THE JOURNAL OF COMPARATIVE NEUROLOGY 202:385-396 (1981)

Neurons and Glia in Cat Superior Colliculus Accumulate [^{3}H]Gamma-Aminobutyric Acid (GABA)

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ABSTRACT We have examined by autoradiography the labeling pattern in the cat superior colliculus following injection of tritiated gamma-aminobutyric acid (GABA). Silver grains were heavily distributed within the zonal layer and the upper 200 μm of the superficial gray. Fewer grains were observed deeper within the superficial gray, and still fewer were found within the optic and intermediate gray layers. The accumulation of label was restricted to certain classes of neuron and glia. Densely labeled neurons were small (8–12 μm in diameter) and located primarily within the upper 200 μm. Dark oligodendrocytes and astrocytes showed a moderate accumulation of label while pale oligodendrocytes and microglia were unlabeled. Label was also selectively accumulated over several other types of profile within the neuropil, including presynaptic dendrites, axons, and axon terminals.

The superior colliculus contains significant quantities of gamma-aminobutyric acid (GABA) and its synthetic enzyme, glutamic acid decarboxylase (GAD) (Okada, '74, '76). Which cells and neural processes within the colliculus contain GABA is unknown but of considerable importance because GABA is an inhibitory neurotransmitter and inhibitory processes are important in generating the receptive field properties of collicular neurons (Dreher and Hoffmann, '73; McIlwain and Fields, '71; Sterling, '71; Sterling and Wickelgren, '69). As a step toward determining which elements in the colliculus are GABA-ergic, we have examined the accumulation patterns of [^{3}H]GABA using light and electron microscope autoradiography. This approach seemed legitimate since neurons identified as GABA-ergic by their GAD content usually show a selective accumulation of exogenous GABA (Brandon et al., '79; Hökfelt and Ljundahl, '72; Iversen and Kelly, '75; Kelly and Dick, '75; McLaughlin et al., '74; Sterling and Davis, '80). Using this technique, we have found GABA to be concentrated largely in the upper superficial gray layer of the superior colliculus, both in the neuropil and in certain small neurons and glia.

MATERIALS AND METHODS

Four normal cats and one with an ablation of cortical areas 17, 18, 19, and the lateral suprasylvian area (LS) were used in this study. Each cat received an initial injection of aminooxyacetic acid (AOAA; 40 mg/kg/hour) to inhibit the catabolism of GABA by GABA transaminase. One hour later, 12.5–25.0 μCi (0.5–1.0 μl) of γ-[2,3,-3H(N)] aminobutyric acid (New England Nuclear, specific activity 32–38 Ci/mmole) in 0.9% saline was injected into the superior colliculus with a micropipette. One hour after the GABA injection, the cat was perfused with a 2% paraformaldehyde-2% glutaraldehyde fixative in 0.1 M phosphate buffer with 0.002% calcium chloride at pH 7.4. Following overnight refrigeration in fixative, the colliculus was cut into 100-μm coronal slices with a vibratome, postfixed for 1 hour in 2% buffered osmium tetroxide, and stained *en bloc* with 0.5% uranyl acetate. Each section was then dehydrated and embedded in Epon 812.

For light microscope autoradiography, semithin (1 μm) sections were mounted on glass slides, coated with Kodak NTB-3 emulsion di-

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0021-9967/81/2023-0385$03.50

luted 1:1 with water, and exposed for 1–2 weeks at 4°C. The slides were developed in D-19 for 2 minutes at 19°C, fixed with Kodak Rapid Fixer, and stained with toluidine blue.

For electron microscope autoradiography, thin sections (silver-pale gold thickness) were mounted on colloidin-coated microscope slides. The slides were then coated with a thin layer of carbon followed by a coat of Ilford L4 emulsion applied to obtain a purple interference color (Kopriwa, '73). The sections were exposed in the dark for 1 week at 4°C, developed in full strength D-19 for 2 minutes at 19°C, and fixed in 24% sodium thiosulfate for 5 minutes. The films were then stripped from the slides onto a water surface and a single-slot formvar-coated grid was placed over each autoradiogram with the aid of a micromanipulator (Davis et al., '79). The colloidin was removed with amyl acetate and the sections stained with 4% uranyl acetate and 0.1% lead citrate.

For quantitative analysis, three ultrathin sections from two animals were systematically scanned with a JEOL JEM-120B electron microscope. Every cell within the zonal, superficial gray, and upper optic layers was photographed at 2,000 × and its area and grain density determined from photographic prints using a Hewlett-Packard 9874A digitizer. The location of each cell was charted with an x-y recorder coupled to the stage controls of the electron microscope (Fig. 1) (Sterling, '73).

RESULTS

Light microscope observations

The selective laminar distribution of silver grains was the most striking feature of the light microscope autoradiograms. Few grains were observed in the pial layer of the colliculus, but grains were intensely distributed within the zonal layer and the upper superficial gray. The deeper superficial gray was more lightly labeled and the fewest grains were seen within the optic and intermediate gray layers (Fig. 2a). This specific distribution must have been independent of the concentration of exogenous GABA because it was found even when the injection was made into the deeper layers of the colliculus (Fig. 2a).

Grains in the superficial gray were heavily distributed over the neuropil and certain cell bodies, most of which were located within the upper 200 μm (Fig. 2b). The selectivity of the GABA accumulation by neurons was impressive in that neurons with intense grain accumulations were immediately adjacent to others which were devoid of grains (Fig. 2b). To study these labeled neurons quantitatively and to determine which elements of the neuropil were labeled, we examined this tissue using electron microscope autoradiography.

Electron microscope observations

Labeling of neurons. The labeling density, size (cross-sectional area), and depth of 248 neurons within the zonal, superficial gray, and upper optic layers were examined with the electron microscope (Table 1). As suggested by the light microscopy, some of these neurons showed a tremendous accumulation of label while others were virtually without grains (Fig. 3a,b). The most intensely labeled neurons were small, ranging from 50 to 150 μm^2 (8–12 μm diameter). No cell with a grain density

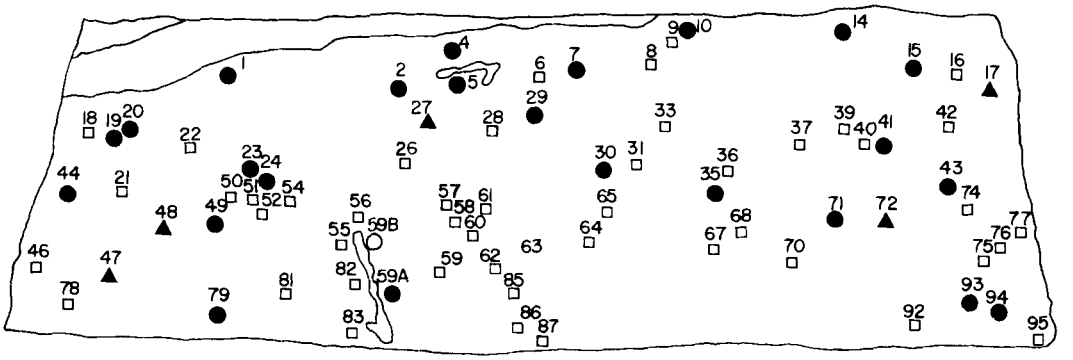

Fig. 1. Distribution of GABA-labeled neurons within the upper 200 μm of the superior colliculus from one of three plotted electron microscope sections. The plot was obtained using an x-y recorder attached to the electron microscope (Sterling, '73). Cells are numbered in the order photographed. Symbols represent labeling density. □ = 0–33 grains/100 μm^2; ▲ = 34–62 grains/100 μm^2; ● = > 63 grains/100 μm^2.

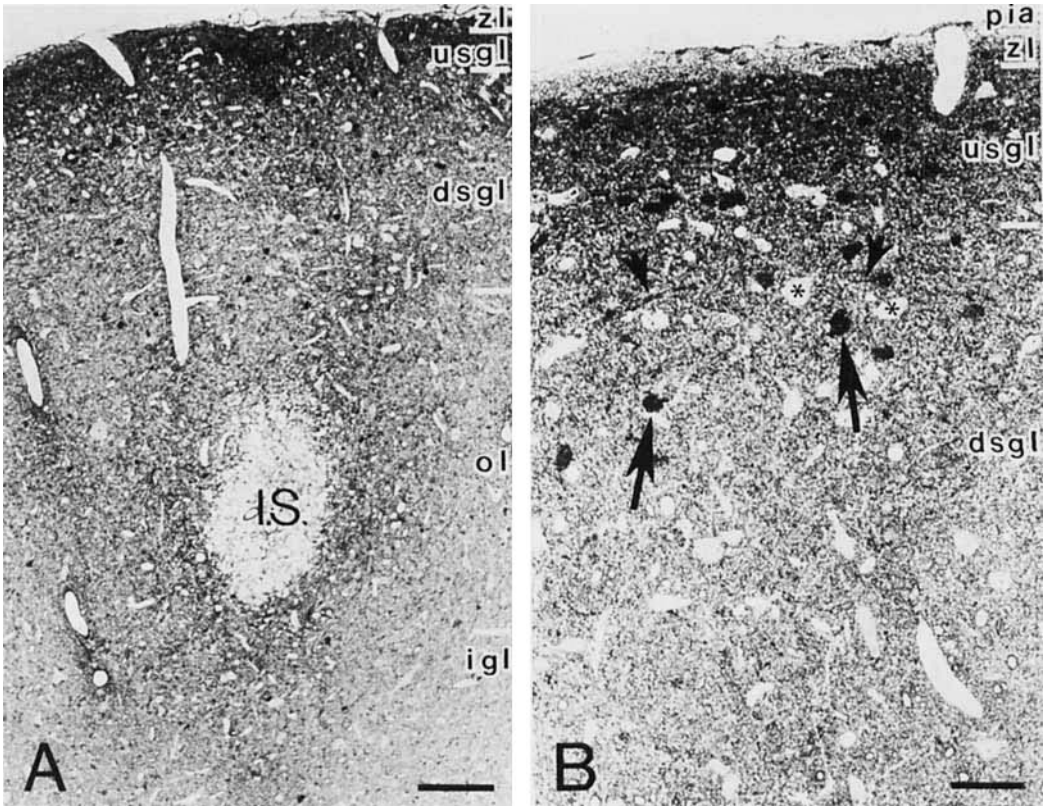

Fig. 2. Light micrographs. A. GABA injection into the optic layer. Neurons and neuropil within the upper portion of the superficial gray are heavily labeled. Labeling in the vicinity of the injection site was sparse. zl, zonal layer; usgl, upper superficial gray layer; dsgl, deep superficial gray layer; ol, optic layer; igl, intermediate gray layer. B. Labeled neurons (large arrows) in the superficial gray. Unlabeled neurons (asterisks) and labeled dendrites (small arrows) were also present. Scale: A, 200 μm; B, 50 μm.

TABLE 1. Neuron labeling in superior colliculus

Cell type	N	Mean labeling density	Mean cell area	Mean depth
Total neurons	248	37.57 ±44.67	120.53 ±71.24	203.29 ±127.08
Neurons with nucleolus	85	38.07 ±46.65	152.83 ±89.53	226.03 ±134.12
Neurons without nucleolus	163	37.31 ±43.74	103.68 ±52.42	191.43 ±121.99

exceeding 50 grains per 100 μm^2 had a cell area greater than 161 μm^2 (Fig. 4). However, many other small cells in the same region accumulated almost no label. Thus, size alone was not a distinguishing characteristic of labeled cells, although none of the largest cells was labeled (Fig. 4).

The most intensely labeled cells were distributed superficially (Fig. 5). Almost all intensely labeled neurons were located within the upper 200 μm of the colliculus and the majority of these were within the upper 100 μm. Only three cells with a grain density exceeding 50 grains per 100 μm^2 were found as

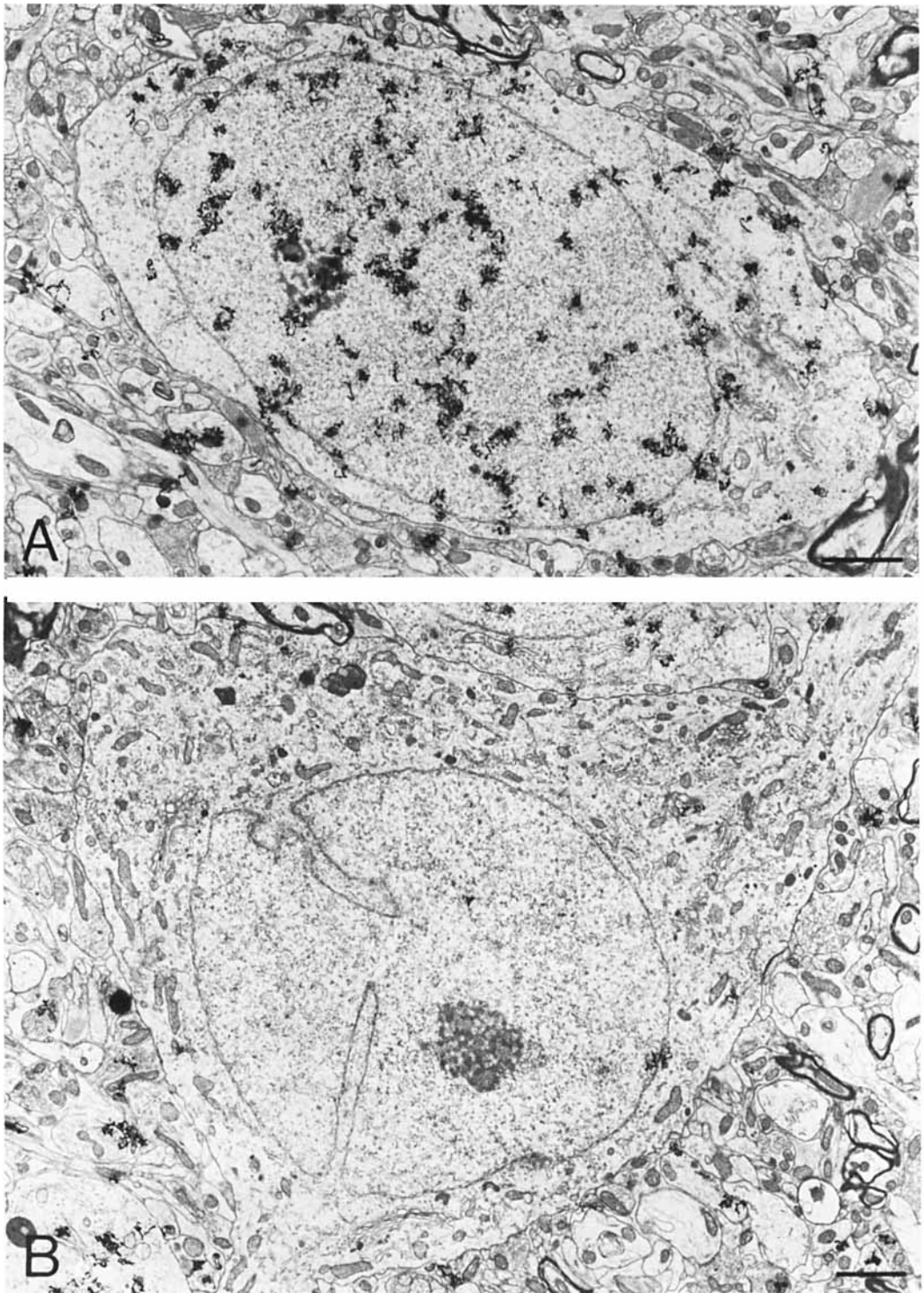

Fig. 3. Electron micrographs. A. Labeled neuron in the upper 100 μm of the superficial gray layer. Grains are over the nucleus as well as the cytoplasm. B. Unlabeled neuron in the upper 100 μm of the superficial gray. Scale: A and B, 2 μm.

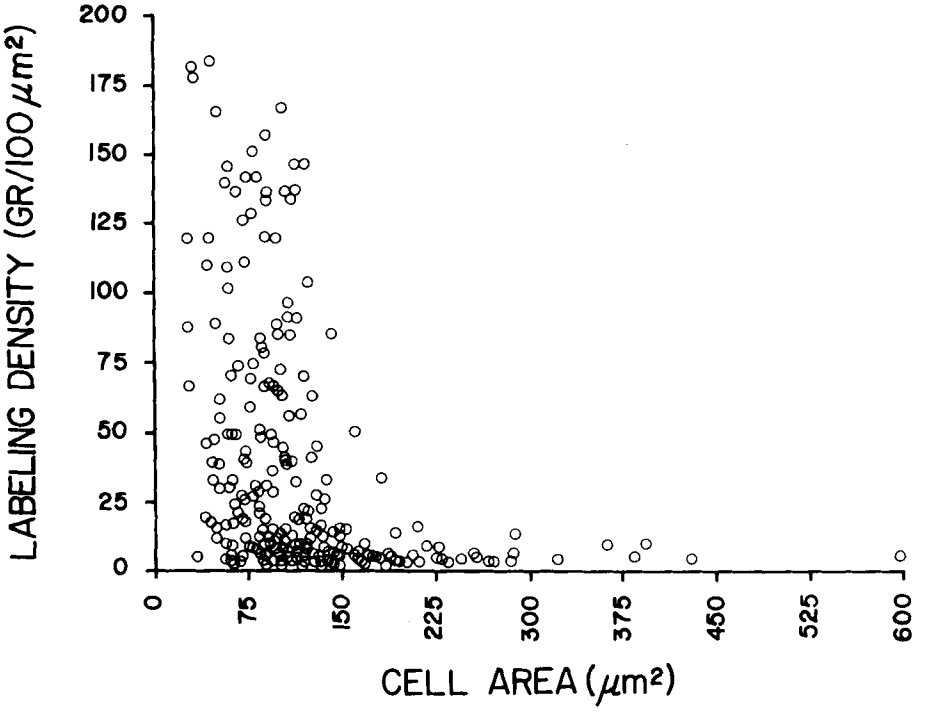

Fig. 4. Labeling density (grains/100 μm^2) vs. cross-sectional area (μm^2) of 248 neurons. Most labeled cells ranged from 50 to 150 μm^2. Other cells of this size range were unlabeled.

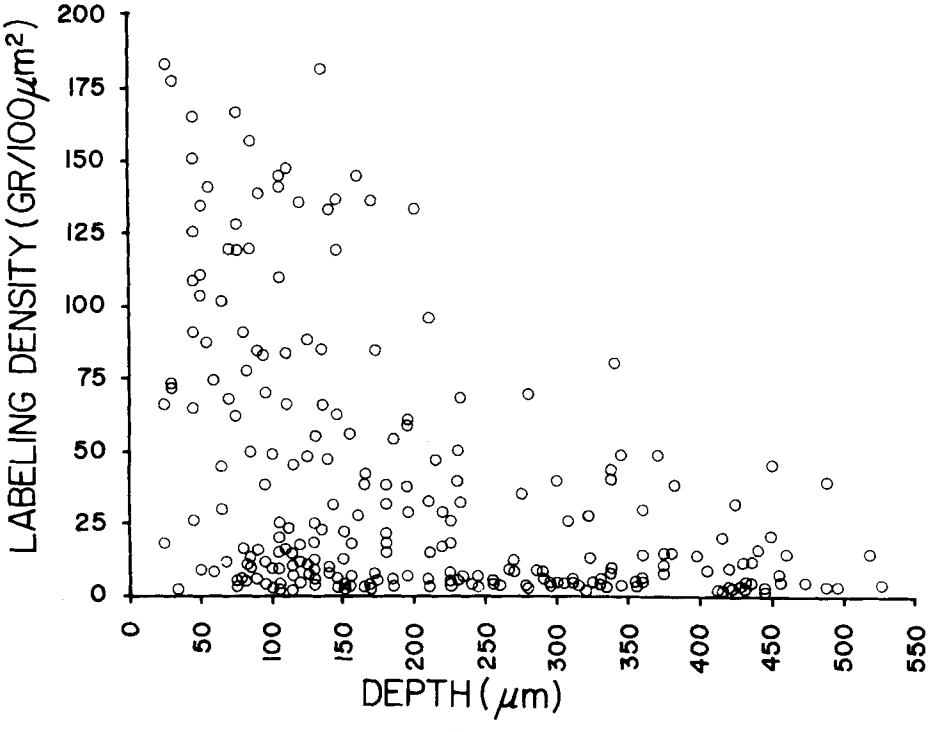

Fig. 5. Relationship of labeling density (grains/100 μm^2) to depth (μm) of 248 neurons within the superior colliculus. Most intensely labeled neurons were located within the upper 200 μm of the superficial gray.

deep as 350 μm beneath the pial surface (Fig. 5).

The morphology of heavily labeled neurons varied. Some cells were fusiform while others were round or pear-shaped. Some had a sparse cytoplasm, while the cytoplasm of others was well-developed. The mitochondria in some well-labeled cells were dark with well-organized cristae, while the mitochondria in others were pale with irregular cristae. Other cytological features, such as chromatin pattern and membrane contour, were also quite variable. This variability suggested that GABA is selectively accumulated by several different classes of neuron, but we could not determine from single electron microscope autoradiograms precisely how the neurons should be subdivided.

Neuropil labeling. Silver grains in the neuropil accumulated over certain synaptic terminals, dendrites, and myelinated axons (Fig. 6). The intensely labeled synaptic terminals were bulbous, with smooth contours and dense accumulations of synaptic vesicles. The synaptic vesicles were usually pleomorphic and the contacts formed by labeled terminals were usually symmetrical (Fig. 6a,b).

Processes classed as dendrites by their large size, postsynaptic position, and the presence of ribosomes, were often conspicuously labeled (Fig. 6c,d,e). Some of these labeled dendrites contained round synaptic vesicles and were presynaptic to other dendrites (Fig. 6d,e). These labeled presynaptic dendrites often extended horizontally, but their cells of origin were not apparent in single sections.

In addition to these grain accumulations in specific neural elements, there was a lighter sprinkling of single grains or small clumps of grains throughout the neuropil. Much of this labeling was due to accumulations of [^{3}H]GABA by fine astrocytic processes (Sterling and Davis, '80).

Glia labeling. We distinguished four classes of glia in the superior colliculus, two of which showed significant accumulations of silver grains. Astrocytes (Peters et al., '76) had round nuclei, smoothly contoured nuclear envelopes, and abundant, pale cytoplasm containing bundles of microfilaments (Fig. 7a). Astrocytic processes, marked by filaments, were tortuously woven through the neuropil. These cells invariably showed substantial accumulations of silver grains (mean grain density of 48.6 grains/100 μm^2, Table 2). One class of oligodendrocyte also showed significant grain accumulations. This class had a large nucleus of regular contour with finely divided chromatin. The cytoplasm was scant, but dark (Fig. 7b). These dark oligodendrocytes had substantial grain accumulations (mean grain density of 51.4 grains/100 μm^2, Table 2). A second class of oligodendrocyte had a nucleus of irregular shape which contained clumps of chromatin, some of which coated the inner leaflet of the nuclear membrane. Its cytoplasm was conspicuously pale (Fig. 7c). These pale oligodendrocytes contained few silver grains (mean grain density of 15.1 grains/100 μm^2, Table 2). Microglia (Peters et al., '76) had irregularly contoured nuclei with large dense clumps of chromatin as well as conspicuous bands of chromatin lining the inner leaflet of the nuclear membrane. The cytoplasm of these glia appeared dense and usually contained large lipofucsin granules and long strands of granular endoplasmic reticulum (Fig. 7d). The microglia also contained few silver grains (mean grain density of 9.3 grains/100 μm^2, Table 2).

The labeling density found in astrocytes and dark oligodendrocytes was uniform as judged by the small standard deviations seen in Table 2. Labeling density in these two classes of glia was also independent of depth within the colliculus (Fig. 8). Glia as deep as 550 μm accumulated the label as intensely as those near the surface of the colliculus. Thus, the labeling density of glia is also independent of a diffusion gradient from the injection site.

DISCUSSION

Tritiated GABA injected into the superior colliculus is accumulated by specific elements that show a distinct laminar distribution. The cells that accumulate GABA most intensely are small neurons, most of which are located within the zonal layer and upper superficial gray. Presynaptic dendrites, certain axon terminals, and two types of glia also accumulate GABA.

These grain accumulation patterns seem to reflect a specific, localized uptake of exogenous GABA. The labeling cannot be due merely to a concentration gradient because the most intense grain accumulations are found not at the site of injection deep in the colliculus, but within the upper superficial gray hundreds of microns away. Furthermore, within the region of most intense accumulation, only certain elements bear grains while others are virtually devoid of them. Finally, the accumulation is confined to two easily identifiable types of glia.

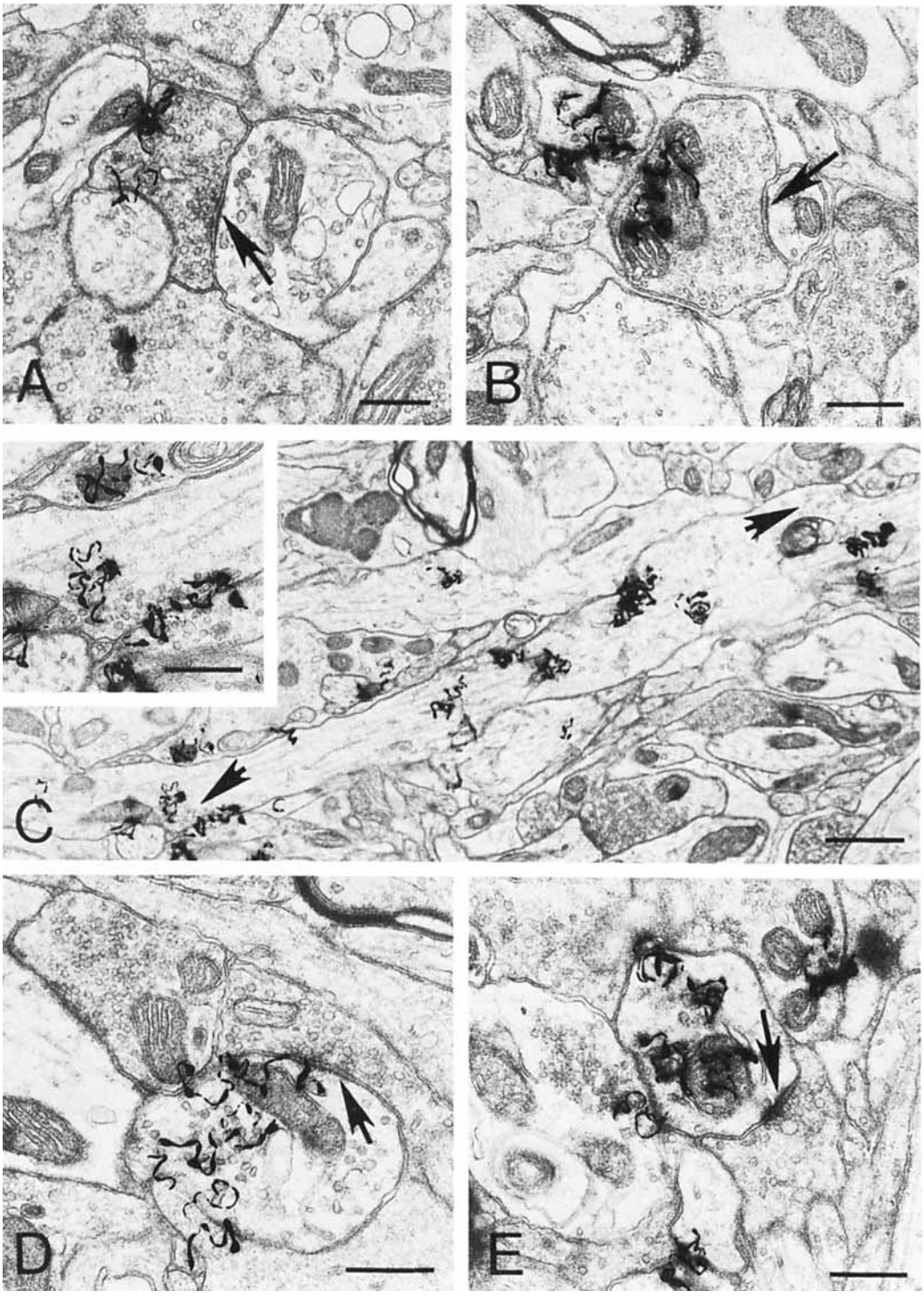

Fig. 6. Electron micrographs. A,B. Labeled synaptic terminals located within the upper 200 μm of the superficial gray. The terminals had pleomorphic synaptic vesicles and symmetric membrane specializations (arrows). C. Longitudinally sectioned labeled dendrite located in the upper 100 μm of the superficial gray. Note the clusters of synaptic vesicles at sites of synaptic contact (arrows and inset). D,E. Examples of transversely sectioned labeled dendrites within the upper superficial gray. Note the loosely dispersed synaptic vesicles in D. Both dendrites are contacted by synaptic endings. Scale: A, B, C (inset), D, E, 0.5 μm; C, 1 μm.

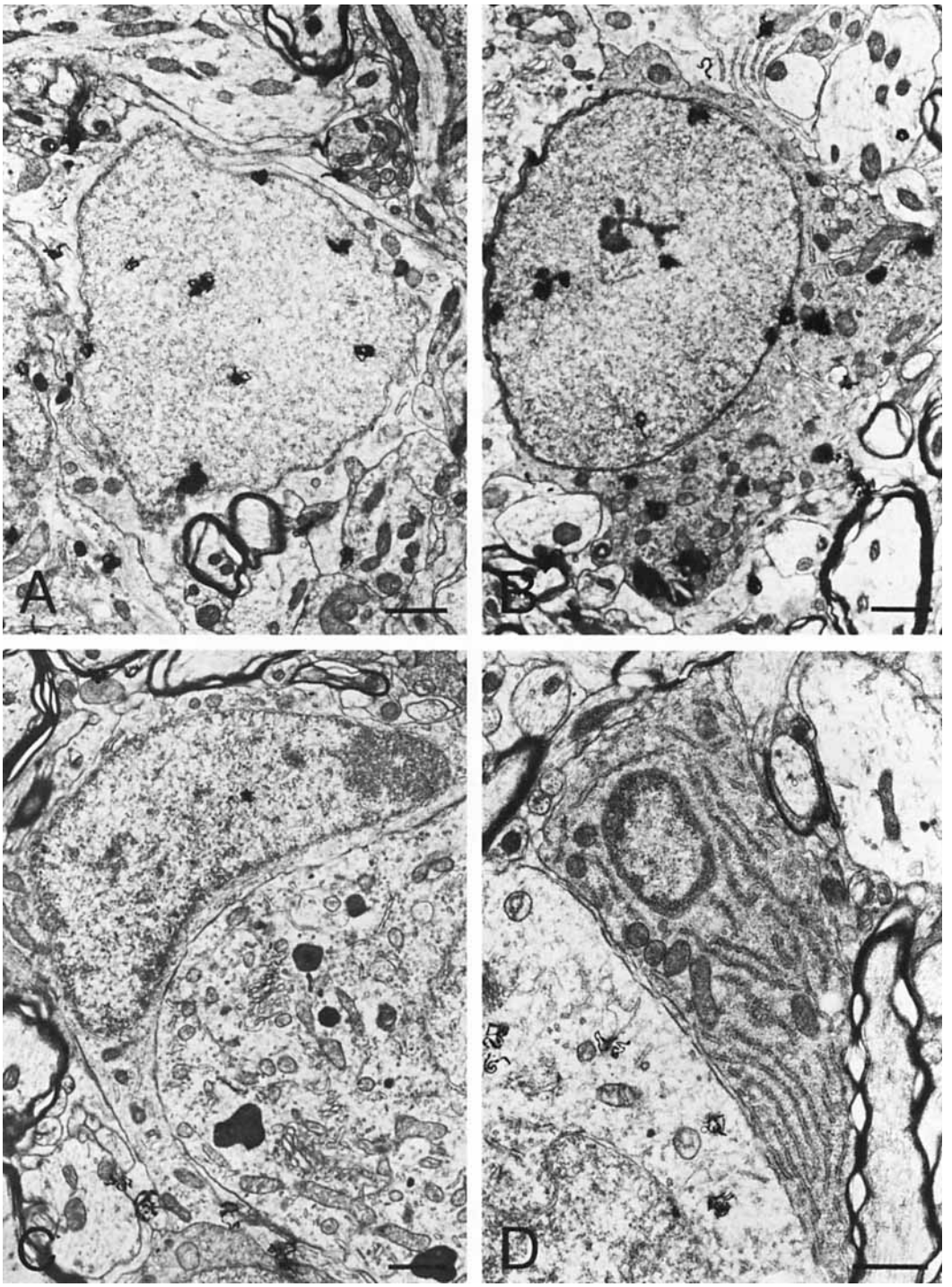

Fig. 7. Four classes of glial cell identified in the cat superior colliculus. A. Astrocyte. B. Dark oligodendrocyte. C. Pale oligodendrocyte. D. Microglial cell. Scale: A–D, 1 μm.

TABLE 2. Glial cell labeling in superior colliculus

Cell type	N	Mean labeling density	Mean cell area	Mean depth
Astrocytes	80	48.59 ±17.69	63.37 ±23.61	159.97 ±128.00
Dark oligodendrocytes	106	55.20 ±16.51	31.79 ± 9.61	247.48 ±144.12
Pale oligodendrocytes	18	15.14 ±11.39	29.88 ±13.04	197.92 ±128.49
Microglia	26	9.29 ± 9.62	25.27 ±12.51	128.37 ± 93.20
Total labeled glia	186	52.36 ±17.29	45.38 ±23.16	209.84 ±143.78
Total unlabeled glia	44	11.68 ±10.66	27.16 ±12.78	156.82 ±113.02
Total glia	230	44.58 ±22.80	41.89 ±22.70	199.70 ±139.77

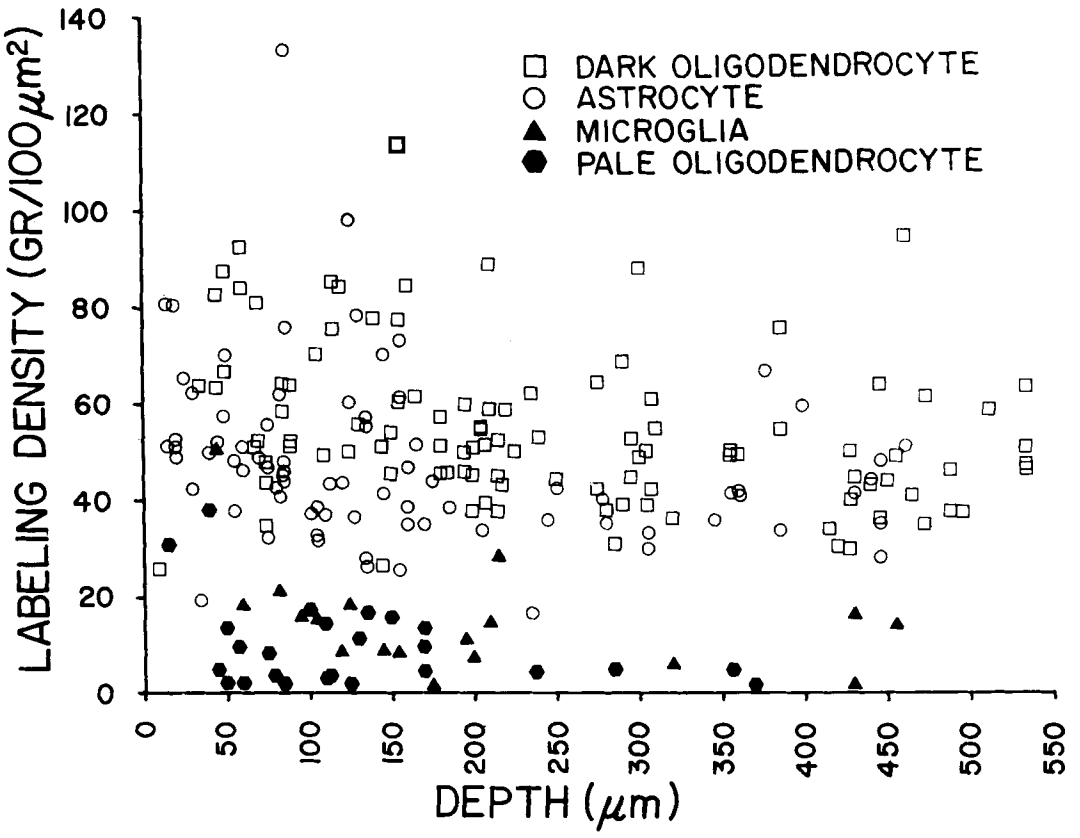

Fig. 8. Distribution of labeling densities (grains/100 μm²) of 230 glia plotted within the upper 550 μm of the superior colliculus. Four classes of glia are identified. The labeling density of individual classes is uniform and independent of depth within the superficial gray layer.

The selective accumulation thus coincides with specific cell morphologies.

GABA as well as other neurotransmitters can be transported both anterogradely and retrogradely (Hunt and Künzle, '76; Streit, '80), and neurons in the upper superficial gray might therefore have been labeled by retrograde transport from the deeper layers. Similarly, boutons in the superficial neuropil might have been labeled by anterograde transport from neurons near the injection site. This idea is attractive because connections have long been suspected between the superficial and deep layers, but evidence for these connections is equivocal (Sprague, '75; Edwards, '80). Although attractive, several arguments oppose this hypothesis. The superficial glia whose processes probably do not extend to the deeper layers accumulate significant amounts of label. They must therefore have been exposed to significant extracellular concentrations of exogenous GABA during the experiment. Furthermore, if deep layer neurons were transporting the GABA anterogradely, significant label would undoubtedly remain in the cell body for the short survival periods used in this study. Yet few labeled cells were observed in the optic and intermediate gray layers near the injection site. For these reasons, we believe the accumulation of GABA occurs by local uptake rather than by axonal transport from a distance. This uptake is reportedly due to a high-affinity membrane transport system which is sodium dependent (Iversen and Kelly, '75; Kelly and Dick, '75; Schon and Iversen, '74).

The accumulation of GABA by glia also apparently results from high-affinity membrane transport (Henn and Hamberger, '71; Iversen and Kelly, '75; Schon and Kelly, '74; Schrier and Thompson, '74). The glial transport can be distinguished from neuronal transport because the uptake rates, transport carriers, and inhibitor specificities differ for the two cell types (Hertz et al., '78; Hösli and Hösli, '79; Schousboe, '79). Glial uptake of GABA is often selective for astrocytes and other glia which are satellite to neurons (Hösli and Hösli, '79; Kelly and Dick, '75; Ribeiro-da-Silva and Coimbra, '80; Schon and Kelly, '74; Schousboe, '79). Both the astrocytes and dark oligodendrocytes labeled in this study are closely associated with neurons. Dark oligodendrocytes are frequently adjacent to neuronal cell bodies, while astrocytic processes often wrap around presynaptic terminals. These two glia are thus likely candidates for removal of extracellular GABA released from nerve terminals, a role they are thought to play in other parts of the nervous system as well (Henn and Hamberger, '71; Iversen and Kelly, '75; Schousboe, '79).

The selective labeling of two separate classes of glia in this study was apparent because these two cell types have such distinctive morphologies. We suspect that GABA is also accumulated by more than one class of neuron. This would explain the variety of cytological characteristics as well as the range of cell sizes and depths at which we found labeled cells. Although it proved impossible to identify the various classes with single sections, three-dimensional reconstructions should allow us to determine the unique cytological features and types of synaptic input of the different labeled cell classes.

At least one of these classes must have presynaptic dendrites. These lie in precisely the zone of the superficial gray that receives significant input from both the retina and visual cortex (Mize and Sterling, '77; Sterling, '73, '75). If GABA proves to be their transmitter, the dendrodendritic junctions formed by these presynaptic dendrites would be ideal for mediating the inhibitory mechanisms activated by retinal and cortical inputs (Sterling, '71; Berman and Sterling, '76).

The idea that collicular GABA-accumulating neurons are actually inhibitory is richly supported by circumstantial evidence. High levels of endogenous GABA and GAD are found in the superior colliculus (Okada, '74, '76). Furthermore, the synaptic terminals which accumulated grains in this study have the same morphologic features as inhibitory, GABA-containing terminals in the cerebellum (Uchizono, '65; McLaughlin et al., '74). Finally, iontophoretic application of GABA onto certain collicular neurons inhibits their responses (Kayama et al., '80; Straschill and Perwein, '71).

These results are consistent with biochemical evidence in other structures showing that neurons which accumulate exogenous GABA are both inhibitory and utilize GABA as a neurotransmitter. In cerebellum, for example, neurons which are known to be inhibitory contain GAD in their somata and synaptic terminals (McLaughlin et al., '74; Ribak et al., '78). GABA transaminase, GABA's degradating enzyme, is found in structures which are postsynaptic to these GAD-labeled synaptic terminals (Barber and Saito, '76; Hyde and Robinson, '76). The same inhibitory cerebellar neurons which are labeled by GAD show a

high-affinity uptake of exogenous GABA (Hökfelt and Ljungdahl, '72; Kelly and Dick, '75; McGeer et al., '75). In this context, our findings clearly support the notion that neurons in the upper layers of the colliculus are inhibitory and suggest the value of reconstructing the connections of these GABA accumulating neurons in more detail.

ACKNOWLEDGMENTS

This study was supported by USPHS Postdoctoral Research Fellowship EY05034 (R.R.M.) and EY00361 (R.F.S.), Research Grant EY00828, and Vision Research Center Grant EY07035 from the National Eye Institute, and USPHS National Institutes of Health Biomedical Research Grant RR-05423. We are grateful to Dan Leonard and Linda Reed for technical assistance. Ms. Bequi Bradford Krell typed the manuscript.

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