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Patterns of γ-Aminobutyric Acid and Glycine Immunoreactivities Reflect Structural and Functional Differences of the Cat Lateral Lemniscal Nuclei

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ABSTRACT

The three nuclei of the cat lateral lemniscus (dorsal, intermediate, and ventral) were distinguished by their immunoreactivities for the putative inhibitory transmitters, γ-aminobutyric acid (GABA) and glycine. Each nucleus had a distinct pattern of somatic and perisomatic labeling. The dorsal nucleus contained mostly GABA-immunoreactive neurons (85%), with moderate numbers of GABA- and glycine-immunoreactive puncta along their somata. The remaining neurons were nonimmunoreactive (15%). The intermediate nucleus contained mostly nonimmunoreactive neurons (82%), and these had numerous glycine-immunoreactive and few GABA-immunoreactive perisomatic puncta. The remaining neurons were immunoreactive for GABA only (10%), glycine only (2%), or both (6%). The ventral nucleus contained mostly glycine-immunoreactive neurons (81%), and about half of these were also GABA-immunoreactive. The remaining neurons were either nonimmunoreactive (8%) or GABA-immunoreactive only (11%). Neurons in the ventral nucleus had fewer immunoreactive perisomatic puncta than neurons in either the dorsal or the intermediate nuclei. These differences in neuronal immunoreactivity and in the relative abundance of GABA-and glycine-immunoreactive perisomatic puncta among the three nuclei of the lateral lemniscus support connectional and electrophysiological evidence that each nucleus has a different functional role in auditory processing. In particular, this study demonstrates that the intermediate nucleus of the cat is cytochemically distinct from the dorsal and ventral nuclei in terms of the somatic and perisomatic immunoreactivity of its neurons for these two important inhibitory transmitters and may provide novel inputs to the inferior colliculus. J. Comp. Neurol. 389:264–276, 1997.

Indexing terms: auditory pathway; inhibitory neurotransmitter; inferior colliculus; lateral lemniscus

The nuclei of the lateral lemniscus consist of three large groups of neurons that are interposed in the ascending pathway to the auditory midbrain. They are designated as the ventral (VNLL), intermediate (INLL), and dorsal (DNLL) nuclei of the lateral lemniscus. Together, these nuclei provide one of the largest inputs to the cat inferior colliculus (IC; see, e.g., Elverland, 1978; Adams, 1979; Brunso-Bechtold et al., 1981; Kudo, 1981; Whitley and Henkel, 1984; Shneiderman et al., 1988). Each nucleus is distinct in its cytoarchitecture, response type, and afferent and efferent connections, and each is expected to make unique contributions to the processing of auditory information in the IC.

Connectional studies indicate that each nucleus has a distinct set of inputs, and their output targets may also

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vary (for reviews, see Irvine, 1986; Oliver and Shneiderman, 1991; Schwartz, 1992; Helfert and Aschoff, 1997). The VNLL and INLL are innervated primarily by contralaterally driven monaural structures. They differ in that the VNLL receives an almost exclusive input from the contralateral ventral cochlear nucleus (VCN), whereas the INLL, in addition to its inputs from the contralateral VCN, also receives considerable inputs from the ipsilateral medial nucleus of the trapezoid body and the VNLL. The principal output target of the VNLL and INLL is the IC on the same side. The DNLL is unique among the three nuclei in receiving inputs primarily from binaural structures, including the superior olivary complex (SOC) and the DNLL of the opposite side. It is also unique, in that it has bilateral projections to the IC as well as reciprocal projections with the opposite DNLL.

Electrophysiological studies indicate that each nucleus responds uniquely to acoustic stimulation. Contralaterally driven monaural response types predominate in the VNLL and INLL, and both nuclei are thought to have an important role in acoustic temporal discrimination (see, e.g., Aitkin et al., 1970; Guinan et al., 1972a,b; Metzner and Radtke-Schuller, 1987; Covey and Casseday, 1991). They are especially prominent in species that rely heavily on echolocation for orientation and object recognition, e.g., bats and dolphins (for reviews, see Covey, 1993a; Casseday and Covey, 1995). Typically, their neurons are broadly tuned with low spontaneous activity and respond robustly to transient stimuli of short duration. In contrast, the DNLL is primarily a binaural nucleus (Aitkin et al., 1970; Brugge et al., 1970; Buckthought et al., 1993; Covey, 1993b; Markovitz and Pollak, 1993, 1994). The preponderance of its neurons respond to interaural time and level differences, which are important for sound localization. Disrupting the crossed projections of the DNLL more than doubles the size of the minimum audible angle, a measure of acoustic spatial discrimination, in trained rats (Ito et al., 1996; Kelly et al., 1996). The effect of the DNLL on spatial acuity is presumably by way of its crossed inhibitory projections to the opposite IC (Li and Kelly, 1992; Faingold et al., 1993; Kidd and Kelly, 1996).

Emerging evidence suggests that the three nuclei of the lateral lemniscus may be the largest source of inhibitory inputs to the IC. Lateral lemniscal neurons contain high levels of two inhibitory transmitters, γ-aminobutyric acid (GABA) and glycine, as determined immunocytochemically (see, e.g., Adams and Mugnaini, 1984; Mugnaini and Oertel, 1985; Thompson et al., 1985; Peyret et al., 1986; Moore and Moore, 1987; Roberts and Ribak, 1987; Aoki et al., 1988; Vater et al., 1992; Winer et al., 1995), and many of these immunoreactive neurons have direct projections to the IC in the rat (González-Hernández et al., 1996) and cat (Shneiderman et al., 1996; unpublished results). Lateral lemniscal neurons are also capable of selectively accumulating exogenous GABA or glycine by way of synaptic uptake mechanisms in their terminations in the IC (Hutson, 1988; Saint Marie and Baker, 1990; Glendenning et al., 1992). Neurochemical studies indicate that the DNLL contributes significantly to the synaptic uptake and release of GABA in the IC (Shneiderman et al., 1993). The purpose of the present study was to differentiate the three subdivisions of the lateral lemniscus by the GABA and glycine immunoreactivities of their constituent neurons and synaptic inputs and to relate these findings to what is known of the connectivities and functions of the three lateral lemniscal nuclei and their principal postsynaptic target, the IC.

MATERIALS AND METHODS

All of the brain tissue used in this study was archival and was from cases that have been reported previously (Saint Marie et al., 1989). Tissue from three cats was used in this study. Briefly, animals were perfused with a combination of 2% paraformaldehyde and 3% glutaraldehyde in 0.12 M phosphate buffer, pH 7.2. Tissue was cut with a Vibratome at 60–80 μm, and every fifth section was processed for plastic embedding. Sections were postfixed in osmium tetroxide (0.5%) and flat embedded in Epon (Polybed 812; Polysciences, Warrington, PA). Semithin (1.5 μm) plastic sections were cut, mounted on glass slides, then deplasticized and deosmicated (after Saint Marie et al., 1989). Adjacent semithin sections were incubated with antibodies to glycine or GABA. Antibodies were double affinity purified from rabbit polyclonal antisera raised against bovine serum albumin (BSA)-glutaraldehyde conjugates of GABA or glycine (Wenthold et al., 1987). Binding was detected by using sheep anti-rabbit as a linker and rabbit peroxidase-antiperoxidase histochemistry. Diaminobenzidine was used as the chromogen for the peroxidase reaction. Immunohistochemical controls in which the antisera were preadsorbed with antigen or in which preimmune or normal rabbit sera were used in place of the GABA or glycine antisera produced no staining and have been described previously (Saint Marie et al., 1989).

Plots were made at ×350 from toluidine blue-stained plastic sections of the lateral lemniscus, and all lemniscal neurons with a nucleus present were noted in camera lucida drawings. Drawings were then compared with the adjacent plastic sections, which had been treated with anti-GABA or antiglycine, to determine the immunoreactivity of the nucleated neurons. This was done either directly by aligning the drawings with the microscope image of the treated sections through the drawing tube (after Saint Marie et al., 1989) or by using a video-imaging program (ImagePro Plus 1.3; Media Cybernetics, Silver Spring, MD). Neurons were classified with each antibody as either negatively labeled, lightly labeled, or medium-to-darkly labeled.

Cell size was determined from video images of cells with a nucleolus in the toluidine blue-stained sections by using the ImagePro Plus 1.3 imaging software. Images were acquired with a Nikon Optiphot II microscope equipped with a Sony 3 CCD three-chip color video camera. To produce the photomicrographs for this report, images were further processed by using Adobe Photoshop (version 3.5; Adobe Systems, Inc., Mountain View, CA) and were printed with a Kodak XLS 8600 dye-sublimation printer. Only sizing and adjustments to image brightness and contrast were used in the preparation of the figures.

RESULTS

GABA and glycine immunoreactivity of the DNLL

One of the most notable features of the DNLL was the prevalence of GABA-immunoreactive (GABA-IR) somata (Fig. 1A). Nearly 85% of the neurons were determined to be GABA-IR, and these ranged from very darkly stained (Fig. 1B) to lightly stained (Fig. 1C). No DNLL neurons were

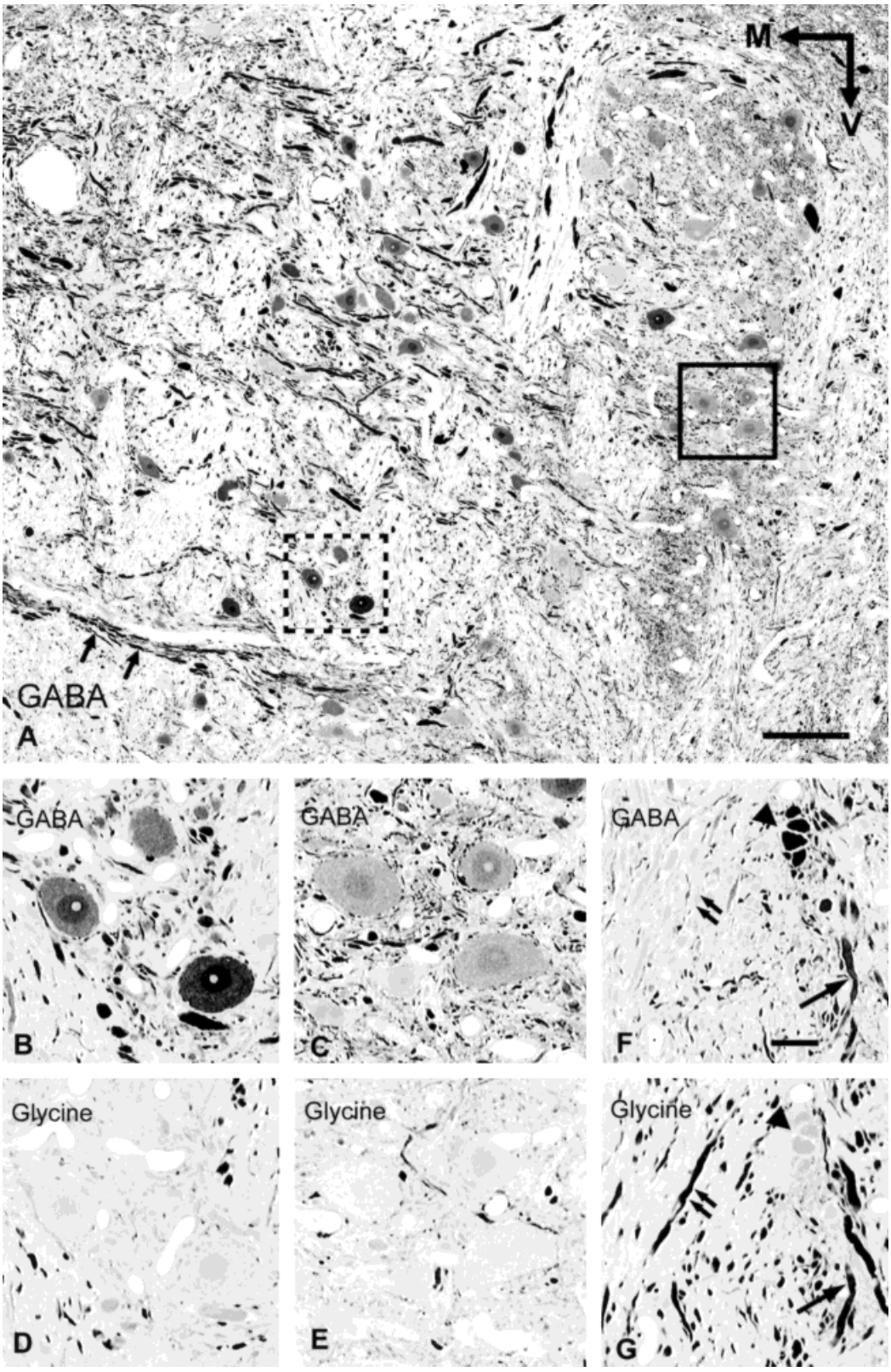

Fig. 1. Immunoreactivity of the dorsal nuclei of the lateral lemniscus (DNLL). **A:** Low magnification of a transverse section shows that nearly all cells are γ-aminobutyric acid (GABA)-immunoreactive (IR) and range in intensity from lightly to darkly stained. Fibers projecting toward the commissure of Probst are darkly stained (arrows). **B:** Enlargement from the dashed outline in the ventromedial part of A. Darkly GABA-IR neurons are found among fascicles of lemniscal fibers in areas with little neuropil and low density of GABA-IR puncta. **C:** Enlargement from the solid outline in the lateral part of A. Lightly GABA-IR neurons are found in the area with few lemniscal fibers, much GABA-IR neuropil, and many perisomatic puncta. **D,E**: Same cells as in B and C show no glycine-IR and few glycine-IR puncta or fibers. **F,G:** GABA-IR and glycine-IR of fibers in the lateral limb of the lateral lemniscus near the boundary between the DNLL and the intermediate NLL (INLL). Fibers can be either GABA-IR only (arrowheads), glycine-IR only (short double arrows), or doubly immunoreactive (long single arrows). V, ventral; M, medial. Scale bars = 100 μm in A, 20 μm in F (also applies to B–E, G).

found to be glycine-immunoreactive (glycine-IR). Nonimmunoreactive neurons accounted for about 15% of all of the neurons in the DNLL. Many were small-to-medium sized (<600 μm^2) and were located in the ventral half of the DNLL. The findings are summarized in Figure 4.

Distinct islands of neuropil were found in the DNLL, and these were most commonly encountered dorsally and laterally in the nucleus. The neuropil consisted of varied components (Fig. 1A,C), including 1) distinct, darkly GABA-IR axons, which were the darkest structures encountered and were present throughout the cellular and noncellular parts of the lemniscus; 2) darkly GABA-IR puncta (usually 1–3 μm in diameter), which were prevalent around neuronal somata and were presumed to represent labeled presynaptic terminals; 3) less darkly GABA-IR profiles, which matched the staining intensity of the GABA-IR neurons, were often found in continuity with these neurons, and were presumed to represent labeled dendrites; and 4) an amorphous background matrix, which presumably represented the profiles of nonimmunoreactive dendrites, lemniscal fibers, and gila.

The fiber population of the DNLL was heterogeneous. Thick, GABA-IR fibers of the commissure of Probst were found medially. These presumably included fibers exiting the nucleus and other fibers entering from the opposite DNLL. The latter fibers appeared to fragment and became thinner as they coursed laterally in the nucleus (Fig. 1A), and they may have contributed to the high density of GABA-IR puncta observed laterally in the DNLL (Fig. 1A,C). Other thick GABA-IR axons appeared to join the fascicles of the lateral lemniscus and to project dorsally toward the IC. The vast majority of lemniscal fibers that penetrated or surrounded the DNLL were nonimmunoreactive, but there were also many that were immunoreactive for GABA, glycine, or both (see, e.g., Fig. 1F,G). In general, there were many more glycine-IR than GABA-IR fibers in the lateral lemniscus. Some of these latter fibers presumably gave rise to the glycine-IR puncta found in the DNLL. Although they were fewer in number, glycine-IR puncta mostly paralleled the distribution of GABA-IR puncta, with the highest density laterally and the lowest density medially.

Shneiderman et al. (1988) reported that there was a dorsoventral organization to the cat DNLL, with larger and more spherical dorsal neurons and smaller and more horizontally elongated ventral neurons. We found similar tendencies in the present material, but we also noted additional mediolateral differences. Neurons located medially in the DNLL tended to exist in isolation or in small clusters embedded among the penetrating fiber fascicles of the lateral lemniscus. They tended to be smaller, to be more darkly GABA-IR, and to be contacted by few GABA-IR or glycine-IR perisomatic puncta. Neurons located laterally in the DNLL, on the other hand, tended to be larger, to be found in highly vascular regions dense with neuropil, and to be contacted by many GABA-IR and some glycine-IR perisomatic puncta.

GABA and glycine immunoreactivity of the VNLL

The VNLL is a comma-shaped nucleus with a bulbous part located ventromedially and caudally and a narrowing tail extending dorsolaterally and rostrally. The most distinctive characteristic of the VNLL was its large population of glycine-IR neurons (Fig. 2A). Approximately 81% of VNLL neurons were glycine-IR; 42% of VNLL neurons were only glycine-IR (Fig. 2C,E), and 39% were immunoreactive for both glycine and GABA (Fig. 2B,D). About 11% of VNLL neurons were only GABA-IR, and an even smaller number (8%) was not immunoreactive for either GABA or glycine. Neurons that were nonimmunoreactive tended to be small and to be located along the medial margin of the VNLL or embedded within the fibers of the medial limb of the lateral lemniscus, especially rostrally. GABA-IR neurons (including the doubly immunoreactive neurons) also tended to be small. Neurons that were only GABA-IR were concentrated rostrally in the VNLL. Those that were doubly immunoreactive were scattered throughout the nucleus among the neurons that were only glycine-IR, but with a concentration laterally and rostrally. Neurons that were only glycine-IR, on average, were slightly larger than the other types and were found throughout the nucleus. They were the most prevalent type caudally in the nucleus. Ventrally in the VNLL, neurons were mostly round or globular in shape, but, proceeding rostrally, neurons in the dorsal parts of the VNLL were more elongated and were horizontally oriented. It was at about this latter location that there was a dramatic transition in somatic and perisomatic immunoreactivity that marked the boundary with the INLL. All of these findings are summarized in Figure 4.

The VNLL was encapsulated by the fibers of the lateral lemniscus, but many more fibers passed through the nucleus as they swept dorsolaterally and rostrally in their ascent to the inferior colliculus. Glycine-IR fibers outnumbered GABA-IR fibers, but both types were found throughout the nucleus and in the enveloping lemniscal fiber tract. A subset of the GABA-IR axons was also glycine-IR. Although the neuropil of the VNLL contained many small immunoreactive profiles, including darkly stained axonal profiles and more lightly stained dendritic profiles, the neurons had fewer labeled perisomatic puncta compared with the other subdivisions of the lateral lemniscus. This difference in perisomatic labeling was particularly striking at the boundary between the VNLL and the INLL.

GABA and glycine immunoreactivity of the INLL

In transverse sections, the INLL consisted of a vertical band of cells that was narrower in the mediolateral dimension than either the DNLL or the VNLL. Similar to the DNLL, clusters of neurons alternated at regular intervals with fascicles of horizontally penetrating fibers of the lateral lemniscus (Fig. 3A). The neurons tended to be smaller and much more horizontally elongated than those in the DNLL. Few were immunoreactive for GABA only (10%), glycine only (2%), or both (6%). Overall, 82% of the neurons in the INLL were not immunoreactive for either GABA or glycine (Fig. 3A–C). Immunoreactive neurons of all kinds were found most frequently along the margins of the nucleus and sometimes beyond, among the lemniscal fibers. Immunoreactive neurons were mostly small, but some large GABA-IR and doubly immunoreactive neurons were found. Nonimmunoreactive neurons, on average, were slightly larger than the other types and were found throughout the nucleus. The findings are summarized in Figure 4.

The organization of the lemniscal fibers changed in the transition from the VNLL to the INLL. Fibers, which were distributed uniformly as they passed through the VNLL,

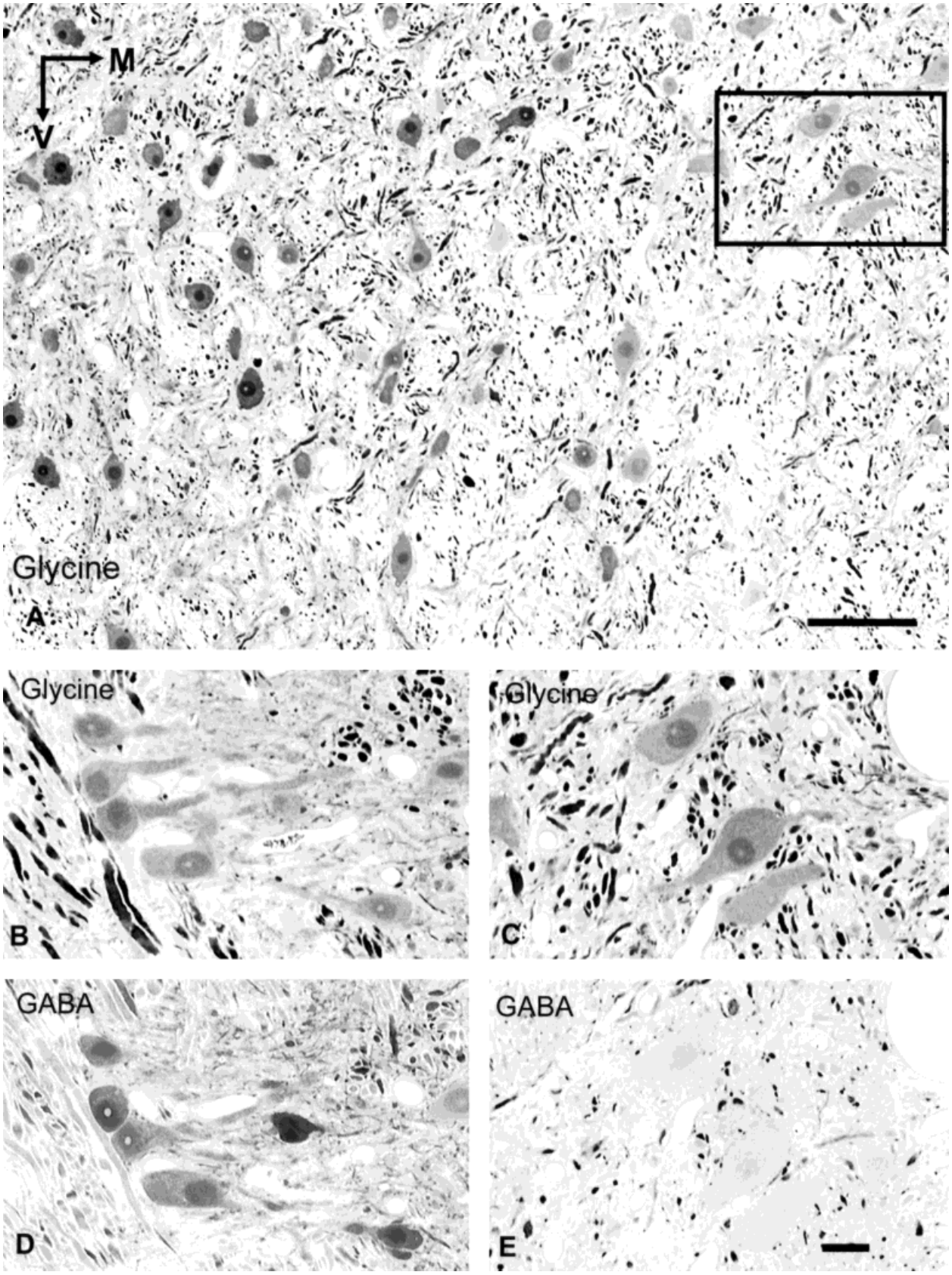

Fig. 2. **A–E:** Immunoreactivity of the ventral NLL (VNLL). Low magnification of a transverse section (A) shows that nearly all cells are glycine-IR and range in intensity from lightly to moderately stained. In addition, many VNLL neurons colocalize both glycine and GABA (compare the same cells in B and D), whereas others are glycine-IR but not GABA-IR (compare the same cells in C and E). Many glycine-IR fibers and puncta are scattered throughout the VNLL (A–C), and some GABA-IR fibers and puncta are also found there (D,E). Scale bars = 100 μm in A, 20 μm in E (also applies to B–D).

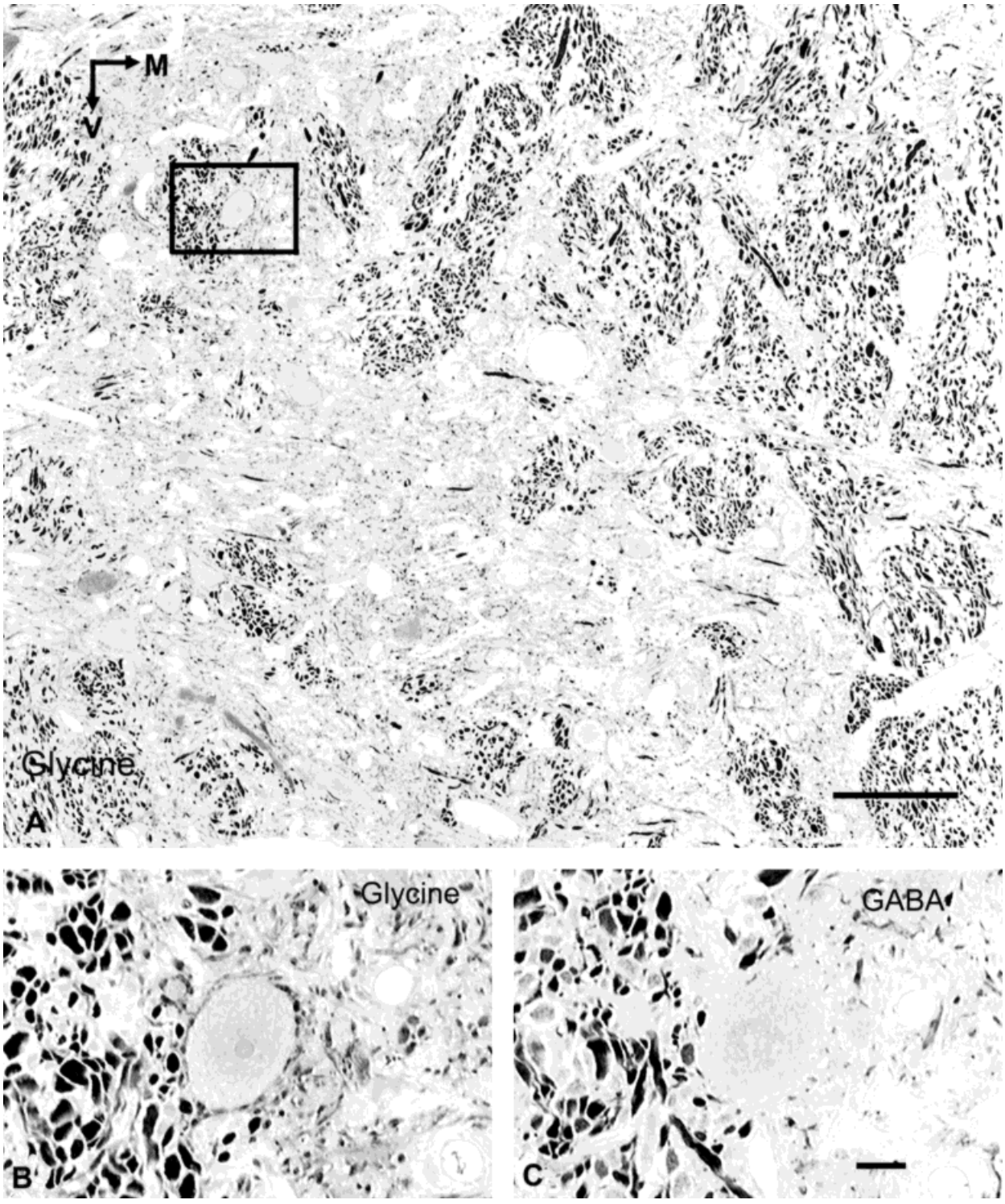

Fig. 3. Immunoreactivity of the INLL. **A:** Low magnification of a transverse section shows that many lemniscal fibers are darkly glycine-IR, but few neurons are glycine-IR. **B:** Enlargement from the box outlined in A. Although most INLL neurons are not glycine-IR, they are densely encrusted with glycine-IR puncta. **C:** The same cells shown B are not GABA-IR and have few GABA-IR perisomatic puncta. Scale bars = 100 μm in A, 10 μm in C (also applies to B).

became decidedly fasciculated as they wove their way through the INLL. Both GABA- and glycine-IR fibers were common among the encapsulating and interweaving fibers of the lemniscus. Glycine-IR fibers, as elsewhere, outnumbered GABA-IR fibers. Also, there was a decided horizontal organization in the INLL that was not present in the

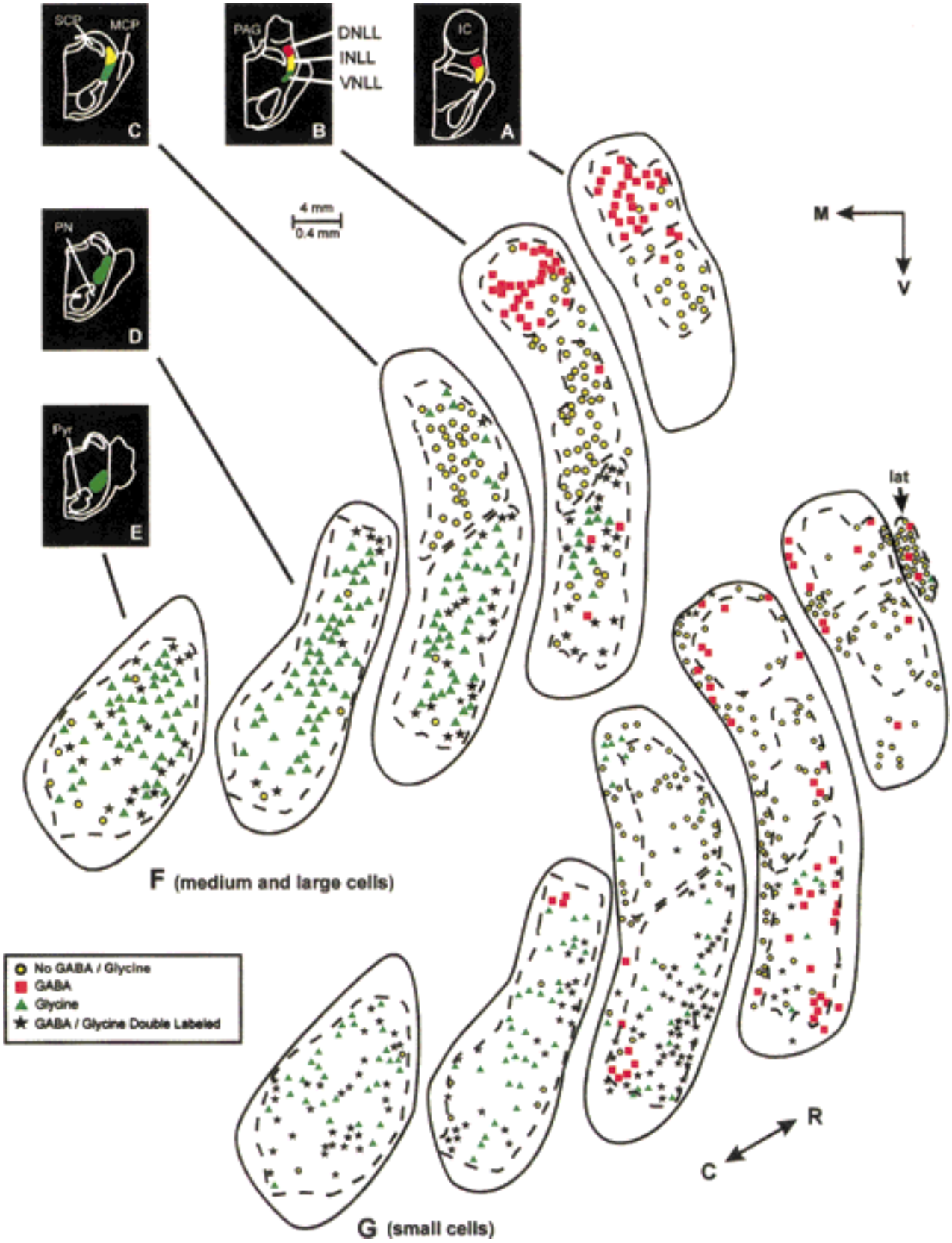

Fig. 4. **A–G:** Distribution of immunoreactive and nonimmunoreactive neurons in the lateral lemniscal nuclei for one transversely sectioned case. Five rostral (A) to caudal (E) levels are represented. F: Distribution of medium-to-large neurons (>300 μm^2) at the designated levels: GABA-IR (red squares), glycine-IR (green triangles), doubly immunoreactive (black stars), and nonimmunoreactive (yellow circles). G: Distribution of small (<300 μm^2) immunoreactive and nonimmunoreactive neurons at the same levels. Solid lines in F and G represent the approximate boundaries of the lateral lemniscus. Dashed lines represent the approximate boundaries of the individual lemniscal nuclei. Most medium and large neurons are confined to the individual nuclei, whereas small neurons are found both within and beyond the nuclear boundaries. lat, Lateral transition area of Shneiderman et al. (1988); IC, inferior colliculus; MCP, middle cerebellar peduncle; PAG, periaqueductal gray; PN, pontine nuclei; Pyr, pyramidal tract; SCP, superior cerebellar peduncle.

VNLL. This included many fine immunoreactive fibers, especially glycine-IR fibers, traversing mediolaterally in the INLL in concert with horizontally oriented cellular and neuropilar areas. The fine fibers undoubtedly gave rise to the most striking feature of the INLL, its abundance of glycine-IR puncta. The nonimmunoreactive INLL neurons were nearly completely enveloped by these glycine-IR puncta (Fig. 3B).

Defining the lateral lemniscal nuclei immunocytochemically

The results indicate that each of the principal subdivisions of the lateral lemniscus has a characteristic pattern of immunoreactivity for GABA and glycine, and these are summarized in Figure 4. Immunoreactive and nonimmunoreactive neurons were plotted from transverse sections at five rostral-to-caudal levels for one case (Fig. 4A–E, respectively). In some of the lemniscal nuclei, a difference in the distribution of small neurons (<300 μm^2) and larger neurons was noted, so the distribution of medium-to-large neurons (Fig. 4F) was plotted separately from that of the small neurons (Fig. 4G). The differences in somatic immunoreactivity revealed clear differences between lemniscal subdivisions. For example, in the DNLL, nearly all of the medium-to-large neurons were GABA-IR (Fig. 4F). Glycine-IR DNLL neurons were nonexistent. Conversely, in the VNLL, the vast majority of neurons, both small and larger, were glycine-IR, and about half of these were also GABA-IR (Fig. 4F,G). The INLL, on the other hand, contained very few neurons, small or larger, that stained for either GABA or glycine (Fig. 4F,G). The boundary between the INLL and the DNLL, which was obvious even in conventionally stained sections, was also obvious in this material, because the INLL contained almost no GABA-IR neurons, and DNLL neurons were almost universally GABA-IR (Fig. 4F). The few nonimmunoreactive neurons along the ventral border of the DNLL (discussed above) were extremely elongated and extended much more medially. They were easily distinguished from the nearby INLL neurons. We also encountered a few nonimmunoreactive neurons in an interstitial area between the DNLL and the INLL that did not appear to be a part of either nucleus. In conventionally stained sections, the boundary between the INLL and the VNLL was not distinct, because neurons in both subdivisions were primarily horizontally elongated at this location. In immunoreacted sections, however, there was a clear boundary between a ventral region (VNLL), where neurons were almost exclusively glycine-IR (many were also GABA-IR), and a more dorsal region (INLL), where most neurons were neither GABA-IR nor glycine-IR (Fig. 4F,G). The boundary between the VNLL and the INLL, as so defined, was not horizontal but, rather, had a strong ventromedial-to-dorsolateral orientation.

Many small neurons were found within the nuclear boundaries and among the enveloping fibers of the lateral lemniscus (Fig. 4G). One such population in an area described as a lateral transitional area between the rostralmost DNLL and the nucleus sagulum (Shneiderman et al., 1988) contained only small, mostly nonimmunoreactive neurons (Fig. 4G, lat). These neurons were distinct from those in the DNLL in morphology and GABA-IR and will be considered in more detail in a separate, forthcoming report on the GABA and glycine immunoreactivity of the nucleus sagulum. Small neurons within the boundaries of the lemniscal nuclei tended to be similar in their range and proportions of immunoreactivities to the medium-to-large neurons of the same nuclear subdivision (compare Fig. 4F with Fig. 4G). Beyond the nuclear boundaries, there was an additional large population of small neurons found among the fibers of the lateral lemniscus. These were especially prevalent in rostral sections and in the medial limb of the lemniscal capsule (see the rostralmost three sections in Fig. 4G). All immunoreactive types were found, but the majority (62%) were nonimmunoreactive. The remainder were immunoreactive for either glycine only (15%), GABA only (10%), or both (13%). There is evidence that some of these extranuclear neurons may not only differ in their morphology and location but also may have a different physiology from those in the main cellular areas of the VNLL and INLL (Batra and Fitzpatrick, 1997).

DISCUSSION

Subdividing the lateral lemniscal nuclei

Classically, dorsal and ventral subdivisions of the mammalian lateral lemniscal nuclei were recognized (Held, 1893; Ramón y Cajal, 1909). Most modern studies recognize the classical dorsal nucleus but subdivide the classical ventral nucleus into intermediate and ventral nuclei, based on differences in cell morphology, response type, and afferent and efferent connections (Aitkin et al., 1970; Brugge et al., 1970; Adams, 1979; Kane and Barone, 1980; Brunso-Bechtold et al., 1981; Glendenning et al., 1981; Irvine, 1986; Aitkin, 1989). The boundary between the INLL and the VNLL is not distinct in Nissl-stained sections, and its positioning has varied from one investigator to another and from species to species. In the cat, as summarized by Irvine (1986), most investigators designate the INLL as a small region of multipolar neurons immediately ventral to the DNLL. This region is synonymous with the dorsal zone of the classical VNLL described by Adams (1979). The present study, based on immunocytochemical evidence, suggests that the cat INLL may be more extensive than just the dorsal zone of the classical VNLL and may include both the dorsal zone and much of Adams' middle zone of the classical VNLL. The latter region contains both multipolar and many horizontally elongated neurons. The cat INLL, as we now define it, includes both the dorsal pole of multipolar cells just ventral to the DNLL and a good part of the narrow, vertical streak of the lateral lemniscal nuclei. The relative size, position, and cellular composition of the cat INLL, as so defined, more closely resemble their counterparts as described in rodents (Willard and Ryugo, 1983) and in bats (Schweizer, 1981; Zook and Casseday, 1982a,b; Covey, 1993a; Winer et al., 1995).

DNLL: Source of binaural inhibition

The GABAergic nature of the mammalian DNLL has been known for over a decade, and this is true for a wide variety of species, including terrestrial mammals (Adams and Mugnaini, 1984; Mugnaini and Oertel, 1985; Thompson et al., 1985; Moore and Moore, 1987; Roberts and Ribak, 1987; Hutson, 1988; Shneiderman et al., 1993; González-Hernández et al., 1996) and bats (Vater et al., 1992; Winer et al., 1995). Most earlier reports recognized that the proportion of GABAergic neurons in the DNLL was considerable, but, until the present report, there has been no direct quantification of this finding. Hutson (1988) approximated the proportion of GABA-immunoreactive

neurons in the cat DNLL by comparing the number of labeled neurons in immunoreactive sections with the number of neurons stained in closely matched, Nissl-stained sections. He reported a range between 61% and 83%. With postembedding immunocytochemistry, we have been able to clearly identify and characterize both immunoreactive and nonimmunoreactive DNLL neurons in the same sections, and we estimate that 85% of DNLL neurons are GABA immunoreactive. Based solely on this immunocytochemical evidence, the DNLL appears to contain at least two major neuronal types, one inhibitory and the other not.

The DNLL is an important site for binaural processing in the central auditory system. In vivo electrophysiological studies have shown that a preponderance of units in the DNLL are binaural and are sensitive to interaural time differences (ITDs) or interaural level differences (ILDs; Aitkin et al., 1970; Brugge et al., 1970; Buckthought et al., 1993; Covey, 1993b; Markovitz and Pollak, 1993, 1994). The importance of the DNLL in sound localization was recently demonstrated by experiments in which the projections of the DNLL were lesioned (Ito et al., 1996; Kelly et al., 1996). This degraded the ability of trained rats to localize sounds in the horizontal plane and more than doubled their minimum audible angle, a measure of spatial discrimination. Presumably, this effect was by way of crossed inhibitory projections of the DNLL to the opposite IC and DNLL. Chemical or electrical stimulation of the DNLL inhibits most neurons in the opposite IC (Faingold et al., 1993), and this inhibition is thought to be mediated by GABAergic inputs to the IC. Best estimates are that the two DNLLs contribute as many as 40% of the inhibitory synapses in the central nucleus of the IC (ICc) (Shneiderman and Oliver, 1989) and 35–50% of the GABA found there (Shneiderman et al., 1993). Pharmacological blockade of the DNLL has been shown to increase spontaneous activity in the opposite IC and to block binaural inhibition in most ILD-sensitive IC neurons (Li and Kelly, 1992; Faingold et al., 1993). Lesions or pharmacological blockade of the DNLL have also shown that the DNLL has an important role in shaping the inhibitory responses of ITD-sensitive neurons in the IC (Kidd and Kelly, 1996) and the auditory cortex (Glenn and Kelly, 1992) on the opposite side.

We also found that the DNLL contained a sizeable population of non-GABAergic (and nonglycinergic) neurons, which make up about 15% of its neurons. These neurons were encountered most frequently in the ventral part of the DNLL (see also Adams and Mugnainin, 1984; Hutson, 1988) and had many more GABAergic perisomatic puncta than the GABA-IR DNLL neurons. They clearly represented a separate neuronal type. The contralateral projections of these neurons could account for some of the excitatory postsynaptic potentials recorded in the DNLL after electrical stimulation of the commissure of Probst in in vitro brain slices (Wu and Kelly, 1995a,b, 1996). Also, it is believed that these neurons give rise to the synaptic endings with excitatory morphologies bilaterally in the ICc and in the contralateral DNLL that were described by Shneiderman and Oliver (1989) and Oliver and Shneiderman (1989).

Regarding the inhibitory puncta that we found in the DNLL, Wu and Kelly (1996) have shown in electrically stimulated rat in vitro slice preparations that projections from the opposite DNLL probably account for most of the extrinsic GABAergic inputs to the DNLL, and lemniscal projections from lower auditory structures probably account for most of the glycinergic inputs to the DNLL. The most likely origins of the glycinergic inputs to the DNLL are the ipsilateral VNLL and LSO, which, together, contribute about 25% of the projections to the DNLL in the cat (Glendenning et al., 1981; Whitley and Henkel, 1984). Both projections have been shown to be largely glycinergic (see, e.g., Hutson, 1988; Saint Marie et al., 1989; Saint Marie and Baker, 1990; Glendenning et al., 1992).

The functional implications of these findings are that DNLL neurons are expected to be inhibited by GABAergic inputs from the opposite DNLL and by glycinergic inputs from the ipsilateral VNLL or LSO and are expected to provide primarily, but not exclusively, GABAergic feedforward inhibition to its targets. The principal targets of the DNLL are the opposite DNLL and the two ICcs (for reviews, see Irvine, 1986; Oliver and Shneiderman, 1991; Schwartz, 1992; Helfert and Aschoff, 1997).

VNLL: Source of monaural inhibition

Based on the absolute numbers of neurons in its projection, the VNLL is probably the single largest source of inhibition in the IC. The VNLL is by far the largest of the lateral lemniscal nuclei, and we have found that 92% of its neurons were immunoreactive for glycine and/or GABA. The large size of the glycine-IR component that we find in cats (81%) was predicted by our earlier studies in chinchillas (Saint Marie and Baker, 1990). Other, brief descriptions of glycine-IR neurons in the VNLL of cats (Adams and Wenthold, 1987), rats (Hunter et al., 1987; Aoki et al., 1988), and bats (Winer et al., 1995), however, did not indicate that the glycinergic population was so extensive. Other sources of glycinergic projections to the IC include those from the ipsilateral lateral superior olive and the periolivary nuclei (Hutson, 1988; Saint Marie et al., 1989; Saint Marie and Baker, 1990; Glendenning et al., 1992), both of which are much smaller in comparison.

In addition to being the largest single source of glycine in the IC, the VNLL could also represent a large extrinsic source of GABAergic inhibition in the IC. The large number of GABA-IR neurons (50%) that we found in the VNLL was consistent with some immunocytochemical studies that localized GABA or glutamate decarboxylase (GAD), the enzyme that synthesizes GABA (Mugnaini and Oertel, 1985; Roberts and Ribak, 1987; González-Hernández et al., 1996), but it was not consistent with other similar studies (see, e.g., Thompson et al., 1985; Moore and Moore, 1987; Vater et al., 1992; Winer et al., 1995). Other sources of GABAergic inhibition in the IC include the DNLL on both sides (see above) and intrinsic GABAergic neurons, which make up 20% of the neurons in the IC (Oliver et al., 1994).

Many VNLL neurons were immunoreactive for both GABA and glycine (39%) in the present study, and we expect that these neurons gave rise to many of the doubly immunoreactive axons that we found in the lateral lemniscus. Such doubly immunoreactive neurons have been described elsewhere in the auditory system (Wenthold et al., 1987; Helfert et al., 1989; Saint Marie et al., 1989, 1991; Osen et al., 1990; Kolston et al., 1992; Winer et al., 1995; Moore et al., 1996; Ostapoff et al., 1997), but the significance of having both transmitter substances in the same neuron is not completely understood. Colocalization is apparently not a problem of cross-reactivity between the two antisera, because many darkly staining GABA-IR or

glycine-IR neurons and axons in the present study did not label with the other antiserum. Rather, it seems that some neurons contain elevated levels of both transmitter substances, and both may be available for release from the presynaptic terminals of these neurons. How the two transmitters might interact is a matter of conjecture. Both may act postsynaptically, one as the primary transmitter and the other as a modulator. For example, it has been shown that GABA can modulate the postsynaptic effect of glycine at some synapses (see, e.g., Werman, 1980), and glycine can modulate the excitatory effects of N-methyl-D-aspartate receptor activation (see, e.g., Fletcher et al., 1990). Alternatively, one or both may act presynaptically to modulate the uptake and/or release of the other (see, e.g., Deisz and Lux, 1985; Thomson and Gähwiler, 1989; Raiteri et al., 1992). Finally, both are known to act postsynaptically by way of fast-acting chloride channels, but their individual effects on channel conductance states and duration of channel openings differ (see, e.g., Hamill et al., 1983; Barker et al., 1986; Bormann et al., 1987). Hence, a picture seems to be emerging in which the two transmitters may act in concert at some synapses to produce a more refined postsynaptic response than would be possible with either transmitter alone.

The VNLL is an important site for monaural processing in the mammalian brain. Its neurons are almost exclusively monaural and are driven by stimulation of the contralateral ear (Aitkin et al., 1970; Guinan et al., 1972a,b; Metzner and Radtke-Schuller, 1987; Covey and Casseday, 1991). This is consistent with connectional data, which show that the VNLL gets most of its inputs from contralaterally driven monaural structures. For example, the VNLL receives most of its inputs from the contralateral anterior and posterior VCN (AVCN and PVCN; from bushy, multipolar, and octopus cells), with the remainder coming from periolivary regions of the ipsilateral SOC (Glendenning et al., 1981; Zook and Casseday, 1985; Covey and Casseday, 1986; Huffman and Covey, 1995; Schofield and Cant, 1997). Multiple tonotopic repesentations have been described in the VNLL of bats (Covey and Casseday, 1991), but, in most other species, there is no immediately obvious tonotopic representation (Aitkin et al., 1970; Guinan et al., 1972a,b; Whitley and Henkel, 1984; Saint Marie et al., 1995; but see Malmierca et al., 1997). The principal targets of the VNLL are the INLL, DNLL, and ICc on the same side (for reviews, see Irvine, 1986; Oliver and Shneiderman, 1991; Schwartz, 1992; Helfert and Aschoff, 1997).

It is believed that the VNLL has an important role in the temporal discrimination of sequential signals. Many of its neurons are broadly tuned with low spontaneous activity and respond phasically and with constant latencies to stimulus onsets (see, e.g., Covey and Casseday, 1991; for review, see Covey, 1993a). Other VNLL neurons have variable latencies and/or have sustained (tonic or chopper) responses to stimuli. It is not clear how these different response types in the VNLL relate to the different types of inputs to the VNLL or to the different morphological and cytochemical types that have been reported. What is becoming clear, however, is that most or all of these different responses types probably provide feed-forward, GABAergic and/or glycinergic, contralaterally driven, monaural inhibition to their main postsynaptic targets, the ipsilateral INLL, DNLL, and ICc.

INLL: Source of monaural excitation?

The INLL was distinguished from the DNLL or VNLL by the general absence of detectable GABA-IR or glycine-IR in the great majority of its neurons (82%). This finding is consistent with other immunocytochemical studies, which examined the INLL as an independent structure either in bats (Vater et al., 1992; Winer et al., 1995) or in rats (Mugnaini and Oertel, 1985). The INLL is hypertrophied in the bat, and both the VNLL and the INLL are much more rigidly organized than in terrestrial mammals. Vater et al. (1992) examined only GABAergic neurons in the horseshoe and mustache bats and reported small groups or clusters of GABAergic neurons in the INLL. These clusters were separated by larger areas with nonimmunoreactive neurons. Winer et al. (1995) described GABA-IR, glycine-IR, doubly immunoreactive, and nonimmunoreactive neurons in the INLL of mustache bats and reported that between 10% and 40% of the neurons were glycine-IR (including fewer than 10% that were also GABA-IR). In the cat, we found that 18% of the neurons were immunoreactive for one or the other of these transmitters. González-Hernández et al. (1996) reported that 51–68% of INLL neurons with projections to the ICc in the rat were GABA-IR. Mugnaini and Oertel (1985), on the other hand, reported that fewer than 15% of the neurons in the rat INLL (their dorsal and middle part of VNLL) were GAD-IR. In a brief report on the glycine-IR of the cat and gerbil, Adams and Wenthold (1987) described a large group of glycine-IR neurons in the VNLL (their ventral division of the VNLL) but apparently few such neurons in the INLL (their dorsal division of the VNLL). Hence, it seems reasonable to conclude that, across several mammalian species, the majority of neurons in the INLL probably do not use either GABA or glycine as a transmitter. The alternative interpretation is that GABA and/or glycine are present in these INLL neurons but at levels below those detected with the present methods. This is a consistent problem with immunocytochemistry. Our experiments are designed to minimize false positives in the selection of our antibody dilutions, but this can lead to increased false negatives and the underestimation of immunoreactive neurons.

The prevalence of glycine-IR puncta that we found in the cat INLL may also be common across species. Winer et al. (1995) showed similarly that most neurons in the bat INLL are heavily encrusted with glycine-IR puncta. The origins of these puncta are most likely the ipsilateral VNLL and the ipsilateral medial nucleus of the trapezoid body (MNTB), which together have been estimated to contribute ~45% of the projection to INLL in the cat (Glendenning et al., 1981; Spangler et al., 1985). The present study has demonstrated than most of the VNLL projection is probably glycinergic (see also Saint Marie and Baker, 1990). Likewise, the projection from the MNTB is widely considered to be almost entirely glycine-IR (Wenthold et al., 1987; Helfert et al., 1989; Saint Marie et al., 1989). Finally, the fewer, but still substantial, numbers of GABA-IR puncta that we found in INLL could have originated from the large number of GABA-IR neurons that we and others have found in the VNLL (see Mugnaini and Oertel, 1985; Roberts and Ribak, 1987; González-Hernández et al., 1996).

The function of the INLL is the least understood of the lemniscal nuclei. Like the VNLL, the INLL is primarily a

monaural nucleus driven by stimulation of the contralateral ear (see, e.g., Covey and Casseday, 1991), and most of its inputs originate from contralaterally driven monaural nuclei, i.e., the contralateral AVCN and PVCN and the ipsilateral MNTB and VNLL (Glendenning et al., 1981; Spangler et al., 1985; Zook and Casseday, 1985; Covey and Casseday, 1986; Huffman and Covey, 1995). Its inputs differ from those of the VNLL, however, in that the INLL has a much larger, presumably inhibitory, input from the ipsilateral MNTB, and its inputs from the AVCN are smaller in proportion to those from the PVCN (Glendenning et al., 1981; Huffman and Covey, 1995). The principal output of the INLL is the ipsilateral ICc (for reviews, see Irvine, 1986; Oliver and Shneiderman, 1991; Schwartz, 1992; Helfert and Aschoff, 1997). The INLL is especially prominent in species that rely heavily on echolocation for orientation and object recognition, e.g., bats and dolphins (for review, see Covey, 1993a). In bats, it is tonotopically organized (Covey and Casseday, 1991), but this is apparently not true in most other species (Aitkin et al., 1970; Guinan et al., 1972a,b; Whitley and Henkel, 1984; Saint Marie et al., 1995). Like the neurons in the VNLL, INLL neurons have little or no spontaneous activity, respond robustly to transient stimuli of short duration, and seem to have an important role in temporal discrimination (for review, see Covey, 1993a).

There is considerable overlap in the response types found in the VNLL and INLL, and it is not yet clear how each of these regions distinguishes itself physiologically. From immunocytochemical studies like the present one, however, it appears that neurons in the INLL must be much more strongly inhibited by contralateral stimulation than neurons in the VNLL. Also, we predict that the output of the INLL would have a much larger excitatory component than either the VNLL or the DNLL. In fact, the INLL may provide the principal excitatory output of the lemniscal nuclei.

CONCLUSIONS

The present results demonstrate that the three nuclei of the cat lateral lemniscus can be clearly distinguished by their relative immunoreactivities for the putative inhibitory neurotransmitters GABA and glycine and that these differences in immunoreactivity probably reflect structural and functional differences among the three nuclei. The DNLL contained the highest proportion of GABA-IR neurons (85%), and its neurons were contacted by moderate numbers of GABA-IR and fewer glycine-IR puncta. The VNLL contained the highest proportion of glycine-IR neurons (81%), and about half of these were immunoreactive for both GABA and glycine. Its neurons had few immunoreactive perisomatic puncta of either kind. The INLL was notable for its general lack of GABA-IR and glycine-IR neurons (18%) and for the fact that its neurons were covered by glycine-IR puncta. The implications of these findings are that each of the three main subdivisions of the lateral lemniscal nuclei has a distinct set of inputs and, by implication, is involved in different forms of acoustic processing. Together, they represent the single largest source of inhibitory inputs to the auditory midbrain, but they should not be considered to be uniformly inhibitory in their postsynaptic effect. The INLL is potentially an important source of monaural excitation in the IC, and minor populations of neurons in the DNLL and VNLL may also be excitatory.

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