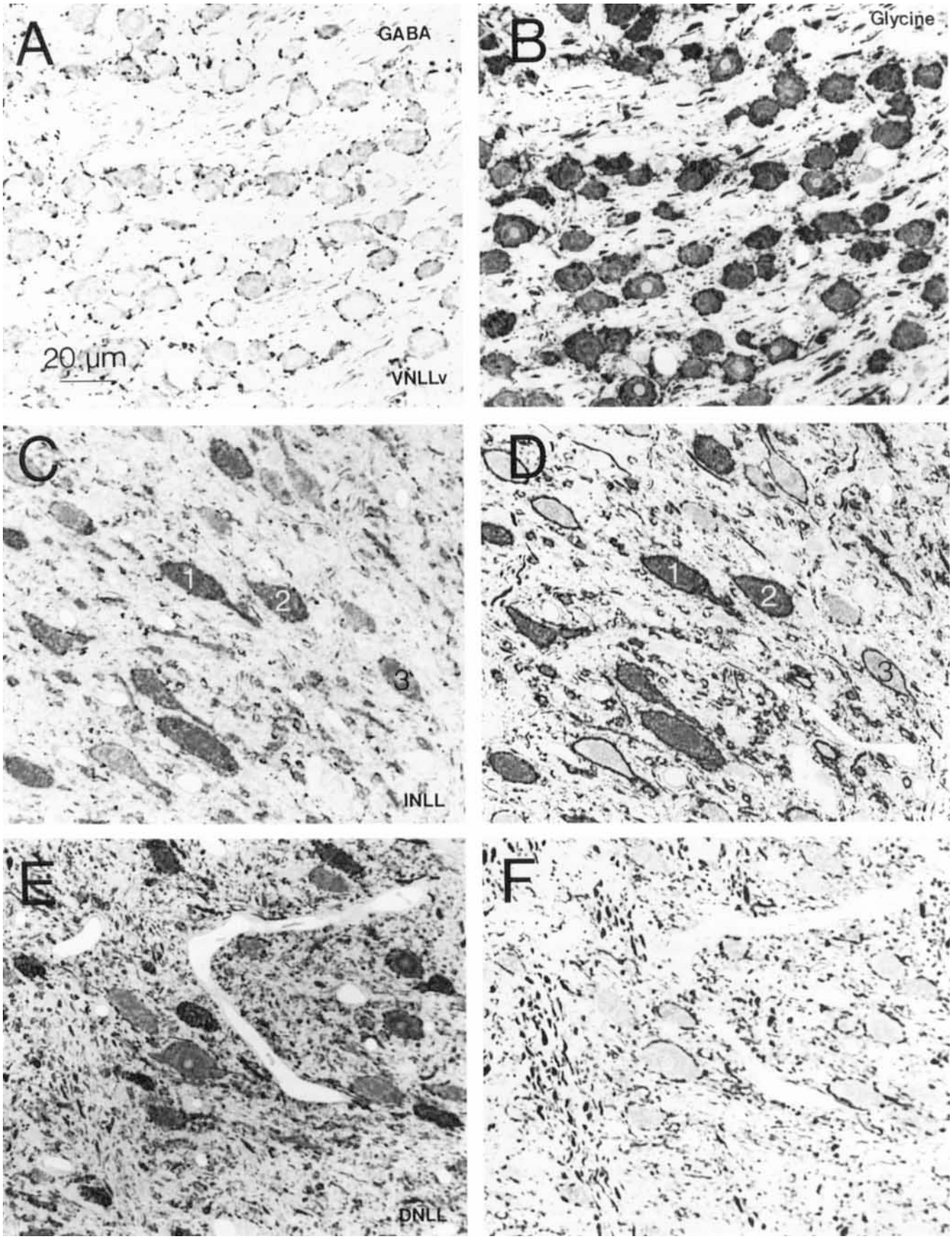


Figure 11. Legend on page 335.

with the preponderance of such cells, had far fewer puncta. No glycinergic neurons or puncta were immunostained in any subdivision.

Auditory cortex

As in the medial geniculate body, auditory cortical immunostaining revealed only GABA+ neurons and puncta. About 16% of cortical neurons were GABA+, ranging from less than 10% in layer II to about 80% in layer I (J.A. Winer, L.A. Bui, J.H. Hong, J.J. Prieto, and D.T. Larue, in preparation). The present values are somewhat lower than those reported in rat (Winer and Larue, 1989) and cat (Prieto et al., 1994a,b) auditory cortex and in primate sensory-motor cortex (Hendry et al., 1987).

The present survey includes characteristic patterns of immunoreactivity in a region that probably corresponds spatially to a subdivision in the primary auditory field, the Doppler-shifted constant frequency area, which has been defined in physiological mapping studies (Suga et al., 1983); other landmarks included the cytoarchitecture and the configuration of blood vessels in unperfused specimens. Measurements from the dorsal and caudal poles of the hemisphere confirmed that these observations were confined to auditory cortex, without specifying their locus more precisely.

Many GABA+ neurons were evident (Fig. 15A), and there were no Gly+ cells (Fig. 15B). Layer I was distinct from the other layers in that all or almost all of the neurons were GAD+ or GABA+ (Fig. 15C:I). Only nonpyramidal neurons were immunostained, a finding that was repeated (with one possible exception) in the deeper layers. Layer I was very thick, comprising 20% of the cortical depth (Fig. 15A:I) compared with about 11% in the rat (Winer and Larue, 1989) and almost 10% in the cat (Winer, 1992). The GABA+ neurons were found in both the superficial and deep portions of layer I, although they were more common in the latter, and cells with round somata as well as neurons with a vertical or horizontal somatodendritic arrangement were seen. The puncta, however, had a different sublaminar pattern: they were concentrated in the superficial half of layer Ia and were appreciably less numerous elsewhere in layer I (Fig. 15C:I). Most were medium-sized and globular in shape, especially those in the deepest three-fourths of the layer, whereas terminals in the upper one-fourth were finer and more granular and tended to fuse with one another, forming a lacy texture. Many of the endings were less than 0.5 μm in diameter and appeared less numerous on neurons than in the neuropil. These patterns resemble those seen in layer I in the rat (Winer and Larue, 1989).

In layer II the proportion of GABA+ neurons was much lower, but these were equally diverse in form and included cells 8–15 μm in diameter; some had a vertical somatodendritic orientation (Fig. 15C:1), and others had lateral processes stained extensively. Clusters of 2–5 GABA+ neurons were relatively common (Fig. 15A:II), and many of these cells were immunostained darkly. The puncta likewise had an organization that alone would have defined this layer: they were much larger than those in layer I and formed conspicuous perisomatic baskets on the densely packed immunonegative cells (Fig. 15C:2), with far fewer endings onto GABA+ neurons.

Layer III GABA+ neurons also varied in size and shape (Fig. 15A:III), ranging from 9 to 15 μm in diameter; their perikaryon was oval and slightly elongated, vertically or horizontally. Some of the largest such cells were comparable in size with the immunonegative pyramidal neurons. These GABA+ cells appeared more dispersed than those in layer II, perhaps as a result of the decreased packing density. Layer III puncta were equal or slightly larger than those in layer II, but sparser; although they were apparent on both GABA+ and GABA− neurons, they formed less often the globular axosomatic rosettes on immunonegative neurons that were characteristic of layer II.

Layer IV was thin and its cytoarchitecture was dominated by nonpyramidal neurons wedged between the pyramidal cell-rich populations above and below it (Fig. 15A:IV). The range of sizes among GABA+ neurons was comparatively narrow, about 8–12 μm, but the cells were just as diverse as those in other layers, and included small oval neurons and larger cells with vertical or lateral orientations. The GAD+ puncta resembled those in layer III in size and shape; perisomatic endings on immunonegative cells were prominent.

Many layer V neurons were GABA+; such cells were concentrated preferentially in the inner half and, as in other layers, included small, medium-sized, and large types (Fig. 15A:V). Among the larger cells, there was a variety of somatodendritic arrangements, with both vertical (Fig. 15D:3) and horizontal (Fig. 15D:2) orientations. The GABA+ puncta formed conspicuous endings about the somata and dendrites of pyramidal cells (Fig. 15D:1) and often outlined the apical trunks as they ascended.

Layer VI GABA+ neurons had a unique arrangement. As in layer II, they were relatively rare; they occurred mainly in layer VIb and were comprised mainly of small or horizontally oriented neurons at the expense of larger cells (Fig. 15A:VI); such neurons could be seen far into the underlying white matter. The GAD+ puncta were fine and granular and resembled those in layer V, although prominent axosomatic or peridendritic concentrations of these endings were unusual. The puncta declined abruptly at the border between layer VIb and the white matter.

In summary, the GABAergic organization of the auditory cortex embodied a wide variety of patterns, ranging from the overwhelmingly GABAergic neuronal population in layer I to the comparatively smaller proportion of such cells in layers II and VI. The puncta likewise had conspicuous interlaminar differences, and those in the granule cell-dominated layers were far larger and coarser than their infragranular counterparts. As in the auditory thalamus, no Gly+ neurons or puncta were present.

DISCUSSION

Technical considerations

Colocalization of GABA and Gly in some neuronal and axonal populations. Postembedding immunostaining in adjacent semithin sections reveals that certain areas of the brain contain neurons and axons that are immunopositive for both glycine and GABA, including the dorsal cochlear nucleus (Wenthold et al., 1987; Osen et al., 1990; Kolston et al., 1992), the cerebellum (Ottersen et al., 1987), the perihypoglossal nuclei (Yingcharoen et al., 1989), and the vestibular nuclei (Walberg et al., 1990). In the present study, serial semithin sections incubated with antisera to GABA or Gly conjugates contain many cell bodies that were immunopositive for both in certain nuclei (such as the dorsal cochlear nucleus, medial superior olive, ventral nucleus of the lateral lemniscus [dorsal part], and the intermediate nucleus of the lateral lemniscus), whereas in

TABLE 2. Patterns of Neuronal Somatic Colocalization of GABA and Glycine in the Mustache Bat Central Auditory Pathway[1]

Nuclei with significant colocalization	Nuclei with some colocalization	Nuclei with no colocalization
dorsal cochlear nucleus	lateral superior olive	medial nucleus of the trapezoid body
posteroventral cochlear nucleus, posterior part	ventral nucleus of the lateral lemniscus, dorsal part	ventral nucleus of the lateral lemniscus, ventral part
medial superior olive		
intermediate nucleus of the lateral lemniscus		dorsal nucleus of the lateral lemniscus
		inferior colliculus

[1]Summary and comparison of the nuclear distribution of neurons colocalizing GABA and glycine in the mustache bat. The findings reported here were confirmed in several specimens and were not contingent on a particular antiserum, specific dilutions, or different periods of incubation. *Significant* means that more than 10% of the neurons shared this feature; *some*, that 5–10% did; and *none*, that no neurons were ever immunostained.

others, such colocalization was not observed (medial nucleus of the trapezoid body, ventral nucleus of the lateral lemniscus [columnar part], dorsal nucleus of the lateral lemniscus, and the inferior colliculus; see Table 2). Axons positive for both substances were seen occasionally in the neuropil of various nuclei, usually where colocalized neurons were present.

Interpreting such colocalization can be problematic. Glycine, the smallest and structurally the simplest amino acid, participates in several metabolic pathways in the central nervous system (Daly and Aprison, 1982) and is likely to be present at some level in all neurons. In addition, evidence regarding glycine's role as a potentiator of glutamate at *N*-methyl-D-aspartate receptors underscores its importance in the extracellular milieu (Daly and Aprison, 1982; Johnson and Ascher, 1987). Despite its broad metabolic role, it was the marked regional concentrations of glycine in the spinal cord that first led to speculation about its neurotransmitter function (Aprison and Werman, 1965). Neurons considered to be glycinergic are thought to have a concentration specific to the neurotransmitter pool several times, and perhaps orders of magnitude, higher than the levels of metabolic and structural glycine. The extraordinarily low background staining seen in our material when using the glycine antiserum at its proper dilution attests to the validity of this precept. Fortunately, GABA has no known role as a metabolic intermediate. Thus, in the medial superior olive and the dorsal cochlear nucleus, where glycine is often detected in the same cells that are also immunopositive for GABA, a plausible interpretation is that the glycine may be of metabolic origin, whereas GABA probably has a transmitter-specific role. In areas such as the intermediate nucleus of the lateral lemniscus, where most GABA+ neurons are also strongly immunoreactive for Gly, it is harder to dismiss the possibility that such cells might use both as transmitters for specific neuronal signaling. It is possible that certain classes of GABAergic neurons contain high levels of metabolic glycine (see Burger et al., 1991). In any event, it is noteworthy that no such neuronal colocalization has been reported using immunostaining in certain regions, namely the cerebral cortex, medial geniculate body, inferior colliculus, and the dorsal nucleus of the lateral lemniscus, each of which has neurons and axons marked consistently and only for GABA, nor in the columnar part of the ventral nucleus of the lateral lemniscus, or in the medial nucleus of the trapezoid body, both of which are immunopositive for glycine exclusively. Thus, if the

Fig. 12. (appears on page 340) GABA (A, C) and glycine (B, D) immunoreactivity in the inferior colliculus, showing both complementarity and segregation. Although there were GABAergic neurons throughout the inferior colliculus, they were sparsest in the lateral lemniscal (LL) entry zone, where the glycinergic puncta and preterminal axons were especially prominent. **A, C:** All subdivisions had many GABAergic neurons, a population that included a wide range of sizes and shapes and some of the largest and the smallest cells. Box in A shows the approximately comparable region for these observations. **B, D:** The glycinergic puncta were most conspicuous in the basalmost one-third of the inferior colliculus, where GABAergic elements were less common. Concentrations of axosomatic endings occurred upon immunonegative cells in the anterolateral division (AlD) and in the lateral lemniscal (LL) entry zone; puncta were more dispersed and granular in the dorsomedial division (D:DmD). Glycinergic puncta were present in moderate numbers to the limits of the dorsal cortex, although they declined in prominence and they formed fewer perikaryal rings dorsal to the lateral lemniscus. In contrast, the GABAergic neurons were concentrated outside the lateral lemniscal entry zone (A, C). Comparatively few GABA+ axosomatic endings were present on the GABAergic cells, though this was not readily evident at this magnification. A: Planapochromat, N.A. 0.04, × 63; B: as in A, darkfield illumination; C, D: planapochromat, N.A. 0.32, × 156. A, C: Plastic embedded section, 1.5-μm thick; GABA (R.J. Wenthold), 1:1,000 dilution, avidin-biotin. B: Vibratome section, 50-μm thick; Gly I (R.J. Wenthold), 1:1,500 dilution, ABC avidin-biotin, free-floating. D: Plastic embedded section, 1.5-μm thick; Gly II (R.J. Wenthold), 1:300 dilution, avidin-biotin. C and D are adjacent sections; box in A denotes their approximate location.

Fig. 13. (appears on page 341) Contrasting patterns of chemical anatomic organization in subdivisions of the inferior colliculus. Each photomicrograph straddles the border of two nuclear regions to illustrate the contrasting puncta patterns, which were most clearly evident in the glycine immunostained material (B, D, F). The dorsal cortex (A) had many more GABA+ puncta than at the junction of the two central nucleus subdivisions (B), whereas the border of the dorsomedial division and the lateral lemniscus (E) had many preterminal fibers and a few comparatively coarse puncta. Moreover, across nuclei (panels A, C, E), there were clear differences in density (see Table 1: INFERIOR COLLICULUS). Planapochromat, N.A. 0.65, × 390. **A:** At the border of the dorsal cortex (DC) and the dorsoposterior division (DpD), the GABA+ puncta were uniformly heavy, and many neurons were immunopositive. 1: GABAergic cell receiving many GABA+ and sparse Gly+ (B:1) endings. 2: Immunonegative neuron targeted by many GABA+ and few Gly+ puncta. **B:** The Gly+ endings, in contrast, were fewer in number, comparatively coarser, and concentrated in the neuropil rather than on somata, as they did in the anterolateral division (D:AlD). They became progressively finer toward the dorsal cortex. No glycinergic cells were present, nor was there neuronal colocalization (Table 2). **C:** Most GABA+ puncta in the dorsoposterior division were fine and granular, both on cell bodies and in the neuropil. 3: A GABA-negative neuron had few GABA+, and many Gly+, endings (D:3). 4: Other GABA− cells received comparable numbers of GABA+ and Gly+ puncta. Within the central nucleus, there was a less marked difference in the GABAergic than in the glycinergic organization (compare with panel D). **D:** Neurons in both the anterolateral (AlD) and dorsoposterior (DpD) divisions were studded with axosomatic endings, and there were many terminals in the neuropil as well. The puncta were most conspicuous on anterolateral division neurons, and they were appreciably larger than GABA+ boutons (C). The border between the anterolateral and dorsoposterior divisions was distinct (dots). **E:** Endings in the ventral part of the dorsomedial division (DmD) were large and coarse, and abundant, thick, preterminal processes, perhaps ascending toward other targets, were also evident. 5: Some GABA+ neurons received both GABA+ and Gly+ axosomatic endings. 6: Other cells received Gly+ puncta chiefly. **F:** At the border of the lateral lemniscal (LL) entry zone and the DmD, there was an abrupt increase in the density of glycinergic axosomatic endings that often encircled GABA+ cells, and ascending immunopositive preterminal trunks were prominent. A, C, E: Plastic embedded section, 1.5-μm thick; GABA (INCstar), 1:5,000 dilution, avidin-biotin. B, D, E: Plastic embedded section, 1.5-μm thick; Gly II (R.J. Wenthold), 1:300 dilution, avidin-biotin.

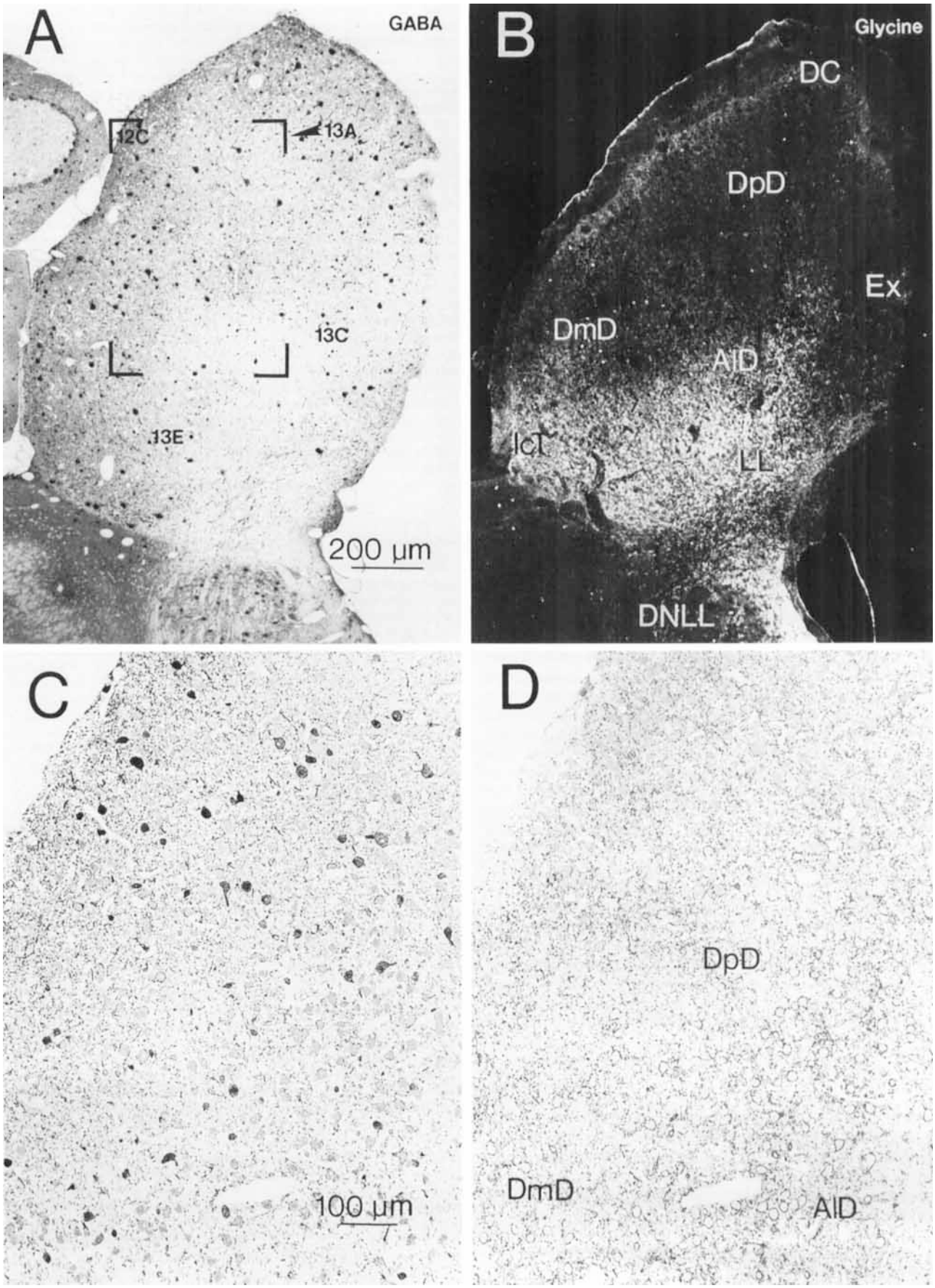


Figure 12. Legend on page 339.

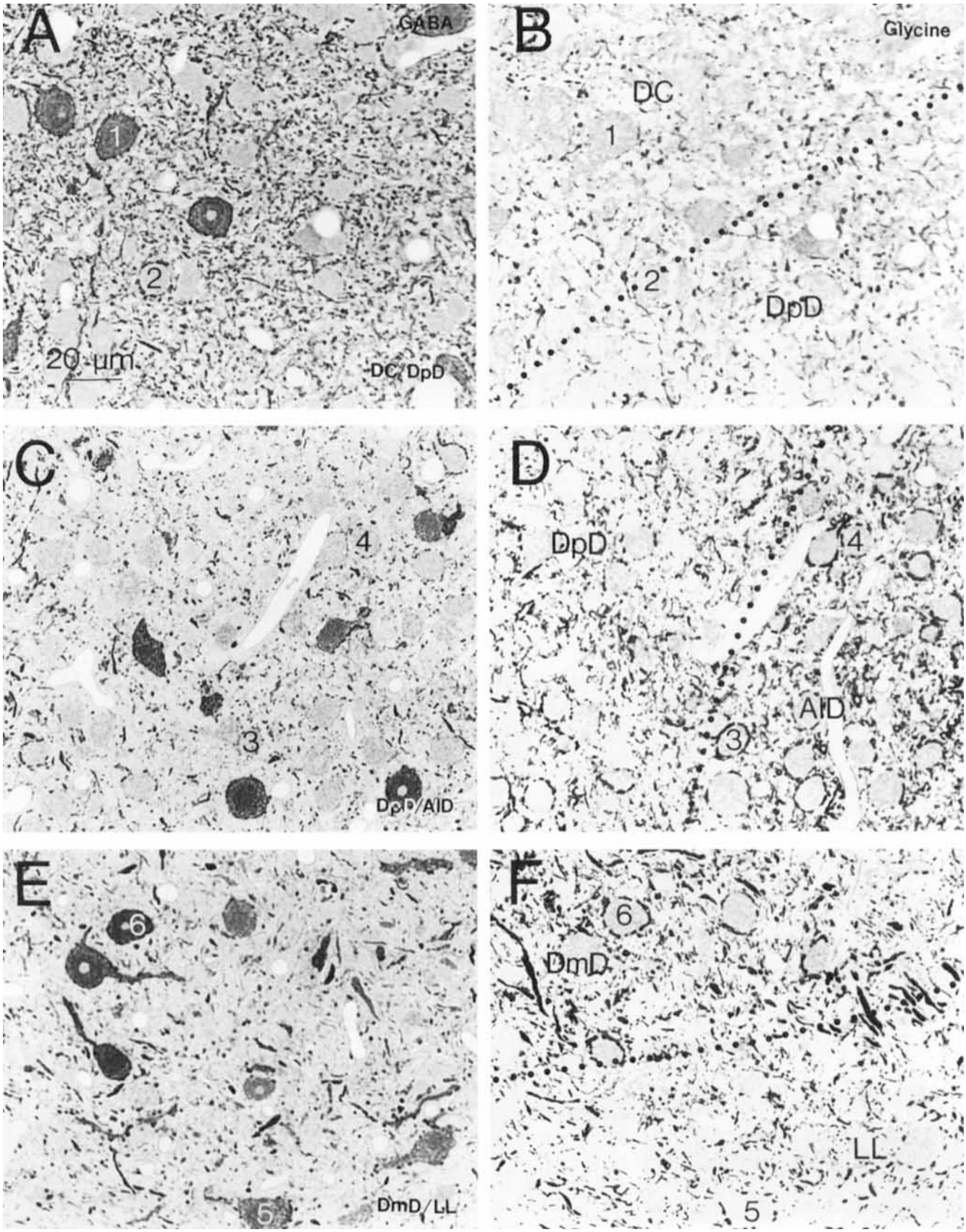


Figure 13. Legend on page 339.

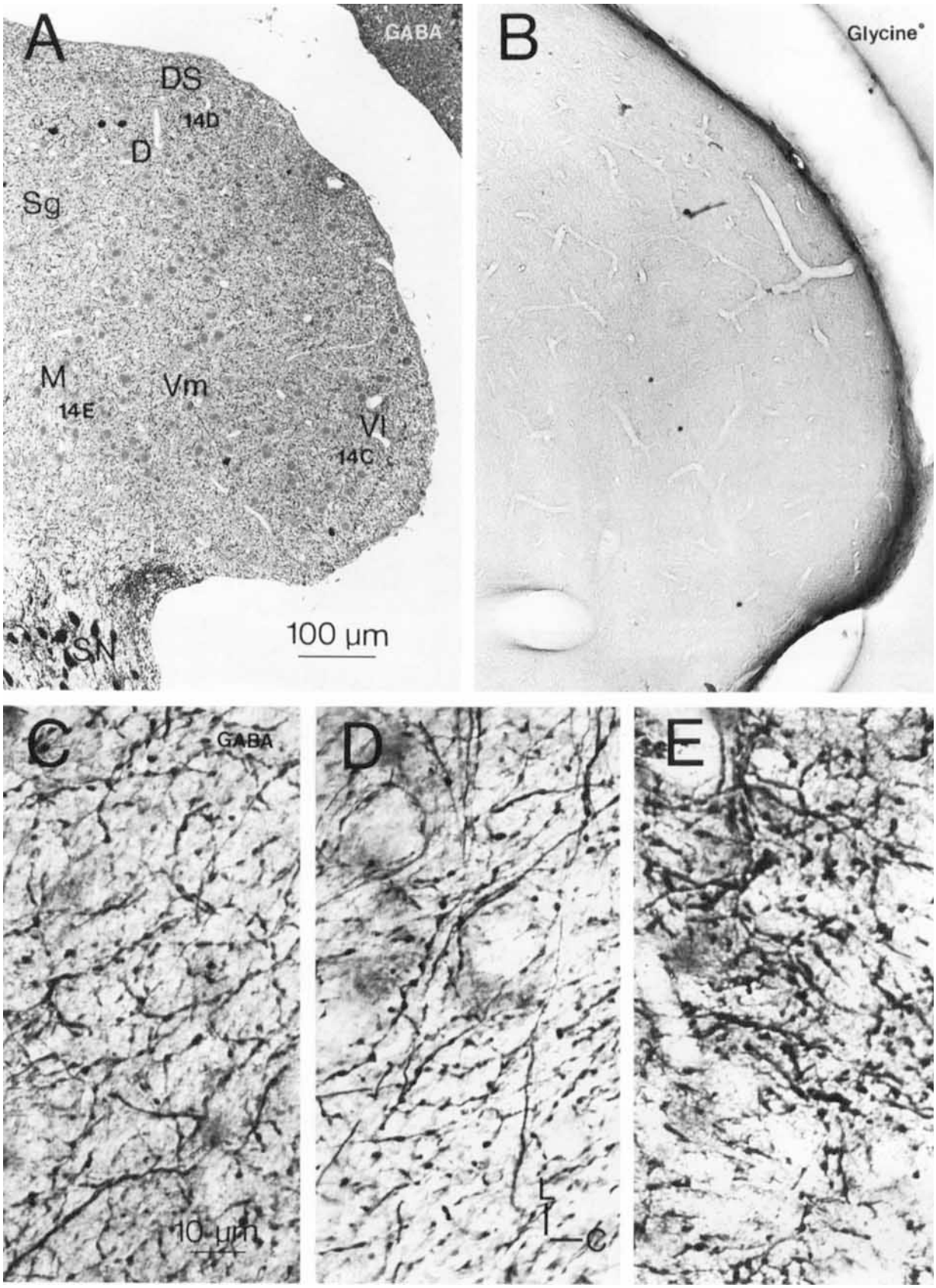

A
GABA
DS
14D
D
Sg
M
14E
Vm
Vl
14C
SN
100 µm
B
Glycine
C
GABA
10 µm
D
E

Figure 14

glycine detected in some GABA-stained neurons is of metabolic origin, the fact that the GABAergic elements in other nuclei are consistently free of this metabolite underscores the marked heterogeneity we have observed in GABAergic cell populations. Perhaps such colocalization represents a functional, as opposed to a metabolic, phenomenon and might thus be relevant physiologically. This seems to be the case in other such instances in the vertebrate and invertebrate nervous systems (Ottersen et al., 1987; Kupfermann, 1991).

With regard to the question of whether antisera to GAD and GABA identify the same populations of neurons, the evidence from biochemical studies suggests a high degree of concordance between them (Katarova et al., 1990). In this material, the results from GABA or GAD immunostaining were virtually identical: each marked the same neuronal group and types of cells, and the distribution of puncta between them was indistinguishable in a section-by-section comparison (material not illustrated; but see Prieto et al., 1994b, Figs. 3, 4).

A second consideration is the technical variability inherent to immunocytochemical studies. Many variables, including appropriate fixation, adequate etching of embedment during deplasticization, concentration of blocking serum, incubation time and temperature, chromogen sensitivity, and the osmolarity of buffer vehicles, all interact. Even with standardized protocols, an empirical approach to the optimal antiserum dilution is most appropriate. This is especially the case in postembedding immunocytochemistry, where dilutions of the same antiserum were varied over a fivefold range with similar results. The technical specifications included in each figure legend are intended to document these procedures and to serve as a point of departure and comparison.

A third technical issue is that some neurons are more darkly immunostained than others. Such differences, especially in thicker, free-floating sections, could reflect the vagaries of immunopenetration. Combined tract-tracing and immunostaining studies suggest that in some structures these differences might reflect chemically specific patterns of projection. Neurons in the dorsal nucleus of the lateral lemniscus that project to the contralateral central nucleus of the inferior colliculus are paler than ipsilaterally projecting cells in the same nucleus (D.T. Larue, T.J. Park, J.A. Winer and G.D. Pollak, unpublished observations). Although this could reflect a metabolic rather than a purely connectional phenomenon, it may still be germane to questions of relative size of axon terminal domains or ipsi- versus contralateral discharge rates.

Inhibitory neural elements are prominent throughout the central auditory pathways

The most striking finding in our survey is the prominence and the diversity of the inhibitory pathways and neural elements recognized by these antisera. These features are especially evident in the brainstem. The size and complexity of the Gly+ pathway is confirmed by the many Gly+ axons that form virtually a continuous network running from the superior olivary and periolivary regions through the nuclei of the lateral lemniscus and terminating in the inferior colliculus. The GABAergic organization is almost certainly as prominent and even more widespread than that of glycine. However, as the GABAergic preterminal axons are not always stained as fully as those for glycine, they are not as evident (compare Figs. 8C,D, 9A,B, 11A,B) and, indeed, they may not participate in as many remote projections as do glycinergic neurons. Nevertheless, the net result is that the glycinergic and GABAergic axons commingle with immunonegative fibers to form a parallel, ascending (and perhaps descending) pathway that comprises a substantial component of the lateral lemniscus.

In addition to the prominence and breadth of inhibitory substrates in this study, the sheer variety in these patterns was equally striking. Almost every conceivable structural arrangement is represented in one nucleus or another. This is demonstrated both by the diversity of inhibitory neurons among nuclei and by the distribution of inhibitory puncta. Some nuclei have largely or only GABAergic neurons (for example, the dorsal nucleus of the lateral lemniscus) or only glycinergic cells (such as the columnar part of the ventral nucleus of the lateral lemniscus). Other nuclei have GABA+ or Gly+ neurons as well as substantial numbers of immunonegative cells. The inferior colliculus contains GABAergic neurons, no Gly+ cells, and many immunonegative cells, whereas the lateral superior olive has many Gly+ neurons, few GABAergic cells, and many GABA–Gly immunonegative neurons.

The concentrations of GABA+ and Gly+ puncta have an equally diverse pattern among nuclei. Some centers were dominated by Gly+ puncta with only few GABA puncta, such as the medial superior olive. Others, such as the ventral part of the ventral nucleus of the lateral lemniscus, were dominated by GABA+ puncta and had far fewer Gly+ puncta. Still others, like the inferior colliculus, had both types of puncta in relative abundance. A few nuclei displayed a pattern of puncta immunoreactivity that was nonuniform within the nucleus, even when the cytoarchitecture did not reveal conspicuous intranuclear differences. The most dramatic examples are the marked density of GABA+ puncta in more medial sectors of the lateral superior olive and the selective concentration of GABA and Gly immunostaining in the dorsolateral and ventromedial parts of the intermediate nucleus of the lateral lemniscus. Such variations of immunoreactivity in these two nuclei correspond to particular tonotopic subregions. In the lateral superior olive, the GABA-rich zone represents cells serving high frequencies. The analogous areas in the intermediate nucleus of the lateral lemniscus are in regions where previous studies find the 60- and 90-kHz components of the mustache bat's echolocation call are (Ross et al.,

Fig. 14. **A:** The distribution of GABAergic neurons and puncta in the medial geniculate body was specific to each subdivision. Most of the GABA+ cells were found in the dorsal division nuclei (D, DS), fewer were seen in the ventral division (Vl, Vm), and none were present in the medial division (M). **B:** There were no glycinergic neurons or puncta (the three stained profiles are red blood cells). Planapochromat, N.A. 0.32, × 156. C–E: The puncta were best seen in thick sections immunostained free-floating for GABA. **C:** The ventral division had a robust array of medium-sized endings more numerous than those in the dorsal nuclei and far finer than those in the medial division. **D:** The dorsal division puncta were comparatively sparse and delicate (except in the suprageniculate nucleus; see also Fig. 4:Sg in Winer et al., 1992). **E:** The medial division had extremely coarse puncta and large-caliber preterminal processes. Semiapochromat, N.A. 1.25, × 984; differential interference contrast (Nomarski) optics. A: Vibratome section, 50-μm thick; GABA (INCstar), 1:5,000 dilution, avidin-biotin, free-floating. B: Vibratome section, 50-μm thick; Gly II (R.J. Wenthold), 1:1,500 dilution, avidin-biotin, free-floating. C–E: Vibratome section, horizontal plane, 50-μm thick; GABA (INCstar), 1:5,000 dilution, avidin-biotin, free-floating.

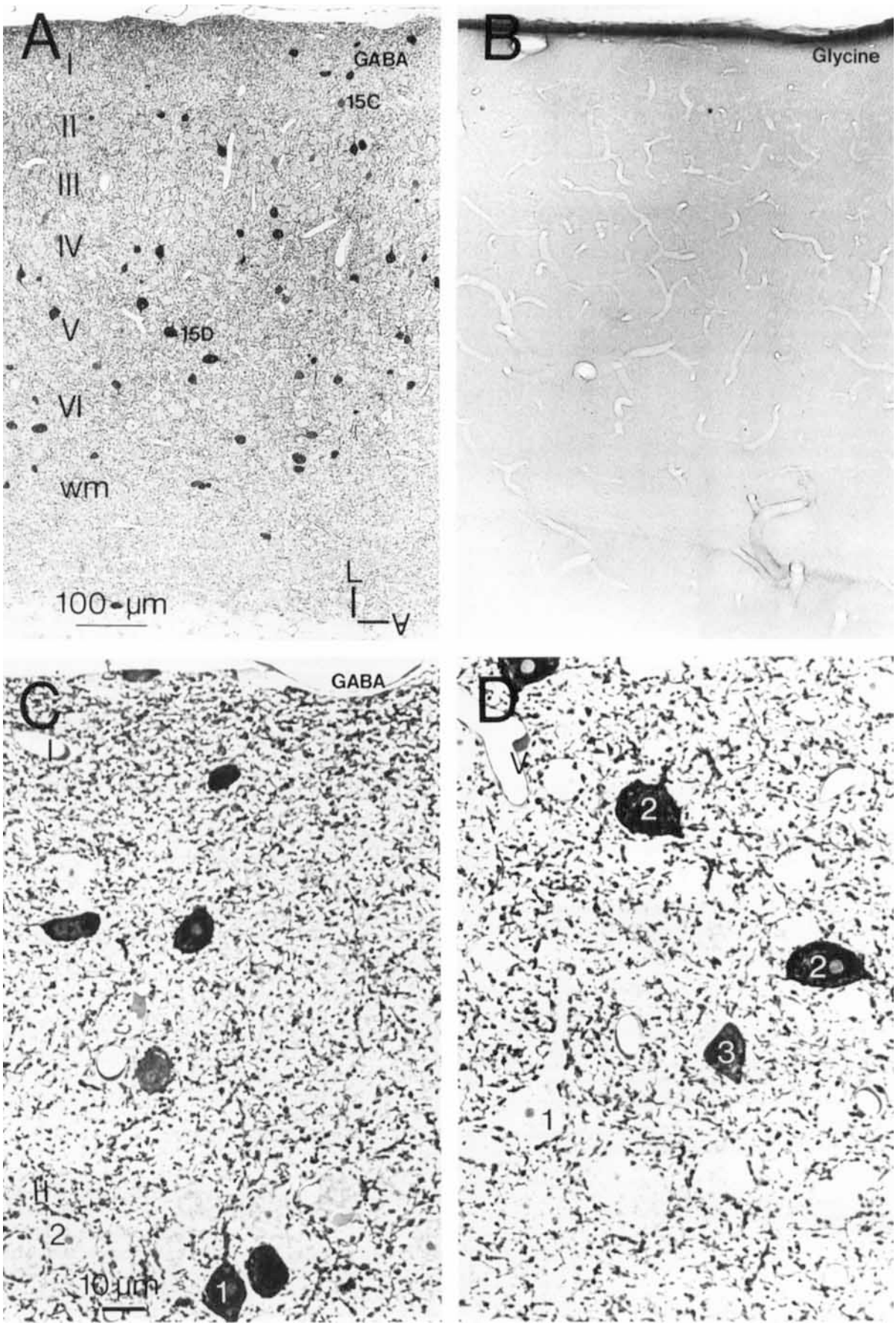

A
GABA
15C
II
III
IV
V
15D
VI
wm
100 µm
L
A
B
Glycine
C
GABA
L
II
2
10 µm
1
D
V
2
2
3
1

Figure 15

1988; Ross and Pollak, 1989). Such frequency-specific subregions could subserve specialized local patterns of inhibition. Analogous neurochemical variations have been reported elsewhere in the nervous system (for example, see Kondo et al., 1994).

The diversity of the inhibitory patterns among nuclei suggest that inhibition plays a role in a range of functions that may be different across nuclei or at various levels of the auditory system. One such example of the spectrum of inhibitory effects is found in the inferior colliculus, where recent iontophoretic studies show that GABAergic inhibition shapes rate-level functions, maximum spike counts, discharge patterns, thresholds, latencies, tuning curves, and binaural properties (Faingold et al., 1989, 1991a,b; Yang et al., 1992; Park and Pollak, 1993a,b; Pollak and Park, 1993). Many of these features are effected by specific circuits: thus, GABAergic influences that shape binaural properties, for example, are distinct from those that modulate rate-level functions, which, in turn, differ from those that influence discharge patterns. Such ubiquitous effects of GABAergic inhibition are not shared by all nuclei. For example, in the posteroventral cochlear nucleus, GABA affects temporal discharge patterns but has little or no impact on rate-level functions or on tuning curves (Palombi and Caspary, 1992).

Substrates for disinhibition

In most of the nuclei we have studied, GABA+ or Gly+ neurons receive axosomatic puncta immunoreactive for either GABA or glycine; both types of endings sometimes interdigitate on the soma. These terminals may have a disinhibitory effect in the cochlear nucleus, the superior olivary complex, the nuclei of the lateral lemniscus, and the inferior colliculus. The most prominent examples are in the three nuclei whose neurons are essentially homogeneous with respect to their neurotransmitter immunoreactivity: the medial nucleus of the trapezoid body, the ventral (columnar) part of the ventral nucleus of the lateral lemniscus (both of which are Gly+), and the dorsal nucleus of the lateral lemniscus (whose cells are GABA+). These three nuclei consist largely, if not exclusively, of inhibitory principal cells with targets up to several millimeters away (Covey and Casseday, 1986; Bledsoe et al., 1990; Saint Marie and Baker, 1990; Adams and Mugnaini, 1984, 1990). The first two nuclei have a strikingly similar arrangement consisting of rows of intensely Gly+ somata that are ringed with small GABA+ puncta and, in the dorsal nucleus of the lateral lemniscus, the GABA+ cells receive both types of axosomatic endings. Such axosomatic inhibition is a hallmark of pyramidal cells and other excitatory projection neurons (DeFelipe and Fariñas, 1992). In the auditory hindbrain, inhibitory projection circuits are far more common than in the cerebral cortex. In the forebrain, GABA+ medial geniculate body cells receive about one-sixth as many GABA+ axosomatic endings as do thalamocortical relay cells (Winer et al., 1993). An analogous picture is seen in the auditory cortex where, among GABA+ neurons, only the basket cells receive abundant axosomatic inhibition from GABA+ puncta (Prieto et al., 1994b). Unlike most cortical GABAergic interneurons, the basket cells project up to a millimeter or more to adjacent cortical areas (reviewed in Prieto et al., 1994a). Other examples of GABA+ projection neurons, such as cerebellar Purkinje cells (Chan-Palay, 1982) and thalamic reticular nucleus cells (Rinvik and Ottersen, 1988) also receive extensive arrays of axosomatic puncta. Perhaps all projection neurons require axosomatic inhibition to regulate their excitability. In every nucleus in which GABA+ or Gly+ neurons receive GABA+ or Gly+ axosomatic puncta, these inhibitory neurons seem to have a remote projection. In the lemniscal auditory pathway, where long-distance inhibitory projection neurons are common, axosomatic disinhibition must play a key role in modulation and control of information.

Speculation on the significance of GABA and glycine in the auditory brainstem

One significant feature of the auditory system is that glycine-mediated processing appears to be largely complete at the level of the inferior colliculus. This is manifest in the lack of Gly+ somata rostral to the intermediate nucleus of the lateral lemniscus and by the absence of Gly+ puncta beyond the inferior colliculus. Both the dorsal nucleus of the lateral lemniscus and the inferior colliculus are distinguished from lower auditory nuclei by their large populations of GABA+ neurons and the virtual absence of glycinergic neurons, although both regions receive substantial Gly+ puncta from ascending sources. The information conveyed from glycinergic origins thus affects the ascending projections that feed forward onto a GABAergic system in the dorsal nucleus of the lateral lemniscus and the inferior colliculus.

Another significant feature of the auditory system, which follows from the discussion above, is that there appears to be no substrate for glycinergic processing in the forebrain. This distinguishes it from lower centers because the forebrain contains neither Gly+ neurons nor any substantial populations of Gly+ terminals, although the literature is not in complete accord with regard to glycinergic immunoreactivity (Pourcho et al., 1992) or the distribution of postsynaptic receptors (see Probst et al., 1986; Naas et al., 1991). The function of these forebrain glycine receptors remains unclear. One hypothesis is that they are not associated with chloride channels but are a subset of *N*-methyl-D-aspartate receptors that might play a facilitative role in glutamatergic transmission (Jansen et al., 1989; Monaghan, 1990; see also Herkenham and McLean, 1986;

Fig. 15. Immunoreactivity in the auditory cortex; the region chosen for analysis came from an area that, in other experiments, contained many corticocollicular projection neurons (unpublished observations by T.J. Park, D.T. Larue, J.A. Winer, and G.D. Pollak). **A:** The number of immunopositive neurons resembled more closely the proportion in the inferior colliculus (Fig. 12A) rather than that in the medial geniculate body (Fig. 14A). Planapochromat, N.A. 0.32, × 156. **B:** There were no glycinergic neurons or puncta. **C:** Each layer had a unique, definitive pattern of immunostaining. Thus, in layer I, nearly all the neurons were immunopositive; in layer II, the proportion of such cells was much lower, and these neurons had diverse sizes and shapes, some with a vertical orientation and few axosomatic endings (1), whereas other, immunonegative cells had far more puncta (2); in layers III and IV, the puncta were appreciably coarser than those in the supragranular layers, and the form of the immunopositive neurons was varied (see A). **D:** In layer V the most conspicuous population of GABAergic neurons was the large multipolar cells (2), but immunopositive neurons with a more vertical somatic orientation (3) were present as well. Many immunonegative pyramidal cell somata (1) were encircled by puncta, a feature that aligned them with such neurons in the cat (Prieto et al., 1994a, b). In layer VI the proportion of GABAergic cells declined relative to that in other layers. Semiapochromat, N.A. 1.0, × 825. A, C: Vibratome sections, 50-μm thick; GABA (INCstar), 1:5,000 dilution, avidin-biotin, free-floating. B, D: Vibratome sections, 50-μm thick, Gly II (R.J. Wenthold), 1:1,500 dilution, avidin-biotin, free-floating.

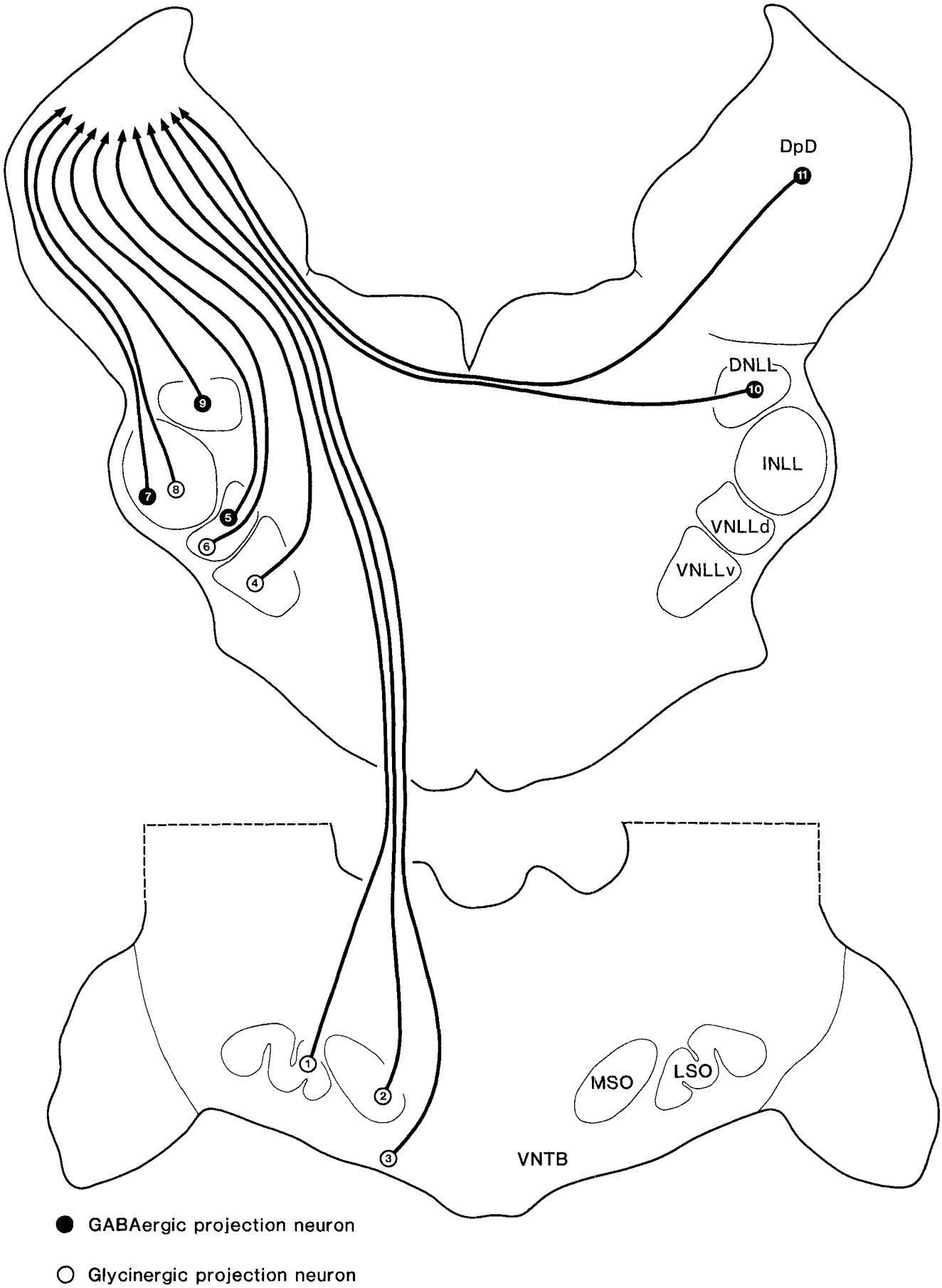

DpD
11
DNLL
10
INLL
VNLLd
VNLLv
9
7
8
5
6
4
1
2
3
MSO
LSO
VNTB
GABAergic projection neuron
Glycinergic projection neuron

Figure 16

Betz, 1991). The presence of so-called "facilitative" glycine receptors does not, in itself, confirm that glycine-mediated neurotransmission is operative, nor can it resolve the mismatch between immunocytochemical and receptor-binding approaches. We propose that GABA is the principal, and perhaps the sole, inhibitory transmitter in the thalamus and cerebral cortex, excluding, of course, peptidergic, aminergic, or other neuroactive substances whose actions may have a longer temporal duration than those attributable to the actions of $GABA_A$ receptors (see Macdonald and Olsen, 1994).

It is not intuitively obvious why more than one inhibitory transmitter is used in the caudal parts of the auditory system or why one apparently suffices rostral to the inferior colliculus. One insight into the nature of glycinergic processing comes from studies of two predominantly glycinergic nuclei, the medial nucleus of the trapezoid body and the columnar part of the ventral nucleus of the lateral lemniscus. A cardinal feature of both centers is that they receive excitatory drive from large calyciform synaptic endings that originate in the cochlear nucleus. Calyciform synapses are highly secure and result in a faithful transfer of information from pre- to postsynaptic cells. In these pathways, then, temporal information is preserved and relayed to their postsynaptic targets as precisely timed inhibitory inputs that appear to use glycine (Covey and Casseday, 1991; Finlayson and Caspary, 1991; Wu and Kelly, 1992a,b; Grothe et al., 1992; Grothe and Sanes, 1993; Grothe, 1994).

Consistent with the notion that glycine is the inhibitory transmitter used in circuits concerned with precise timing are the tuberculoventral neurons, the neurons in the dorsal cochlear nucleus that project to the ventral cochlear nucleus. These neurons are also glycinergic and have been proposed as constituting the inhibitory component of a pathway in which the temporal control of inhibition plays a critical role in monaural echo suppression (Wickesberg and Oertel, 1990; Wickesberg et al., 1991).

Functional differences almost certainly exist between the GABAergic and glycinergic innervation of brainstem centers. One difference might be that much of the GABAergic inhibition in several brainstem auditory nuclei is from descending sources. The lateral superior olive and columnar portion of the ventral nucleus of the lateral lemniscus, for example, also receive GABAergic endings in abundance. The noteworthy feature is that there are no known lower nuclei that provide substantial afferent GABAergic input to either the lateral superior olive or the columnar part of the ventral nucleus. The possibility remains that the innervation arises from intrinsic GABAergic cells. However, it seems unlikely that the relatively few GABAergic neurons present in the lateral superior olive could provide GABAergic input that is so robust. The same reasoning applies to the ventral nucleus. It is possible, then, that GABA puncta in the olivary complex and lateral lemniscal nuclei have a rostral origin and thus are descending, although the combined tract-tracing and immunocytochemical studies are not yet available. Such a prospect is consistent with the connectional evidence in several species, which shows a prominent array of descending projections toward the olivary, periolivary, and associated tegmental regions. These patterns of connectivity, furthermore, could affect the excitability of cochlear nucleus neurons (Kane and Finn, 1977; Kane and Conlee, 1979; Spangler et al., 1987; Henkel and Shneiderman, 1988; Caicedo and Herbert, 1993). At present, the evidence for any descending connections in the mustache bat is only circumstantial given the concentration of GABAergic cell populations in the dorsal part of the ventral nucleus of the lateral lemniscus, the dorsal nucleus of the lateral lemniscus, and in the inferior colliculus itself. Although speculative, this hypothesis suggests a convergence of glycinergic feedforward and GABAergic feedback inhibition onto single neurons in the lateral superior olive. However, several periolivary nuclei, such as the ventromedial periolivary nucleus, and the lateral and ventral nuclei of the trapezoid body, are also candidate sources for such an input. In other systems, descending, pontospinal glycinergic pathways may play an analogous role in the control of motoneuronal excitability in the somatic sensory pathway (Holstege and Bongers, 1991). Such an arrangement would stand in sharp contrast to the organization embodied by GABAergic forebrain neurons, whose projections are believed in most systems to terminate within a few hundred micrometers of their origin, a pattern that virtually defines the locus of action of so-called Golgi type II neurons (Morest, 1971, 1975; Winer, 1992).

Neuronal targets of inhibitory projections

Because each auditory center in the present survey has so specific an arrangement of GABAergic or glycinergic elements, it seems appropriate to ask whether this principle might extend to include specific types of neurons, such as pyramidal or fusiform cells, and whether an equally precise pattern of inhibitory innervation is likewise particular to them. Only for a few neuronal types in the auditory brainstem does an adequate morphological profile exist that would permit such comparisons (see Ostapoff and Morest, 1991). In what may be the most thoroughly documented instance in the neuraxis—the cortical pyramidal cell—the different kinds of inhibitory inputs seem to be segregated spatially on one part or another of the axonal, perikaryal, or dendritic membrane (for a summary, see DeFelipe and Fariñas, 1992). The axon initial segment receives specific synaptic endings from presynaptic chandelier cells (Somogyi, 1977). The perikaryon is the target of a particular set of large inhibitory terminals whose source is large multipolar basket cells (DeFelipe et al., 1986). Smaller GABAergic boutons terminate in discrete clusters elsewhere on the somatic surface, and even more circumscribed terminals form tiny aggregates along dendritic bifurcations, often

Fig. 16. Schematic view of several inhibitory ascending components of the caudal auditory brainstem and midbrain, which terminate in the inferior colliculus. This picture reflects the results from combined tract-tracing and immunolocalization studies in the mustache bat and other species, which revealed that multiple, independent sources of inhibitory input converge upon the subdivisions of the central nucleus, including the dorsoposterior division (DpD; Larue et al., 1991; Park et al., 1991). Intrinsic and descending, possibly inhibitory, connections have been omitted for purposes of clarity. Some of these projections follow lemniscal routes, whereas others do not. Whether and to what degree individual midbrain neurons receive one or more such input remains to be determined. The particular species and source for these observations are documented below; the numbers correspond to these keyed to the putative projection neurons illustrated. 1: Mustache bat (Park et al., 1991); cat (Hutson et al., 1987; Saint Marie et al., 1989). 2, 3: Mustache bat (unpublished observations by T.J. Park, D.T. Larue, G.D. Pollak, and J.A. Winer). 4–8: Mustache bat (Larue et al., 1991); for VNLL in the guinea pig (Saint Marie and Baker, 1990). 9: Mustache bat (Park et al., unpublished observations); cat (Hutson, 1988). 10: Mustache bat (Park et al., unpublished observations); cat (Adams and Mugnaini, 1984). 11: Mustache bat (Park et al., unpublished observations).

near the largest trunks (Somogyi et al., 1985; Somogyi and Soltész, 1986). Much of the apical dendrite receives an assortment of heterogeneous GABAergic endings along its inter- and intralaminar course (reviewed in Prieto et al., 1994a,b). These terminals are most numerous in layer I, where the distal dendritic arbors divide and ramify among the finest neocortical GABA+ endings (Winer and Larue, 1989). Although many of these different synaptic endings probably represent GABAergic terminals, other varieties also exist, and some have spatial arrangements that are equally specific. For example, basket-like serotonergic axosomatic endings (Mulligan and Törk, 1988) complement the patchy distribution of input from large multipolar cell endings onto the perikaryon. Analogous patterns exist with regard to the density of GABAergic axosomatic endings on GABA-positive and GABA-negative medial geniculate neurons, the former receiving more than six times the number of puncta (Winer et al., 1993). There is spatial segregation of inhibition that may be unique for each type of neuron. How far this principle might apply to the auditory brainstem remains an open question.

Species-specific patterns of neuronal organization

Many studies suggest that the primary features of auditory brainstem organization are conserved among mammalian species at least through the level of the inferior colliculus. This concept is well illustrated by the lateral superior olive. In all mammals, a stereotyped neuronal organization is present; the chief connections arise from spherical bushy cells of the anteroventral cochlear nucleus (Cant and Casseday, 1986) and from principal cells in the medial nucleus of the trapezoid body (Spangler et al., 1985). The output of the lateral superior olive is directed bilaterally to the dorsal nucleus of the lateral lemniscus and the central nucleus of the inferior colliculus (Irvine, 1986). Superior olivary neurochemical organization is dominated in mammals by a characteristic pattern of moderate numbers of glycinergic cells, few or no GABAergic neurons, and many neurons that are immunonegative for GABA or glycine (Saint Marie et al., 1989; Adams and Mugnaini, 1990); the guinea pig may be exceptional in having many more GABAergic neurons (Helfert et al., 1989). The ipsilateral projections to the central nucleus of the inferior colliculus include both glycinergic and nonglycinergic components, whereas the projections from the contralateral lateral superior olive are all glycine negative and, presumably, excitatory (Park et al., 1991; for data on the cat, see Hutson et al., 1987; Saint Marie et al., 1989; Saint Marie and Baker, 1990; Glendenning et al., 1992; see also Sanes, 1990). Finally, almost all lateral superior olivary cells, regardless of species, are excitatory–inhibitory physiologically, and thus are the same binaural type (Caird and Klinke, 1983; Covey et al., 1991). Although it would be possible to propose a similar list of attributes for most nuclei in our series, we do not intend to suggest that there are no species differences (Covey and Casseday, 1991; Grothe et al., 1992; Vater et al., 1992a). However, the prevailing view is one of striking similarity and continuity of organization across these and related dimensions.

The robustness of interspecific parallels should be evaluated against the proposition that different behavioral functions probably use both common and species-specific circuitry. It is difficult otherwise to account for the fourfold differences between the guinea pig and cat in the proportion of dorsal cochlear nucleus tuberculoventral neurons colocalizing GABA and Gly (reviewed in Kolston et al., 1992). Such species differences may have functional consequences that remain to be characterized physiologically.

An entirely different picture emerges at the level of the auditory thalamus. In the mustache bat, there is a precipitous decline in the number of GABAergic neurons relative to the proportion of such cells in the auditory midbrain or cerebral cortex. This paucity of GABA+ medial geniculate neurons (Winer et al., 1992) is a feature that this species shares with many rodents but not with carnivores (Rinvik et al., 1987) or primates (Smith et al., 1987), nor with horseshoe bats (Vater et al., 1992a). Nevertheless, the form and relative density of GABAergic puncta are largely conserved across species. Comparative differences in the number of GABAergic neurons in the medial geniculate body may be a pivotal evolutionary and functional feature relevant to specie-specific signal processing. The disparate immunochemical profile of the medial geniculate across species stands in marked contrast not only to the brainstem auditory nuclei, which are much more stereotyped in their organization, but also to that of the auditory cortex; several species have a similar (although not an identical) number of GABAergic neurons in each layer of the auditory cortex (see Winer, 1992). The widest range of differences seems to be in the auditory thalamus (Winer, 1991; Pollak et al., 1995). These species-specific patterns imply that the role of interneurons in animals having many or few GABAergic intrinsic cells cannot be the same.

The pivotal functional role of the auditory thalamus is manifest in a recent study showing that the medial geniculate is the first site where species-specific combination-sensitive neurons are constructed in the mustache bat (Olsen and Suga, 1991a,b). The physiological and anatomic findings suggest that GABAergic local circuit neurons may not have any substantial role in the genesis of combination sensitivity because they are relatively few in number and sparsely distributed, although they are most concentrated in nuclei with the greatest number of combination-sensitive cells. It suggests further that some type of connectional convergence from the inferior colliculus (or elsewhere) might be the decisive factor.

If, as seems plausible, the combination sensitivity of auditory thalamic neurons is indeed generated by the convergence of projections from the inferior colliculus, it follows that the midbrain plays a pivotal role in processing species-specific features. The projections to the mustache bat's inferior colliculus, its basic neuronal organization, and almost all of the response properties of its neurons, are comparable with those of other mammals (Zook and Casseday, 1982a,b; Frisina et al., 1989; Ross and Pollak, 1989; Park and Pollak, 1993a; Pollak and Park, 1993). Moreover, the pattern of immunoreactivity in the mustache bat's central nucleus is very similar, at least in its broad outlines, to that of the cat (Oliver et al., 1994) and rat (Moore and Moore, 1987; Aoki et al., 1988), and to other species that have been studied (Thompson et al., 1985; Roberts and Ribak, 1987). It can plausibly be asserted that the central nucleus of the inferior colliculus, as well as many of the auditory nuclei below it, are largely conserved among mammals in terms of their major cell types, the primary inputs, their gross patterns of immunocytochemical organization, and the response properties of their neurons. One prospective source of evolutionary plasticity is the projections of the inferior colliculus; in the mustache bat, collicu-

lar projections from two or more isofrequency contours (or from other sites) could converge on thalamic cells (Olsen, 1986; Wenstrup et al., 1994). In conjunction with the arrangement of the intrinsic circuits, this could underlie species-specific features, such as combination sensitivity and delay tuning.

The hypothesis that the brainstem auditory system is highly conserved and that species-specific features emerge initially in the targets of the inferior colliculus from a unique combination of projections can be extended further because this pattern seems to occur in other bats as well as in nonmammalian species. The little and big brown bats and the horseshoe bat also have combination-sensitive neurons, but these cells have properties that are different from those of the mustache bat (Sullivan, 1986; Berkowitz and Suga, 1989; Schuller et al., 1991). In owls, the space map emerges in the neurons of the external nucleus from the appropriate convergence and integration of projections from the central nucleus of the inferior colliculus (see Konishi et al., 1988). This map in the barn owl, in turn, is different from that of the great horned owl (Volman and Konishi, 1990), although both representations are formed from the projections of the central nucleus of the inferior colliculus. Similar processes also occur in the amphibian auditory system. The so-called "mating-call detectors," which require the combination of appropriate frequencies, occur not in the midbrain, but arise only in the diencephalon, due, presumably, to the convergence of projections from the torus semicircularis, the amphibian homologue of the inferior colliculus (Fuzessery and Feng, 1983).

GABA and glycine in central auditory, somatic sensory, and visual systems

The present findings also bear on the differences and similarities in the internal organization of inhibitory circuitry within nuclei in other sensory modalities. The abundance of inhibitory projections suggests that the auditory system is singular among the special senses (see Fig. 16); no such analogous arrangement could be proposed for the visual, somatic sensory, vestibular, olfactory, gustatory, or nociceptive modalities, nor in the reticular system. Thus, the auditory brainstem has a series of massive parallel inhibitory projection pathways that must be as important for neural processing as are the classically defined and much better known excitatory parallel pathways. Neither the dorsal column nuclei nor the retina appear to contain appreciable numbers of GABAergic or glycinergic neurons that project to either the ventrobasal complex or lateral geniculate body, respectively. The spinal cord, however, contains both GABAergic and glycine-immunoreactive elements (Todd and Sullivan, 1990), some of which appear to modulate motoneuronal excitability (Schneider and Fyffe, 1992); whether any of these inhibitory projections arise remotely is unknown.

The forebrain auditory system, in contrast, departs from this brainstem pattern and in doing so shares a fundamental continuity of organization with other sensory systems. Indeed, only in the forebrain is there a conspicuous parallel between modalities with respect to a GABAergic projection of extrinsic origin. In this instance, the thalamic reticular nucleus—all of whose neurons are GABAergic in every species so far studied (Mugnaini and Oertel, 1985; Winer et al., 1992)—projects to every part of the medial geniculate complex (Conley et al., 1991), on the ventrobasal complex (Ohara et al., 1989), and onto the dorsal nucleus of the lateral geniculate body (Ohara et al., 1980, 1983), and even to the hypothalamus (Barone et al., 1994). Another feature in common is the neurochemical pattern of thalamocortical connectivity. No GABAergic diencephalic special sensory neurons project to the cortex in any sensory modality or species so far examined. Forebrain and retrothalamic GABAergic circuits thus differ in that the telencephalic GABAergic circuits must represent more or less intrinsic connections, as in the auditory thalamus, whereas some retrothalamic circuits project remotely, as in the case of the dorsal nucleus of the lateral lemniscus or more caudal brain stem centers.

This is not to say that the medullopontine auditory pathway is the only neural system that has inhibitory projection neurons, but only that this feature distinguishes it from the pathways of the other special senses. Long-distance inhibitory projections are shared with other central neural systems, such as the cerebellar Purkinje cell GABAergic projection (Mugnaini and Oertel, 1985) and the dopaminergic nigrostriatal system (Bolam and Smith, 1990), and there is a substantial GABAergic projection from the inferior colliculus to the medial geniculate body (Paydar et al., 1994), to mention just three. Although these circuits have in common with the auditory system the feature of inhibitory projection neurons, this attribute does not appear to represent a common or simple template of inhibitory circuitry, but rather denominates many patterns, each unique to a particular nucleus and, presumably, subserving specific functions.

The preceding account should be viewed as a preliminary attempt to articulate a general framework for the actions of GABA or Gly. It should be noted, however, that the emerging multiplicity of subtypes for $GABA_A$ receptors (Burt and Kamatchi, 1991; Fritschy et al., 1992) and the complexity of glycine-modulated postsynaptic receptor domains (Zucker and Ehinger, 1992) suggest that this initial effort may be too limited to capture completely the diversity of the neural substrates for inhibition.

ACKNOWLEDGMENTS

We thank Drs. D.E. Schmechel, W.H. Oertel, and E. Mugnaini for their contribution of GAD antiserum and pre-immune serum. Dr. R.J. Wenthold generously donated antisera to GABA and Gly. Dr. R.L. Saint Marie provided advice on postembedding immunocytochemistry for which we are grateful. Ms. D. Lambert, Mr. J. Shin, and Ms. T.N. Boerner supplied able secretarial assistance. Ms. L.A. Bui, Ms. M. Jung, and Ms. J. Li kindly shared postembedded material from their experiments. This research was supported by United States Public Health Service grants R01 DC02319-15 (J.A.W.) and R01 DC00068 (G.D.P.), and by University of California Faculty Research Awards. Preliminary accounts of this work have appeared elsewhere (Pollak and Winer, 1989; Larue et al., 1991; Park et al., 1991).

LITERATURE CITED

Adams, J.C. (1981) Heavy metal intensification of DAB-based HRP reaction product. J. Histochem. Cytochem. *29*:775.

Adams, J.C., and E. Mugnaini (1984) Dorsal nucleus of the lateral lemniscus: A nucleus of GABAergic projection neurons. Brain Res. Bull. *14*:585–590.

Adams, J.C., and E. Mugnaini (1990) Immunocytochemical evidence for inhibitory and disinhibitory circuits in the superior olive. Hearing Res. *49*:281–298.

Aitkin, L. (1986) The Auditory Midbrain. Structure and Function in the Central Auditory Pathway. Clifton, NJ: Humana Press.

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.

Alger, B.E. (1985) GABA and glycine: Postsynaptic actions. In M.A. Rogawski and J.L. Barker (eds): Neurotransmitter Actions in the Vertebrate Nervous System. New York: Plenum Publishing Corp., pp. 33–69.

Alloway, K.D., R.J. Sinclair, and H. Burton (1988) Responses of neurons in somatosensory cortical area II of cats to high-frequency vibratory stimuli during iontophoresis of a GABA antagonist and glutamate. Somatosens. Motor Res. *6:*109–140.

Aoki, E., R. Semba, H. Keino, K. Kato, and S. Kashiwamata (1988) Glycine-like immunoreactivity in the rat auditory pathway. Brain Res. *442:*63–71.

Aprison, M.H., and R. Werman (1965) The distribution of glycine in cat spinal cord and roots. Life Sci. *4:*2075–2083.

Babb, T.L., J.K. Pretorius, W.R. Kupfer, and P.H. Crandall (1989) Glutamate decarboxylase-immunoreactive neurons are preserved in human epileptic hippocampus. J. Neurosci. *9:*2562–2574.

Barone, F.C., J.-T. Cheung, and M.J. Wayner (1994) GABA inhibition of lateral hypothalamic neurons: Role of reticular thalamic afferents. Brain Res. Bull. *33:*699–708.

Berkowitz, A., and N. Suga (1989) Neural mechanisms of ranging are different in two species of bats. Hearing Res. *41:*255–264.

Betz, H. (1991) Glycine receptors: Heterogeneous and widespread in the mammalian brain. Trends Neurosci. *14:*458–461.

Bledsoe, S.C., Jr., C.R. Snead, R.H. Helfert, V. Prasad, R.J. Wenthold, and R.A. Altschuler (1990) Immunocytochemical and lesion studies support the hypothesis that the projection from the medial nucleus of the trapezoid body to the lateral superior olive is glycinergic. Brain Res. *517:*189–194.

Bolam, J.P., and Y. Smith (1990) The GABA and substance P input to dopaminergic neurones in the substantia nigra of the rat. Brain Res. *529:*57–78.

Boos, R., F. Müller, and H. Wässle (1990) Actions of excitatory amino acids on brisk ganglion cells in the cat retina. J. Neurophysiol. *64:*1368–1379.

Burger, P.M., J. Hell, E. Mehl, C. Krasel, F. Lottspeich, and R. Jahn (1991) GABA and glycine in synaptic vesicles: Storage and transport characteristics. Neuron *7:*287–293.

Burt, D.R., and G.L. Kamatchi (1991) $GABA_A$ receptor subtypes: From pharmacology to molecular biology. Fed. Am. Soc. Expl. Biol. *5:*2916–2923.

Caicedo, A., and H. Herbert (1993) Topography of descending projections from the inferior colliculus to auditory brainstem nuclei in the rat. J. Comp. Neurol. *328:*377–392.

Caird, D., and R. Klinke (1983) Processing of binaural stimuli by cat superior olivary complex neurons. Exp. Brain Res. *52:*385–399.

Cant, N.B., and J.H. Casseday (1986) Projections from the anteroventral cochlear nucleus to the lateral and medial superior olivary nuclei. J. Comp. Neurol. *247:*457–476.

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.

Chan-Palay, V. (1982) Neurotransmitters and receptors in the cerebellum: Immunocytochemical localization of glutamic acid decarboxylase, GABA-transaminase, and cyclic GMP and autoradiography with ^{3}H-muscimol. In S.L. Palay and V. Chan-Palay (eds): The Cerebellum—New Vistas. Berlin: Springer-Verlag, pp. 554–586.

Conley, M., A.C. Kupersmith, and I.T. Diamond (1991) The organization of projections from subdivisions of the auditory cortex and thalamus to the auditory sector of the thalamic reticular nucleus in *Galago*. Eur. J. Neurosci. *3:*1089–1103.

Covey, E., and J.H. Casseday (1986) Connectional basis for frequency representation in the nuclei of the lateral lemniscus of the bat, *Eptesicus fuscus*. J. Neurosci. *6:*2926–2940.

Covey, E., and J.H. Casseday (1991) The monaural nuclei of the lateral lemniscus in an echolocating bat: Parallel pathways for analyzing temporal features of sound. J. Neurosci. *11:*3456–3470.

Covey, E., M. Vater, and J.H. Casseday (1991) Binaural properties of single units in the superior olivary complex of the mustached bat. J. Neurophysiol. *66:*1080–1093.

Daly, E.C., and M.H. Aprison (1982) Glycine. In A. Lajtha (ed): Handbook of Neurochemistry, vol. 3: Metabolism in the Nervous System. New York: Plenum Press, pp. 467–499.

DeFelipe, J., and I. Fariñas (1992) The pyramidal neuron of the cerebral cortex: Morphological and chemical characteristics of the synaptic inputs. Prog. Neurobiol. *39:*563–607.

DeFelipe, J., S.H.C. Hendry, and E.G. Jones (1986) A correlative electron microscopic study of basket cells and large GABAergic neurons in the monkey sensory-motor cortex. Neuroscience *17:*991–1009.

Faingold, C.L., G. Gehlbach, and D.M. Caspary (1989) On the role of GABA as an inhibitory neurotransmitter in inferior colliculus neurons: Iontophoretic studies. Brain Res. *500:*302–312.

Faingold, C.L., C.A. Boersma-Anderson, and D.M. Caspary (1991a) Involvement of GABA in acoustically-evoked inhibition in inferior colliculus. Hearing Res. *52:*201–216.

Faingold, C.L., G. Gehlbach, and D.M. Caspary (1991b) 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 Pathways. New York: Raven Press, Ltd., pp. 223–251.

Finlayson, P.G., and D.M. Caspary (1991) Low-frequency neurons in the lateral superior olive exhibit phase-sensitive binaural inhibition. J. Neurophysiol. *65:*598–605.

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.

Fritschy, J.-M., D. Benke, S. Mertens, W.H. Oertel, T. Bachi, and H. Möhler (1992) Five subtypes of type A γ-aminobutyric acid receptors identified in neurons by double and triple immunofluorescence staining with subunit-specific antibodies. Proc. Natl. Acad. Sci. USA *89:*6726–6730.

Fuzessery, Z.M., and A.S. Feng (1983) Mating call selectivity in the thalamus and midbrain of the leopard frog (*Rana p. pipiens*): Single and multiunit analysis. J. Comp. Physiol. A. *150:* 333–344.

Glendenning, K.K., B.N. Baker, K.A. Hutson, and R.B. Masterton (1992) Acoustic chiasm V: Inhibition and excitation in the ipsilateral and contralateral projections of LSO. J. Comp. Neurol. *319:*100–122.

Grothe, B. (1994) Interaction of excitation and inhibition in processing of pure tone and amplitude-modulated stimuli in the medial superior olive of the mustached bat. J. Neurophysiol. *71:*706–721.

Grothe, B., and D.H. Sanes (1993) Binaural inhibition by glycinergic afferents in the medial superior olive. J. Neurophysiol. *69:*1192–1196.

Grothe, B., M. Vater, J.H. Casseday, and E. Covey (1992) Monaural interaction of excitation and inhibition in the medial superior olive of the mustached bat: An adaptation for biosonar. Proc. Natl. Acad. Sci. USA *89:*5108–5112.

Helfert, R.H., J.M. Bonneau, R.J. Wenthold, and R.A. Altschuler (1989) GABA and glycine immunoreactivity in the guinea pig superior olivary complex. Brain Res. *501:*269–286.

Hendry, S.H.C., H.D. Schwark, E.G. Jones, and J. Yan (1987) Numbers and proportions of GABA-immunoreactive neurons in different areas of monkey cerebral cortex. J. Neurosci. *7:*1503–1519.

Henkel, C.K., and A. Shneiderman (1988) Nucleus sagulum: Projections of a lateral tegmental area to the inferior colliculus in the cat. J. Comp. Neurol. *271:*577–588.

Herkenham, M., and S. McLean (1986) Mismatches between receptor and transmitter localizations in the brain. In C.A. Boast, E.W. Snowhill, and C.A. Altar (eds): Quantitative Receptor Autoradiography. New York: Alan R. Liss, Inc., pp. 137–171.

Holstege, J.C., and C.M.H. Bongers (1991) A glycinergic projection from the lower brainstem to spinal motoneurons. An ultrastructural double labeling study in rat. Brain Res. *566:*308–315.

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, Florida State University, Tallahassee, FL.

Hutson, K.A., K.K. Glendenning, and R.B. Masterton (1987) Biochemical basis for the acoustic chiasm? Proc. Soc. Neurosci. *13:*548 (abstract).

Irvine, D.R.F. (1986) The auditory brainstem. A review of the structure and function of auditory brainstem processing mechanisms. In H. Autrum, E.R. Perl, R.F. Schmidt, H. Shimazu, and W.D. Willis (eds): Progress in Sensory Physiology, vol. 7. Berlin: Springer-Verlag, pp. 1–279.

Jansen, K.L.R., R.L.M. Faull, and M. Dragunow (1989) Excitatory amino acid receptors in the human cerebral cortex: A quantitative autoradiographic study comparing the distribution of [^{3}H]TCP, [^{3}H]glycine,

L-[^{3}H]glutamate, [^{3}H]AMPA and [^{3}H]kainic acid binding sites. Neuroscience *32*:587–607.

Johnson, J.W., and P. Ascher (1987) Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature *325*:529–531.

Kane, E.S., and J.W. Conlee (1979) Descending inputs to the caudal cochlear nucleus of the cat: Degeneration and autoradiographic studies. J. Comp. Neurol. *187*:759–784.

Kane, E.S., and R.C. Finn (1977) Descending and intrinsic inputs to dorsal cochlear nucleus of cats: A horseradish peroxidase study. Neuroscience *2*:897–912.

Katarova, Z., G. Szabo, E. Mugnaini, and R.J. Greenspan (1990) Molecular identification of the 62 kD form of glutamic acid decarboxylase from the mouse. Eur. J. Neurosci. *2*:190–202.

Kolston, J., K.K. Osen, C.M. Hackney, O.P. Ottersen, and J. Storm-Mathisen (1992) An atlas of glycine- and GABA-like immunoreactivity and colocalization in the cochlear nuclear complex of the guinea pig. Anat. Embryol. *186*:443–465.

Kondo, H., T. Hashikana, K. Tanaka, and E.G. Jones (1994) Neurochemical gradient along the monkey occipito-temporal cortical pathway. NeuroReport *5*:613–616.

Konishi, M., T.T. Takahashi, H. Wagner, W.E. Sullivan, and C.E. Carr (1988) Neurophysiological and anatomical substrates of sound localization in the owl. In G.M. Edelman, W.E. Gall, and W.M. Cowan (eds): Auditory Function. Neurobiological Bases of Hearing. New York: John Wiley & Sons, pp. 721–745.

Kupfermann, I. (1991) Functional studies of cotransmission. Physiol. Revs. *71*:683–732.

Kuwabara, N., and J.M. Zook (1991) Classification of the principal cells of the medial nucleus of the trapezoid body. J. Comp. Neurol. *314*:707–720.

Larue, D.T., T.J. Park, G.D. Pollak, and J.A. Winer (1991) Glycine and GABA immunostaining defines functional subregions of the lateral lemniscal nuclei in the mustache bat. Proc. Soc. Neurosci. *17*:301 (abstract).

Macdonald, R.L., and R.W. Olsen (1994) $GABA_A$ receptor channels. Ann. Rev. Neurosci. *17*:569–602.

Masland, R.H. (1988) Amacrine cells. Trends Neurosci. *11*:405–410.

Monaghan, D.T. (1990) Glycine modulation of NMDA receptors: Autoradiographic studies. In O.P. Ottersen and J. Storm-Mathisen (eds): Glycine Neurotransmission. Chichester: John Wiley & Sons, pp. 219–237.

Moore, J.K., and R.Y. Moore (1987) Glutamic acid decarboxylase-like immunoreactivity in brainstem auditory nuclei of the rat. J. Comp. Neurol. *260*:157–174.

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 relations of Golgi type II cells in the medial geniculate body of the cat. J. Comp. Neurol. *162*:157–194.

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 immunocytochemistry. 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.

Mulligan, K.A., and I. Törk (1988) Serotoninergic innervation of the cat cerebral cortex. J. Comp. Neurol. *270*:86–110.

Naas, E., K. Zilles, H. Gnahn, H. Betz, C.-M. Becker, and H. Schröder (1991) Glycine receptor immunoreactivity in rat and human cerebral cortex. Brain Res. *561*:139–146.

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

Ohara, P.T., A.J. Sefton, and A.R. Lieberman (1980) Mode of termination of afferents from the thalamic reticular nucleus in the dorsal lateral geniculate nucleus of the rat. Brain Res. *197*:503–506.

Ohara, P.T., A.R. Lieberman, S.P. Hunt, and J.-Y. Wu (1983) Neural elements containing glutamic acid decarboxylase (GAD) in the dorsal lateral geniculate nucleus of the rat: Immunohistochemical studies by light and electron microscopy. Neuroscience *8*:189–211.

Oliver, D.L., J.A. Winer, G.E. Beckius, and R.L. Saint Marie (1994) Morphology of GABAergic neurons in the cat inferior colliculus. J. Comp. Neurol. *340*:27–42.

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.

Osen, K.K., O.P. Ottersen, and J. Storm-Mathisen (1990) Colocalization of glycine-like and GABA-like immunoreactivities: A semiquantitative study in the dorsal cochlear nucleus of the cat. In O.P. Ottersen and J. Storm-Mathisen (eds): Glycine Neurotransmission. Chichester: John Wiley & Sons, pp. 417–451.

Ostapoff, E.-M., and D.K. Morest (1991) Synaptic organization of globular bushy cells in the ventral cochlear nucleus of the cat: A quantitative study. J. Comp. Neurol. *314*:598–613.

Ottersen, O.P., S. Davanger, and J. Storm-Mathisen (1987) Glycine-like immunoreactivity in the cerebellum of rat and Senegalese baboon, *Papio papio*: A comparison with the distribution of GABA-like immunoreactivity and with [^{3}H]glycine and [^{3}H]GABA uptake. Exp. Brain. Res. *66*:211–221.

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.

Palombi, P.S., and D.M. Caspary (1992) $GABA_A$ receptor antagonist biculline alters response properties of posteroventral cochlear nucleus neurons. J. Neurophysiol. *67*:738–746.

Park, T.J., D.T. Larue, J.A. Winer, and G.D. Pollak (1991) Glycine and GABA in the superior olivary complex of the mustache bat: Projections to the central nucleus of the inferior colliculus. Proc. Soc. Neurosci. *17*:300 (abstract).

Park, T.J., and G.D. Pollak (1993a) GABA shapes sensitivity to interaural intensity disparities in the inferior colliculus: Implications for encoding sound location. J. Neurosci. *13*:2050–2067.

Park, T.J., and G.D. Pollak (1993b) GABA shapes a topographic organization of response latency in the mustache bat's inferior colliculus. J. Neurosci. *13*:5172–5187.

Paydar, S., R.L. Saint Marie, D.L. Oliver, D.T. Larue, and J.A. Winer (1994) GABAergic projections from the inferior colliculus to the medial geniculate body in the cat. Proc. Soc. Neurosci. *20*: 976.

Pollak, G.D. (1988) Time is traded for intensity in the auditory system. Hearing Res. *36*:107–124.

Pollak, G.D., and J.H. Casseday (1989) The Neural Basis of Echolocation in Bats. Springer Series in Zoophysiology, vol. 25. Berlin: Springer-Verlag, pp. 1–143.

Pollak, G.D., and T.J. Park (1993) The effects of GABAergic inhibition on monaural response properties of neurons in the mustache bat's inferior colliculus. Hearing Res. *65*:99–117.

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).

Pollak, G.D., T.J. Park, D.T. Larue, and J.A. Winer (1992) The role inhibitory circuits play in shaping spatial receptive fields of neurons in the mustache bat's inferior colliculus. In R.N. Singh (ed): Nervous Systems: Principles of Design and Function. New Delhi: Wiley Eastern, Ltd., pp. 271–290.

Pollak, G.D., J.A. Winer, and W.E. O'Neill (1995) Perspectives on the functional organization of the mammalian auditory system: Why bats are good models. In A.N. Popper and R.R. Fay (eds): Springer Handbook of Auditory Research, vol. 4: Hearing by Bats. New York: Springer-Verlag (in press).

Pourcho, R.G., D.J. Goebel, L. Jojich, and J.C. Hazlett (1992) Immunocytochemical evidence for the involvement of glycine in sensory centers of the rat brain. Neuroscience *46*:643–656.

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

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

Probst, A., R. Cortés, and J.M. Palacios (1986) The distribution of glycine receptors in the human brain. A light microscopic autroradiographic study using [^{3}H]strychnine. Neuroscience *17:*11–35.

Rinvik, E., and O.P. Ottersen (1988) Demonstration of GABA and glutamate in the nucleus reticularis thalami: A postembedding immunogold labeling investigation in the cat and baboon. In M. Bentivoglio and R. Spreafico (eds): Cellular Thalamic Mechanisms. Amsterdam: Excerpta Medica, pp. 321–337.

Rinvik, E., O.P. Ottersen, and J. Storm-Mathisen (1987) Gamma-aminobutyrate-like immunoreactivity in the thalamus of the cat. Neuroscience *21:*787–805.

Roberts, R.C., and C.E. Ribak (1987) GABAergic neurons and axon terminals in the brainstem auditory nuclei of the gerbil. J. Comp. Neurol. *258:*267–280.

Ross, L.S., and G.D. Pollak (1989) Differential ascending projections to aural regions in the 60 kHz contour of the mustache bat's inferior colliculus. J. Neurosci. *9:*2819–2834.

Ross, L.S., G.D. Pollak, and J.M. Zook (1988) Origin of ascending projections to an isofrequency region of the mustache bat's inferior colliculus. J. Comp. Neurol. *270:*488–505.

Saint Marie, R.L., and R.A. Baker (1990) Neurotransmitter-specific uptake and retrograde transport of [^{3}H]glycine from the inferior colliculus by ipsilateral projections of the superior olivary complex and nuclei of the lateral lemniscus. Brain Res. *524:*244–253.

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

Saint Marie, R.L., C.G. Benson, E.-M. Ostapoff, and D.K. Morest (1991) Glycine immunoreactive projections from the dorsal to the anteroventral cochlear nucleus. Hearing Res. *51:*11–28.

Sanes, D.H. (1990) An in vitro analysis of sound localization mechanisms in the gerbil lateral superior olive. J. Neurosci. *10:*3494–3506.

Schneider, S.P., and R.E.W. Fyffe (1992) Involvement of GABA and glycine in recurrent inhibition of spinal motoneurons. J. Neurophysiol. *68:*397–406.

Schofield, B.R., and N.B. Cant (1991) Organization of the superior olivary complex in the guinea pig. I. Cytoarchitecture, cytochrome oxidase biochemistry, and dendritic morphology. J. Comp. Neurol. *314:*645–670.

Schuller, G., E. Covey, and J.H. Casseday (1991) Auditory pontine grey: Connections and response properties in the horseshoe bat. Eur. J. Neurosci. *3:*648–662.

Shneiderman, A., and D.L. Oliver (1989) EM autoradiographic study of the projections from the dorsal nucleus of the lateral lemniscus: A possible source of inhibitory inputs to the inferior colliculus. J. Comp. Neurol. *286:*28–47.

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.

Somogyi, P. (1977) A specific axo-axonal neuron in the visual cortex of the rat. Brain Res. *136:*345–350.

Somogyi, P., and I. Soltész (1986) Immunogold demonstration of GABA in synaptic terminals of intracellularly recorded, horseradish peroxidase–filled basket cells and clutch cells in the cat's visual cortex. Neuroscience *19:*1051–1065.

Somogyi, P., T.F. Freund, A.J. Hodgson, J. Somogyi, D. Beroukas, and I.W. Chubb (1985) Identified axo-axonic cells are immunoreactive for GABA in the hippocampus and visual cortex of the cat. Brain Res. *332:*143–149.

Spangler, K.M., W.B. Warr, and C.K. Henkel (1985) The projections of principal cells of the medial nucleus of the trapezoid body in the cat. J. Comp. Neurol. *238:*249–262.

Spangler, K.M., N.B. Cant, C.K. Henkel, G.R. Farley, and W.B. Warr (1987) Descending projections from the superior olivary complex to the cochlear nucleus of the cat. J. Comp. Neurol. *259:*452–465.

Suga, N. (1988) Auditory neuroethology and speech processing: Complex-sound processing by combination-sensitive neurons. In G.M. Edelman, W.E. Gall, and W.M. Cowan (eds): Auditory Function: Neurobiological Bases of Hearing. New York: John Wiley & Sons, pp. 679–720.

Suga, N., W.E. O'Neill, K. Kujirai, and T. Manabe (1983) Specificity of combination-sensitive neurons for processing of complex biosonar signals in auditory cortex of the mustached bat. J. Neurophysiol. *49:*1573–1626.

Sullivan, W.E. (1986) Processing of acoustic temporal patterns in barn owls and echolocating bats: Similar mechanisms for the generation of neural place representations of auditory space. Brain Behav. Evol. *28:*109–121.

Thompson, G.C., A.M. Cortez, and D.M.-K. Lam (1985) Localization of GABA immunoreactivity in the auditory brainstem of guinea pigs. Brain Res. *339:*119–122.

Todd, A.J., and A.C. Sullivan (1990) Light microscope study of the coexistence of GABA-like and glycine-like immunoreactivities in the spinal cord of the rat. J. Comp. Neurol. *296:*496–505.

Vater, M., M. Kössl, and A.K.E. Horn (1992a) GAD- and GABA-immunoreactivity in the ascending auditory pathway of horseshoe and mustached bats. J. Comp. Neurol. *325:*183–206.

Vater, M., H. Habbicht, M. Kössl, and B. Grothe (1992b) The functional role of GABA and glycine in monaural and binaural processing in the inferior colliculus of horseshoe bats. J. Comp. Physiol. A *171:*541–553.

Venter, J.C. (1984) Evolution and structure of neurotransmitter receptors. In J.C. Venter, C.M. Fraser, and J. Lindstrom (eds): Monoclonal and Anti-Idiotypic Antibodies: Probes for Receptor Structure and Function. New York: Alan R. Liss, Inc., pp. 117–139.

Volman, S.F., and M. Konishi (1990) Comparative physiology of sound localization in four species of owls. Brain Behav. Evol. *36:*196–215.

Walberg, F., O.P. Ottersen, and E. Rinvik (1990) GABA, glycine, aspartate, glutamate and taurine in the vestibular nuclei: An immunocytochemical investigation in the cat. Exp. Brain Res. *79:*547–563.

Wenstrup, J.J., D.T. Larue, and J.A. Winer (1994) Projections of physiologically defined subdivisions of the inferior colliculus in the mustached bat: Targets in the medial geniculate body and extrathalamic nuclei. J. Comp. Neurol. *346:*207–236.

Wenthold, R.J. (1987) Evidence for a glycinergic pathway connecting the two cochlear nuclei: An immunocytochemical and retrograde transport study. Brain Res. *415:*183–187.

Wenthold, R.J., and C. Hunter (1990) Immunocytochemistry of glycine and glycine receptors in the central nervous system. In O.P. Ottersen and J. Storm-Mathisen (eds): Glycine Neurotransmission. Chichester: John Wiley & Sons, pp. 391–416.

Wenthold, R.J., D. Huie, R.A. Altschuler, and K.A. Reeks (1987) Glycine immunoreactivity localized in the cochlear nucleus and superior olivary complex. Neuroscience *22:*897–912.

Wickesberg, R.E., and D. Oertel (1990) Delayed, frequency-specific inhibition in the cochlear nuclei of mice: A mechanism for monaural echo suppression. J. Neurosci. *10:*1762–1768.

Wickesberg, R.E., D. Whitlon, and D. Oertel (1991) Tuberculoventral neurons project to the multipolar cell area but not to the octopus cell area of the posteroventral cochlear nucleus. J. Comp. Neurol. *313:*457–468.

Winer, J.A. (1991) Anatomy of the medial geniculate body. 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. 293–333.

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

Winer, J.A., and D.T. Larue (1988) Anatomy of glutamic acid decarboxylase immunoreactive neurons and axons in the rat medial geniculate body. J. Comp. Neurol. *278:*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 D.K. Morest (1984) Axons of the dorsal division of the medial geniculate body of the cat: Study with the rapid Golgi method. J. Comp. Neurol. *224:*344–370.

Winer, J.A., J.J. Wenstrup, and D.T. Larue (1992) Patterns of GABAergic immunoreactivity define subdivisions of the mustached bat's medial geniculate body. J. Comp. Neurol. *319:*172–190.

Winer, J.A., S.K. Khurana, J.J. Prieto, and D.T. Larue (1993) GABAergic axosomatic endings preferentially target non-GABAergic neurons in the cat medial geniculate body. Proc. Soc. Neurosci. *19:*1422 (abstract).

Wu, S.H., and J.B. Kelly (1992a) Binaural interaction in the lateral superior olive: Time difference sensitivity studied in mouse brain slice. J. Neurophysiol. *68:*1151–1159.

Wu, S.H., and J.B. Kelly (1992b) Synaptic pharmacology of the superior olivary complex studied in mouse brain slice. J. Neurosci. *12:*3084–3097.

Yang, L., G.D. Pollak, and C. Resler (1992) GABAergic circuits sharpen tuning curves and modify response properties in the mustache bat's inferior colliculus. J. Neurophysiol. *68:*1760–1774.

Yingcharoen, K., E. Rinvik, J. Storm-Mathisen, and O.P. Ottersen (1989) GABA, glycine, glutamate, aspartate and taurine in the perihypoglossal nuclei: An immunocytochemical investigation in the cat with particular reference to amino acid colocalization. Exp. Brain Res. *78:*345–357.

Zook, J.M., and J.H. Casseday (1982a) Cytoarchitecture of auditory system in lower brainstem of the mustache bat, *Pteronotus parnellii.* J. Comp. Neurol. *207:*1–13.

Zook, J.M., and J.H. Casseday (1982b) Origin of ascending projections to the inferior colliculus in the mustache bat, *Pteronotus parnellii.* J. Comp. Neurol. *207:*14–28.

Zook, J.M., and J.H. Casseday (1987) Convergence of ascending pathways at the inferior colliculus of the mustache bat, *Pteronotus parnellii.* J. Comp. Neurol. *261:*347–361.

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.

Zucker, C.L., and B. Ehinger (1992) Heterogeneity of receptor immunoreactivity at synapses of glycine-utilizing neurons. Proc. R. Soc. Lond. B *149:*89–94.