

# Vertical Organization of $\gamma$ -Aminobutyric Acid-accumulating Intrinsic Neuronal Systems in Monkey Cerebral Cortex<sup>1</sup>

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## Abstract

Light and electron microscopic methods were used to examine the neurons in the monkey cerebral cortex labeled autoradiographically following the uptake and transport of [<sup>3</sup>H]- $\gamma$ -aminobutyric acid (GABA). Nonpyramidal cell somata in the sensory-motor areas and primary visual area (area 17) were labeled close to the injection site and at distances of 1 to 1.5 mm beyond the injection site, indicating labeling by retrograde axoplasmic transport. This labeling occurred preferentially in the vertical dimension of the cortex. Prior injections of colchicine, an inhibitor of axoplasmic transport, abolished all labeling of somata except those within the injection site. In each area, injections of superficial layers (I to III) produced labeling of clusters of cell somata in layer V, and injections of the deep layers (V and VI) produced labeling of clusters of cell somata in layers II and III. In area 17, injections of the superficial layers produced dense retrograde cell labeling in three bands: in layers IVC, VA, and VI. Vertically oriented chains of silver grains linked the injection sites with the resulting labeled cell clusters. In all areas, the labeling of cells in the horizontal dimension, i.e., on each side of an injection, was insignificant. Electron microscopic examination of labeled neurons confirms that the neurons labeled at a distance from an injection site are nonpyramidal neurons, many with somata so small that they would be mistaken for neuroglial cells light microscopically. They receive few axosomatic synapses, most of which have symmetric membrane thickenings. The vertical chains of silver grains overlie neuronal processes identifiable as both dendrites and myelinated axons, but unmyelinated axons may also be included. The clusters of [<sup>3</sup>H]GABA-labeled cells are joined to one another and to adjacent unlabeled cells by many junctional complexes, including puncta adherentia and multi-lamellar cisternal complexes.

We conclude that groups of GABA-transporting neurons are likely to use GABA as a transmitter and form an inhibitory,

bidirectional system of connections that join together cells in superficial and deep layers of functional cortical columns; intrinsic, horizontal GABAergic connections are either far less significant in the organization of the cerebral cortex or are not labeled by this method. It is possible that the retrogradely labeled somata in both the superficial and deep layers are those demonstrated in other studies (Hendry, S. H. C., E. G. Jones, and P. C. Emson (1984) *J. Neurosci.* 4: 2497-2517) to show immunoreactivity both for neuropeptides and for markers of GABAergic transmission.

Anatomical studies of the cerebral cortex in a wide variety of mammals have revealed two major patterns of intrinsic connectivity: vertical connections which join together cells in two or more laminae, and horizontal connections which link together cells within a single lamina (Nauta et al., 1973; Fisker et al., 1975; Butler and Jane, 1977; Creutzfeldt et al., 1977; Colonnier and Sas, 1978; Jones et al., 1978; Rockland et al., 1982; Rockland and Lund, 1983). The vertical connections appear to form one of the principal bases of the columnar functional organization of the cortex (see Jones, 1981). Connections linking the principal thalamic receiving layers to layers above and below them are perhaps the major links in ensuring that all cells within a column share similar receptive field properties. Convergence of vertically organized inputs appears responsible, at least in part, for the additional functional properties that distinguish superficial and deep cortical cells from those lying at the heart of the thalamic terminal layers (e.g., Gilbert and Wiesel, 1979). Horizontal connections, in contrast, would form the basis for interactions between functional columns.

Two major groups of cells make intracortical connections. Axons of pyramidal cells project out of the cortex (Gilbert and Kelly, 1975; Lund et al., 1975; Wise, 1975; Jones and Wise, 1977), but these axons also give rise to elaborate systems of horizontal and recurrent collaterals as they descend toward the white matter (Ramón y Cajal, 1911; Feldman, 1984). Recent experiments have shown that in the visual and sensory-motor cortex these collaterals can terminate at intervals in concentrated clusters (Gilbert and Wiesel, 1983; J. DeFelipe, M. Conley, and E. G. Jones, submitted for publication) and form complex lattice-like patterns (Rockland et al., 1982; Rockland and Lund, 1983). The reverse of this pattern has been shown in studies of retrograde labeling from a single injection site in the sensory-motor cortex: multiple patches or strips of pyramidal cells project to a single cortical focus (Jones et al., 1978).

Axons of nonpyramidal cells remain within the cortex in most areas. They make up the second group of elements that establish intracortical connections. The nonpyramidal cells can be subdivided into those with significant populations of dendritic spines and those without. In addition, the non-spiny type can be further subdivided into several classes, based on morphological criteria, in particular,

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the shape and distribution of their axonal ramifications (Valverde, 1971; Jones, 1975; Fairén et al., 1984). Because of their highly stereotyped axonal arborizations, each type of nonpyramidal cell can be predicted to exert its effects either locally, vertically across laminae, or horizontally within one or more laminae.

Efforts to determine the functional characteristics of the horizontal and vertical circuit elements of the cortex have employed a number of techniques. Among these are the intracellular injection of markers into physiologically characterized neurons (Gilbert and Wiesel, 1979; Parnavelas et al., 1983; Martin and Whitteridge, 1984) and the immunocytochemical staining of neurons for specific neurotransmitter substances or their synthesizing enzymes (Emson and Hunt, 1984; Houser et al., 1984). These studies have positively identified two morphological classes of non-spiny, nonpyramidal cells as GABAergic: chandelier cells, the axons of which terminate on axon initial segments of pyramidal cells, and basket cells, the axons of which terminate primarily on somata of pyramidal cells (Peters et al., 1982; Hendry et al., 1983; Freund et al., 1983; DeFelipe et al., 1985b). Another method for identifying transmitter-specific intrinsic neurons and their connections relies on the probably selective capacity of neurons to take up with high affinity and axoplasmically transport substances used by them as neurotransmitters (Cuénot et al., 1982). The cells of origin of certain putatively GABAergic pathways have thus been labeled by the retrograde transport of tritiated GABA (Streit et al., 1979), although the specificity of the method has sometimes been disputed (e.g., Zucker et al., 1984). The uptake of [<sup>3</sup>H]GABA in the cerebral cortex has been used to examine the distribution of GABAergic neurons (Hökfelt and Ljungdahl, 1972; Chronwall and Wolff, 1978; Hendry and Jones, 1981; Wolff and Chronwall, 1982); and in a series of reports on presumed [<sup>3</sup>H]GABA transport, the idea has been advanced that systems of GABAergic axons synaptically link superficial and deep layers of the mammalian visual cortex (Somogyi et al., 1981, 1983a). In the present study we have used an inhibitor of axoplasmic transport in several experiments to prove unequivocally that many cortical neurons are indeed labeled by the axoplasmic transport of [<sup>3</sup>H]GABA, and we have used light and electron microscopic methods to examine the neurons in the first somatic sensory, primary motor, and primary visual areas of monkey cortex labeled by retrograde transport of [<sup>3</sup>H]GABA. We have looked for patterns of organization that are common to all areas as well as those that distinguish one functional area from the others.

## Materials and Methods

Six adult cynomolgus monkeys (*Macaca fascicularis*) were used in this study. Each animal was anesthetized by an intramuscular injection of ketamine and maintained on intravenous injections of Nembutal. A solution of [<sup>3</sup>H]GABA (specific activity, 40.2 Ci/mmol; New England Nuclear) in normal saline was injected by applying air pressure through glass micropipettes at multiple sites in the pre- and post-central gyri and in the occipital cortex. Three of the monkeys were injected intravenously with the GABA transaminase inhibitor, amino-oxyacetic acid (40 mg/kg of body weight), 40 min before the [<sup>3</sup>H]GABA injections. [<sup>3</sup>H]GABA for injection was evaporated and reconstituted in normal saline (final activity, 20 or 40  $\mu$ Ci/ $\mu$ l). The injections were made at different angles and depths with respect to the central and lunate sulci in order to affect particular layers without contamination of the overlying layers of the cortex. As a further guide to localization, multiunit activity was recorded via a silver wire inserted in the [<sup>3</sup>H]GABA solution. Layer IV and the layer VI-white matter junction could usually be identified in this way. Single or multiple deliveries of isotope solution, each consisting of 0.025 to 0.06  $\mu$ l, were made. Multiple injections were commonly made at intervals or continuously along a track as the pipette was withdrawn.

One monkey received multiple injections of colchicine (10  $\mu$ g/ $\mu$ l) into the sensory-motor and primary visual areas of the cortex 2 days before injection of [<sup>3</sup>H]GABA. The subsequent injections of [<sup>3</sup>H]GABA were aimed at closely adjacent sites. This animal was also pretreated with amino-oxyacetic acid.

Fifteen minutes after the last injection of [<sup>3</sup>H]GABA and a maximum of 90 min after the first injection, the monkeys were perfused with a solution of 2.5% glutaraldehyde and 1.0% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2. The brains were removed and immersed in the same fixative at 4°C for about 4 hr. Then, blocks of cortex containing the injection sites were

removed and processed in one of two ways. Thin blocks from four hemispheres were Golgi impregnated and gold toned (Fairén et al., 1977). These blocks were then postfixed in 2% osmium tetroxide in phosphate buffer, stained in the block with 2% uranyl acetate in 70% ethanol, dehydrated, and flat-embedded in Spurr's resin between microscope slides coated with silicone. Later, serial 1.5- $\mu$ m-thick sections were cut from these blocks with glass knives and were mounted by heating onto sheets cast from Spurr's resin and having the same dimensions as a microscope slide.

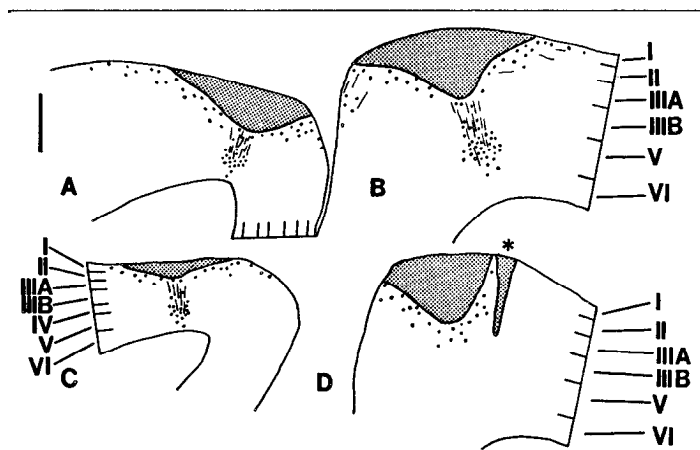
The second group of blocks was cut serially as 25- $\mu$ m-thick frozen sections and mounted on gelatinized slides so that, for every pair of sections, the apposed cut surfaces faced upward. All of the semithin sections from gold-toned blocks and all of the frozen sections were coated with Kodak NTB2 emulsion and exposed at 4°C for 4 to 12 weeks. The sections were then developed in Kodak D19 and fixed, and the frozen sections were stained through the emulsion with thionin. Semithin sections used for autoradiography were not counterstained, but additional semithin sections from parts of the blocks remaining after the original autoradiographic semithin sections had been taken were stained with methylene blue and azure II (Richardson et al., 1960). The distribution of autoradiographically labeled cell bodies was plotted onto projection drawings of the sections and composite drawings were made by superimposing individual plots of 10 to 20 serial frozen section autoradiographs. Semithin section autoradiographs that displayed labeling of somata and neuropil were photographed, resectioned without removing the emulsion at 65 to 70 nm (DeFelipe and Fairén, 1982) on a Porter-Blum MT2B Ultramicrotome, and then collected on Formvar-coated single slot grids. The new thin sections were stained with lead citrate (Reynolds, 1963) and examined on a Zeiss EM-9S or a Philips EM 300 electron microscope. Additional blocks of sensory and motor cortex from the animals injected with [<sup>3</sup>H]GABA were stained by the rapid Golgi technique without subsequent gold toning or autoradiography. These were examined light microscopically.

## Results

### Light microscopy

#### General considerations

As described previously (Chronwall and Wolff, 1980; Hendry and Jones, 1981), cortical injection sites of [<sup>3</sup>H]GABA can be divided into a central dense core in which many cells and the neuropil are heavily labeled, and a peripheral zone in which a relatively small number of labeled cells are surrounded by a progressively more lightly stained neuropil (Figs. 1 and 2). Outside the peripheral zone, separated from the injection site by distances of 1 to 1.5 mm, additional, heavily labeled cell bodies are often found. These cell bodies are frequently grouped together in dense clusters and are



**Figure 1.** Camera lucida drawings of sagittal sections showing injections of [<sup>3</sup>H]GABA (stippling) into the superficial layers of the sensory-motor cortex. Many labeled cells (dots) are found adjacent to the injection site in SI (A and C) and in the primary motor area (B). Vertical chains of silver grains (lines) run between the injection sites and clusters of labeled cells in the deep layers in A to C. In D, both the vertical, autoradiographic labeling and the labeling of clusters of deeply situated cells are abolished by prior injection of colchicine (asterisk). Sections are from monkeys untreated (C) and pretreated (A, B, and D) with amino-oxyacetic acid. Bar = 1 mm.

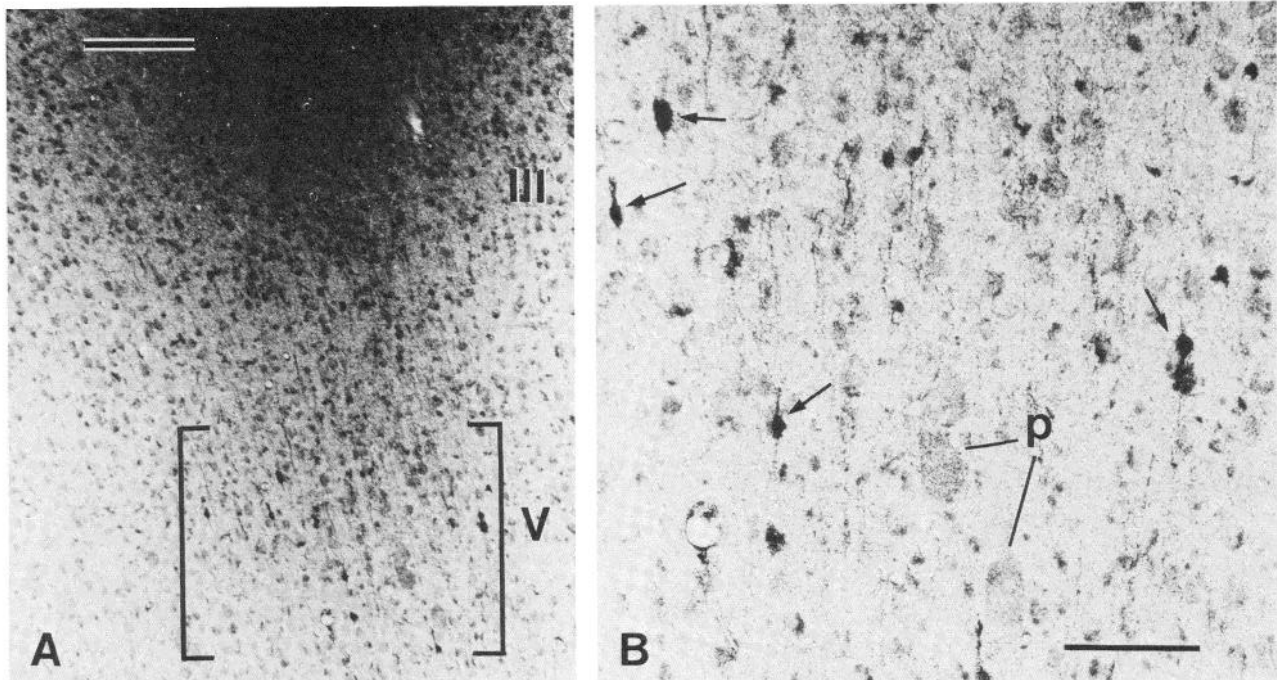


Figure 2. Photomicrographs of a [ $^3\text{H}$ ]GABA injection and retrograde cell labeling in motor cortex of a monkey pretreated with amino-oxyacetic acid. The injection of [ $^3\text{H}$ ]GABA is in layers I to IIIA (A). Many labeled cells are found around the injection and, at some distance, in layer V. Bar = 200  $\mu\text{m}$ . B shows a higher magnification of the area bracketed in A. Small autoradiographically labeled cells (arrows) are intermingled with background-labeled, thionin-counterstained pyramidal cells (p). Bar = 100  $\mu\text{m}$ .

most apparent directly above or below an injection site and, rarely, to the sides of an injection site (Figs. 2 and 4). Because they lie well outside the zone of obvious diffusion of the injected [ $^3\text{H}$ ]GABA, these cells are considered to be labeled by the retrograde transport and somal accumulation of the tritiated amino acid. Our experiments in which axoplasmic transport was inhibited support this interpretation (see below). In addition to labeling of cell bodies at a distance, many sections contained vertical aggregations of silver grains, ascending from deep or descending from superficial injection sites (see Fig. 4B). Chains of silver grains were also found parallel to the pial surface in layers I and II, but these were less conspicuous and occurred only when the superficial layers were, themselves, heavily involved in an injection.

The same general pattern of labeling is seen in monkeys pretreated with amino-oxyacetic acid as in those left untreated. The prior processing of the tissue used for semithin sections by Golgi impregnation with gold toning produced no changes in the autoradiographic labeling pattern when compared with the frozen section autoradiographs.

#### Identification of labeled neurons

Outside the central dense core of an injection site, all neurons labeled autoradiographically possessed the morphological features characteristic of non-spiny nonpyramidal cells. The vast majority of [ $^3\text{H}$ ]GABA-accumulating cells had rounded or oblong cell bodies, none gave rise to a typical apical dendrite, and no labeled dendritic spines were visible on labeled segments of their dendrites. By resectioning semithin section autoradiographs and examining them in the electron microscope, we found that somata and proximal dendrites of [ $^3\text{H}$ ]GABA-accumulating cells lacked spines and received both asymmetrical (type I) and symmetrical (type II) synapses (Gray, 1959; Colonnier, 1968), a feature that characterizes the majority of nonpyramidal cells (Jones and Powell, 1970; Sloper and Powell, 1979).

#### Vertical connectivity

**Sensory motor cortex.** Many injections of [ $^3\text{H}$ ]GABA were placed to affect maximally only two or three layers. When injections were

made into the superficial layers (I to IIIA) of either the first somatic sensory area (SI) or the primary motor area (area 4), clusters of heavily labeled cells were found in the layers deep to the injection site, as much as a millimeter from the zone of greatest diffusion (Figs. 1 and 2, A and B). These are considered to be labeled by retrograde axoplasmic transport. Vertically oriented chains of silver grains ran from the injection site to clusters of labeled cell bodies in layer V. A small number of [ $^3\text{H}$ ]GABA-labeled cells were also found in layer VI and scattered in layers IIIB, IV, and in the white matter subjacent to layer VI. The number of cells in layers V, VI, and the subjacent white matter were greatest following large injections that included layer IIIA (Fig. 1, A and B), but labeled neurons were still found in layers V and VI below injections restricted to layers I and II (Fig. 1C).

Injections of [ $^3\text{H}$ ]GABA that were placed close to the needle track left by a prior injection of colchicine produced intense labeling of neurons in the peripheral zone surrounding the densely labeled injection core and in the core itself (Fig. 1D). This somal labeling was heavier than the somal labeling in the core and peripheral zones of the injection sites of non-colchicine-pretreated animals. However, in no case where the colchicine and [ $^3\text{H}$ ]GABA injections were made within a millimeter of one another in layers I to III did any labeling of cell somata occur in layers V or VI (Fig. 1D).

By introducing the injection pipette at an acute angle to the cortical surface or down the bank of the central sulcus, it was possible to make injections of [ $^3\text{H}$ ]GABA that affect the deep cortical layers without contamination of the layers lying immediately superficial to the injection site. Injections in middle layers (IIIB to V) of the sensory-motor cortex (Figs. 3A and 4A) resulted in retrograde labeling of somata mostly in layer II and in the adjacent part of layer IIIA (Fig. 4, B and C). A few cells were also labeled in layer I, but in contrast to the retrograde labeling seen after injections of layers I to IIIA, none were found in layers V or VI. With injections of layers V or VI, clusters of heavily labeled cells were seen again in the overlying superficial layers, particularly layers II and IIIA (Fig. 3B). Numerous chain-like aggregates of silver grains were again found to run along radial lines joining the injection site to the region of labeled cells in the superficial layers (Fig. 4B). Figure 3C illustrates the labeling of

cell bodies resulting from three injections of [<sup>3</sup>H]GABA in the posterior bank of the central sulcus. The injection sites vary in depth: one includes layers IV and V, one includes layers V and VI, and one includes layer VI and subjacent white matter. In the composite figure (Fig. 3C) it is clear that, regardless of depth, each injection results in labeling of cell bodies in layers II and IIIA.

In all cases of deep injections the cells labeled by retrograde transport lie along radial lines, directly superficial to the injection site. Few or no cells were retrogradely labeled in layers V and VI following injections largely confined to layers III and/or IV. The radial arrangement of the retrogradely labeled cells is particularly clear superficial to the large injection site shown in Figures 3B and 5A. After this and

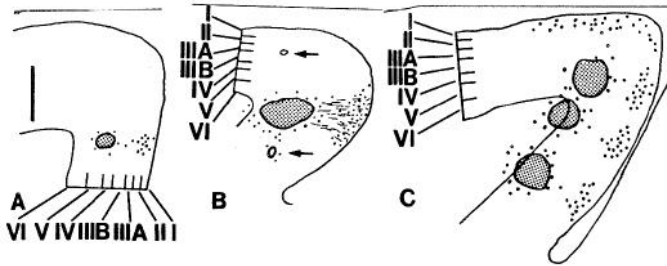


Figure 3. Camera lucida drawings of injections of [<sup>3</sup>H]GABA in the middle and deepest layers of the somatic sensory cortex. Injections (stippling) in area 1 (A and B) produce labeling of neuronal clusters (dots) in layers II and IIIA. C is a composite drawing of three injections from a single penetration down the posterior bank of the central sulcus. Each injection (stippling) is in the deep layers and produces cell labeling (dots) in layers II and IIIA. Sections are from monkeys pretreated (A and C) and untreated (B) with amino-oxyacetic acid. Bar = 1 mm.

similarly placed and sized injections, no cells are labeled with [<sup>3</sup>H]GABA to the sides of the injections, at distances equal to the distance between the injection and the superficially placed, labeled somata (Fig. 5, B and C).

Our results in the sensory-motor areas thus indicate that layer V is the main source of putative GABAergic inputs to layers I to IIIA, that layers II and IIIA are the main sources of putative GABAergic inputs to layers V and VI, and that layers IIIB and IV, although receiving inputs from layers I to IIIA, receive few or no putative GABAergic inputs from layers V and VI.

**Visual cortex.** Injection of [<sup>3</sup>H]GABA into the primary visual area (area 17) gave rise to labeled cells in situations similar to those seen in the sensory-motor cortex: injections in deep layers (IV to VI) produced retrograde cell labeling in layer II and the upper half of layer III, and injections in the superficial layers (I to III) produced cell labeling in the deeper layers (V and VI) (Fig. 6). However, a major difference between the visual and sensory-motor areas was the presence of far more significant retrograde cell labeling in layer IV of the visual area after superficial injections. Unlike the sensory motor areas, injections located in layers III and IVA in area 17 not only resulted in heavy cell somata labeling in layers V and VI, as in the sensory motor cortices, but also led to cell somal and neuropil labeling in layer IVC (Fig. 7). An injection confined to layer IVA alone specifically labeled a small, dense patch of cell somata in layer IVC. The [<sup>3</sup>H]GABA-accumulating cells in layer IVC and in the adjacent part of layer V appeared uniform in size, with somata 8 to 10 μm in diameter, like those retrogradely labeled in other layers.

All of the results in the three areas examined are summarized in Table 1.

**Horizontal connectivity.** In the sensory-motor cortex, some but not all injections of [<sup>3</sup>H]GABA in layers I to III produced fiber-like chains of autoradiographic labeling that spread laterally mainly in

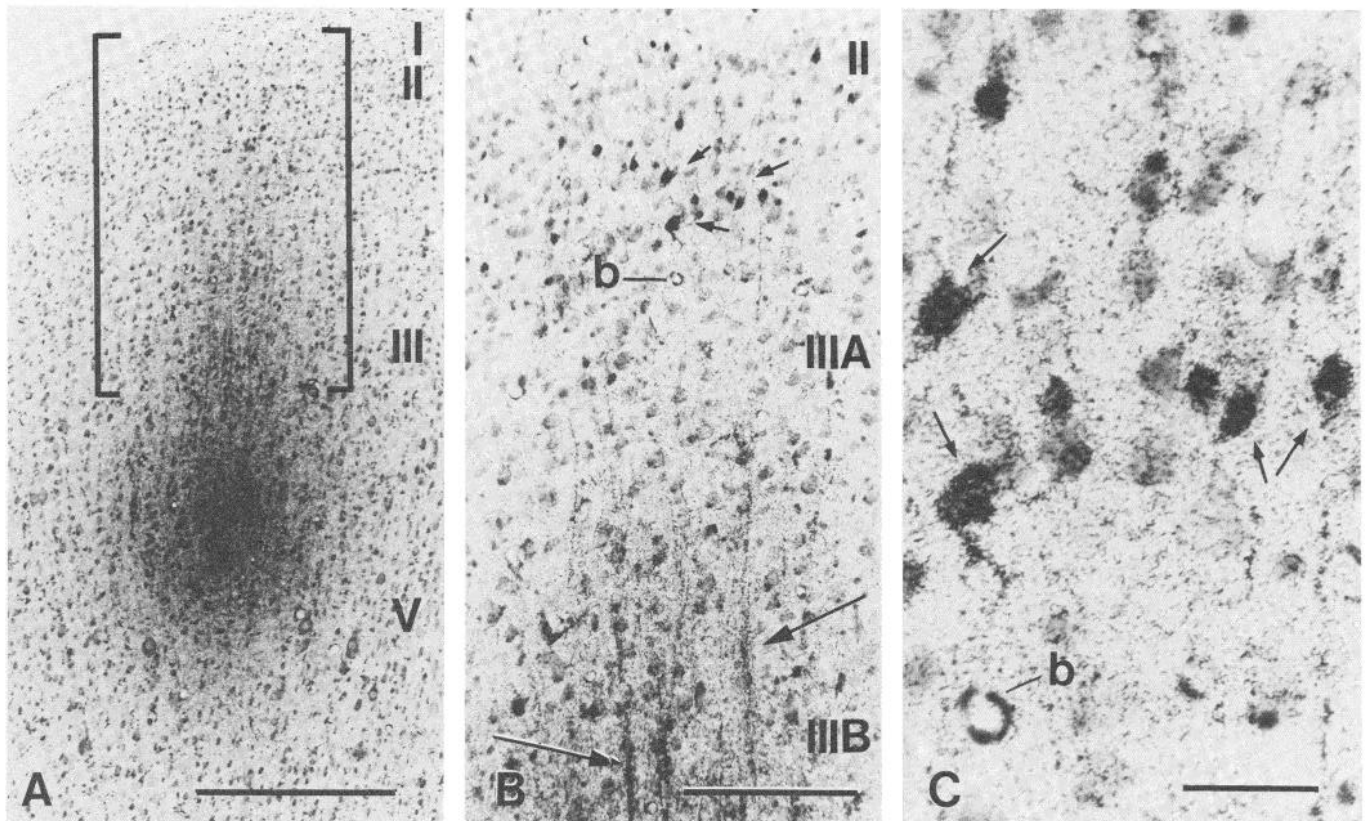


Figure 4. Photomicrograph (A) of an injection of [<sup>3</sup>H]GABA into layers IIIB to V of area 4 and the accompanying cell labeling (B and C). Bar = 500 μm. B is a higher magnification of the area bracketed in A. Vertical chains of silver grains (large arrows) are found issuing from the injection site. A large number of cells (small arrows) are found in layer II above a blood vessel (b) and are separated from the injection by a gap in layer IIIA. Bar = 200 μm. C is a higher magnification of the upper part of B, showing the same cluster of labeled cells (arrows) and the same blood vessel (b) as in B, from a monkey pretreated with amino-oxyacetic acid. Bar = 30 μm.

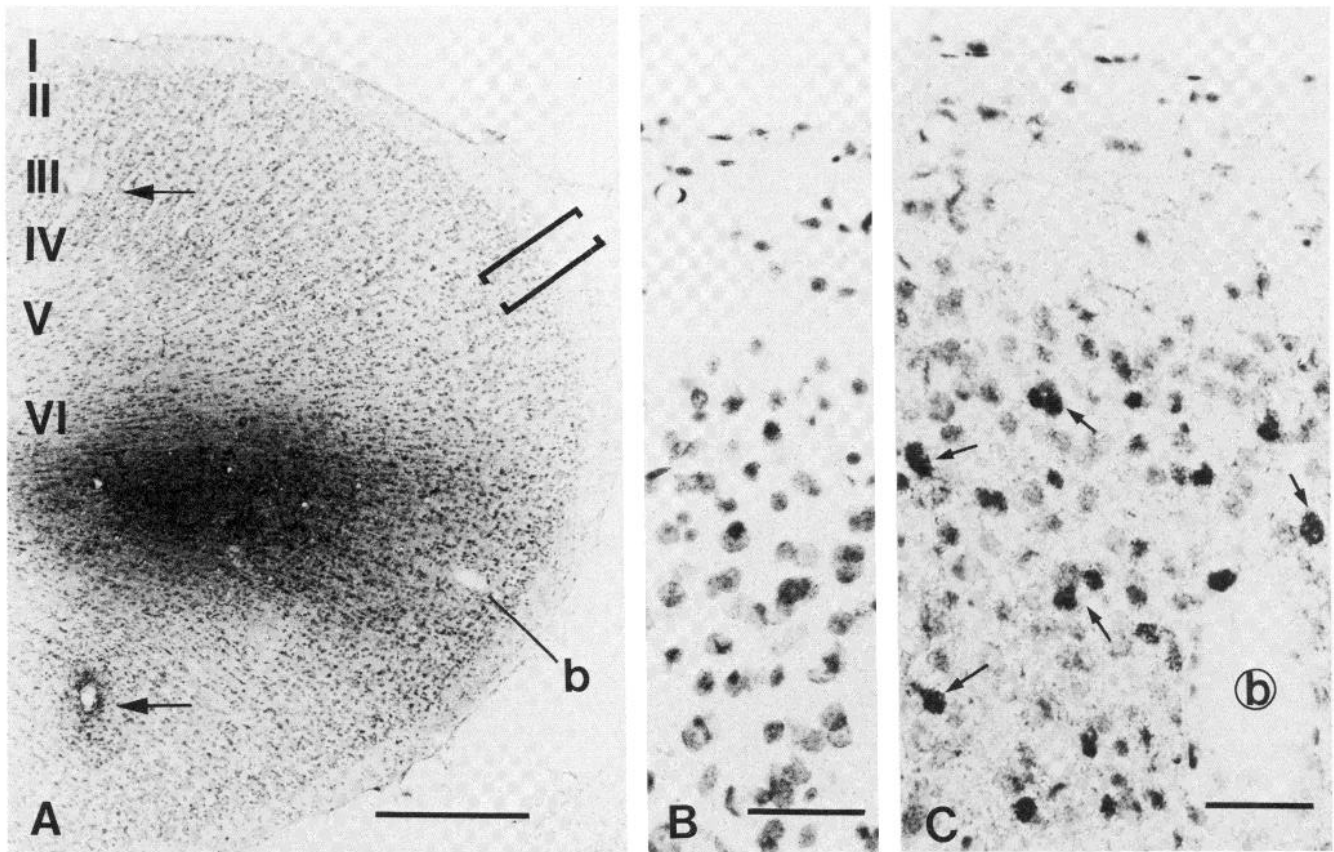


Figure 5. Photomicrographs of a  $[^3\text{H}]\text{GABA}$  injection and cell labeling in area 1. A, Photomicrograph of the injection in layers V and VI made along a vertical pipette track (arrows). Dorsal is up, and anterior is to the left. Labeled cells near the blood vessel (b) are shown in C. Bar = 500  $\mu\text{m}$ . B is a higher magnification of the area bracketed in A. Only thionin-stained cells are found. Bar = 50  $\mu\text{m}$ . C is a magnification of the part of A near the blood vessel (b), showing autoradiographically labeled cells (arrows). Bar = 50  $\mu\text{m}$ .

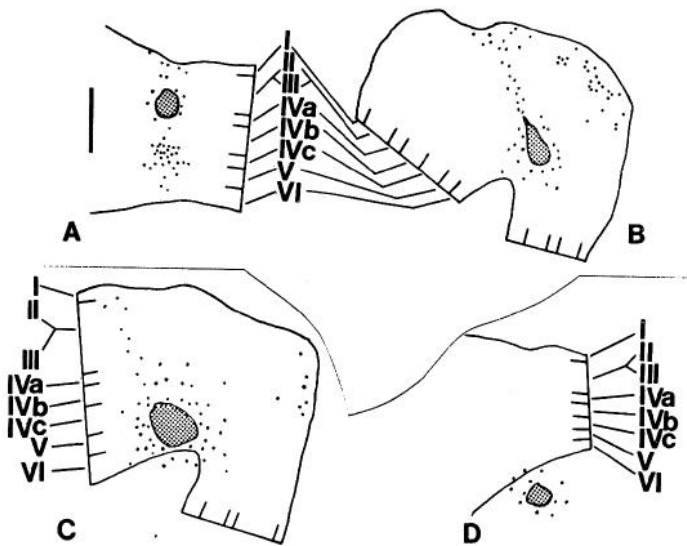


Figure 6. Camera lucida drawings of injections of  $[^3\text{H}]\text{GABA}$  (stippling) in area 17 and the resultant labeling of cell bodies (dots). Injections in superficial layers (A) produce labeling of cells in layers IVC, VA, and VI. Injections into layers V and VI (B and C) produce labeling of cells in layers I and III. Injections into the white matter label nearby cells only (D). The animal was not pretreated with amino-oxyacetic acid. Bar = 1 mm.

layer I, just below the pial surface. Accompanying this labeling, scattered somata were also labeled in layers I and II. We consider that this lateral spread of labeling can be explained by the direct diffusion of  $[^3\text{H}]\text{GABA}$  over the cortical surface during withdrawal of

the micropipette, because in these cases the pia mater showed a heavy accumulation of silver grains over a large extent. Horizontal fiber-like aggregations of silver grains sometimes spread out horizontally for short distances on either side of an injection site in deeper layers, but very few or no labeled cells could be detected among them. No labeled neurons in the lateral dimension could be found in any layer further than approximately 600  $\mu\text{m}$  beyond an injection site. Neurons labeled in the horizontal domain were relatively small (less than 15  $\mu\text{m}$  somal diameter) and never formed clusters, as found for the cells retrogradely labeled in the vertical dimension. In order to examine further the lateral extent of retrograde labeling, we exposed serial semithin sections for autoradiography after trimming a block already known to contain an injection in layer III B of area 4. This layer is known to contain a large population of the large, GABAergic basket cells (Jones and Hendry, 1984), which have been described as having long horizontal axons (Jones, 1975). In this experiment we did not find labeled neurons farther than 600  $\mu\text{m}$  from the core of the injection site in the horizontal dimension.

In the visual cortex a pattern of lateral labeling similar to that in the sensory motor areas was seen. However, when injections of  $[^3\text{H}]\text{GABA}$  were located in layers V and VI, the lateral spread of labeled cells was more extensive than in the superficial layers and the most extensive of the three areas studied. Some labeled somata in layer VI of area 17 were as much as 700  $\mu\text{m}$  from the injection site, but they were relatively few and never formed clusters.

Thus, the lateral extent of cell somata retrogradely labeled by uptake and transport of  $[^3\text{H}]\text{GABA}$  is small. It appears that horizontal (intralaminar) connectivity demonstrated by this method is insignificant in comparison with the heavy vertical (interlaminar) connectivity established by cells with somata labeled in both superficial and deep layers.

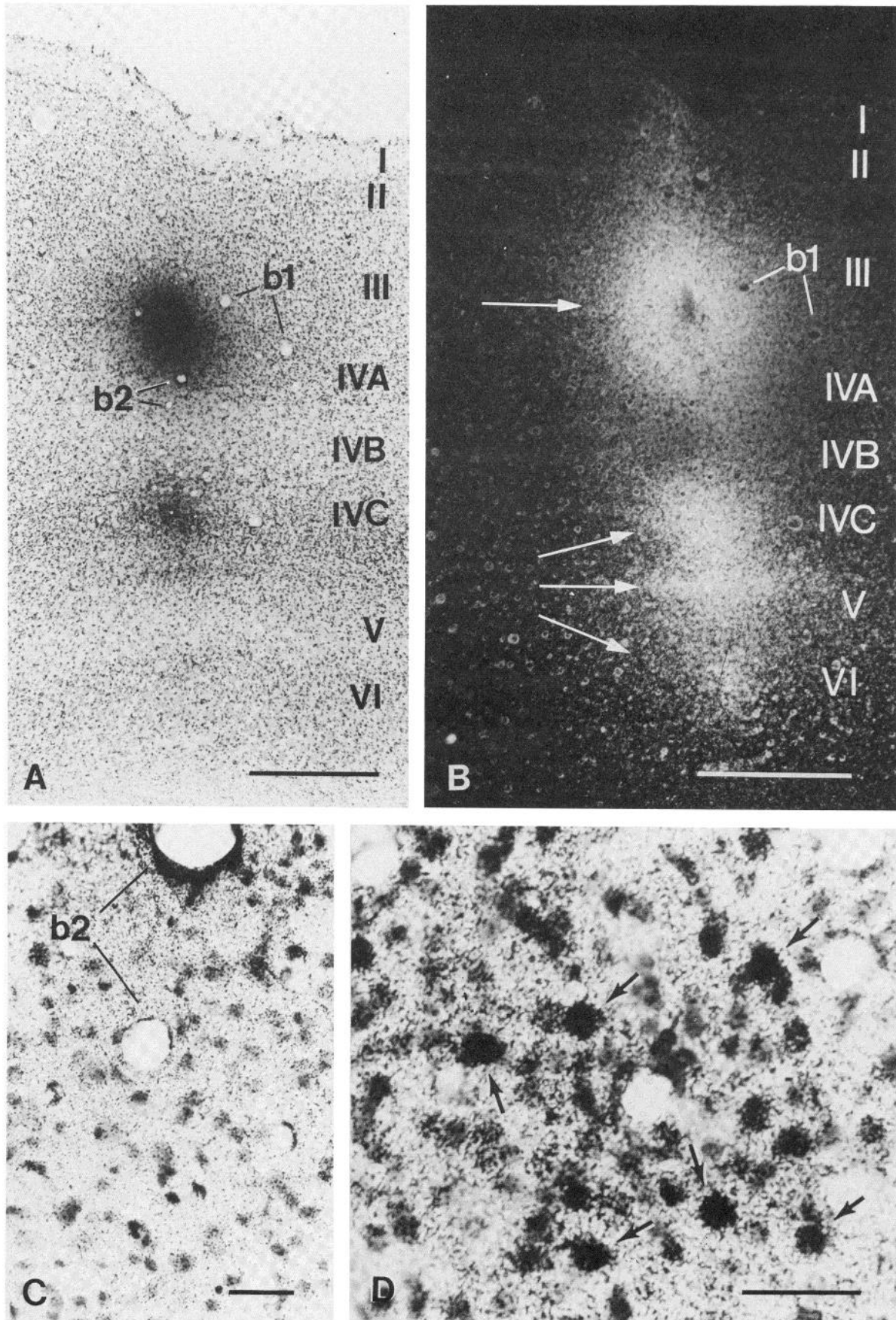


Figure 7. Photomicrographs of  $[^3\text{H}]\text{GABA}$  injection and cell labeling in area 17. Brightfield (A) and darkfield (B) photomicrographs of an injection primarily in layers III and IVA flanked by blood vessels (*b1* and *b2*). The injection as seen in B (single arrow) produces three very distinct bands of cell labeling indicated by arrows in layers IVC, VA, and VI. Lamination is according to the system of Brodmann (1905) as modified by Lund (1973). Bars = 500  $\mu\text{m}$ . Higher magnification photomicrographs (C and D) of A show few autoradiographically labeled cells around two blood vessels (*b2*) in layer IVB (C) and many labeled cells (arrows) in layer IVC (D). The animal was not pretreated with amino-oxyacetic acid. Bars (C and D) = 30  $\mu\text{m}$ .

TABLE I

Summary of the laminar distribution of retrogradely labeled somata after injections of [<sup>3</sup>H]GABA in the three areas examined; first somatic sensory area (SI), primary motor area (area 4), and primary visual area (area 17)

Site of Injection	Layers Containing Retrogradely Labeled Somata						
	I	II	III A	III B	IV	V	VI
SI							
I-II				+	+	+++	+
I-III A				+	++	+++++	+
IV-VA	+	+++	+				
IV-VI	+	+++++	++				
V-VI	+	+++	++				
Area 4							
I-III A				++		+++	+
III B-VA	+	+++++	++				
Area 17					IVC		
III-IV A					+++++	+++	+
IVC-V	+	+++	++				
V-VI	+	++	+				

<sup>a</sup> The relative numbers of labeled cells in each layer are indicated by the number of crosses.

Electron microscopy of [<sup>3</sup>H]GABA-accumulating cells

**Cell bodies.** In the somatic sensory cortex 5 neurons in layer III B (Fig. 8) and 22 in layer V, all labeled by retrograde transport of [<sup>3</sup>H]GABA from injections in layers I and II (Fig. 9A), were identified in autoradiographs of serial semithin sections. All were from animals pretreated with amino-oxyacetic acid. These neurons were then resectioned as ultrathin sections and examined in the electron microscope. All of the labeled cell bodies were small (7 to 12 μm in diameter) (Figs. 8A, 9, B and C, 11, A and B, and 12C) and possessed nuclei containing clumped chromatin and with highly folded nuclear envelopes (Figs. 10A and 11E). A general lack of cytoplasmic organelles, especially of rough endoplasmic reticulum, was a conspicuous feature of the cells, causing them to closely resemble astrocytes. However, unlike astrocytes, axon terminals formed synaptic contacts on the labeled cells and the somata gave rise to obvious dendrites. The number of terminals was small, rarely larger than 1 or 2 on a single thin section, and in some cases more than 10 serial ultrathin sections had to be examined to identify a single synaptic contact. All of the axosomatic synapses onto the labeled cells were of the symmetrical type (Figs. 8C and 11D). No unequivocal asymmetrical synapses were found on the cell bodies. Of the 27 cells labeled in layers V or VI by superficial layer

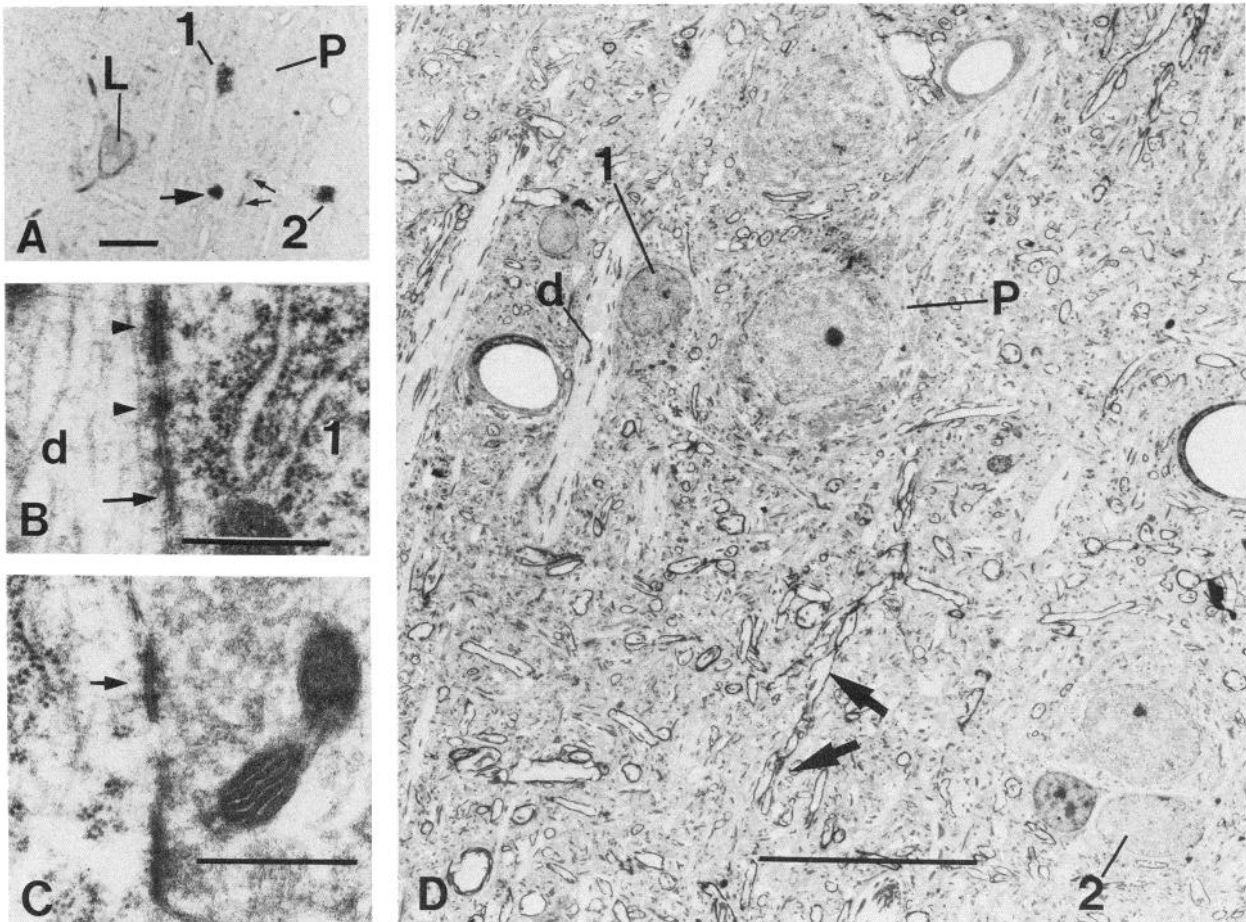
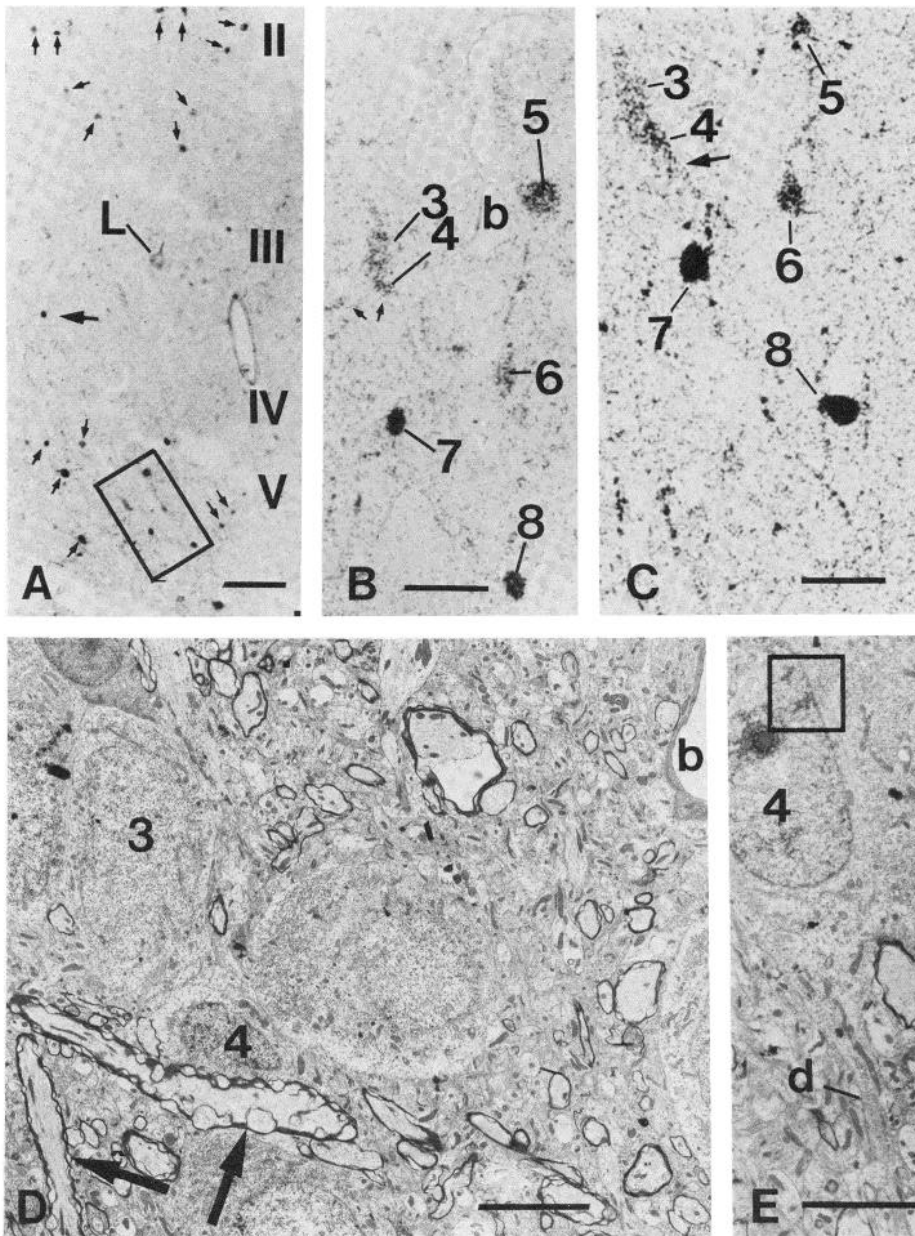


Figure 8. Light and electron micrographs of two cells (1 and 2) in layer III B of area 1 labeled autoradiographically by an injection of [<sup>3</sup>H]GABA into layers I and II. A, Photomicrograph of a semithin section from which the thin section shown in D was cut, showing the labeled cells, a Golgi-impregnated nonpyramidal cell (L) and an unlabeled pyramidal cell (P). The small arrows indicate a chain of silver grains. The large arrow indicates an artifact. Bar = 30 μm. B, Higher magnification of cell 1 as seen in A and D and its association with the apical dendrite (d) of a pyramidal cell also seen in A and D. The junction between the two is characterized by puncta adherentia (arrowheads) and an additional zone of specialized contact (arrow). Bar = 0.5 μm. C, Synapse (arrow) of an axon terminal onto the soma of cell 1. The synapse was clearly symmetric when compared with axodendritic asymmetric contacts (e.g., Fig. 12E). Bar = 0.5 μm. D, Electron micrograph of 60-nm-thick sections cut from the semithin section in A. The two [<sup>3</sup>H]GABA-labeled cells (1 and 2) and the unlabeled pyramidal cell (P) seen in A are all indicated. Arrows indicate the part of the neuropil seen labeled as a chain of grains in A and revealed in D as a small myelinated axon. Note the small size of cells 1 and 2 and their superficial similarity to neuroglial cells. Bar = 200 μm.



**Figure 9.** Serial light and electron micrographs of a cluster of neurons in layer V of area 1, labeled by the injection of [ $^3\text{H}$ ]GABA in layers I and II. **A**, Semithin section containing numerous labeled neurons (*small arrows*) around the injection site in layer II and others labeled by retrograde transport in layer V; one other labeled cell occurs in layer III (*large arrow*), near a Golgi-stained nonpyramidal cell (*L*). *Bar* = 100  $\mu\text{m}$ . **B** and **C**, Consecutive 1.5- $\mu\text{m}$  semithin sections of the region shown boxed in **A**. Six [ $^3\text{H}$ ]GABA-labeled neurons (3 to 8) and radially oriented chains of silver grains are found in each section. *Bars* (**B** and **C**) = 30  $\mu\text{m}$ . **D** and **E**, Electron micrographs of the cells 3 and 4 after resectioning the semithin sections illustrated in **B** and **C**. In **D**, *arrows* indicate the same two unlabeled myelinated axons indicated by *small arrows* in **B**. *b*, same blood vessel. A dendritic process arising from the lower part of cell 4 in **E** was also radiolabeled (*arrow* in **C**). For the boxed area, see Figure 10, **C** and **D**. *Bars* (**D** and **E**) = 5  $\mu\text{m}$ .

injections and examined in the electron microscope, 19 were members of clusters made up of two or more neurons having membranes which were closely apposed over a considerable distance. Within a cluster, the [ $^3\text{H}$ ]GABA-labeled neurons were often associated with the somata or dendrites of other unlabeled nonpyramidal cells and with the somata or dendrites of pyramidal cells (Figs. 8D, 9D, 10A, and 11E). In Figure 9, serial light and electron micrographs are shown of neurons in layer V of area 1 of the first somatic sensory cortex labeled autoradiographically following an injection of [ $^3\text{H}$ ]GABA into layers I and II. The labeled neurons in this group (Figs. 9, B and C, and 12C) were closely associated with one or more other neurons. In Figure 9, D and E, labeled neuron 3 was found to be part of the cluster that included a second, labeled neuron (neuron 4) and two large unlabeled neurons.

Although we did not find typical gap junctions between any of the elements of a cluster, in all cases the apposed plasma membranes of adjacent elements showed contacts resembling puncta adherentia (Figs. 8B and 10B) and other junctional specializations that consisted of a close apposition between membranes, with particu-

larly narrow (5 to 9 nm wide) clefts containing electron-dense material. In some cases a thin electron-dense coating was associated with the cytoplasmic face of one or both of the membranes (Figs. 8B and 10B) and sometimes a multilamellar subsurface cisternal complex (Fig. 10, C and D) was associated with one of the plasma membranes.

**Processes.** Dendrites (either directly labeled or traced in continuity with the somata of cells labeled by retrograde transport of [ $^3\text{H}$ ]GABA) were examined in the electron microscope and were found to contain large bundles of microtubules (Fig. 11, E and F). The cytoplasm of the dendrites is electron dense in comparison with that of the dendrites of unlabeled neurons. Although we did not make a quantitative survey, there appeared to be a larger number of synapses on the more distal dendrites (those greater than 20  $\mu\text{m}$  from the cell body). In both distal and proximal dendritic regions the synapses were of both symmetrical and asymmetrical types (Fig. 11F), but there was a predominance of asymmetrical synapses on the distal portions of the dendrites. None of the labeled dendrites possessed dendritic spines in the series of sections examined.



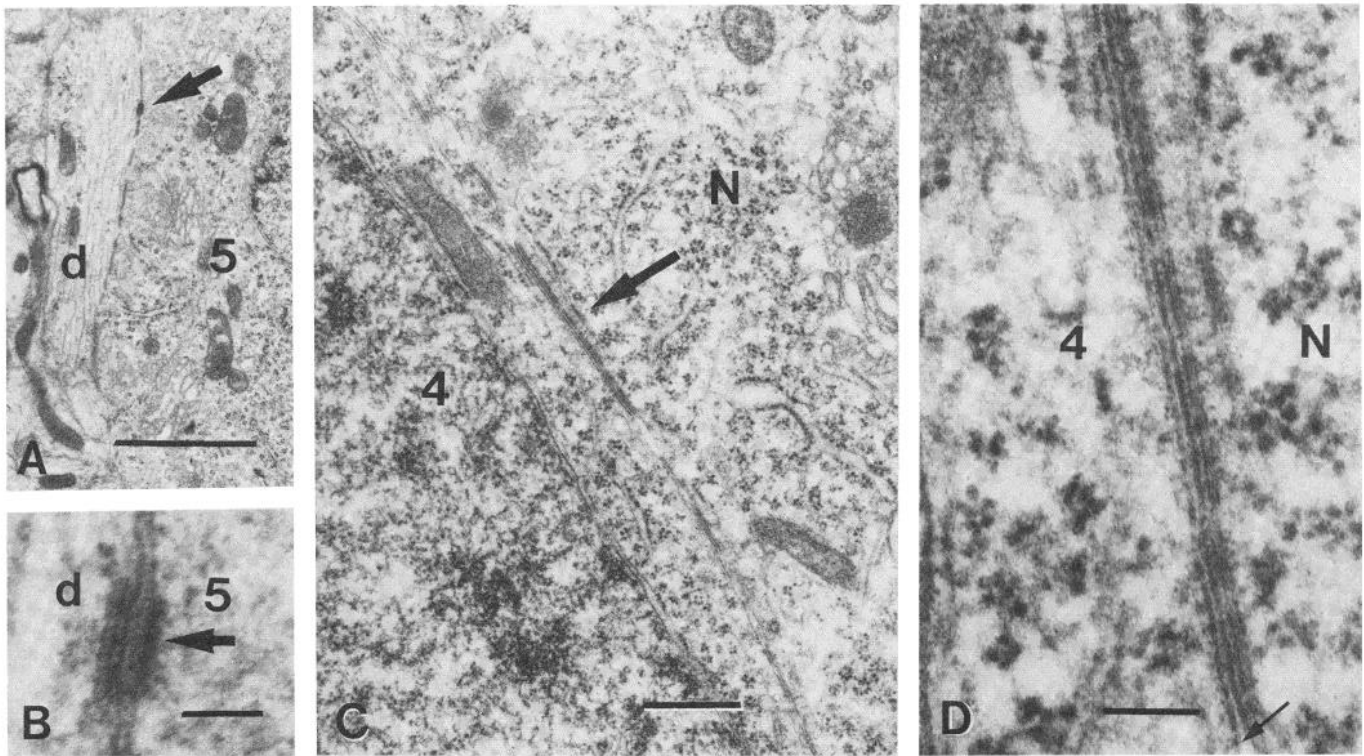


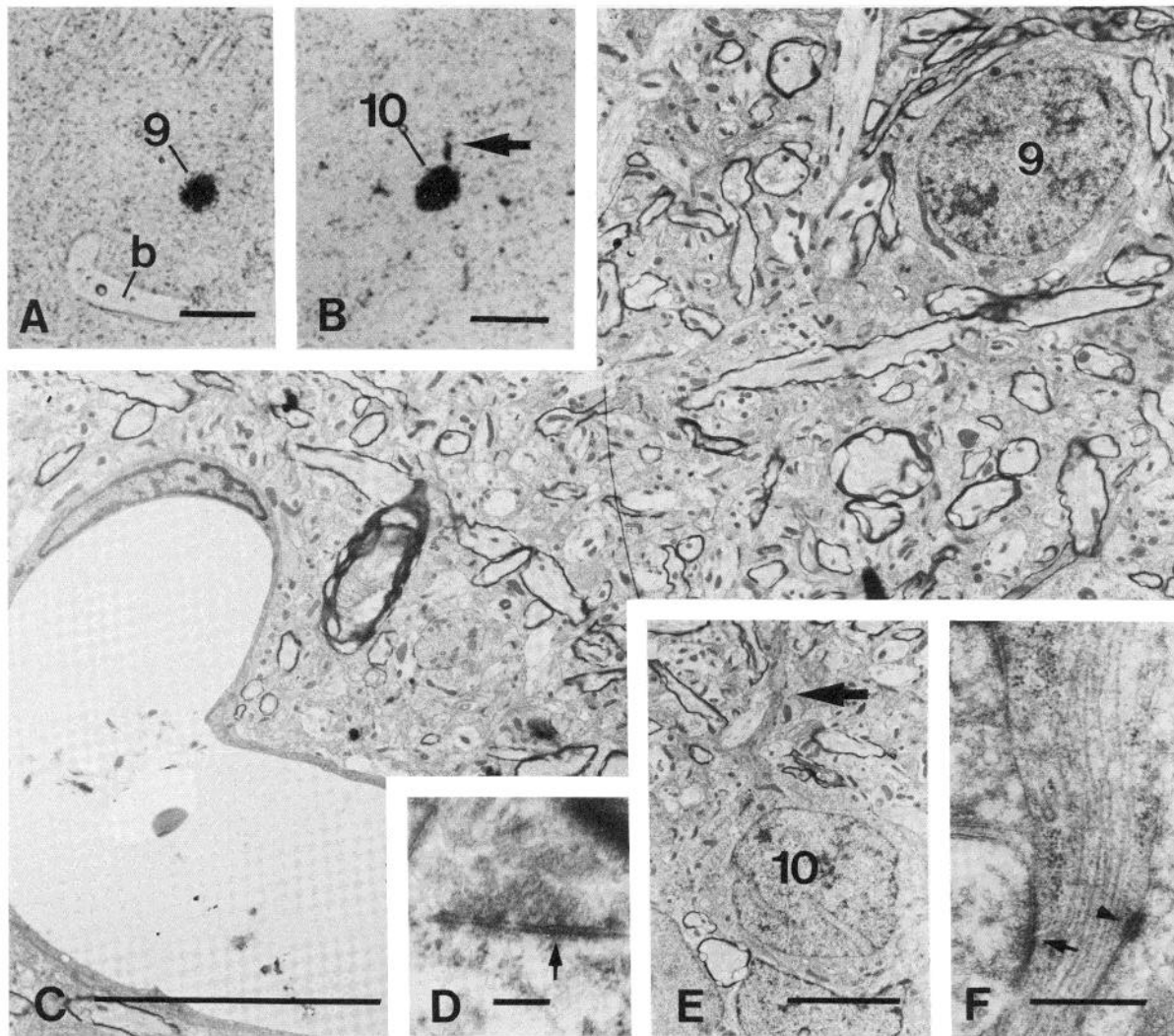
Figure 10. Electron micrographs of specialized junctions formed by [ $^3\text{H}$ ]GABA-labeled neurons. A, Junction (arrow) between a dendrite (d) and labeled neuron 5 of Figure 9. Bar = 2  $\mu\text{m}$ . B, Higher magnification of the junctional complex indicated by the arrow in A. This complex includes a punctum adherens (arrow). Bar = 0.1  $\mu\text{m}$ . C and D, Low (C) and high (D) magnification electron micrographs of a multilaminar subsurface complex at the junction formed between cell 4 (boxed area in Fig. 9E) and an unlabeled neuron (N). The extracellular space is indicated by an arrow in D. Bars = 0.5  $\mu\text{m}$  in C and 0.1  $\mu\text{m}$  in D.

Semithin sections displaying vertically oriented chains of silver grains running between an injection site and cell somata retrogradely labeled in superficial or deep layers were resectioned and examined in the electron microscope after carefully noting the position of individual chains in relation to other landmarks. Two types of autoradiographic labeling could be distinguished, one consisting of a single row of grains (Fig. 12, B and C) and the other of multiple parallel rows of grains (Fig. 13A). As shown in Figure 12, directly beneath the emulsion containing single rows of silver grains we found either myelinated axons (Fig. 12A) or unmyelinated profiles, either demonstrably dendrites (Fig. 12, D and E) or not distinguishable as either dendrites or unmyelinated axons. The same two groups of profiles were found both in labeled bundles descending from a superficial injection and in labeled bundles ascending from a deep injection. Multiple rows of silver grains were found to overlie bundles of profiles consisting of dendrites and myelinated axons and processes not distinguishable as either dendrites or unmyelinated axons (Fig. 13C). The myelinated axons of the labeled bundles were all of small caliber, measuring 0.5 to 1.3  $\mu\text{m}$  in diameter. They could not be followed to their terminations or to their origins. The dendrites of the labeled bundles possessed the same characteristic bundles of microtubules, dense cytoplasm, and sparse distribution of symmetric and asymmetric synaptic contacts on dendrites seen to arise directly from labeled cell bodies, and they lacked dendritic spines (compare Fig. 12, D and E, with Fig. 11, E and F). Thus, the vertically oriented labeling seems to be formed, in part, by dendrites arising from the retrogradely labeled cells, but whether the myelinated axons of the bundles also arise from the retrogradely labeled cells could not be determined. Furthermore, it is not clear what contribution to the bundles is made by unmyelinated axons.

## Discussion

Several lines of evidence suggest that GABAergic neurons of the cerebral cortex are selectively labeled by the high affinity uptake of

[ $^3\text{H}$ ]GABA: (1) [ $^3\text{H}$ ]GABA is taken up preferentially by cortical terminals that contain glutamic acid decarboxylase (GAD), the enzyme marker of GABAergic neurons (Neal and Iversen, 1969); (2) cortical neurons taking up [ $^3\text{H}$ ]GABA in tissue culture are also GAD immunoreactive (Neale et al., 1983); (3) many of the morphological cell types in the intact mammalian cerebral cortex that are labeled by [ $^3\text{H}$ ]GABA uptake (Hökfelt and Ljungdahl, 1972; Chronwall and Wolf, 1978; Hendry and Jones, 1981; Somogyi et al., 1981, 1983a; Hamos et al., 1983; Harondi et al., 1983) have also been demonstrated to be GAD immunoreactive (Ribak, 1978; Hendrickson et al., 1981; Hendry et al., 1983; Houser et al., 1983); (4) axon terminals accumulating [ $^3\text{H}$ ]GABA *in situ* (Hamos et al., 1983; Harondi et al., 1983) and those stained immunocytochemically for GAD (e.g., Ribak, 1978; Hendry et al., 1983) both form symmetric synaptic contacts in the cortex; and (5) [ $^3\text{H}$ ]GABA is selectively accumulated and retrogradely transported in the axons of known populations of GABAergic neurons such as those in the pars reticulata of the substantia nigra (Streit et al., 1979). Both the light and electron microscopic findings of the present study demonstrate only nonpyramidal neurons lacking dendritic spines to be labeled, a finding that is consistent with those made on GABAergic neurons identified immunocytochemically. We conclude, provisionally, that the neurons labeled directly and by retrograde transport in the present study are probably GABAergic. However, our data do not exclude the possibility that some population of GABAergic neurons is left unlabeled by the injection of [ $^3\text{H}$ ]GABA or that certain non-GABAergic neurons may be positively labeled by our methods. The observation that injections of [ $^3\text{H}$ ]GABA into the visual cortex produce labeling of cells above or below the site of injection has been reported by Somogyi et al. (1981, 1983a). This labeling was attributed to the uptake by axon terminals and retrograde axoplasmic transport of [ $^3\text{H}$ ]GABA to the cell bodies of putatively GABAergic neurons. Such a mechanism of transmitter-specific transport has been strongly suggested to exist for several populations of neurons including those



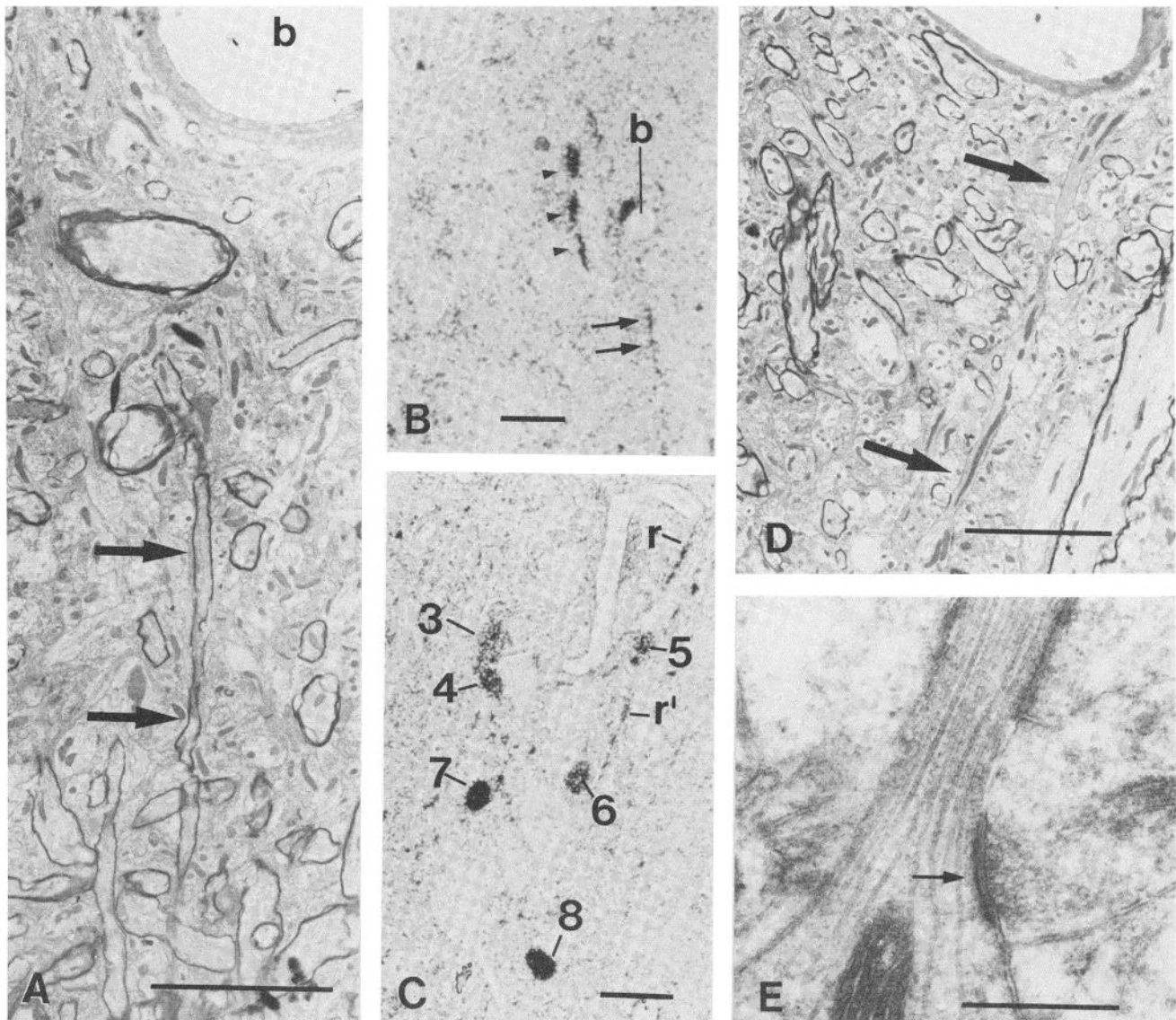
**Figure 11.** Light and electron micrographs of two [ $^3\text{H}$ ]GABA-labeled cells. *A* and *B*, Labeled neurons (9 and 10) found in layer V outside the boxed area of Figure 9. *Bar* = 20  $\mu\text{m}$ . *C* and *D*, Electron micrographs of labeled neuron 9. The cell body 9 was identified by reference to the blood vessel (*b*) seen in *A* and *C*. *Bar* = 10  $\mu\text{m}$ . The synaptic contacts (*D*) formed by axon terminals onto the cell body are symmetric (*arrow*). *Bar* = 0.2  $\mu\text{m}$ . *E* and *F*, Electron micrographs of labeled neuron 10 giving rise to a dendrite (*arrow* in *E*). *Bar* (*E*) = 5  $\mu\text{m}$ . *F*, Higher magnification of *E* showing a synapse onto the dendrite (*arrow*). The *arrowhead* indicates a punctum adherens. *Bar* = 0.5  $\mu\text{m}$ .

thought to use transmitters other than GABA (Cuénod et al., 1982). In the present study, neurons in the subdivisions of the sensory-motor area in addition to those in the primary visual area were labeled by injections of [ $^3\text{H}$ ]GABA at distances of 1 to 1.5 mm superficial or deep to an injection site. When axoplasmic transport was blocked by prior local injection of colchicine, the distant labeling of cells was abolished. These experiments, therefore, provide a strong measure of support for the idea that labeling of cell bodies above and below the site of [ $^3\text{H}$ ]GABA injection results from retrograde transport of the amino acid. The electron microscopic findings of this study indicate that the radial fasciculi of the cortex between the site of the injection and the patches of distant labeled cells are labeled and that the labeled fasciculi contain bundles of dendrites, myelinated axons, and possibly unmyelinated axons. Although no myelinated axon was traced to its parent soma, the consistent presence of these elements indicates that they are probably a route through which the [ $^3\text{H}$ ]GABA is transported. It is also possible that some of the cells may have been labeled by retrograde dendritic transport since dendrites, too, were elements of the labeled fascicles. Apart from the colchicine experiments, perhaps the strongest evidence in our study for retrograde transport of [ $^3\text{H}$ ]GABA is furnished by the experiments in which labeling of somata occurred

in layers V and VI after injections of layers I to IIIA but not after injections of layers IIIB and IV. This would imply that more significant amounts of [ $^3\text{H}$ ]GABA for retrograde transport are taken up by the terminal ramifications of ascending axons than by these axons *de passage* or by vertical dendrites.

Labeling of cell bodies at close proximity to the injection site persisted when colchicine was administered and appears, then, to arise from a mechanism independent of axoplasmic transport. Direct, high affinity uptake of [ $^3\text{H}$ ]GABA by cell bodies is the most likely explanation, although high affinity uptake by local axon terminals and dendrites, with diffusion to local somata, could account for some of the residual somal labeling.

In the postcentral gyrus, where the radial arrangement of the cells is particularly clear, labeling of neurons always occurred superficial to injections of [ $^3\text{H}$ ]GABA placed in deep layers and deep to those placed in superficial layers but never at distances equivalent to these distances on each side of the injection (e.g., Fig. 5). We regard this as indicative of the fact that the retrograde labeling is constrained by a columnar anatomical substrate. This is supported by the common occurrence of radiolabeled vertical profiles that proved on resectioning of semithin autoradiographic sections to be single and bundled dendrites, and myelinated and possibly unmyelinated ax-

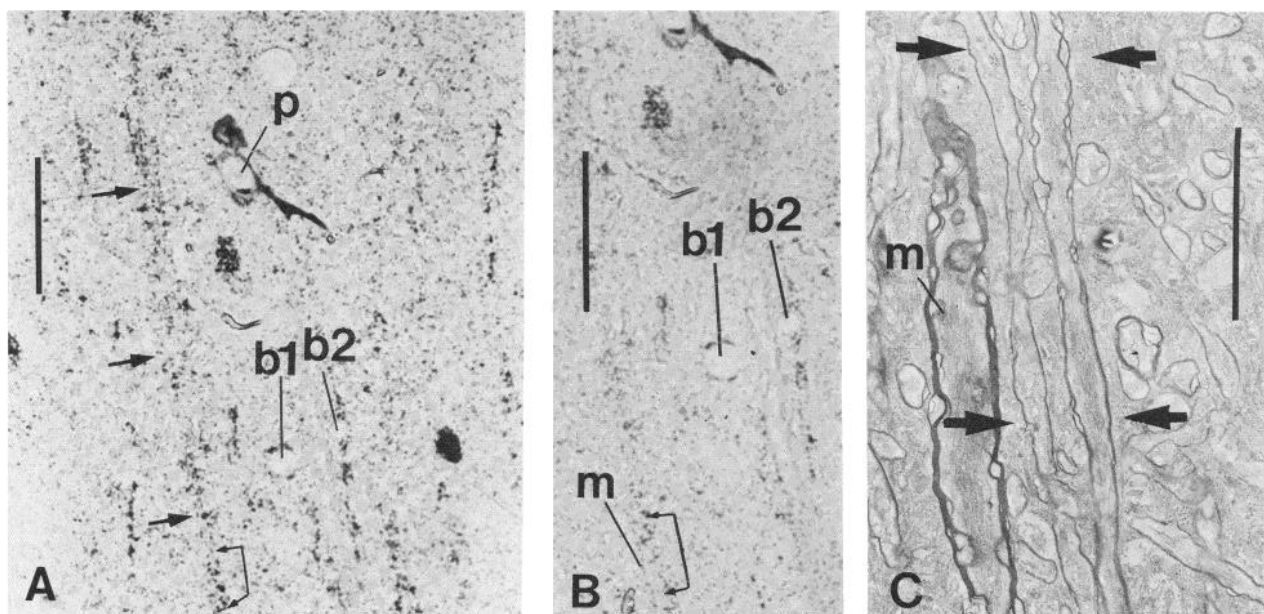


**Figure 12.** Correlative light and electron micrographs of elements in the neuropil labeled by an injection of [ $^3\text{H}$ ]GABA. **A**, Electron micrograph of myelinated axon (arrows) underlying the single chain of silver grains indicated by arrows in the semithin section autoradiograph shown in **B**. Underlying the three chains of silver grains (arrowheads in **B**) were dendritic profiles (not shown); **b**, same blood vessel as in **B**. Bars =  $\sim 5 \mu\text{m}$  (A) and  $20 \mu\text{m}$  (B). **C** lies between semithin autoradiographic sections shown in Figure 9, **B** and **C**. Radially oriented chains of silver grains are seen in all three sections (arrows, arrowheads, and **r**). Bar =  $30 \mu\text{m}$ . **D**, A dendritic process (arrows) found underlying the radiolabeled neuropil beneath **r** at upper right in **C**. A similar dendrite was found beneath **r'** at middle right in **C**. Bar =  $5 \mu\text{m}$ . **E**, Higher magnification of an asymmetric synapse on the dendrite shown in **D**. Bar =  $0.5 \mu\text{m}$ .

ons. This intense vertical labeling contrasts strongly with the lack of similar labeling in the horizontal dimension. Some of the dendrites of the labeled vertical bundles clearly arise from retrogradely labeled cell somata, and this, in turn, appears to indicate that the cells of both the superficial and deep groups also have preferred radial dendritic as well as axonal arborizations.

Our results in the sensory-motor areas indicate that there are two main populations of putatively inhibitory interneurons that project vertically: one population, mainly in layer V, is a source of inputs to layers I to IIIA; the second population in layer II and the adjacent part of layer IIIA is a main source of input to layers V and VI. The same two populations are seen in area 17, but in this area, there is a third population in layer IVC which projects vertically to layers III and IVA. These putatively inhibitory vertical connections must now be seen as a system of interlaminar connections additional to those formed by the putatively excitatory spiny nonpyramidal neurons (e.g., Lund, 1984) and by the collaterals (also thought to be excitatory) of pyramidal cell axons.

Under our experimental conditions, only nonpyramidal cells were labeled retrogradely after injections of [ $^3\text{H}$ ]GABA. Of the morphologically distinct classes of nonpyramidal cells (Valverde, 1971; Jones, 1975; Fairén et al., 1984), several have been identified as GABAergic by their immunoreactivity for GAD or GABA (Peters et al., 1982; Freund et al., 1983; Hendry et al., 1983; DeFelipe et al., 1985) and by their high affinity uptake of [ $^3\text{H}$ ]GABA (Hendry and Jones, 1981; Somogyi et al., 1981). It has been suggested (by Somogyi et al., 1981) that cells with somata in the superficial cortical layers labeled by [ $^3\text{H}$ ]GABA injections in deep layers are the class of double bouquet cells (Ramón y Cajal, 1911; Jones, 1975). This interpretation is based on Golgi data showing the double bouquet somata to exist in layers II and III and to give off tightly bundled vertical axons that terminate in layer V. The double bouquet cell is thus a prime candidate for the type labeled by specific retrograde transport of [ $^3\text{H}$ ]GABA from the deep layers. It has to be pointed out, however, that the evidence for this is at best circumstantial: double bouquet cells can only be positively identified by their axons (Jones, 1975),



**Figure 13.** Correlative light and electron micrographs of elements in the neuropil labeled by an injection of [ $^3\text{H}$ ]GABA in layers I and II of area 1. **A**, Photomicrograph of a semithin section autoradiograph, 1.5  $\mu\text{m}$  thick, from layer V. Arrows indicate radiolabeled vertical neuropil. *p*, a gold-toned pyramidal cell. *b1, b2*, blood vessels. Bar = 50  $\mu\text{m}$ . **B**, Photomicrograph of the same semithin section shown in **A** but taken at a focal plane deep to the silver grains in order to facilitate further the identification of profiles in the electron microscope. *m*, an unlabeled myelinated axon. *b1, b2*, same blood vessels shown in **A**. Bar = 50  $\mu\text{m}$ . **C**, Electron micrograph taken after resectioning of the same semithin section shown in **A** and **B**. *m*, the same large unlabeled myelinated axon shown in **B**. Underlying the chains of silver grains of the bracketed regions in **A** and **B** are found a bundle of small myelinated axons (between arrows). Bar = 5  $\mu\text{m}$ .

and these axons have not yet been specifically shown to be stained immunocytochemically for GAD or GABA nor to be specifically involved in the retrograde transport of [ $^3\text{H}$ ]GABA. As demonstrated in Golgi preparations, the long descending bundles of axon collaterals formed by double bouquet cells in the monkey cortex give off multiple terminal boutons as they descend (Jones, 1975). This suggests that the axons are unmyelinated in their descent. It is possible that such axons are included in the unmyelinated profiles labeled in descending bundles after injections of [ $^3\text{H}$ ]GABA in the superficial layers. If this is so, however, the labeled myelinated axons in the bundles remain unaccounted for.

Golgi studies on the cortex of primates have revealed few non-spiny, non-pyramidal neurons with somata situated in the deep cortical layers, and with ascending axonal systems comparable to those descending from the double bouquet cells. The classical cell type of layers V and VI, with a vertical ascending axon, is that described originally by Martinotti (1889) and Ramón y Cajal (1891) and shown in a drawing from one of our own preparations in Figure 14B. The laminar position, small somal size, and ascending axon of this cell make it a candidate for that labeled by retrograde axoplasmic transport of [ $^3\text{H}$ ]GABA. It is not known, however, whether the axons of such cells are myelinated, or whether the cells are immunoreactive for GAD or GABA. Small cell somata immunoreactive for markers of GABA transmission have been observed, however, in layers V and VI of the monkey cortex (Hendry et al., 1983).

An additional non-spiny cell type whose soma may be labeled by retrograde transport of [ $^3\text{H}$ ]GABA has been observed by us in the monkey sensory-motor cortex (Fig. 14A). The somata of these cells are found in layers IIIB and IV and the axon forms a tight bundle of ascending collaterals ending in a focused tuft of terminal branches in layers II and IIIA. Such cells might be represented by the population of somata retrogradely labeled in small numbers in layers III and IV of the sensory-motor cortex and in larger numbers in layer IVc of the visual cortex, after injections of [ $^3\text{H}$ ]GABA in superficial layers. Again, however, there is no direct correlation of this cell type with GABA- or GAD-immunoreactive cells, and it is not known whether its axon is myelinated or not.

A major group of cells that needs to be considered as candidates for [ $^3\text{H}$ ]GABA-specific retrograde labeling in both the superficial and deep cortical layers is demonstrably immunoreactive for both GAD and GABA. These are the peptide-containing neurons of the monkey cortex (Hendry et al., 1984a, b; Somogyi et al., 1984; Jones and Hendry, 1985). Neurons immunoreactive for the peptides cholecystokinin octapeptide, somatostatin, and/or neuropeptide Y have small somata situated in all layers but concentrated in layers II and IIIA and in layer VI and the subjacent white matter. All of the peptide-containing cells that we have studied in monkeys have elongated processes, not readily distinguishable as axons or dendrites at the light microscopic level, that extend vertically across several laminae and form distinct plexuses in the supra- and infragranular layers. We have pointed out the essential similarity of all peptidergic cortical cells, despite superficial differences in their morphology (Hendry et al., 1984a). In many respects the deeply situated cells of this type resemble the Martinotti cells (Fig. 14B). Virtually all of the cells immunoreactive for the three peptides mentioned above are also immunoreactive for GAD or GABA (Hendry et al., 1984b). The likelihood that the somata of some or all of these cells can be retrogradely labeled by [ $^3\text{H}$ ]GABA transported through their elongated processes (whether they be dendritic or axonal) seems strong. It has to be noted, however, that more peptidergic cell somata are found in layer VI than in layer V in which the larger number of retrogradely labeled somata is found after supragranular injections of [ $^3\text{H}$ ]GABA.

The very small number of neurons labeled by horizontal (intralaminar) transport of [ $^3\text{H}$ ]GABA was surprising because of the clear evidence that the large basket cells of the sensory-motor cortex are GABAergic (Hendry et al., 1983). These cells possess long, horizontally oriented axon branches stretching across the cortex for 1 to 3 mm (Marin-Padilla 1969; Jones, 1975). The failure to label these neurons by injections of [ $^3\text{H}$ ]GABA at a distance of 1 mm could result from one of the finer details of the cells' axonal morphology: collaterals of intracellularly injected basket cells in the visual cortex are richly distributed within the dendritic field of the cells, but outside that field they give rise to relatively small terminal arborizations

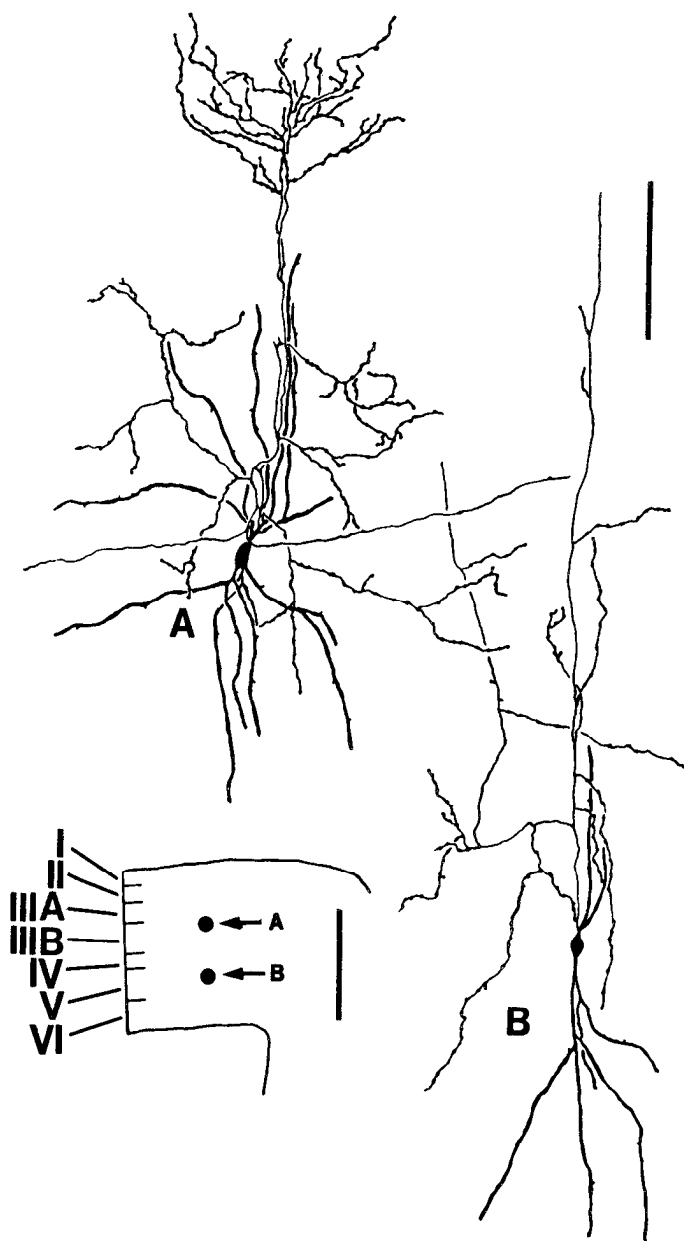


Figure 14. Camera lucida drawings of two examples of cells with ascending axons (A and B) in area 1-2. Note that the axon of cell A gives rise to a very focal axonal arborization in layers above the cell body. Cell B resembles the peptidergic neurons described by Hendry et al. (1948a) in the same areas. Material is from rapid Golgi impregnations of adult monkey cortex. The inset is a camera lucida drawing of the section to illustrate the localization (dots) of cells A and B (arrows). Large bar, 100  $\mu\text{m}$  (drawings). Small bar: 1 mm (inset).

(Gilbert and Wiesel, 1979; Somogyi et al., 1983b) which might, therefore, accumulate relatively insignificant amounts of [ $^3\text{H}$ ]GABA for retrograde transport. Alternatively, these cells may simply not be retrogradely labeled by the method because of differences in uptake mechanisms.

Physiological studies in the visual cortex of the cat suggest that the tangential spread of intracortical inhibition following afferent stimulation extends across only a small number of orientation columns (Toyama et al., 1981) or over a distance of no more than 400  $\mu\text{m}$  (Hess et al., 1975). This tangential, intracortical, inhibitory spread is similar to the extent of monosynaptic inhibitory effects described with intracortical microstimulation by Asanuma and Rosén (1973) in the motor cortex of the cat, although polysynaptic effects occurred

over distances up to 1 mm from the stimulation site. Similar horizontal inhibitory effects have not been described in the cortex of the primate, although activation of one place- and modality-specific column in the monkey somatic sensory area is accompanied by inhibition of neighboring columns (Mountcastle and Powell, 1959).

Another obvious feature of the cells retrogradely labeled in the vertical dimension by [ $^3\text{H}$ ]GABA was their tendency to form clusters in which cell somata were linked by appositional specializations. Gap junctions have been reported to exist between neuronal processes in the monkey sensory-motor cortex (Sloper, 1972; Sloper and Powell, 1978). Although we did not find typical gap junctions between the labeled cells, the functional specializations observed were consistently found in large numbers. These specializations adopted several configurations which included puncta adherentia, close contacts, and a specialization associated with subsurface cisternal complexes. We know of no data to indicate how these specializations contribute to neuronal function nor whether they are specifically associated with neuronal groups that include GABAergic interneurons.

The vertical (or columnar) anatomical organization of the cortex first suggested by Lorente de Nó (1938) and Mountcastle (1957) has tended to dominate thinking on the intrinsic organization of the cortex, and our findings are strongly supportive of this type of organization. Recently, the horizontal intrinsic organization of the cortex has also received attention (Fisken et al., 1975; Künzle, 1976; Creutzfeldt et al., 1977; Jones et al., 1978; Rockland and Pandya, 1979; Rockland et al., 1982; Rockland and Lund, 1983). If our findings on the rather restricted horizontal extent of putatively GABAergic cells implies that the known GABAergic basket cells have less significant long distance connections than previously assumed from the anatomy, then pyramidal cells, the excitatory, output cells of the cortex, may have an important role in the formation of intracortical horizontal connectivity. Many pyramidal cells have profuse horizontal collaterals that have probably rarely been satisfactorily revealed in Golgi studies (e.g., Gilbert and Wiesel, 1983; J. DeFelipe, M. Conley, and E. G. Jones, submitted for publication); injections of horseradish peroxidase or of [ $^3\text{H}$ ]leucine and [ $^3\text{H}$ ]proline in the cerebral cortex reveal, by retrograde or anterograde axonal transport, a pattern of intracortical connectivity characterized by multiple clusters of pyramidal cells or of terminal axonal ramifications separated by gaps. Long-range horizontal activity, therefore, may be primarily effected by axon collaterals of excitatory pyramidal cells. Of course, pyramidal cell axons traverse many layers in the vertical dimension as they descend toward the white matter, giving off collateral ramifications as they do so. In this sense they are also interlaminar neurons. However, their collaterals usually spread very widely within a lamina, so that the effect is presumably widely dispersed in the horizontal dimension.

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