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γ-Aminobutyric Acid and Glycine in the Baboon Cochlear Nuclei: An Immunocytochemical Colocalization Study With Reference to Interspecies Differences in Inhibitory Systems

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ABSTRACT

Previous studies of the cochlear nuclei in cat, rat, and guinea pig have demonstrated neural structures that are enriched in the inhibitory neurotransmitter amino acids γ-aminobutyric acid (GABA) and glycine. In these mammals, inhibitory terminals are widely distributed throughout the nuclear complex, but somata of inhibitory neurons are concentrated in the dorsal cochlear nucleus, in granule cell regions, and in the cap area. Because these are the subdivisions that undergo the most pronounced phylogenetic changes in primates, we wanted to see whether the inhibitory systems are influenced by changes in cytoarchitecture. Therefore, we applied light microscopic postembedding immunostaining and optical densitometry to the cochlear nuclei of an anthropoid primate, the Senegalese baboon (*Papio anubis*). Our results demonstrate that, in baboon 1) glycinergic neurons and axons in the ventral cochlear nucleus seem to form a commissural system similar to that of other mammals; 2) the tuberculoventral system appears to be unchanged in morphology but exhibits a higher level of colocalization of GABA with glycine; 3) there is a reduction of the granule/cartwheel cell system, which is reflected in lesser numbers of inhibitory cartwheel, Golgi, and molecular layer stellate cells; 4) the cap area is larger than in rodents and carnivores and contains many neurons that colocalize GABA and glycine; and 5) throughout the nuclear complex, a higher proportion of the inhibitory terminals colocalize GABA and glycine. We conclude that modulation of the ascending auditory pathway in baboon is likely to differ from that in rodents and cat.

Indexing terms: amino acids, auditory pathways, neurotransmitters, phylogeny, primates

At the central synapse of the eighth nerve in the cochlear nuclear complex (CNC), auditory signals are acted upon by a number of brainstem inhibitory circuits. In a functional sense, it has long been clear that inhibition plays an important role in the evoked patterns of activity of cochlear nucleus neurons. Interaction of excitation and inhibition is particularly clear in responses of neurons in the dorsal cochlear nucleus (DCN; Evans and Nelson, 1973; Goldberg and Brownell, 1973; Voigt and Young, 1980, 1990; Young and Voigt, 1982; Oertel and Wu, 1989; Zhao and Evans, 1992).

Evidence from physiological experiments indicates that many of the inhibitory influences that shape the responses of neurons in the cochlear nuclei are mediated by the amino acids GABA and glycine (Caspary et al., 1979, 1984, 1987; Wu and Oertel, 1986; Walsh et al., 1990; Zhao and Evans, 1991; Kakehata et al., 1992; Zhang and Oertel, 1993). In addition, immunocytochemical studies have demonstrated the presence of abundant GABA- and glycine-positive neural elements in the CNC (Mugnaini, 1985; Peyret et al., 1986, 1987; Wenthold et al., 1986, 1987; Adams and Mugnaini, 1987; Moore and Moore, 1987; Roberts and Ribak, 1987; Aoki et al., 1988; Saint Marie et al., 1989,

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1991; Osen et al., 1990; Kolston et al., 1992; Vater et al., 1992; Ottersen et al., 1995). In fact, the question has been raised as to whether all, or virtually all, of the nonprimary terminals in the cochlear nuclei are characterized by these two inhibitory neurotransmitters (Saint Marie et al., 1993).

The immunocytochemical studies cited above have shown a similar pattern of distribution of inhibitory elements in the CNC across nonprimate mammalian species. In each case, GABA- and glycine-positive neuronal somata were seen to be concentrated in the DCN and in granule cell regions of the complex. A similar pattern of concentration of GABA and glycine has been shown with biochemical analysis (Fisher and Davies, 1976; Godfrey et al., 1977, 1978) and in studies of the distribution of GABA and glycine receptors (Altschuler et al., 1986; Frostholm and Rotter, 1986; Sanes et al., 1987; Glendenning and Baker, 1988; Wenthold et al., 1988; Juiz et al., 1989).

This concentration of inhibitory elements in the DCN and in granule cell areas has implications for primates, because these subregions of the CNC undergo progressive change in cytoarchitecture across the primate order (Moskowitz, 1969; Moore and Osen, 1979; Moore, 1980). Although primates have been shown to be similar to nonprimate species in the mode of branching of the auditory nerve in the cochlear nuclei (Moskowitz and Liu, 1972) and in the pattern of projections from the cochlear nuclei to higher brainstem nuclei (Strominger, 1973; Strominger et al., 1977), the morphologic changes in the dorsal nucleus and in granule cell regions raise the possibility of differences in nonprimary auditory circuits. Variation in these inhibitory systems could result in species-specific modulation of activity in the main ascending pathway, with implications for auditory behavior.

GABA-containing neurons and terminals have been shown to be present in the CNC of the monkey (Thompson et al., 1985), but there is not enough available information to determine whether inhibitory systems homologous to those in nonprimates are present in primates and, if so, whether they differ in size, organization, or neurochemistry. In an attempt to address this issue, we have carried out the present study in an anthropoid primate, the Senegalese baboon. The same procedures for postembedding immunostaining and optical densitometry that were previously used in cat (Osen et al., 1990), guinea pig (Kolston et al., 1992), and rat (Ottersen et al., 1995) were employed. The antisera used had the same properties, and all studies stressed quantification of the density of immunostainings and determination of colocalization. The present results, therefore, should be directly comparable to those in the nonprimate species. Some of these results have been presented previously in abstract form (Moore and Osen, 1990). Because, to our knowledge, there is no published description of the anatomy of the baboon cochlear nuclei, a brief description of their cytoarchitecture has been included.

MATERIALS AND METHODS

Animals, perfusion, and general histology

Four adult Senegalese baboons (*Papio anubis*) of both sexes were perfused under pentobarbitol anesthesia (30 mg/kg body weight). A brief flush with phosphate buffer (0.1 M, pH 7.4, at 4°C) containing 2% dextran (MW 70,000) was followed by a fixative consisting of 2.5% glutaraldehyde and 1% formaldehyde (fresh from paraformaldehyde) in the same buffer (10 liters at room temperature). The perfusion was initiated within a few seconds of opening the thorax, and the neck was stiff within 2 minutes. The brain was removed and stored in fixative at 4°C for several weeks.

Tissue blocks were dissected out of the brainstem immediately prior to immunocytochemistry. Partial removal of the cerebellum allowed visualization of the caudal end of the complex and orientation of a block of tissue containing the nuclei. The block was cut by hand with a razor blade into slices about 0.5 mm in thickness. The slices were made in the traditional transverse plane or in planes oriented with respect to the long (strial) axis of the complex. The "tangential" plane of section is parallel to the strial axis and lateral surface of the complex and, thus, is a tilted sagittal plane (Fig. 1). The "transstrial" plane of section is perpendicular to the long axis of the complex and is, in effect, a tilted horizontal plane (Fig. 2).

Postembedding immunocytochemistry

The slices were rinsed overnight in phosphate buffer (0.1 M, pH 7.4). On the following day, they were treated with 1% OsO_4 in phosphate buffer for 45 minutes, dehydrated in graded ethanols and propylene oxide, and embedded in

Abbreviations

ab	ascending branches of cochlear nerve fibers	lr	lateral recess
AVCN	anteroventral cochlear nucleus	m	mantle
cap	cap area	ml	molecular layer
cereb	cerebellum	mp	multipolar cell
CNC	cochlear nuclear complex	mz	marginal zone
cnr	cochlear nerve root	oc	octopus cell
co	commissural cell	oca	octopus cell area
cw	cartwheel cell	p	pyramidal cell
d	dendrite	PVCN	posteroventral cochlear nucleus
das	dorsal acoustic stria	sgrl	superficial granule cell layer
db	descending branches	sph	spherical cell
DCN	dorsal cochlear nucleus	spha	spherical cell area
ep	ependymal layer	st	molecular layer stellate cell
g	glial cell	tb	trapezoid body
gi	giant cell	tch	tela choroidea
gl	globular cell	tv	tuberculoventral cell
gr	granule cell	v	blood vessel
ias	intermediate acoustic stria	VCN	ventral cochlear nucleus
icp	inferior cerebellar peduncle	vnr	vestibular nerve root
lam	lamina of granule cells		

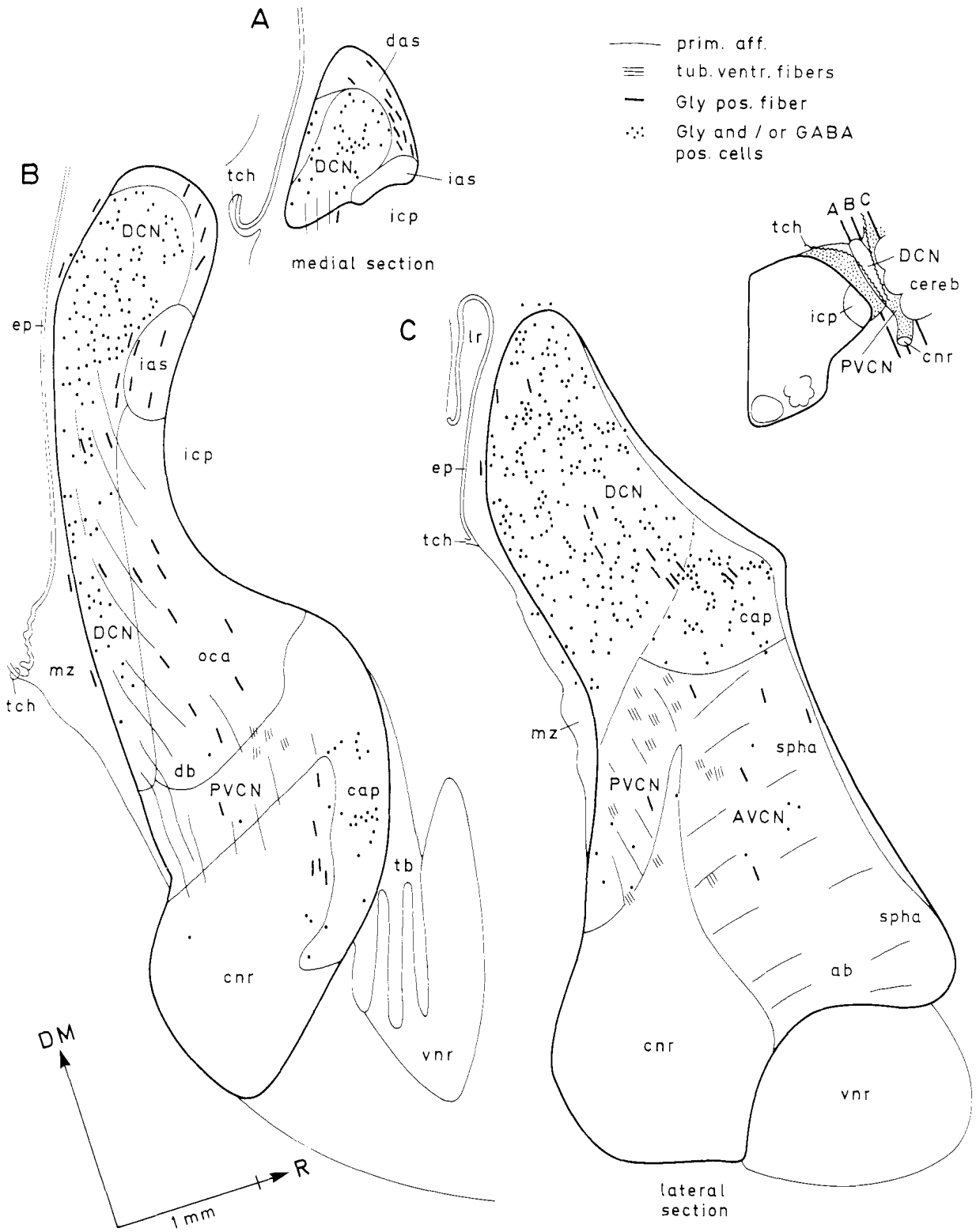


Fig. 1. **A–C:** Camera lucida drawings of sections in the tangential plane: A is the most medial level, and C is the most lateral level (see **inset**, in which the surface of the brainstem is stippled, except where it is covered with ependyma). For orientation, DM is dorsomedial, and R is rostral. Each drawing was made by successively superimposing three adjacent 0.5 μm plastic sections, one stained with toluidine blue, one with γ-aminobutyric acid (GABA) antiserum, and one with glycine (GLY) antiserum. Each immunoreactive GABA- and/or GLY-positive cell that could be recognized in all three sections was registered as a dot. The density of immunoreactive cells is much higher in the dorsal cochlear nucleus (DCN) and the cap area (cap) than in the ventral cochlear nucleus (VCN). Note that the cap continues ventrally lateral to the VCN (in B). Fascicles of fine-diameter tuberculoventral fibers, which label for both GABA and GLY, are represented by clusters of short lines. They are found in the central part of the VCN in the vicinity of the cochlear nerve root (cnr), but not in the octopus or spherical cell areas (oca and spha, respectively). Solitary segments of heavier lines illustrate large-diameter GLY-positive axons, presumably commissural, which converge on the dorsal acoustic stria (das). The course of the ascending and descending branches (ab and db, respectively) of the immunonegative cochlear nerve fibers is indicated by single thin lines. For additional abbreviations, see list.

epoxy resin (Durcupan ACM, Fluka). Serial 0.5 μm sections were cut on an ultramicrotome and mounted on gelatinized glass slides together with a 0.5 μm section of a resin-embedded "sandwich" of amino acid-glutaraldehyde-rat brain protein conjugates for assessing the specificity and quality of the immunoreaction (Ottersen, 1987).

The immunolabeling was performed according to a procedure (Ottersen, 1987; Kolston et al., 1992) based on that of Somogyi et al. (1984). This protocol involved immersion of the mounted sections in 1) sodium ethanolate, 2) sodium periodate, 3) normal swine serum (DAKO), 4) primary antiserum overnight at 4°C (diluted as below), 5) sheep or swine anti-rabbit IgG (DAKO), 6) rabbit peroxidase-anti-peroxidase complex (DAKO), 7) diaminobenzidine/H_2O_2, and 8) osmium tetroxide. The sections were thoroughly rinsed between each step of the procedure.

The primary antisera were raised and purified as originally described by Storm-Mathisen et al. (1983), diluted in Tris-phosphate-buffered saline, and preadsorbed with soluble glutaraldehyde complexes of possibly cross-reacting amino acids (AA-G, 100–300 μM with respect to the amino acid) at 4°C for 18–24 hours prior to use. Glycine antiserum 31 (Dale et al., 1986) was diluted 1:30 and was preadsorbed with GABA-G and β-Ala-G. GABA antiserum 25 (Ottersen and Storm-Mathisen, 1984) was diluted 1:100 and was preadsorbed with Gly-G and Glu-G. To study possible colocalization in individual cell somata, one out of three adjacent sections was labeled for glycine, a second was labeled for GABA, and a third was stained with toluidine blue. The quality of the immunostaining was very similar to that presented by Osen et al. (1990) in cat and by Kolston et al. (1992) in guinea pig, and, similar to those studies, the test sections exhibited immunostaining only of the homologous conjugates. Adsorption controls with AA-G (300 μM) of the amino acid that was used for the immunization showed suppression of the immunostaining both in tissue and test sections.

Optical densitometry

Optical density (OD) was measured with a Nikon P1 photometer (aperture 0.2 mm; objective ×40; measured area 7.8 μm^2). Measurements were taken from the cytoplasm of individual neurons that could be identified in all of the three adjacent GABA-, glycine-, and toluidine blue-stained sections. Each neuron was given OD values for GABA and glycine. Photomontages of the sections at ×200 were used as reference maps. The photometer was adjusted to read zero OD (100% transmission) through the glass slide outside the section. The results were expressed in scatter plots. Because the OD (= −log transmission) is always higher in tissue than in the empty glass slide, "zero zones" of GABA and glycine density were defined in the plots, with upper limits corresponding to the upper ODs of the presumptively immunonegative pyramidal and giant cells in the DCN. Measurements were made from two pairs of GABA- and glycine-stained sections from the dorsal

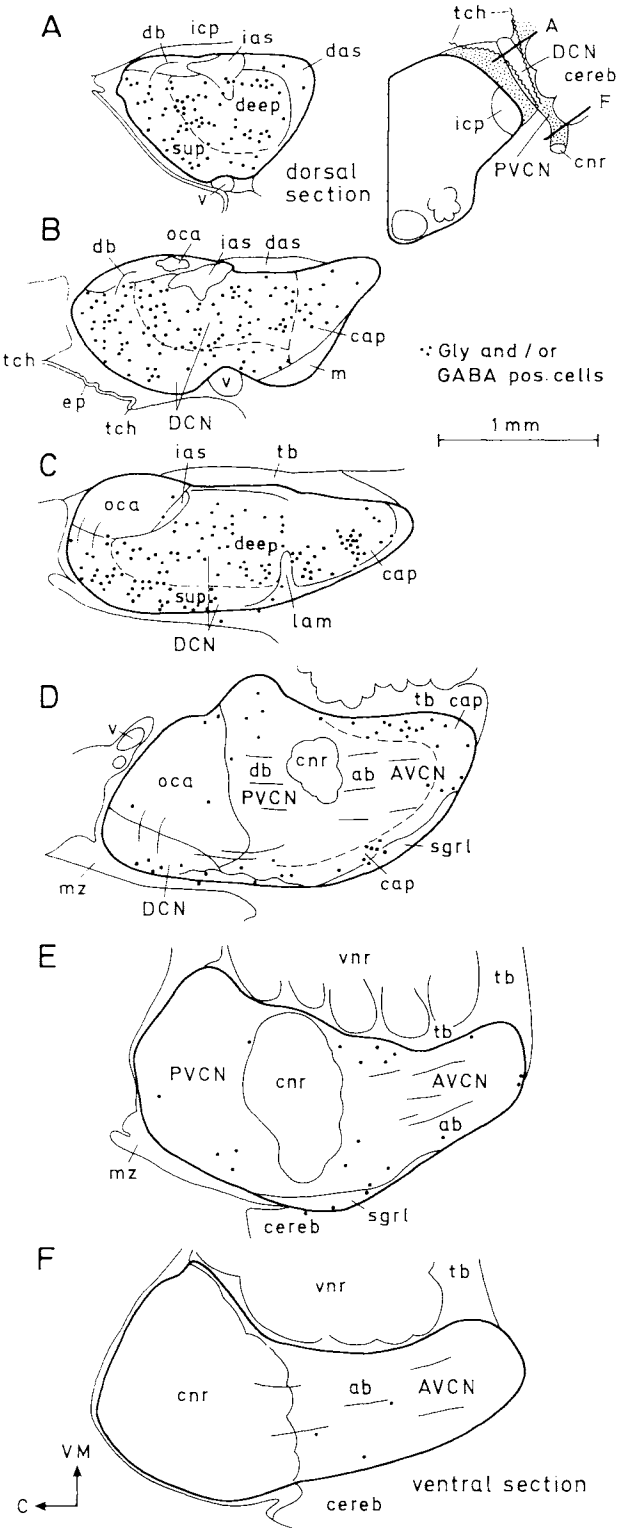


Fig. 2. **A–F:** Camera lucida drawings at regularly spaced levels (A and F are shown in **inset**; brain surface indicated as in Fig. 1). The direction of the immunonegative cochlear fibers (thin lines) is indicated for orientation. GABA- and/or GLY-immunopositive cells (dots) are abundant in the DCN and in the cap area. The scattered immunopositive cells in the anteroventral cochlear nucleus (AVCN) and the posteroventral cochlear nucleus (PVCN) proper are probably commissural cells. Immunopositive axons are not illustrated, because most are cut across in this plane of section. Rudiments of the superficial granule cell layer (sgrl) and lamina (lam) are seen at levels C–E. For additional abbreviations, see list.

nucleus (160 and 135 cells, respectively) and two pairs from the cap area (52 cells in each), all from the same series of transstrial sections.

RESULTS

Topography and general plan of structure

The CNC of the baboon is situated on the dorsolateral aspect of the brainstem, immediately caudal to the cerebellar peduncles. Similar to man and other anthropoid primates, the complex is almost completely covered by the cerebellum. The only superficial surfaces are the caudalmost edges of the DCN and ventral cochlear nucleus (VCN) together with the cochlear nerve as it enters the VCN caudoventrally (Fig. 1, inset). This free surface of the complex is covered by pia mater, except for a small portion of the DCN, which is subjacent to the ependyma of the lateral recess (Figs. 1A–C, 2A,B). The long (strial) axis of the baboon CNC is tilted about 30° from dorsomedial to ventrolateral with respect to the sagittal plane (Fig. 1, inset). The CNC measures about 6 mm along the strial axis, 2 mm rostrocaudally, and 1 mm mediolaterally (without correction for shrinkage during fixation).

We consider the CNC to consist of three main subdivisions, namely, the VCN, the DCN, and the cap area. These three regions are discussed separately in the following sections on anatomical organization and immunostaining. Generally speaking, the intrinsic structure of the VCN conforms to the general mammalian pattern, whereas the DCN shows features typical of anthropoid primates in its rudimentary granule cell population and indistinct lamination (Moore, 1980). The cap area is well developed, which is another feature that is typical of higher primates (Moore and Osen, 1979).

VCN. The VCN in the baboon shows the same distribution of cochlear nerve fibers and cell types as has been described previously in cat (Osen, 1969) and in guinea pig (Moore, 1986; Hackney et al., 1990). Spherical cells are found rostrally, octopus cells are found caudally, and a mixture of globular and multipolar cells are found in the central region of the nucleus, within and around the cochlear nerve root (Fig. 1B,C). Fascicles of thin to medium-sized fibers, which are presumably part of the tuberculoventral system, course vertically between the DCN and the VCN (Figs. 1B,C, 3A).

The *anteroventral* cochlear nucleus (AVCN) is roughly rectangular, measuring 3 mm along its strial axis, 1.5 mm caudorostrally, and 0.7 mm mediolaterally (Fig. 1B,C). The spherical cell area is not sharply delimited, but it seems to occupy approximately the rostral half of the AVCN. The ascending branches of the cochlear fibers run from caudal to rostral in evenly spaced, parallel fascicles (Fig. 3A, arrows). Single tangential sections like the one shown in Figure 3A, which is cut parallel to the strial axis of the nucleus, may span the entire height of the AVCN and, hence, contain a representation of the full apicobasal extent of the spiral ganglion.

The *posteroventral* cochlear nucleus (PVCN) is horn shaped and has a diameter of 2 mm at its base near the cochlear nerve root. The tip of the PVCN is formed by the octopus cell area, which reaches far up under the DCN (Figs. 1B, 2B–D). The border between the octopus cell area and the remainder of the PVCN is sharp and is situated about 0.5 mm from the caudal edge of the nerve root. Descending cochlear branches converge as they course caudodorsally through the PVCN. They become densely packed in the octopus cell area, from which they enter the DCN (Fig. 2A–D, 3B).

DCN. The DCN in baboon resembles that of man, in that it is long and slender (Fig. 1). It measures about 4 mm along its strial axis, 1.5 mm rostrocaudally, and 0.7 mm mediolaterally. The caudal part is covered superficially by a marginal zone of varying thickness. This zone is poor in myelin and cells and is difficult to distinguish from the underlying DCN in conventionally stained sections (Fig. 3B). The DCN is less distinctly layered than in nonprimate mammals. However, in contrast to the situation in man, superficial and deep zones can be defined (Figs. 2A–C, 3B). The superficial zone almost certainly corresponds to layers 1 and 2 in other species. Whether the deep zone, as we define it, is entirely homologous to layers 3 and 4 of cat and guinea pig is less clear.

The *superficial* DCN consists of a vestigial molecular layer and a well-developed pyramidal cell region. The molecular layer is clearly distinguishable only at caudal levels, where the DCN is subpial or subependymal (Fig. 3B). It contains few myelinated fibers and only scattered small to medium-sized cells. A few small clusters of granule cells occur along the outer rim of the molecular layer, but almost no cells of this type are found at deeper levels. The pyramidal cell layer is composed of large and medium-sized cells that are interposed between fascicles of descending cochlear branches. We interpret the larger neurons as pyramidal cells, although they have a less distinct radial orientation than pyramidal cells in nonprimates. The cochlear nerve, which is identifiable because of the uniform size of its axons, enters the DCN caudally and medially. Instead of making the usual sharp bend into the central region of the DCN (e.g., cat: Osen, 1970; guinea pig: Hackney et al., 1990), the fibers take a straighter dorsal course through the pyramidal cell region. This is similar to the situation in humans (Moore and Osen, 1979), where cochlear axons run parallel to the strial axis. However, we are not sure of the direction of the terminal axon segments (parallel or orthogonal to the surface) in either baboon or man.

The *deep* DCN is defined here as the region deep to the fasciculated cochlear branches. Because the fascicles are distinct only in the caudal half of the DCN, the border between the superficial and deep zones had to be set rather arbitrarily at rostral levels of the nucleus. The deep portion of the baboon DCN consists of a dense network of myelinated fibers that contain numerous medium-sized to large neurons, which are presumably homologous to tuberculoventral cells (vertical or corn cells) and giant cells in other species.

Cap area. In baboon, as in humans, the cap area is a relatively large saddle-shaped region that sits over the top of the VCN and tapers down its medial and lateral sides (Fig. 1B,C). It is not sharply bounded from the VCN, but it differs in the absence of fascicles of cochlear nerve axons and a greater frequency of small cells. The superficial granule cell layer, which, in nonprimate mammals, covers the whole lateral surface of the cap area, is very poorly developed in the baboon (Fig. 2D,E). The cap area also has an indistinct boundary against the deep DCN; however, here, the border is marked laterally by a vestigial granule cell lamina (Fig. 2C). The cap is thickest (about 200 μm)

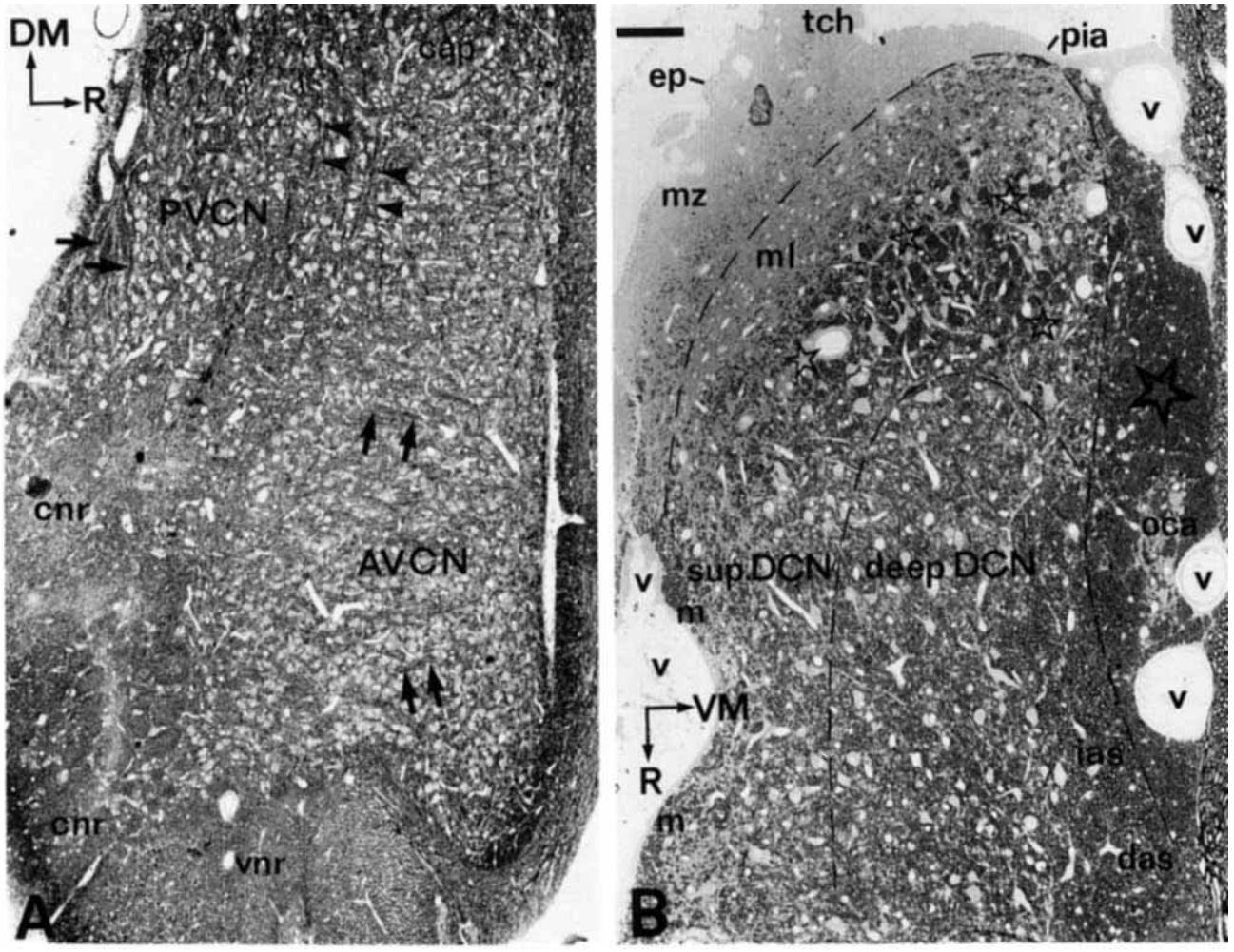


Fig. 3. Photomicrographs of toluidine blue-stained 0.5 μm plastic sections. For orientation, DM is dorsomedial, VM is ventromedial, and R is rostral. **A:** Illustration of a tangential section through the VCN, which includes the cochlear nerve root (cnr), the cap area (cap), the PVCN, and the AVCN (same level as Fig. 1C). Ascending and descending branches of the cochlear nerve fibers are indicated by arrows, and fascicles of tuberculoventral fibers are indicated by arrowheads. **B:** The DCN and dorsal tip of the octopus cell area (oca) in a transstrial section (same level as Fig. 2B). The caudal part of the DCN is subjacent to the ependyma (ep) of the lateral recess and the marginal zone (mz), which contains scattered myelinated fibers. More rostrally, the lateral surface of the nucleus is covered by a thin mantle of myelinated fibers (m). The DCN is divided into a superficial and a deep part (dashed lines). Caudally, deep to the ependyma, the superficial DCN consists of a thin molecular layer (ml) and a thicker pyramidal cell region. Descending branches of the cochlear nerve (large star) are densely packed in the octopus cell area on the medial side of the nucleus. From this position, fascicles of primary axons (small stars) fan out into the pyramidal cell layer. The deep DCN contains small and medium-sized cells lying in a meshwork of myelinated fibers. The dorsal and intermediate acoustic striae (das and ias, respectively) are seen medial to the DCN. For additional abbreviations, see list. Scale bar = 250 μm.

just anterior to the DCN, where it is contiguous with the deep zone of the nucleus (Figs. 1C, 2B,C).

Fiber tracts. The three ascending pathways from the CNC, namely, the trapezoid body and the intermediate and dorsal acoustic striae, are all well developed in baboon. The trapezoid body emanates from the rostral pole of the VCN, as in man; however, instead of coursing dorsally, it takes a more ventral route (compare Fig. 1B with Moore and Osen, 1979, their Fig. 3B,C). The largest part of the trapezoid body courses rostral to the vestibular nerve root, whereas axons from the more caudal VCN interdigitate with vestibular fibers (Figs. 1B, 2E). The intermediate and dorsal acoustic striae merge on the medial side of the DCN. The intermediate stria, which is composed of large-diameter axons of octopus cells, is located most caudally, close to the octopus cell area (Fig. 2A,B). A thin fiber mantle on the rostrolateral aspect of the DCN (m in Figs. 2B, 3B) may represent fibers taking an aberrant superficial course to the stria.

Immunostaining

Glycine and GABA immunoreactivity are found in a subset of neuronal cell bodies, fibers, and terminal-like puncta that are perisomatic or distributed in the neuropil. In the pairs of adjacent 0.5 μm sections, colocalization of GABA and glycine can be tested with confidence in cell somata and cross-cut axons, especially when the axons are large or fasciculated. For cell somata, the visual rating of staining intensity was supplemented with densitometry. In

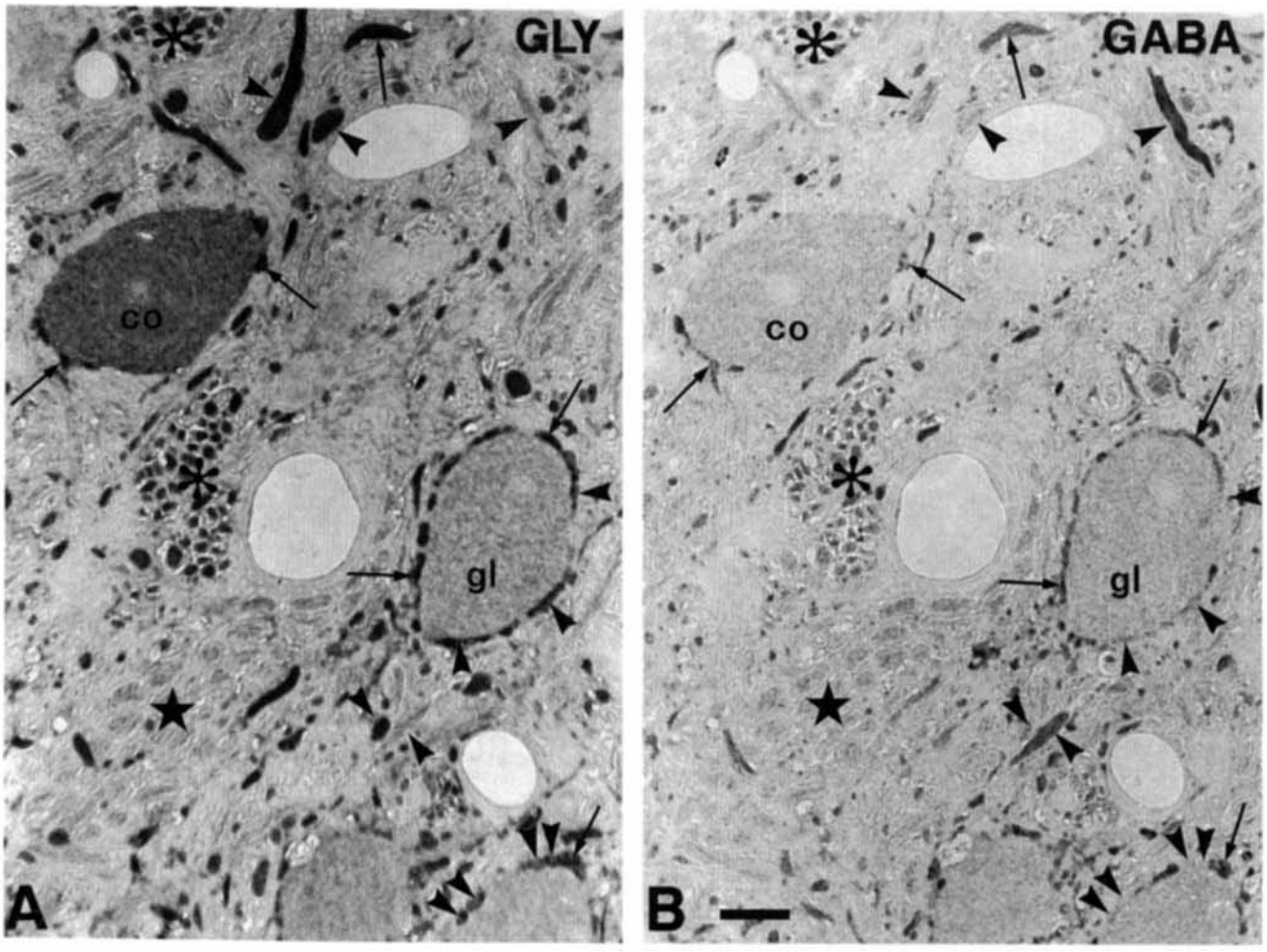


Fig. 4. **A,B:** A transstrial section through the AVCN immediately rostral to the cochlear nerve root (same level as Fig. 2D). Examples of fibers and terminal-like puncta are indicated by arrowheads when they are single labeled for either GLY or GABA and by arrows when they are double labeled. The soma of a globular cell (gl) is outlined by both double- and single-labeled puncta (the latter is mostly GLY positive). The single GLY-labeled cell (co) is presumably a commissural neuron. It shows only a few largely double-labeled perisomatic puncta. The large single GLY-labeled fibers (arrowheads) are probably axons of the commissural cells. The neuropil contains two types of fasciculated fibers, the immunonegative ascending cochlear branches (stars) and the double-labeled tuberculoventral fibers (asterisks). There are also scattered small fibers that are either double labeled or single labeled for GLY or GABA. For additional abbreviations, see list. Scale bar = 10 μm.

the case of nerve terminals, one must allow for a certain error rate with respect to double labeling; thus, only estimates of the degree of colocalization can be made. Figures 4–9 and 11–13 illustrate immunoreactivity for glycine and GABA as seen in photomicrographs of adjacent 0.5 μm sections that were treated with either of the two antisera. Most structures, except for those smaller than 0.5 μm, are likely to be present in both sections.

Immunoreactive cell bodies. The majority of immunoreactive cells are found in the DCN and the cap area (Figs. 1, 2). The VCN contains only scattered immunopositive cells, because most immunostained cells along the medial and lateral margins of the VCN appear to belong to the ventral extensions of the cap area. The cells of the superficial DCN differ from those of the deep DCN in size, distribution, and staining quality. Immunostained cells in the cap area resemble, but are not identical to, those of the deep DCN.

In the VCN, the few immunopositive cells form a distinct class, which we interpret as commissural neurons (Fig. 4A,B). They are about the same size as the immunonegative VCN neurons and are distinguished from them by the glycine-positive immunoreaction of their cell somata and thick dendritic roots. They are GABA negative as a rule, although a few also appear to be faintly stained with the GABA antiserum. They are typically situated in the central region of the VCN and are generally absent from the octopus and spherical cell areas, but they may also occur close to and within the cap area. The only other immunostained somata in the VCN are occasional small neurons that are intensely reactive for GABA.

In the superficial DCN, the largest (pyramidal) and smallest (granule) cells are immunonegative. Between these two extremes, all small to medium-sized cells are immunoreactive. Most of the immunostained cells colocalize glycine and GABA, but the molecular layer also contains a small population of single GABA-labeled cells, which are probably molecular layer stellate cells (Figs. 5, 6, 7A–D). By analogy to cat and guinea pig, the double-labeled cells appear to be Golgi cells, which are strongly stained for GABA (Fig.

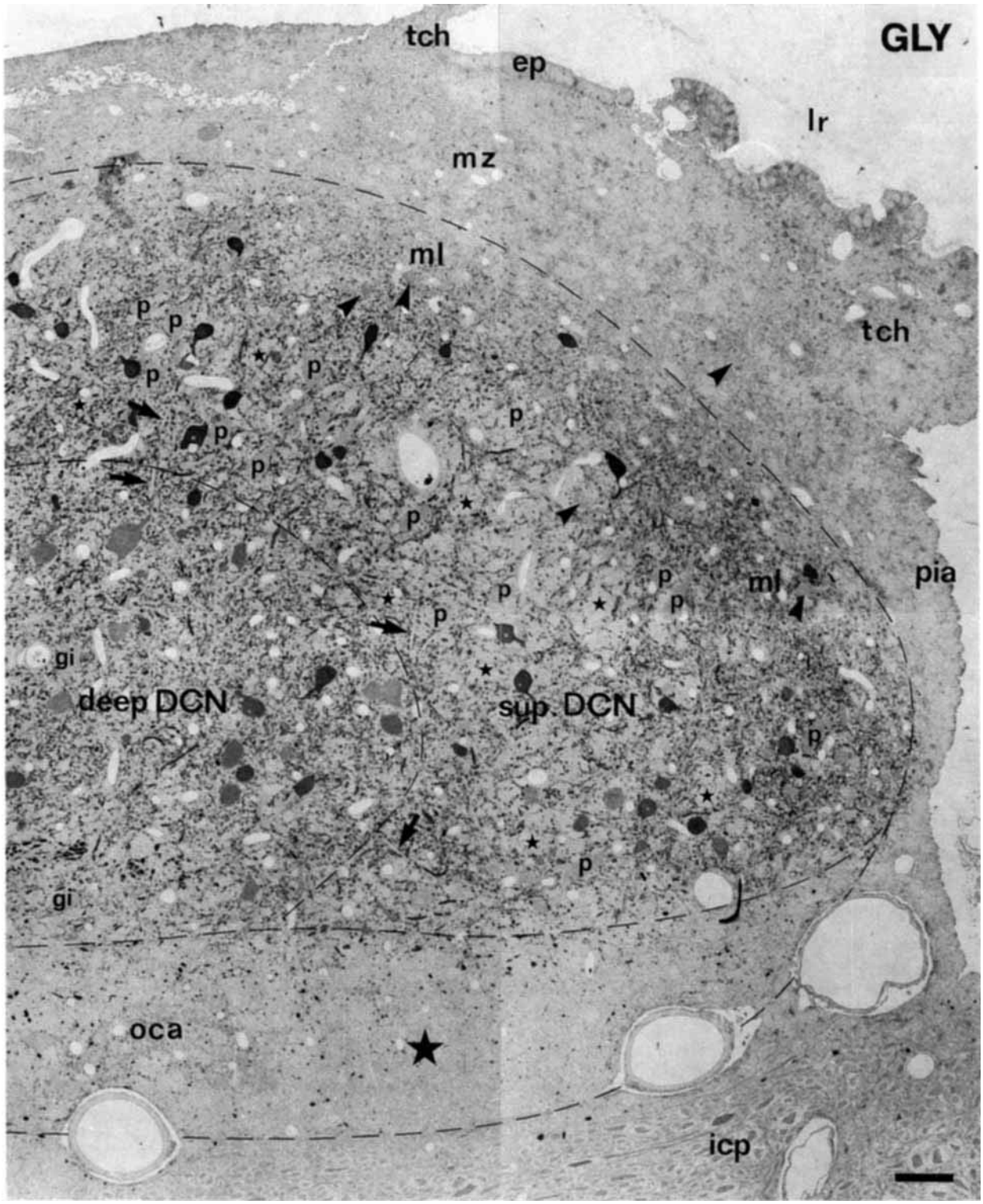


Fig. 5. Montage of survey photomicrographs of transstrial sections through the DCN (same level as Fig. 2B) immunoreacted for GLY. Examples of fibers and cell bodies are indicated by arrowheads when they are single labeled for either GLY or GABA and by arrows when they are double labeled. Dashed lines demarcate the marginal zone (mz), the superficial DCN, the deep DCN, and the oca. Immunonegative descending cochlear branches (large star) are densely packed in the oca and form fascicles (small stars) among the pyramidal cell bodies (p). Pyramidal cells are immunonegative, as are the scattered giant cells (gi) in the deep DCN. Many small and medium-sized cells throughout the DCN are strongly stained for GLY. All parts of the DCN show numerous GLY-positive puncta, some of which outline the pyramidal cell dendrites (arrows). For additional abbreviations, see list. Scale bar = 50 μm.

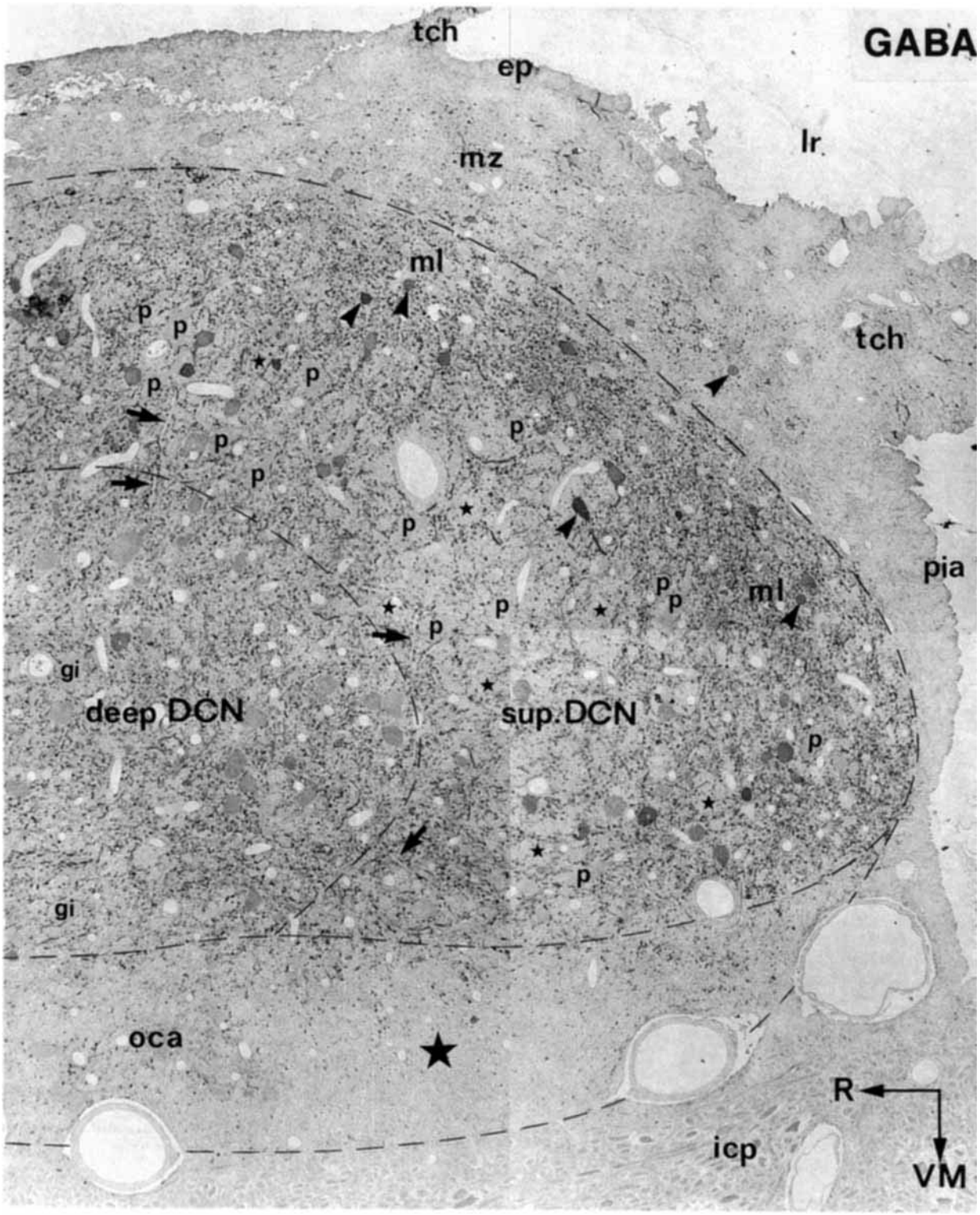
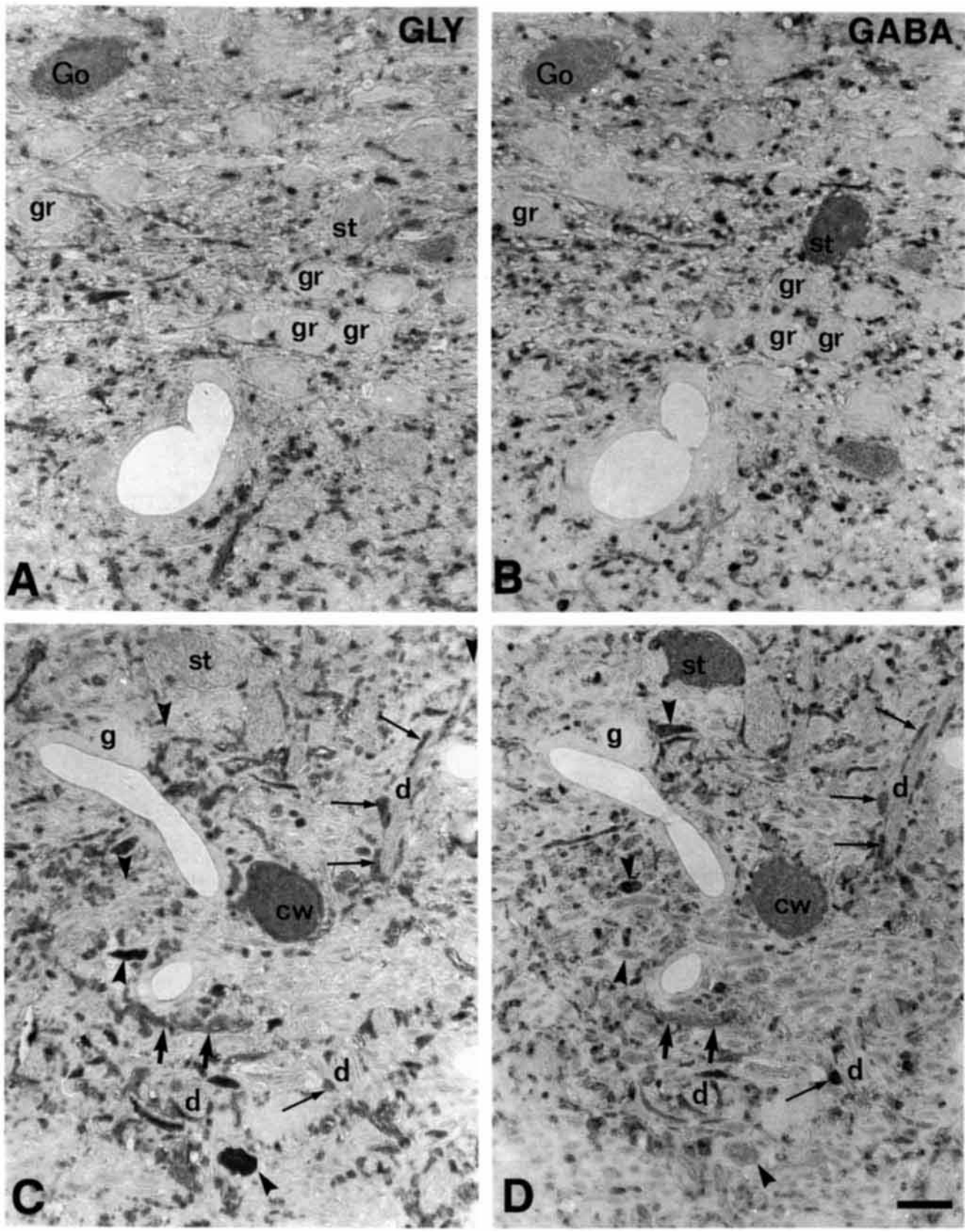


Fig. 6. Montage of survey photomicrographs of transtrial sections through the DCN (same level as Fig. 2B) corresponding to Figure 5, but treated for GABA. For orientation, VM is ventromedial, and R is rostral. Examples of fibers and cell bodies are indicated by arrowheads when they are single labeled for either GLY or GABA and by arrows when they are double labeled. Dashed lines demarcate the mz, the superficial DCN, the deep DCN, and the oca. The small and medium -sized cells throughout the DCN, which are strongly stained for GLY (Fig. 5). are more faintly stained for GABA. The superficial zone contains scattered small cells that are strongly stained for GABA: in the molecular layer (ml), they tend to be single labeled. All parts of the DCN contain GABA-positive puncta, but they are most densely concentrated in the outer molecular layer. For additional abbreviations, see list.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D

Figure 7

7A,B), and cartwheel cells, which are more strongly stained for glycine (Figs. 7C,D, 8A,B). Structurally, the double-labeled thick dendrites in the neuropil resemble spiny cartwheel cell dendrites. The immunoreactive cells are evenly distributed throughout the superficial DCN, with no trace of an aggregation of cartwheel cells at the deep border of the molecular layer, as is typical in cat (Osen et al., 1990) and in guinea pig (Kolston et al., 1992).

In the deep DCN, there are scattered immunonegative cells that are of the same size as the pyramidal cells (Figs. 5, 6, 8A–D). Despite their moderate size, they are denoted here as giant cells because of their immunonegativity and their resemblance to this cell type, as identified in cat and guinea pig (Osen et al., 1990; Kolston et al., 1992). All other deep DCN neurons are immunoreactive. They appear to form a continuum from medium to large, with the largest cells being approximately the same size as the giant cells (Figs. 5, 6, 8C,D). They are all double labeled as a rule, with a higher level of glycine staining than GABA and with the highest levels of glycine seen in the smallest cells. In addition, there are a few small cells with a strong GABA staining and with faint-to-negative glycine staining, similar to those seen in the VCN and the superficial DCN. All immunoreactive cells in the deep DCN have been designated here as tuberculoventral cells, although the presence of other cell types cannot be absolutely excluded.

The cap area contains both immunonegative and immunopositive cells. The immunoreactive cells are mostly small to medium sized, with a few larger cells lying adjacent to the VCN. The vast majority are double labeled with stronger glycine than GABA labeling (Fig. 9). Segments of similarly stained thick dendrites can also be identified in the neuropil (Fig. 9C,D). Cap cells resemble those of the deep DCN, but they tend to be somewhat smaller. A few large cells are single labeled for glycine and resemble commissural neurons of the VCN.

Densitometry was applied to cell bodies in the DCN and cap area. The OD of the glycine and GABA immunostaining showed both considerable spread within each cell type and overlap between the various types (Fig. 10B,C). The densitometric data nonetheless exhibit definite trends that support our subjective evaluation. In the DCN, these trends conform with previous findings in cat (Osen et al., 1990), which we have used as a reference (Fig. 10A). In the scatter plots, the "zero zones" of GABA and glycine OD were defined according to the upper OD levels that were recorded in the clusters of presumably excitatory pyramidal and giant cells (see Materials and Methods). Thus, the zero zones include the spread of "metabolic" amino acid levels as well as "background," nonantigen-related staining, depending on factors such as protein concentration. Pyramidal cells in baboon regularly showed a higher background labeling with the glycine antiserum than did giant cells.

The immunoreactive cells of the DCN in baboon can be viewed as four partly overlapping clusters. Those of the superficial DCN (Fig. 10B, solid circles) form three separate clusters. One cluster of cells shows medium-to-high glycine and low-to-medium GABA density, similar to cartwheel cells in cat (compare to Fig. 10A). A second cell cluster with low-to-negative glycine and medium GABA density may be molecular layer stellate cells. A third cell cluster with medium-to-high densities of both glycine and GABA may represent Golgi cells, even though their glycine density is relatively higher than in cat. Occasional single glycine-labeled cells of unknown type are also observed.

The immunopositive cells of the baboon deep DCN, which are designated here as tuberculoventral cells, form the fourth cell cluster with low-to-moderate glycine density and generally low GABA density (Fig. 10B, open circles). In agreement with the visual rating, the peak OD levels for both antisera are lower in tuberculoventral cells than in neurons of the superficial DCN, but there is a considerable overlap with the cartwheel cell cluster. In fact, our superficial/deep DCN border may not entirely separate the two cell types, and some superficial cells could be tuberculoventral (see, e.g., the two unlabeled cells in Fig. 8A,B). Cells of the baboon deep DCN have a range of glycine levels that is roughly similar to that in cat, but they differ in that nearly all neurons show some degree of GABA staining.

Cap cells (Fig. 10C) formed two clusters. One of these consisted of immunonegative cells with OD values similar to DCN pyramidal and giant cells. A broader cluster of cells showed middle-to-high values for glycine and low-to-middle levels for GABA. The ODs for the immunopositive cap cells appeared to be in the upper range or slightly higher than those for the tuberculoventral cells, even in sections stained in the same experiment.

Immunoreactive axons. The main afferent and efferent fiber tracts of the CNC were largely immunonegative. The fibers of the cochlear nerve showed no immunostaining. In the trapezoid body, only scattered immunoreactive fibers appeared to be present. No distinct bundles of glycine- and/or GABA-immunoreactive fibers could be seen to join the medial side of the CNC, as do the descending trapezoidal fibers in guinea pig (Kolston et al., 1992). The dorsal acoustic stria showed a restricted population of glycine-positive fibers of various calibers, ranging from about 1–6 μm in diameter (Fig. 11A,B). Small numbers of thin (about 1 μm) GABA-positive axons were also present. Within the CNC, it was possible to identify two conspicuous systems of immunoreactive axons, apparently representing the commissural and tuberculoventral projections.

Thick, single, glycine-labeled axons (about 6 μm in diameter) were seen as scattered segments in the central and caudal parts of the VCN (Figs. 4A,B, 12A,B). It is our impression, although it is unproven, that these axons arose from the glycine-positive neurons in the central part of the VCN and that they coursed dorsally through the octopus cell area, the cap area, and the DCN, including its outer fiber mantle, en route to the dorsal acoustic stria (Fig. 1). Within the stria, they constituted the largest diameter axons in the population of glycine positive axons described above (Fig. 11A,B). These axons were interpreted by us as commissural fibers.

Fig. 7. Details from the superficial DCN. Examples of fibers and terminal-like puncta are indicated by arrowheads when they are single labeled for either GLY or GABA and by arrows when they are double labeled. **A,B:** Photomicrographs from the outer part of the molecular layer in a standard transverse plane which show a group of immunonegative granule cells (gr), a single GABA-labeled small cell (st; presumably a molecular layer stellate cell), and a medium-sized double-labeled cell (Go; possibly a Golgi cell). **C,D:** Photomicrographs from the deep part of the molecular layer in a tangential section (same level as Fig. 1B). A thick dendrite (thick arrows) is moderately labeled for GLY and faintly labeled for GABA, and it may belong to a cartwheel cell (cw). The single GABA-labeled cell (st) may be a molecular layer stellate cell. Immunonegative dendrites (d), presumably of pyramidal cells, are outlined by double-stained puncta (thin arrows). Arrowheads denote axons that are either GLY or GABA immunoreactive. Small unstained profiles are glial cells (g). Scale bar = 10 μm.

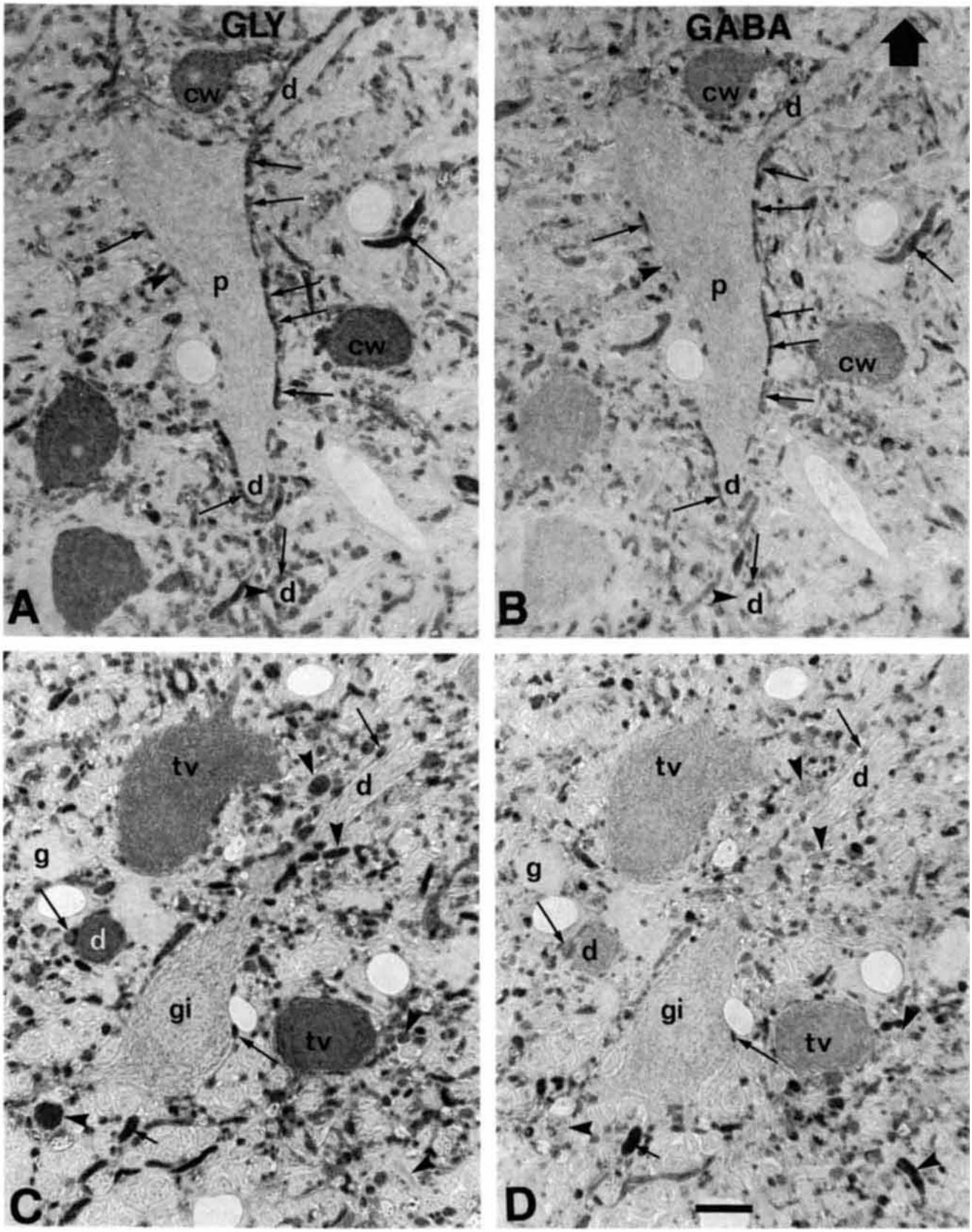


Figure 8

Fasciculated double-labeled axons forming bundles of 20–30 axons with diameters from 1 to 3 μm were cut obliquely in tangential sections (Fig. 1B,C) and across in transstrial sections (Figs. 4A,B, 12A,B). The axon fascicles were present in the central part of the VCN but were not seen in the spherical or octopus cell areas (however, see Fig. 13C,D, which includes a fascicle of tuberculoventral fibers in the outskirts of the latter area). They were most prominent in glycine-stained sections, but most of the axons were also positive for GABA. We interpreted the fascicles as tuberculoventral axons.

Immunoreactive terminal-like structures. With the exception of the octopus cell area, all parts of the CNC showed a dense punctate immunostaining of the neuropil. These puncta may represent cross-cut, small dendrites or axons or may be axon terminals. Without ultrastructural verification, immunoreactive puncta can only be identified as synaptic terminals with a reasonable degree of certainty when apposed to cell bodies or identifiable dendrites. The following description, therefore, will be restricted mainly to perisomatic puncta, with occasional cases of well-defined peridendritic terminals. One basis for confidence in this interpretation is the similarity in size (about 1-2 μm) and distribution of our puncta to inhibitory synaptic terminals revealed electron microscopically in similarly treated material of the guinea pig CNC (Hackney et al., 1995; Osen et al., 1995). This size implies that testing of colocalization should be feasible in 0.5 μm sections, particularly when there is a sequence of differently labeled terminals on the surface of a cell body. However, cases of misinterpretation due to the presence of two or more contiguous profiles cannot be excluded. An exact estimate of the relative proportions of single GABA-, single glycine-, and double-labeled terminals would require investigation of multiple serial sections at the ultrastructural level.

In the VCN, all cells, with the exception of octopus cells, commissural cells, and occasional other cells (see, e.g., Fig. 12A,B, star), are contacted by numerous immunoreactive perisomatic terminals. Of the perisomatic arrays of terminals observed on globular cells (Fig. 4A,B), multipolar cells (Fig. 12A,B), and spherical cells (Fig. 12C,D), the double-labeled category seems to be the most frequent, and the single GABA-labeled category seems to be the least frequent. Commissural neurons receive a variable number of perisomatic terminals, but they usually have less than other VCN cell types, and those present seem to be mostly double labeled (Fig. 4A,B). The octopus cell area is characterized by a far less immunoreactive neuropil than that seen in the remaining VCN (Fig. 13A,B). At low magnification, the area stands out as a blank spot in any immunostained section. At high power, only scattered double-labeled terminals are seen on the somata and thick dendrites of octopus cells (Fig. 13C,D). These dendrites are identifiable even when they are seen as isolated segments because of the virtual absence of other cell types in the area.

In the superficial DCN, the neuropil shows a relatively dense punctate staining in both GABA and glycine sections, but GABA-stained puncta extend more superficially towards the marginal zone (Figs. 5, 6). Granule cells, molecular layer stellate cells, Golgi cells, and cartwheel cells all receive a few perisomatic terminals in both types of section, but the terminals are too scattered for the evaluation of colocalization (Figs. 7, 8A,B). The somata and stem dendrites of the immunonegative pyramidal cells are outlined by numerous terminals, the majority of which seem to colocalize glycine and GABA (Fig. 8A,B). In the marginal zone outside the DCN, punctate GABA staining around groups of mainly immunonegative small cells forms typical cerebellar glomeruli, possibly consisting of ectopic cell groups from the cerebellar cortex (Fig. 6).

In the deep DCN, the neuropil shows a dense punctate staining in both the glycine and the GABA sections, but corresponding puncta in adjacent sections are difficult to identify (Fig. 8C,D). A few terminals, which are mostly double labeled, are apposed to the somata and stem dendrites of both immunonegative and immunopositive cells, particularly the former. Thus, the majority of terminals apparently contact more distal dendrites.

In the cap area, the neuropil is distinguishable from that of the adjacent VCN by its uniformly dense distribution of puncta, which do not congregate into perisomatic arrays (Fig. 9). The lack of such arrays suggests that the majority of glycine- and/or GABA-positive terminals in the cap are axodendritic.

Fig. 8. Details from the DCN. Examples of fibers and terminal-like puncta are indicated by arrowheads when they are single labeled for either GLY or GABA and by arrows when they double labeled. **A,B:** Photomicrographs from the pyramidal cell region of the superficial DCN in a tangential section (same level as Fig. 1C). The large arrow in B points to the surface of the nucleus. An immunonegative pyramidal cell (p) and its apical and basal stem dendrites (d) are outlined by puncta, which are mostly double labeled (arrows). Single-labeled puncta are marked by arrowheads. Two probable cartwheel cells (cw) are strongly stained for GLY and moderately stained for GABA. Two slightly larger double-labeled cells (lower left) are less reactive for GABA and may be tuberculoventral cells located in the pyramidal cell region. **C,D:** Photomicrographs from the deep DCN in a transstrial section (same level as Figs. 5, 6). Giant cells (gi) and glial cells (g) are immunonegative, whereas tuberculoventral cells (tv) show moderate or strong GLY and lighter GABA immunoreactivity. Scattered mostly double-labeled terminal-like puncta (long arrows) are seen on the surface of somata and stem dendrites (d) of both types of cell. Axons that are double labeled (short arrows) and single labeled (arrowheads) are abundant in the neuropil, but most of the remainder of the neuropil structures are immunonegative. Scale bar = 10 μm.

DISCUSSION

Figure 14 illustrates the distribution in the baboon CNC of GABA- and glycine-immunostained somata and of the two distinctive types of immunopositive axons. On the basis of several types of evidence, baboon immunoreactive structures can be grouped into four inhibitory systems whose cells of origin lie within the CNC, namely, the commissural projection, the tuberculoventral projection, the granule/cartwheel cell system, and the cap area. Each of these systems will be considered separately in the subsequent discussion.

In nonprimate mammals, descending fibers form a fifth inhibitory system that acts on CNC neurons. Studies involving uptake and release of radiolabeled GABA and glycine from the guinea pig CNC have identified periolivary cell groups as a major source of the centrifugal projection (Bergman et al., 1989; Benson and Potashner, 1990; Ostapoff et al., 1990). In immunostained sections of the guinea pig CNC, this projection can be seen as numerous single and fasciculated axons running in the trapezoid body and up the medial side of the VCN, many of them following the route of the centrifugal bundle of Lorente de Nó (1933) to the DCN (Kolston et al., 1992, their Figs. 4–6, 14E,F). Most

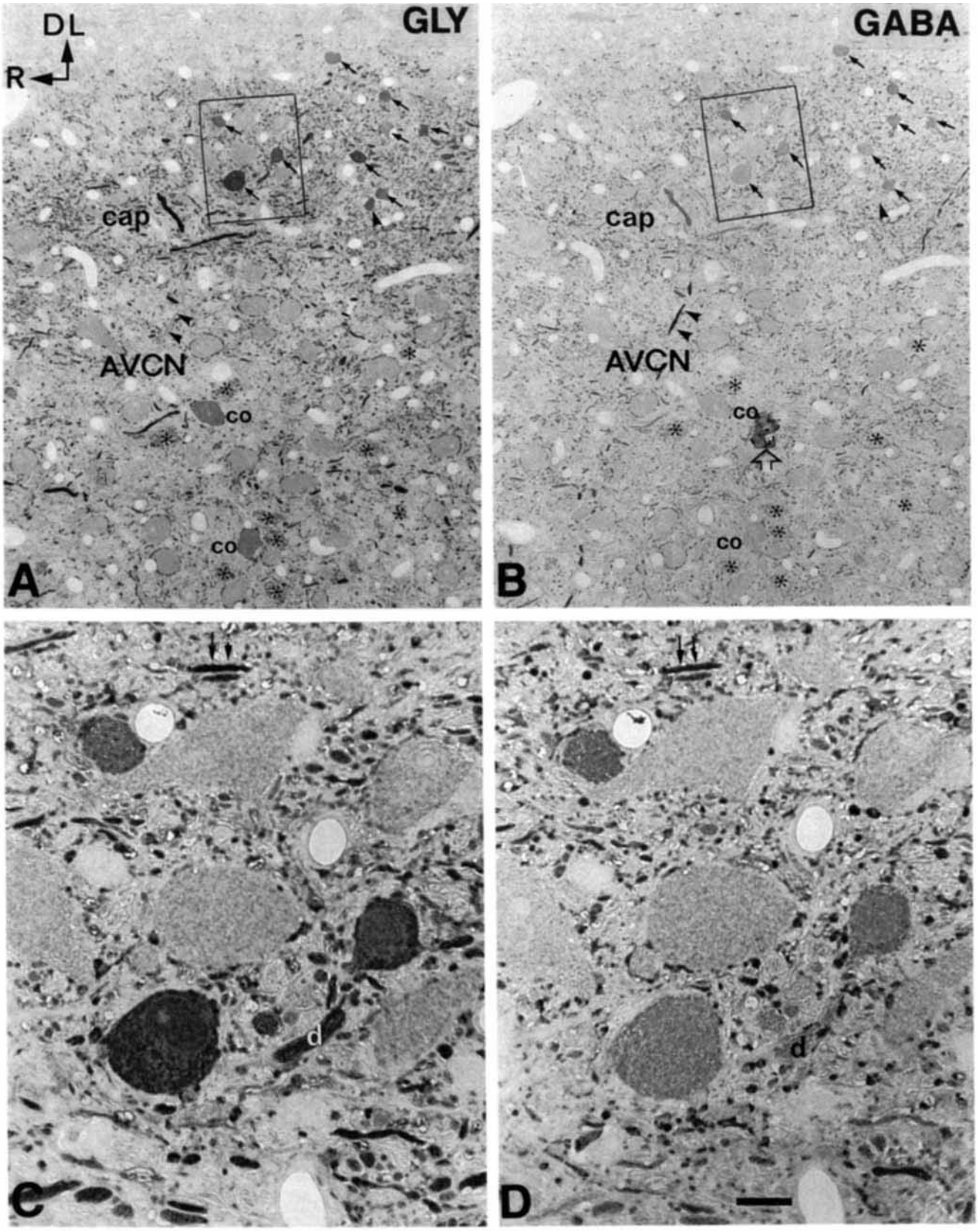


Figure 9

of these axons are single glycine-labeled, but double and single GABA-labeled fibers are also present. In nonprimates, these descending projections contribute significantly to the GABA-glycine profile of the CNC, as demonstrated by the fact that lesions transecting the efferent pathways reduce uptake and release of GABA and glycine from the CNC tissue slices (Potashner et al., 1985; Staatz-Benson and Potashner, 1988) and reduce CNC content of GABA by about 50% (Godfrey et al., 1988).

Given the substantial size of the descending projection in nonprimates, it is surprising that, in baboon, we have found only scattered immunoreactive axons in the trapezoid body. It seems unlikely that these descending fibers are present in baboon but follow a different route to the CNC, because we observe no increase in the number of immunostained axons in the acoustic striae and subpeduncular route. The apparent absence of this descending projection in baboon is all the more unexpected, because the periolivary region is a large component of the superior olivary complex in closely related species of cercopithecoid monkeys; in fact, periolivary cell groups are prominent in all primates, including prosimians, monkeys, apes, and man (Moore and Moore, 1971). The findings in baboon, however, are consistent with the fact that we were unable to identify the centrifugal bundle of Lorente de Nó in myelin-stained material from the human CNC (Moore and Osen, 1979). Further immunocytochemical studies of the primate brainstem, including the superior olivary complex, are needed to test the apparent lack of this sizeable inhibitory projection in the baboon.

Commissural projection

In baboon, we observe large, single glycine-labeled neurons scattered throughout the central region of the VCN surrounding the nerve root and occasionally in the overlying cap area (Fig. 14). We also observe a number of thick glycine-positive axons running along the strial axis of the CNC between the central region of the VCN and the dorsal acoustic stria. These structures are very similar in location and frequency to glycine-positive cells and axons found in cat, guinea pig, and rat (Osen et al., 1990; Kolston et al., 1992: Ottersen et al., 1995).

There are several reasons why we believe that these cells and axons constitute the source of the commissural projection to the contralateral CNC. First, contralaterally projecting CNC cells that are enriched in glycine have been demonstrated by combined horseradish peroxidase transport and immunolabeling (Wenthold, 1987) and by uptake of radiolabeled glycine (Benson and Potashner, 1990). Second, in rat, we have been able to follow the thick glycine-labeled fibers along the acoustic stria towards the midline, where they cross deep to the olivocochlear bundle (Ottersen et al., 1995). The fibers may not simply join the acoustic stria on the opposite side, because silver-impregnation studies following midline lesions have shown only a small population of thin degenerating fibers in the acoustic stria on both sides (Osen et al., 1984). Perhaps the thick axons divide into thinner fibers before entering the CNC by way of the dorsal stria, subpeduncular pathway, and trapezoid body, as suggested by the work of Cant and Gaston (1982).

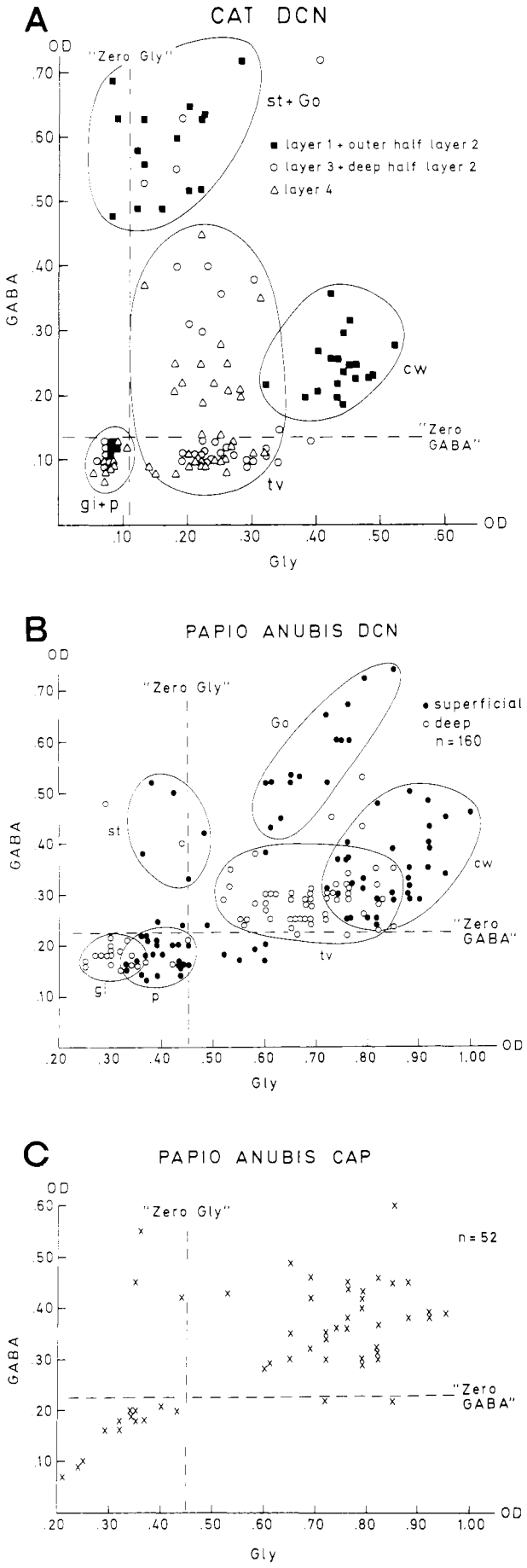
The glycine-positive commissural cells are likely to be the D-stellate cells in mouse brain slices (Oertel et al., 1990) that are characterized by extensive but sparsely branched dendrites and thick, dorsally directed axons. They may also be the onset choppers observed in cat (Smith and Rhode, 1989). Both of these cell types are characterized by axons that give off widespread collaterals within the VCN and DCN before entering the strial pathway. Within the contralateral CNC, commissural axons branch widely and terminate preferentially in areas of projection neurons, i.e., throughout the VCN and in layer 2 of the DCN (Cant and Gaston, 1982). Thus, the commissural system can distribute glycine-labeled terminals broadly within the CNC bilaterally.

By providing a monosynaptic connection to the contralateral CNC, this glycine-positive commissural projection may be responsible for the short latency crossed inhibition demonstrated in the CNC of chinchilla (Mast, 1970), cat (Young and Brownell, 1976), and guinea pig (Evans and Zhao, 1993). Studies in guinea pig (Zhao, 1995) have confirmed that short latency inhibition from contralateral stimulation is a glycinergic phenomenon, but these studies have also shown a degree of tonal specificity that is difficult to reconcile with the highly divergent commissural projection observed in morphological studies. Our present observations suggest that some type of crossed inhibition occurs in baboon. Because commissural cells and axons are not distinctive in standard histological material, the presence of the system in man could only be confirmed by some type of labeling for glycine or related substances.

Tuberculoventral projection

In baboon, we observe distinctive fascicles of immunolabeled axons throughout the central region of the VCN, surrounding the nerve root area (Fig. 14). These fascicles are identical in size and location to those observed in cat and guinea pig (Osen et al., 1990; Kolston et al., 1992). We believe that the fascicles are composed of axons of a population of neurons in the deep DCN that have been designated tuberculoventral cells by Oertel and Wu (1989) because of their highly specific projection to the VCN. These cells have also been referred to as vertical cells, corn cells, fan cells, or simply layer 3 cells (Lorente de Nó, 1933, 1981; Osen, 1983; Moore, 1986). All of the various nomenclatures refer to a population of medium-sized neurons in the deep DCN that are characterized by dendritic arbors flattened in the isofrequency planes of the nucleus. One basis for regarding the deep DCN cells and the axon fascicles in the VCN as a single system are the results of studies in cat in which DCN lesions were followed by degeneration of similarly fasciculated axons running from the deep DCN into the central VCN (Osen, unpublished observations). A second reason for regarding these struc-

Fig. 9. **A,B:** Survey views of the cap area and the subjacent part of the AVCN in a transstrial section (same level as Fig. 2D). Examples of fibers and cell bodies are indicated by arrowheads when they are single labeled for either GLY or GABA and by arrows when they are double labeled. The cap contains a higher concentration of immunoreactive cells (double labeled; marked by arrows) and neuropil puncta. A single GLY-labeled small cell is marked by a single arrowhead. The AVCN contains scattered large single GLY-labeled commissural cells (co) and fascicles of double-labeled tuberculoventral fibers (asterisks). A solitary GABA-labeled fiber is marked by the double arrowheads. The open arrow indicates an artifact. **C,D:** High-power views of the boxed field in the cap area in A and B. The immunostained neurons and their dendrites (d) show a strong GLY and a faint GABA staining. Both immunopositive and immunonegative cells show few perisomatic puncta. The neuropil itself, however, exhibits dense punctate staining in both the GLY and GABA sections. Longitudinally sectioned axons colocalize GLY and GABA (arrows). Scale bar = 10 μm.



tures as a unified system is the covariance in levels of inhibitory neurotransmitters occurring across species in the deep DCN neurons and the bundles of axons. In rat and guinea pig, about 10% of the tuberculoventral cells and fibers are colabeled for glycine and GABA (Saint Marie et al., 1991; Kolston et al., 1992; Ottersen et al., 1995) compared to about 35–40% in cat (Osen et al., 1990) and nearly 100% in baboon (Fig. 14).

Studies in mouse slice preparations have shown that tuberculoventral cells in the DCN project to bands of neurons in the VCN that are innervated by the same subset of auditory nerve axons (Wickesberg and Oertel, 1988; Wickesberg et al., 1991). Thus, a group of tuberculoventral cells and their target neurons in the VCN lie in corresponding isofrequency planes of the two nuclei. The frequency specificity of the circuit has been confirmed by retrograde transport studies in cat (Snyder and Leake, 1988) and bat (Feng and Vater, 1985) and by anterograde labeling of individual tuberculoventral axons in mouse brain slices (Oertel and Wu, 1989; Zhang and Oertel, 1993).

In addition to their projection to the VCN, tuberculoventral cells have been shown to give off axon collaterals with a planar distribution in the DCN (Lorente de Nó, 1981; Oertel and Wu, 1989; Zhang and Oertel, 1993). Type II units, which have been recorded mainly in the deep DCN, are believed to be tuberculoventral neurons (Young and Voigt, 1982). In cross-correlation studies, inhibitory troughs are seen in the activity of type IV units, identified as pyramidal and giant cells, immediately following periods of activity of type II cells in the same isofrequency plane (Voigt and Young, 1990). This frequency-specific "lateral" or "side-band" inhibition of DCN type IV cells has been shown in guinea pig to be a glycinergic phenomenon (Zhao and Evans, 1990). Thus, tuberculoventral neurons in nonprimates appear to provide frequency-specific inhibition with one synaptic delay to projection neurons in both the DCN and VCN.

Because of the morphological similarity of the baboon tuberculoventral system to that of other mammals, it seems likely that baboon tuberculoventral cells could provide the same type of short latency inhibition to neurons in the VCN

Fig. 10. Scatter plots comparing the optical density (OD) of GABA and GLY immunostaining of individual cells (n = number of cells). **A:** Photomicrograph from the cat DCN (modified after Osen et al., 1990, their Fig. 6). **B:** Photomicrograph from the baboon DCN (A serves as a reference for B, although they are from different labeling experiments). Cells are coded for locations in DCN layers (see keys) more arbitrarily in baboon because of the less distinct lamination of the nucleus. Clusters of presumptively homologous cells are outlined by hand and are marked for cell type (cw, cartwheel cells; gi, giant cells; Go, Golgi cells; p, pyramidal cells; st, molecular layer stellate cells; tv, tuberculoventral cells). The upper OD values recorded in the presumptively excitatory pyramidal and giant cells (lower left) were taken as "biological zero" reference levels (dashed lines). Cells with OD exceeding these levels are defined as immunopositive. In baboon, all tuberculoventral cells appear to be double labeled for GABA and GLY, whereas, in cat, a substantial proportion of these cells are only GLY labeled. In baboon, Golgi cells form a cluster that is separate from the molecular layer stellate cells. The identity of the five superficial GABA-negative and moderately GLY-positive cells in baboon is unclear. **C:** Photomicrograph from the cap area of the same baboon and from same labeling experiment as in B. Judging by the reference zero levels, which have been transferred from B, the vast majority of immunonegative cells in the cap area are double labeled and form one wide cluster that is distinct from that of the immunonegative cells. A few cells are single labeled for either GABA or GLY.

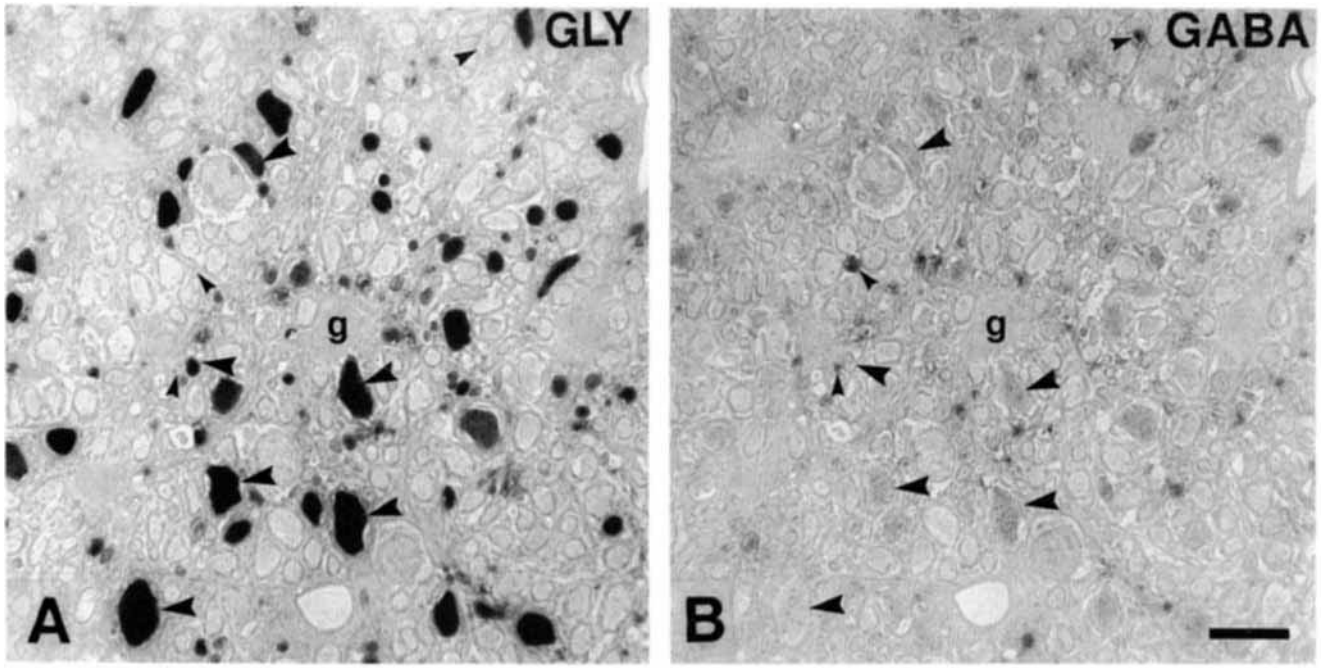


Fig. 11. **A,B:** Photomicrographs of the dorsal acoustic stria cut transstrially. Scattered among the immunonegative strial fibers are solitary GLY-positive fibers of various calibers (large arrowheads) and a few thin GABA-positive fibers (small arrowheads). Scale bar = 10 μm.

and DCN. It is not clear whether the cytoarchitectonic changes in the baboon DCN, which blur its laminar organization, also affect the orientation of its isofrequency planes. Changes of this sort have gone considerably further in the human DCN, in which cochlear nerve axons and dendritic arbors are oriented parallel, rather than orthogonal, to the nuclear surface (Moore and Osen, 1979; Adams, 1986). Such a radical shift should affect the geometry of linkage of isofrequency planes in the DCN and VCN; however, at present, there is no information whatsoever on the structure or even the existence of a tuberculoventral system in man.

Granule-cartwheel cell system

In nonprimate mammals, the granule-cartwheel cell system is a complex set of interconnected neurons with a ground plan of circuitry similar to that of the cerebellar cortex. Granule cells in all parts of the CNC project into the molecular layer of the DCN, where their axons form the parallel fibers (Mugnaini et al., 1980a). Some of the small neurons in the superficial DCN that colocalize glycine and GABA are likely to be Golgi cells (Wenthold et al., 1986, 1987; Osen et al., 1990; Kolston et al., 1992; Ottersen et al., 1995), which are assumed to form inhibitory axon terminals in mossy fiber glomeruli (Mugnaini et al., 1980b). Molecular layer stellate cells, which are possible homologues of cerebellar stellate/basket cells, are mostly single labeled for GABA (Mugnaini, 1985; Wenthold et al., 1986, 1987; Osen et al., 1990; Kolston et al., 1992; Ottersen et al., 1995). They receive input from parallel fibers, and their short axons contact the dendrites of pyramidal and cartwheel cells in the molecular layer (Wouterlood et al., 1984). Their terminals may account for the higher density of GABA puncta in the outer DCN than in the rest of the CNC. Cartwheel cells show homologies with cerebellar Purkinje cells in their spiny dendrites (Wouterlood and Muganini, 1984) and in their content of cell markers PEP-19 and cerebellin (Mugnaini and Morgan, 1987; Mugnaini et al., 1987). They label for both GAD/GABA and glycine (Mugnaini, 1985; Wenthold et al., 1986, 1987; Osen et al., 1990; Saint Marie et al., 1991; Kolston et al., 1992; Ottersen et al., 1995), and they project onto pyramidal cell somata and basal dendrites (Berrebi and Muganini, 1991).

In primates, there is a steady dissolution of this complex intrinsic circuitry. The series of changes begins with a failure of inward migration and maturation of granule cells, a process that is incipient in prosimians and is more pronounced in monkeys (Moskowitz, 1969; Moore, 1980). Concomitantly, the granule cell population dwindles, possibly due to the failure of cell maturation, with the result that the number of granule cells in layer 2 of the DCN, the lamina, and the superficial layer of the VCN is very reduced in New and Old World monkeys (Moore, 1980). The present findings in baboon are in agreement with previous observations in other simian species. They also provide insight into the cascade of morphological changes precipitated by reduction of the granule cell population. Layer 1 of the baboon DCN is thinner than in nonprimate species, presumably due to a reduced number of granule cell axons forming parallel fibers. The loss of parallel fiber input may account for the less systematic radial orientation of the pyramidal cell somata and dendrites in baboon and for the superficial displacement of fascicles of the cochlear nerve. In nonprimate species, cochlear nerve descending branches course through layer 3 of the DCN in close relationship to pyramidal cell basal dendrites (Osen, 1970; Hackney et al., 1990); however, in baboon, fascicles of cochlear nerve axons are located in layer 2, surrounding pyramidal cell somas.

In addition to loss of granule cells, the present findings indicate that the number of granule cell-related interneurons is reduced. Judging by the densitometric data, the

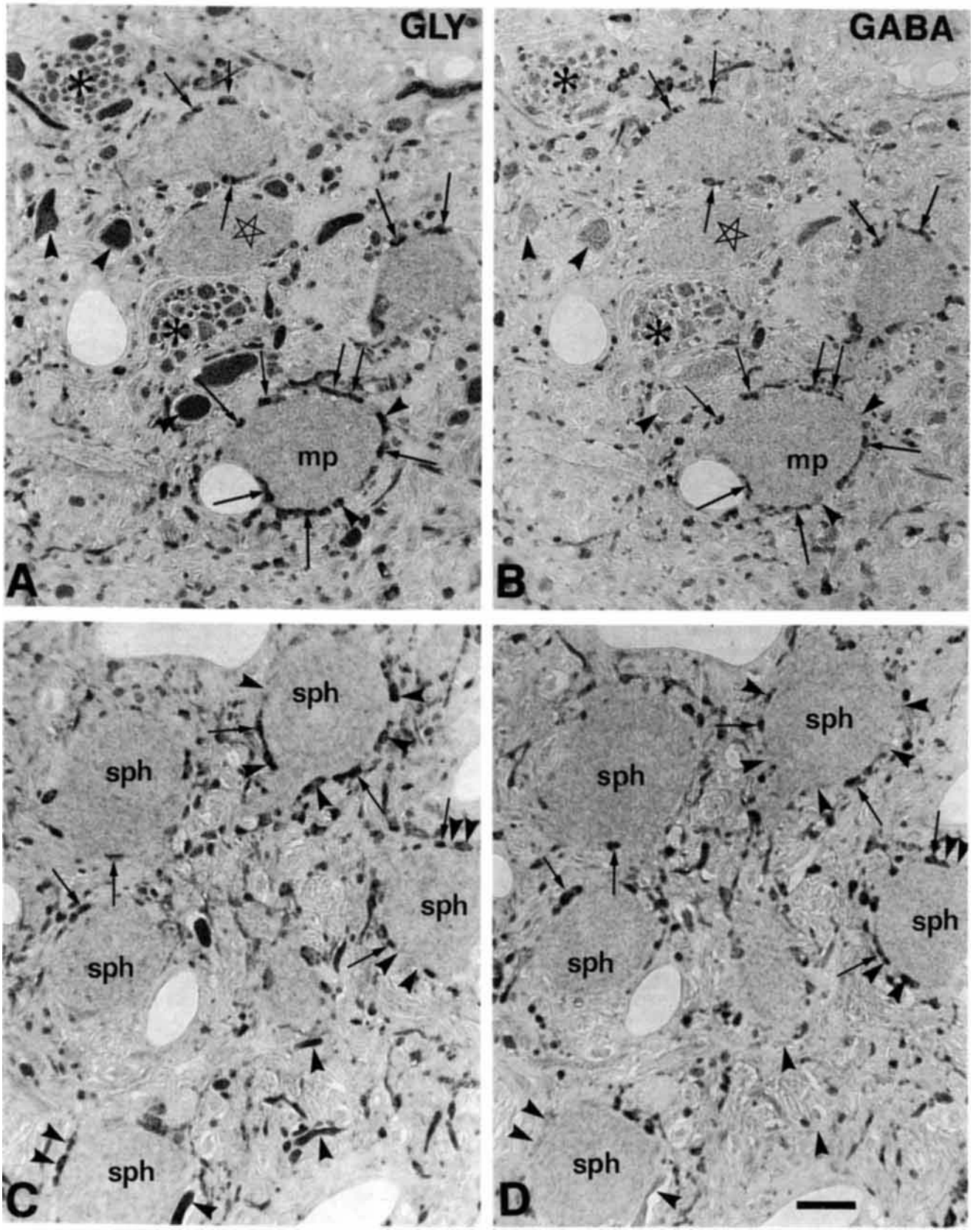


Figure 12

superficial DCN in baboon contains neurons that have typical immunochemical properties of Golgi cells (strong glycine and GABA labeling), molecular layer stellate cells (single GABA labeling), and cartwheel cells (strong glycine labeling, light GABA labeling), but all three types are relatively less numerous than in cat (Osen et al., 1990), rat (Ottersen et al., 1995), and guinea pig (Wenthold et al., 1986, 1987; Saint Marie et al., 1991; Kolston et al., 1992).

Further reduction of the granule/cartwheel system to the point of virtual absence of the superficial DCN occurs in apes and in man (Moore and Osen, 1979; Moore, 1980; Johnson et al., 1994). A similar loss of granule cells and lamination of the DCN has been noted in seals, sea lions, manatees, cetaceans (Osen and Jansen, 1965; Johnson et al., 1994), and in several species of bat (Vater et al., 1992; Johnson et al., 1994). Johnson and coworkers point out that DCN lamination is maintained in terrestrial species but disappears in species whose original adaptation is aquatic, aerial, or arborial. Possibly, the system deals with the position of sound sources relative to the ground substrate, as suggested by the role of the DCN in localization of sound in terms of elevation (Sutherland, 1991). However, even with a return to a terrestrial mode of life by *Homo sapiens*, the human granule/cartwheel cell system in man remains vestigial, indicating that it is not a significant factor in human auditory function.

Cap area

Homologues of the cap area of Osen (1969) have been described in several species of primate and nonprimate mammals by Fuse (1913; his laterale Zellgebiet), in cat by Lorente de Nó (1933; his anterior and posterior lateral nuclei and internal marginal layer), and in porpoise by Osen and Jansen (1965; their pars dorsomedialis and pars dorsolateralis). The cap area sits on top of the VCN, extending down either side like a saddle. Along the medial and lateral margins, the cap is not sharply bounded from either the VCN or the overlying areas of granule cells (Cant, 1993). The cap area also abuts on the deep zone of the DCN. Where the two regions are contiguous, the border is difficult to define because of the similarities in structure and immunostaining. Because the cap area appears to form a bridge between the VCN and DCN, we have chosen to regard it as a separate subdivision rather than classifying it as part of either nucleus.

Investigation of the cap area is facilitated by immunostaining, because this area is rather inconspicuous in normal histological sections but presents a uniformly dense distribution of labeled cells and puncta in immunostained material (rat: Moore and Moore, 1987; guinea pig: Kolston et al., 1992; cat: Adams and Mugnaini, 1987; Osen et al., 1990). In baboon, as in other species, glycine and GABA puncta are densely distributed throughout the neuropil but are not clustered into perisomatic arrays of the type that characterize the VCN. This is in agreement with observations that many synaptic terminals in the cap area contain flattened or pleomorphic vesicles and contact dendritic shafts (Cant, 1993). Immunostained cells are numerous: in fact, virtually all immunopositive neurons in the CNC that are not part of the three previously described intrinsic systems (commissural, tuberculoventral, and granule/cartwheel) are located in the cap area. In rodents, the immunopositive cells are mostly glycinergic, whereas, in baboon, most colocalize GABA to a variable degree (Fig. 14). These cells could conceivably form double-labeled terminals throughout the cochlear complex; however, at present, it is not possible to say where these cells exert their influence or even whether their axons terminate within the CNC or outside it.

The cap area is larger in cat than in rodents, and its increased size in baboon is a reflection of a trend to increasing size of the area in higher primates (Fuse, 1913; J.K. Moore, unpublished observations). The cap area is strikingly large in the human CNC (Moore and Osen, 1979) and in the porpoise, where it constitutes a large fraction of the volume of the CNC (Osen and Jansen, 1965). Thus, the size of the cap area appears to parallel both forebrain size and total brain size. Although we still have essentially no understanding of the function of the cap area, its growth in size indicates that it plays an increasingly important role in audition in cetaceans and anthropoid primates, including man.

SUMMARY

It is clear from the foregoing discussion that there is no single trend toward development or regression of inhibitory systems in the baboon CNC. Rather, the systems vary independently. One region of concentration of inhibitory elements, the cap area, is larger in baboon than in nonprimate mammals. The commissural projection and tuberculoventral projections are largely unchanged in their morphological organization, but a higher level of GABA is present in tuberculoventral neurons and axons in baboon than in nonprimates. The granule/cartwheel system shows definite signs of regression, and the descending inhibitory projections cannot be identified in our material.

To the extent that these changes have occurred, a somewhat different constellation of inhibitory influences acts upon neurons of the baboon CNC. At a cellular level, this is reflected in a relative increase in double-labeled perisomatic puncta at the expense of single glycine- and GABA-labeled puncta. In cat, pyramidal cells exhibit a segregation of GABA-positive, double-labeled, and glycine-positive terminals on the apical dendrites, soma, and basal dendrites, respectively (Osen et al., 1990). In contrast, in baboon, the double-labeled variety dominate on all parts of these cells. This is also the case for the giant cells of the deep DCN, which, in cat, are studded with glycine-immunoreactive terminals. In the baboon VCN as well, double-labeled terminals on spherical cells, globular cells, and multipolar cells seem relatively more numerous than in other species. The few inhibitory terminals on octopus cells and commissural cells are also largely double labeled.

Fig. 12. **A,B:** Photomicrographs of a transstrial section through the PVCN (same level as Fig. 2D). Examples of fibers and terminal-like puncta are indicated by arrowheads when they are single labeled for either GLY or GABA and by arrows when they are double labeled. A typical multipolar cell (mp) is contacted by many double-labeled (arrows) and a few single GLY-labeled perisomatic puncta (arrowheads). Of the three other cells, which are cut eccentrically, two have an assortment of perisomatic puncta similar to those of the multipolar cell, whereas the third cell (star) shows only a few immunoreactive puncta on its surface. The neuropil contains segments of solitary, thick, single GLY-positive fibers (arrowheads), which are probably commissural axons. Thinner fasciculated fibers (asterisks), which are more heavily labeled for GLY than for GABA, are tuberculoventral axons. **C,D:** Photomicrographs from a tangential section through the AVCN (same level as Fig. 1C) showing five spherical cells (sph) with both double-labeled (arrows) and single-labeled (arrowheads) perisomatic puncta. Scale bar = 10 μm.

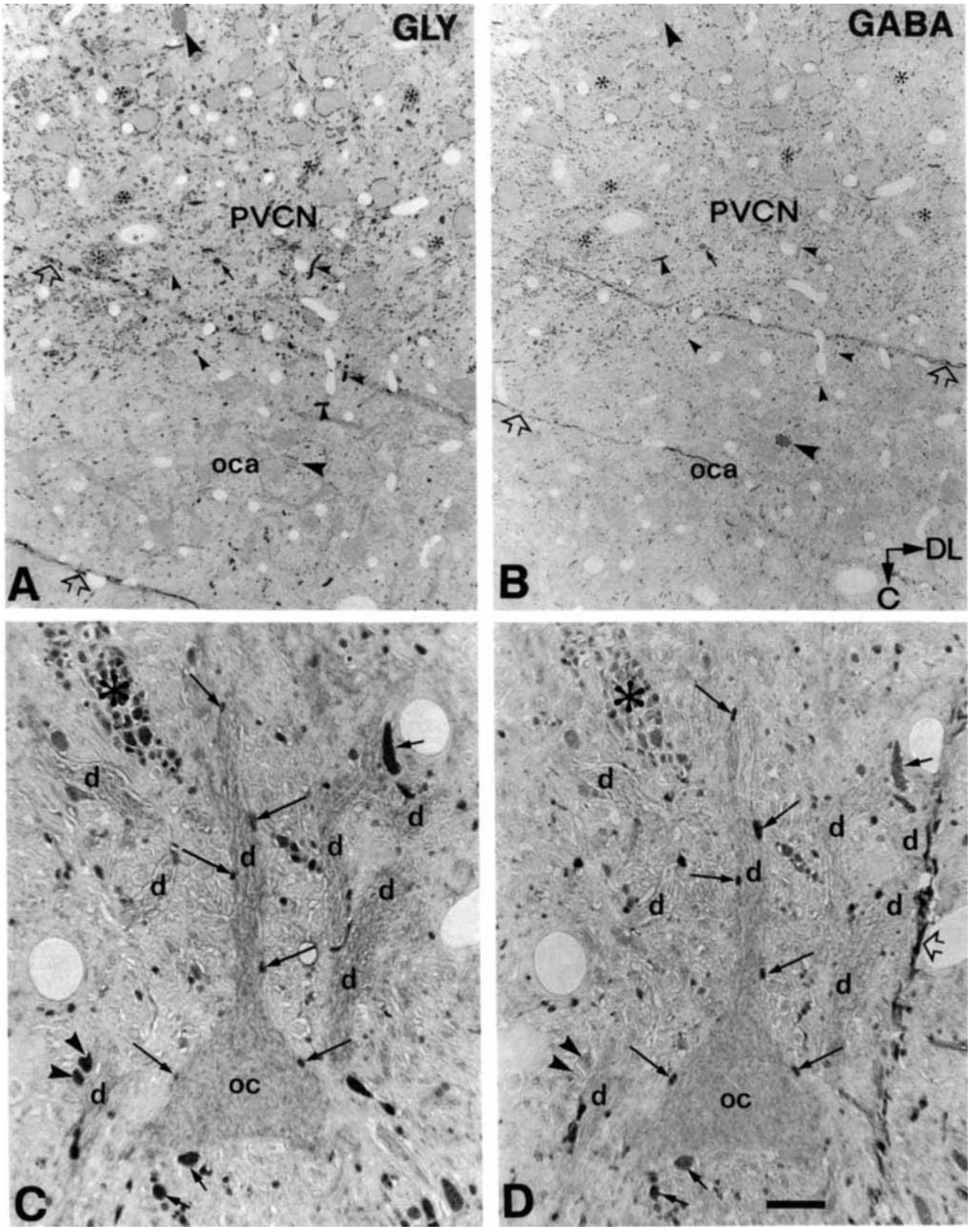


Figure 13

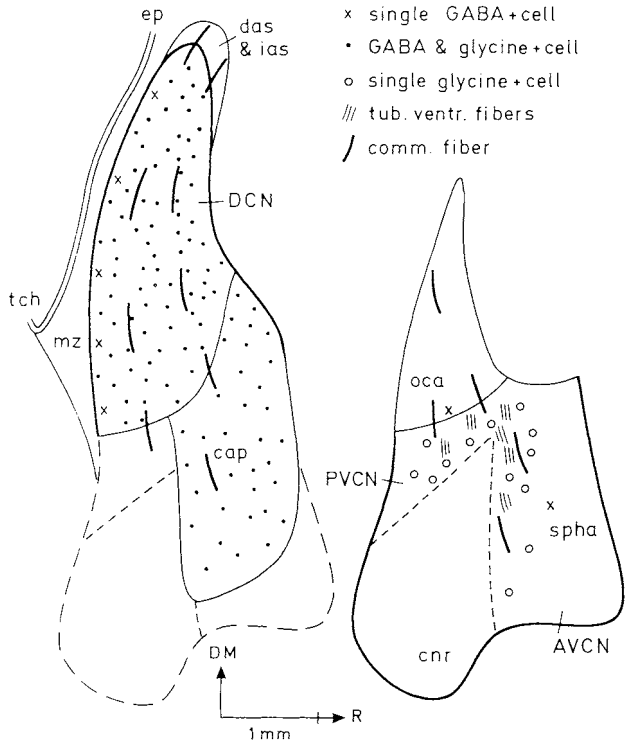


Fig. 14. A summary diagram showing the distribution of GABA- and/or GLY-immunoreactive cell bodies, solitary commissural axons, and tuberculoventral fiber fascicles in the baboon cochlear nuclei (see key). The complex is represented in the tangential plane: DM is dorsomedial, and R is rostral. **Right:** The deeper ventral nucleus (PVCN and AVCN) and cochlear nerve root (cnr). **Left:** The more superficially situated DCN and cap area. Fascicles of double-labeled tuberculoventral axons course obliquely downward through the central part of the ventral nucleus. This central region also contains single GLY-labeled cells, which are probably commissural neurons. Thick line segments represent GLY-positive commissural axons. The DCN and cap both contain numerous double-labeled cells. A limited number of single GABA-labeled cells are present in the molecular layer of the DCN, and a small number of single GABA-labeled cells are found in the VCN. For additional abbreviations, see list.

The shift to a higher proportion of terminals that colocalize GABA and glycine is consistent with the changes that have occurred in baboon inhibitory systems. Although the cartwheel cell population is reduced, and centrifugal fibers are not found, potential sources of double-labeled terminals are augmented in baboon by the enlarged cap area and the higher GABA levels in tuberculoventral neurons. With the apparent absence of centrifugal projections, the only source of purely glycinergic terminals would seem to be commissural axons, and the only source of purely GABAergic terminals should be the few small GABA-positive cells scattered throughout the complex. Thus, it seems clear that modulation of the ascending auditory pathway of the baboon differs from that occurring in cat and rodents and that, in ways that are not yet well understood, this will affect the baboon's ability to process environmental sound.

Fig. 13. **A,B:** Survey views of the transitional zone between the oca and the remainder of the PVCN in a transstrial section (same level as Fig. 2D). Note the sharp boundary between the two areas with regard to density of immunoreactive puncta, showing conspicuously less GLY and GABA immunoreactivity in the oca. Outside the oca, the neuropil contains single-labeled axons (mostly GLY positive; small arrowheads) and fascicles of double-labeled tuberculoventral fibers (asterisks). A few small neurons are single labeled for either GLY or GABA (large arrowheads). Open arrows indicates artifacts. **C,D:** A single octopus cell (oc) and isolated segments of thick octopus cell dendrites (d). Widely spaced double-labeled puncta on the soma and dendrites are indicated by arrows. The neuropil contains scattered double-labeled axons (short arrows) and single GLY-labeled axons (arrowheads). The field is from the outskirts of the oca and includes a fascicle of tuberculoventral axons, which are generally not present in the oca. Scale bar = 10 μm.

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