Distribution of GABAergic Neurons and Axon Terminals in the Macaque Striate Cortex

D. FITZPATRICK, J. S. LUND, D. E. SCHMECHEL, AND A. C. TOWLES

Department of Anatomy and Psychology (D.F.) and VA Medical Center and Department of Medicine (D.E.S.), Duke University, Durham, North Carolina 27710; Department of Psychiatry (J.S.L.), University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261; and Department of Neurology (A.C.T.), Cornell University Medical Center, New York, New York 10021

ABSTRACT

Antisera to glutamic acid decarboxylase (GAD) and γ-aminobutyric acid (GABA) have been used to characterize the morphology and distribution of presumed GABAergic neurons and axon terminals within the macaque striate cortex. Despite some differences in the relative sensitivity of these antisera for detecting cell bodies and terminals, the overall patterns of labeling appear quite similar. GABAergic axon terminals are particularly prominent in zones known to receive the bulk of the projections from the lateral geniculate nucleus; laminae 4C, 4A, and the cytochrome-rich patches of lamina 3. In lamina 4A, GABAergic terminals are distributed in a honeycomb pattern which appears to match closely the spatial pattern of geniculate terminations in this region. Quantitative analysis of axon terminals that contain flat vesicles and form symmetric synaptic contacts (FS terminals) in lamina 4C, and in lamina 5 suggest that the prominence of GAD and GABA axon terminal labeling in the geniculate recipient zones is due, at least in part, to the presence of larger GABAergic axon terminals in these regions.

GABAergic cell bodies and their initial dendritic segments display morphological features characteristic of nonpyramidal neurons and are found in all layers of striate cortex. The density of GAD and GABA immunoreactive neurons is greatest in laminae 2-3A, 4A, and 4C. The distribution of GABAergic neurons within lamina 3 does not appear to be correlated with the patchy distribution of cytochrome oxidase in this region; i.e., there is no significant difference in the density of GAD and GABA immunoreactive neurons in cytochrome-rich and cytochrome-poor regions of lamina 3.

Counts of labeled and unlabeled neurons indicate that GABA immunoreactive neurons make up at least 15% of the neurons in striate cortex. Layer 1 is distinct from the other cortical layers by virtue of its high percentage (77–81%) of GABAergic neurons. Among the other layers, the proportion of GABAergic neurons varies from roughly 20% in laminae 2-3A to 12% in laminae 5 and 6.

Finally, there are conspicuous laminar differences in the size and dendritic arrangement of GAD and GABA immunoreactive neurons. Lamina 4C, and lamina 6 are distinguished from the other layers by the presence of populations of large GABAergic neurons, some of which have horizontally spreading dendritic processes. GABAergic neurons within the superficial layers are significantly smaller and the majority appear to have vertically oriented dendritic processes. These results provide support for the idea that GABAergic neurons make up a significant proportion of the neurons within
Neurons that utilize the neurotransmitter γ-aminobutyric acid (GABA) appear to play an important role in generating many of the receptive field properties that distinguish neurons in the striate cortex from those in the lateral geniculate nucleus. When the inhibitory effects of GABA are blocked by the administration of the potent GABA antagonist bicuculline, the selective responses of cortical neurons for the orientation, length, direction of movement and velocity of a visual stimulus are abolished or diminished (Sillito, '75, '77a, '79; Sillito and Versiani, '77; Patel and Sillito, '78; Tsumoto et al., '79).

GABAergic neurotransmission within the striate cortex, as in other regions of neocortex, is thought to be mediated largely by a morphologically diverse class of non-pyramidal neurons that share the feature of having relatively spineless dendritic processes. Evidence for the selective role of aspiny neurons in cortical inhibition is derived from the observations that neurons with this morphology (1) accumulate 3H-GABA (Chronwall and Wolff, '78; Hamos et al., '83; Hendry and Jones, '81; Somogyi et al., '84); (2) are immunoreactive for glutamic acid decarboxylase (GAD), the synthesizing enzyme for GABA (Ribak, '78; Peters and Fairen, '78; Freund et al., '83; Houser et al., '83; Somogyi et al., '83b); (3) are immunoreactive for GABA itself (Ottersen and Storm-Mathisen, '84; Somogyi and Hodgson, '85); and (4) have terminals that contain flat vesicles and make symmetric synaptic contacts, characteristics thought to be indicative of inhibitory transmission (Uchizono, '65; Peters and Fairen, '78; Somogyi and Cowey, '81).

While a general consensus has been reached on the morphological class of neurons responsible for GABAergic transmission in the striate cortex, considerably less is known about the numbers of these neurons and their distribution within the macaque striate cortex. The existing information on the precise laminar and columnar segregation of cell types and connections within the monkey striate cortex provides a valuable structural and functional framework within which to examine the distribution of GABAergic neurons. For example, the projections from the magnocellular and parvocellular layers of the lateral geniculate nucleus (LGN) to the striate cortex terminate in separate tiers of lamina 4C (4Cv and 4Cp, respectively; Hubel and Wiesel, '72; Hendrickson et al., '78). Differences in the number and types of GABAergic neurons in these layers may have important implications for understanding the way striate cortex processes the information derived from these two pathways. The discovery that lamina 3 has distinct cytochrome-oxidase-rich patches that are distinguished from the surrounding regions in their response properties and in their connections (Horton and Hubel, '81; Livingstone and Hubel, '82, '84; Fitzpatrick et al., '83a) provides another set of functional landmarks for examining the distribution of GABAergic neurons. Indeed, there is already some evidence that the cytochrome-oxidase-rich regions of lamina 3 are particularly rich in GABAergic neurons and terminals (Hendrickson et al., '81; Carroll and Wong-Riley, '85).

In the present study, immunocytochemical markers for GAD and GABA were used to characterize the morphology, number, and distribution of presumed GABAergic neurons and axon terminals in the macaque striate cortex. The main purpose of this study was to establish how the normal distribution pattern of GABAergic neurons and terminals relates to the well-defined laminar and columnar subdivisions of the monkey striate cortex. It is our view that this information constitutes a useful first step in understanding how neurons and terminals involved with GABAergic neurotransmission are arranged with respect to the terminal fields of functionally different geniculocortical pathways and to the cortical cells that give rise to extrastriate and brainstem projections.

**MATERIALS AND METHODS**

**Immunocytochemistry**

A total of five macaque monkeys (*Macaca fascicularis*) were used in these experiments. Animals were deeply anaesthetized and perfused through the heart with a 0.9% saline solution followed by one of the following fixatives: 4% paraformaldehyde, 0.1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4 (two animals); 0.5% zinc salicylate dissolved in 10% formalin in water as described by Mugnaini and Dahl ('83) (one animal); or a mixture of paraformaldehyde, lysine, and sodium periodate (PLP) made according to the protocol of McLean and Nakane ('74) (two animals). Except for the animals perfused with the PLP mixture, the fixative was flushed out with a solution of 10% sucrose in 0.1 M phosphate buffer, pH 7.4.

Sections from the striate cortex were cut at 15–40 μm on an Oxford vibratome and collected in 0.01 M phosphate buffer, pH 7.6. Selected sections were processed using GAD or GABA immunocytochemistry, cytochrome oxidase histochemistry (Wong-Riley, '79) or Nissl stains (either thionin or toluidine blue).

The antiserum developed by Oertel et al. ('81a) was used for the demonstration of GAD immunoreactivity. Details of the purification of GAD and the preparation of the antiserum have been published (Oertel et al., '81a, b). The PLP fixative was superior to other fixatives for labeling cortical cell bodies with this antiserum, but labeled terminals were found with all three fixatives. In some cases, prior to immunocytochemical processing, sections were run through an increasing and decreasing series of alcohol solutions (10, 25, 50%) for 3 minutes each. This procedure significantly increased the penetration of the antiserum; in the thinner sections, terminals appeared through the depth of the section. However, immersion in alcohol solutions significantly...
reduced the intensity and number of labeled cell bodies, so this method was of limited value in assessing the distribution of GAD immunoreactive neurons.

Sections were incubated in primary antiserum diluted 1:1,500–1:2,000 in PBS containing 1% normal rabbit serum for 2–24 hours. Linking antiserum (biotinylated antigoat) was used at a dilution of 1:50 and the avidin-biotin complex buffer, pH 7.4. The brain was postfixed in the same solution, and this was routinely used to enhance penetration. Sections were incubated in the anti-GAD serum. Control sections showed no labeling of cell bodies or terminals.

The antiserum produced by Lauder et al. (’86) was used for the demonstration of GABA immunoreactivity. This antiserum was raised in rats against GABA conjugated to keyhole limpet hemocyanin with glutaraldehyde and it shows no cross-reactivity with glutamate, aspartate, $\beta$-alanine, taurine, or glycine. Immunoreactive staining was completely abolished by absorption with the GABA conjugate. The most robust staining of cell bodies and dendrites was achieved by fixation with the 4% paraformaldehyde, 0.1% glutaraldehyde mixture. The intensity of GABA immunoreactivity in cell bodies was not affected by alcohol pretreatment, and this was routinely used to enhance penetration. Sections were incubated in primary antiserum diluted at 1:2,000 in PBS containing 1% normal rat serum (NRS) for 12–24 hours. Linking antisera (biotinylated antirat) was used at a dilution of 1:50 and the ABC complex was localized with DAB as described above. In some cases, the silver intensification procedure described by Liposits et al. (’84) was used to enhance the staining of GABA immunoreactive terminals.

Electron microscopy

One macaque monkey was deeply anaesthetized and perfused with 0.9% saline followed by a solution of 2.0% paraformaldehyde, 0.05% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. The brain was postfixed in the same solution overnight. Blocks of tissue (1–2 mm) from area 17 were rinsed in phosphate buffer and placed in 2% osmium tetroxide in 0.1 M phosphate buffer for 1 hour. Following a brief rinse in phosphate buffer, the tissue was dehydrated and embedded in Polybed 812.

In order to compare the size of axon terminals that contained flat vesicles and made symmetric synaptic contacts (FS terminals) in lamina 4C and in lamina 5, 1–2-μm-thick sections were cut, stained with toluidine blue, and viewed with the light microscope. Drawings were made to illustrate the 4C-5 border, and blocks were trimmed to include 4C and lamina 5. Rims of thin sections were cut, placed on 0.125% Formvar-coated 2-mm slot grids, and stained with uranyl acetate and lead citrate.

Measurements of the size of FS terminal profiles from lamina 4C and lamina 5B were made from electron micrographs taken at a magnification of 15,000 with the aid of a graphics tablet and computer.

Analysis of the distribution of GABAergic neurons

The laminar distribution of GAD and GABA immunoreactive neurons was analyzed by drawing all of the labeled neurons in a 500-μm-wide strip extending from the pia to the white matter at a magnification of 540× with a camera lucida. This strip of cells was then divided into sequential 65- or 70-μm horizontal sectors and the number of cells in each of these rectangular sectors (500 × 65 or 70 μm) was counted. Multiple nonoverlapping strips were drawn from nearby areas of the same section and the cell counts from all the sectors of a given depth were added together to achieve a reasonable sample size from each depth. Sample collection continued until the bin with the smallest number of cells (usually from the vicinity of lamina 4B) exceeded 20 neurons. Only those neurons in which a nucleus could be discerned were counted.

The proportion of GABAergic neurons as a function of depth was determined by counting GABA immunoreactive and non-immunoreactive neurons from 15–20-μm counterstained sections by using procedures identical to those described above. This was done using only the sections that had undergone alcohol pretreatment to enhance antiserum penetration. Since alcohol pretreatment is incompatible with GAD immunoreactive labeling of cell bodies, this issue was not addressed with the GAD antiserum.

The relationship between the distribution of GABA and GAD immunoreactive neurons and the cytochrome-rich patches of lamina 3 was analyzed with the aid of a Videometrics M-100 graphics system attached to an IBM PC-XT. A custom-designed program superimposed a square box (0.08 μm2 in area) on a video image of the tissue section. This box served as the sample window in which cell counts were made and it could be positioned to include the cytochrome-rich or cytochrome-poor regions of the tissue section. The size of the sample window was automatically adjusted to the objective magnification so that it enclosed 0.08 μm2 of tissue area regardless of magnification. This allowed the borders of the cytochrome-rich regions to be seen at low power (where the boundaries appear sharper), a sample region to be chosen, and counts to be made of the number of GAD or GABA immunoreactive neurons in the sample region at higher power (so that cell nuclei could be detected). Typically, the process consisted of (1) choosing a sample site on a section treated for cytochrome oxidase with a 4× objective and drawing both the borders of the region to be sampled and the pattern of blood vessels on the video image with the graphics tablet; (2) replacing the cytochrome oxidase section with an adjacent section treated for GAD or GABA immunocytochemistry and aligning the blood vessel patterns; (3) changing the objective to 20× and enlarging the sample window proportionally; and (4) counting the number of cells within the borders of the sample region. Cells falling on the border of the sample region were included in the sample.

The cross-sectional area of the neurons within a given layer was also measured with the computer graphics system. The borders of the neurons were drawn at a magnification of 1,050× on the video monitor. To ensure an accurate representation of the size distribution of cells within a layer, samples were taken throughout the depth of each layer. Statistical methods for comparison of samples were taken from Hays and Winkler (’71).

RESULTS

GABAergic axon terminals

Sections processed for GAD immunoreactivity and those processed for GABA immunoreactivity show a distinct punctate pattern of labeling that is distributed throughout
Fig. 1. Darkfield photomicrograph showing the distribution of GAD immunoreactive axon terminals in a coronal section through macaque striate cortex. GAD immunoreactive axon terminals are prominent in zones that receive direct projections from the LGN: laminae 4C, 4A and the patches in lamina 3. Scale bar = 150 μm.

the neuropil or directly apposed to cell bodies. While some of these immunoreactive elements may be dendritic processes or fibers cut in cross section, the vast majority are likely to be axon terminals, and we have chosen to apply this term to the punctate immunoreactive staining. In general, the GAD antiserum produced more robust staining of axon terminals than the GABA antiserum, but both antisera produced comparable patterns of labeled terminals throughout the cortex.

GAD and GABA immunoreactive axon terminals are found in all layers of striate cortex from layer 1 to the deep aspect of layer 6, but are particularly striking in the zones that have been shown to receive the bulk of the projection from the LGN: laminae 4C and 4A (Fig. 1). GAD and GABA immunoreactive terminals are also more prominent in the geniculate recipient, cytochrome-rich patches of lamina 3, a finding first reported by Hendrickson et al. (81). The coincidence between the cytochrome-rich and GAD immunoreactive patches can be seen in Figure 2, which shows photomicrographs from adjacent tangential sections through lamina 3A that have been processed for cytochrome oxidase and for GAD immunoreactivity.

The precision with which the prominent GAD immunoreactive terminals match the pattern of geniculate terminations is exemplified by the intricate pattern of terminations that is found in lamina 4A. The projections from the LGN to lamina 4A have been shown to terminate in a weblike or honeycomb pattern when viewed in tangential sections (Hendrickson et al., '78; Blasdel and Lund, '82), and this pattern is reflected in the distribution of cytochrome oxidase staining in this region. Tangential sections through lamina 4A show that GAD immunoreactive terminals are also distributed in a honeycomb pattern and that this pattern is strikingly similar to the pattern of cytochrome oxidase staining (Fig. 3). While the photomicrographs of Figure 3 are from adjacent sections, the honeycomb pattern of cytochrome oxidase staining appears to be shifted to the left of the prominent GAD terminal distribution. This shift is due to the difficulty in achieving perfectly tangential sections through a layer as thin as 4A. Never-
Fig. 2. Comparison of the distribution of prominent GAD immunoreactive terminal staining and cytochrome oxidase staining in adjacent tangential sections through lamina 3 of macaque striate cortex. a: GAD immunocytochemistry (darkfield illumination). b: Cytochrome oxidase histochemistry (brightfield illumination). Cytochrome-oxidase-rich patches are distinguished by prominent GAD immunoreactive terminal staining. Scale bar = 300 μm.

theless, the similarity in the size and shape of the two patterns provides strong evidence that they are coincident. The phrase ‘prominence of terminal staining’ has been used to refer to the difference in the quality of terminal staining that distinguishes the geniculate recipient regions from the others. At first glance, it might be assumed that this difference is simply a reflection of the density of GABAergic terminals. But when viewed at higher-power magnification, GABAergic axon terminals appear to vary in size, and many of the GAD and GABA immunoreactive terminals in the geniculate-recipient regions appear larger in caliber than those in regions that do not receive geniculate terminals (Fig. 4). This apparent difference can be seen between lamina 4C and the immediately adjacent laminae (4B and 5) and between the cytochrome-rich and cytochrome-poor regions of laminae 3 and 4A. A difference in size of this magnitude would be difficult to quantify at the light microscopic level, so the size of putative GABAergic terminals was examined with the electron microscope.

It is generally accepted that GAD and GABA immunoreactive terminals in the cortex have a distinctive morphology. They contain flattened or pleomorphic vesicles and are associated with symmetric synaptic thickenings (Ribak, '78; Hendry et al., '83; Somogyi et al., '83b). For the sake of convenience, profiles with this morphology will be referred to as FS (flat-symmetrical) profiles. If the population of GABAergic axon terminals in the geniculate-recipient regions are larger than those in other regions, there should be a detectable difference in the cross-sectional area of the FS profiles in these regions. To test this idea, the cross-sectional area of FS profiles in lamina 4C were measured and compared with the cross-sectional areas of FS profiles in lamina 5. Typical electron micrographs of FS profiles and profiles with round vesicles and asymmetric contacts (RA profiles) from lamina 4C of macaque striate cortex are shown in Figure 5.

The distributions of area measurements for 158 FS profiles from lamina 4C and 124 FS profiles from lamina 5B are shown in Figure 6 (a and b). Large profiles (> 0.6 μm²) appear to make up a greater proportion of the FS profiles in lamina 4C than their counterparts in lamina 5, and conversely, small profiles (< 0.4 μm²) make up a larger proportion of the FS profiles in lamina 5 than their counterparts in lamina 4C. The difference between the two distributions is best seen in Figure 6c, where the values for each bin in the lamina 5 distribution have been subtracted from the corresponding bins in the lamina 4C distribution. Statistical analysis supports the interpretation that the profiles in lamina 4C are significantly larger than their...
counterparts in lamina 5 (lamina 4C, mean = 0.49 µm², SD = 0.3; lamina 5: mean = 0.35 µm², SD = 0.2; Kolmogorov-Smirnov two-sample test: P < .001). These distributions probably underestimate the actual size of FS boutons since many of the profiles with small cross-sectional areas are parts of larger boutons that have not been sectioned through their centers. Despite this limitation, the results show that FS profiles in lamina 4C, are, on average, 40% larger in cross-sectional area than their counterparts in lamina 5.

**GABAergic cell bodies**

**Laminar differences in size and morphology.** Both antisera labeled neurons that were distributed across all layers of striate cortex. However, the staining for GABA was much more robust and this staining often continued into the proximal portions of dendritic processes. Examples of the morphology of the GABA immunoreactive neurons from different layers of striate cortex are shown in Figures 7 and 8. GABA immunoreactive neurons have smooth or occasionally beaded dendrites that are displayed in multipolar or bitufted dendritic arrangements. Each layer contains a morphologically heterogeneous population of GABAergic neurons that differ in cell size as well as dendritic configuration. In general, the GABA immunoreactive neurons in the superficial layers appear smaller in size than those in other layers and have predominantly vertically oriented dendritic fields. Larger GABA immunoreactive neurons with horizontally oriented dendritic processes are most common in lamina 4C, and in lamina 6.

General impressions about the size distribution of GABAergic neurons were confirmed by measurements of the cross-sectional area of GAD and GABA immunoreactive cell bodies in different layers (Table 1). The GABA immunoreactive population as a whole shows a considerable variation in size, from 25 to 200 µm² in cross-sectional area. While each layer has a range of cell sizes, the largest GAD and GABA immunoreactive neurons are found in lamina 4C, and in lamina 6. Indeed, these are the only two layers that contain immunoreactive neurons with soma areas greater than 140 µm². At the same time, neurons in laminae 2 and 3A are distinctly smaller than those in the other layers; in our sample of 214 neurons from 2-3A, none were found to exceed 110 µm² in area.

The sizes of GABA and GAD immunoreactive neurons in the portions of lamina 4C that receive their projections from the magnocellular and parvocellular layers of the LGN were examined in more detail. Figure 9 shows histograms of the soma area of GABA and GAD immunoreactive neurons in 4C, and 4C, from two different macaque monkeys.
perfused with fixatives that were optimal for each antiserum. In both cases, the distribution of GABAergic neurons in 4C, has a broader range than that of 4C, and includes large neurons that are not found within 4C,. These differences are statistically significant (Kolmogorov-Smirnov two-sample test $P < .001$).

The largest GAD and GABA immunoreactive neurons in lamina 4C, are not distributed evenly throughout the layer but appear more frequently near the lamina 4B border. The size of the neurons in 4C was plotted as a function of their distance from the top of 4C, (the top of 4C being defined as the upper boundary of the zone of prominent GABA immunoreactive axon terminals), and the results of this analysis are presented in Figure 10 (total 316 neurons). This plot shows that a population of large GABA immunoreactive neurons ($> 120 \mu m^2$) is clustered in the upper 20% of lamina 4C (or the upper half of 4C,). These large GABA immunoreactive neurons make up a small percentage of the total population of GABA immunoreactive neurons in lamina 4C, (roughly 7%; nine out of 135), but their restriction to the upper half of 4C, is significant since there are other lines of evidence that the upper part of 4C, is distinct from the lower part (see Discussion).

Density and proportion of GABAergic neurons across cortical layers. While GABA and GAD immunoreactive neurons are found in all layers of striate cortex, there are obvious differences in their density across the cortical depth. GAD and GABA immunoreactive neurons are more dense in laminae 2-3, 4A, and 4C, than in the other laminae. The density of GABA and GAD immunoreactive neurons as a function depth is depicted in the histograms of Figures 11a and 12. A low-power photomicrograph showing the laminar distribution of GABA immunoreactive neurons is shown in Figure 13.

The fluctuation in the density of GABAergic neurons across cortical layers resembles the fluctuation in the density of unlabeled neurons and this relationship is depicted in Figure 11b. In general, regions with a high density of
GABA immunoreactive neurons also appear to have a high density of unlabeled neurons (4Cp, 4A, 2-3A) while zones with a low density of GABA immunoreactive neurons (4B and 5) contain fewer unlabeled neurons. The similarity in the fluctuations in the density of labeled and unlabeled populations across the cortical depth is reflected in the fact that layers that differ markedly in their total density of neurons have similar percentages of GABA immunoreactive neurons. For example, lamina 4Cp, which contains the greatest density of neurons, has a percentage of GABA immunoreactive neurons that is not significantly different from lamina 4B or 4Ccy-layers that contain far fewer total neurons (Fig. 11c, Table 2).

However, the fluctuation in the density of the GABA immunoreactive population is not a simple function of the density of the unlabeled population. If this were true, then the percentage of GABA immunoreactive neurons should be constant across all cortical layers, and, as shown in Figure 8c and Table 2, this was not the case. GABA immunoreactive neurons make up approximately 15% of all the neurons in striate cortex, but layer 1 is clearly distinct from the other layers in containing a very high proportion (>75%) of GABA immunoreactive neurons. This finding is consistent with previous reports in other species and in other cortical areas (Schmechel et al., '84; Gabbott and Somogyi, '86; Lauder et al., '86; Lin et al., '85).

Fig. 5. Examples of profiles with flat vesicles and symmetric synaptic contacts (FS) and those with round vesicles and asymmetric synaptic contacts (RA) from lamina 4C. Arrows point to sites of synaptic contact. Scale bar = 0.2 μm.

Differences between other layers in the proportion of GABA immunoreactive neurons are modest by comparison with lamina 1. In general the proportion of GABA immunoreactive neurons is highest in the superficial layers, decreases through the middle layers, and is lowest in the deep layers. In both monkeys that were examined, lamina 3A had the greatest proportion of GABA immunoreactive neurons (roughly 20%), and this value was almost twice that found in lamina 5 and lamina 6. These differences are statistically significant (lamina 3 vs. lamina 5: \( \chi^2 = 10.9, \) d.f. = 1, \( P < .01 \); lamina 3 vs. lamina 6: \( \chi^2 = 8.8, \) d.f. = 1, \( P < .01 \)).

Areal distribution of GABAergic neurons within lamina 3. One question that remains to be addressed is whether the patchy distribution of GABAergic axon terminals in lamina 3 is associated with a patchy distribution of GABAergic cell bodies. This issue was examined by comparing adjacent tangential sections through lamina 3 that had been reacting for cytochrome oxidase and for GABA immunocytochemistry (Fig. 14). There are no obvious signs of a clustered or patchy distribution of GABA immunoreactive neurons that might correspond to the pattern of cytochrome-oxidase-rich patches. A similar result was found with the GAD antiserum, and an example of the relationship between GAD immunoreactive cell bodies and the cytochrome-oxidase-rich patches is shown in Figure 15. From the photo-
Fig. 6. Cross-sectional area of FS profiles from lamina 4C (a) and lamina 5 (b) of macaque striate cortex. The distributions are significantly different and the nature of this difference is best seen in c, where the bins from the lamina 5 distribution have been subtracted from the lamina 4C distribution. Lamina 4C has a greater percentage of FS terminals that are larger than 0.5 \( \mu m^2 \) in cross-sectional area.

Fig. 7. Camera lucida drawings of GABA immunoreactive neurons from different layers of macaque striate cortex. All neurons were drawn at the same magnification but the widths of the layers are not drawn to scale.
Fig. 8. Photomicrographs of GABA immunoreactive neurons in different laminae of macaque striate cortex. a-e: Lamina 2-3. f,g: Lamina 4C. h,i: Lamina 4C. j-l: Lamina 6. Scale bar = 20 μm.
GABAergic neurons in the cytochrome-rich and cytochrome-poor regions of lamina 3, this difference must be small. To test this possibility, the number of GABA immunoreactive neurons in equal-size samples of cytochrome-rich and cytochrome-poor zones was determined. A total of 1.3 mm² of the cytochrome-rich patches and 1.3 mm² of the cytochrome-poor zones were examined and 1,595 GABA immunoreactive neurons were counted. There was considerable variation in the number of GABA immunoreactive neurons found in the samples from both regions. The average density of GABA immunoreactive neurons was 586 neurons/mm² (SD = 90) in the cytochrome-rich regions and 624 neurons/mm² (SD = 107) in the cytochrome-poor zones. The slightly greater number of GABA immunoreactive neurons found in the cytochrome-poor regions (6%) is not statistically significant (Kolmogorov-Smirnov two-sample test: \( P = .49 \)). Thus, while there is a difference in the GABAergic axon terminals in

### Table 1. Cross-Sectional Area Measurements of GABA and GAD Immunoreactive Neurons in Macaque Striate Cortex

<table>
<thead>
<tr>
<th>Layer</th>
<th>GABA Neurons</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-3A</td>
<td>214</td>
<td>53.0</td>
<td>19.3</td>
<td>24.3-107.7</td>
<td></td>
</tr>
<tr>
<td>3B-4A</td>
<td>196</td>
<td>73.6</td>
<td>18.6</td>
<td>36.9-132.7</td>
<td></td>
</tr>
<tr>
<td>4B</td>
<td>175</td>
<td>64.7</td>
<td>18.6</td>
<td>27.6-134.8</td>
<td></td>
</tr>
<tr>
<td>4C</td>
<td>219</td>
<td>87.2</td>
<td>34.1</td>
<td>21.0-183.3</td>
<td></td>
</tr>
<tr>
<td>4C</td>
<td>249</td>
<td>73.8</td>
<td>18.0</td>
<td>27.4-126.3</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>176</td>
<td>56.9</td>
<td>27.5</td>
<td>24.1-131.8</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>191</td>
<td>72.7</td>
<td>26.8</td>
<td>33.2-160.5</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Layer</th>
<th>GAD Neurons</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-3A</td>
<td>100</td>
<td>60.8</td>
<td>16.3</td>
<td>28.5-104.3</td>
<td></td>
</tr>
<tr>
<td>3B-4A</td>
<td>97</td>
<td>62.0</td>
<td>18.5</td>
<td>31.8-118.6</td>
<td></td>
</tr>
<tr>
<td>4B</td>
<td>107</td>
<td>64.3</td>
<td>19.2</td>
<td>33.6-117.3</td>
<td></td>
</tr>
<tr>
<td>4C</td>
<td>176</td>
<td>98.7</td>
<td>34.7</td>
<td>22.1-197.2</td>
<td></td>
</tr>
<tr>
<td>4C</td>
<td>196</td>
<td>72.0</td>
<td>17.5</td>
<td>35.6-132.5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>99</td>
<td>61.3</td>
<td>22.5</td>
<td>30.3-122.7</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>105</td>
<td>70.3</td>
<td>33.4</td>
<td>26.2-193.3</td>
<td></td>
</tr>
</tbody>
</table>

1Measurements (μm²) are from two different macaque monkeys perfused with fixative optimal for each antiserum.

**DISTRIBUTION OF SOMA SIZE IN LAMINA 4C**

![Graphs](image-url)

Fig. 9. Distribution of cross-sectional area measurements for GAD and GABA immunoreactive neurons in laminae 4C, and 4C, of macaque striate cortex.
the cytochrome-rich and cytochrome-poor regions, this difference is not correlated with a difference in the density of GABAergic cell bodies.

**DISCUSSION**

In this study antisera to GAD and GABA have been used to show that GABAergic neurons constitute a significant proportion of the neurons in macaque striate cortex and that there are differences between laminae in the number and sizes of GABAergic cell bodies and axon terminals. While GAD and GABA immunoreactivity was not examined in tissue from the same animal, the laminar distribution of labeled neurons was found to be quite similar in tissue that was fixed optimally for each antiserum (compare Figs. 11a and 12). Both antisera also showed similar laminar differences in the size of labeled neurons and in the size and/or density of immunoreactive terminals.

However, there were differences in the total density of labeled cell bodies and terminals stained by these antisera: the number of cell bodies labeled and the intensity of cell body labeling seemed greater with the GABA antiserum than with the GAD antiserum. Conversely, axon terminal labeling generally appeared more robust with the GAD antiserum than with the GABA antiserum. These differences in staining intensity do not appear to be unique to the particular antisera that were used in this study. Somogyi et al. ('84a) used a different set of GAD and GABA antisera in the study of GABAergic neurons in cat striate cortex and also noted the increased effectiveness of GABA antiserum for labeling cell bodies. Hendry and Jones ('86) applied still-another GABA antiserum to macaque striate cortex, and their photomicrographs show cell body and terminal staining levels comparable to the GABA antiserum used in the present study. Whatever the reasons for these differences, the overall similarities in the pattern of GAD and GABA immunoreactivity strengthen the argument that the labeled cells and terminals are involved in GABAergic neurotransmission.

**GABAergic axon terminals**

In the present report we have noted the coincidence in the distribution of prominent GABAergic axon terminals...
and the pattern of termination of axons from the LGN. A prominence of GABAergic axon terminals in the geniculate-recipient regions of the striate cortex has also been found in the cat and squirrel monkey (Fitzpatrick et al., '83b; Bear et al., '85). At the very least, this suggests that there is some particular specialization in the inhibitory circuitry associated with the geniculate-recipient regions that may play a crucial role in the transfer of information from LGN axons to neurons within the striate cortex.

The specialized nature of the GAD immunoreactive terminals within the geniculate-recipient zones of macaque striate cortex was first noted by Hendrickson et al. ('81) and interpreted as an increase in their density. While the present results do not contradict the idea that GAD-immunoreactive axon terminals in these regions may be more numerous, they do suggest that, as a population, they are larger in size than the axon terminals in the regions that do not receive geniculate afferents. A difference in the size and density of the GAD immunoreactive terminals has also been noted between lamina 4 and other layers of macaque somatic cortex (Houser et al., '84).

Fig. 12. Distribution of GAD immunoreactive neurons in 70-μm steps through macaque striate cortex.

Fig. 13. Photomicrograph showing the distribution of GABA immunoreactive neurons in a coronal section through macaque striate cortex. Scale bar = 150 μm.
TABLE 2. Numbers of GABA Immunoreactive Neurons in Different Layers of Macaque Striate Cortex

<table>
<thead>
<tr>
<th>Layer</th>
<th>No. of GABA and unlabeled neurons</th>
<th>No. of GABA neurons</th>
<th>% GABA neurons</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21</td>
<td>17</td>
<td>81.0</td>
</tr>
<tr>
<td>2</td>
<td>330</td>
<td>43</td>
<td>13.0</td>
</tr>
<tr>
<td>3A</td>
<td>445</td>
<td>86</td>
<td>19.3</td>
</tr>
<tr>
<td>3B</td>
<td>516</td>
<td>79</td>
<td>15.3</td>
</tr>
<tr>
<td>4A</td>
<td>409</td>
<td>77</td>
<td>18.8</td>
</tr>
<tr>
<td>4B</td>
<td>231</td>
<td>33</td>
<td>14.3</td>
</tr>
<tr>
<td>4C</td>
<td>434</td>
<td>59</td>
<td>17.2</td>
</tr>
<tr>
<td>4C</td>
<td>614</td>
<td>92</td>
<td>15.0</td>
</tr>
<tr>
<td>5</td>
<td>335</td>
<td>30</td>
<td>11.6</td>
</tr>
<tr>
<td>6</td>
<td>473</td>
<td>57</td>
<td>12.1</td>
</tr>
<tr>
<td>Total</td>
<td>3,717</td>
<td>582</td>
<td>(Avg.) 15.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Layer</th>
<th>No. of GABA and unlabeled neurons</th>
<th>No. of GABA neurons</th>
<th>% GABA neurons</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17</td>
<td>13</td>
<td>76.5</td>
</tr>
<tr>
<td>2</td>
<td>175</td>
<td>31</td>
<td>17.7</td>
</tr>
<tr>
<td>3A</td>
<td>314</td>
<td>67</td>
<td>21.3</td>
</tr>
<tr>
<td>3B</td>
<td>381</td>
<td>55</td>
<td>14.4</td>
</tr>
<tr>
<td>4A</td>
<td>299</td>
<td>47</td>
<td>15.7</td>
</tr>
<tr>
<td>4B</td>
<td>167</td>
<td>24</td>
<td>14.4</td>
</tr>
<tr>
<td>4C</td>
<td>254</td>
<td>33</td>
<td>13.0</td>
</tr>
<tr>
<td>4C</td>
<td>395</td>
<td>52</td>
<td>13.2</td>
</tr>
<tr>
<td>5</td>
<td>217</td>
<td>28</td>
<td>13.4</td>
</tr>
<tr>
<td>6</td>
<td>323</td>
<td>26</td>
<td>8.9</td>
</tr>
<tr>
<td>Total</td>
<td>2,540</td>
<td>379</td>
<td>(Avg.) 14.9</td>
</tr>
</tbody>
</table>

In the original description of GAD immunoreactivity in macaque striate cortex (Hendrickson et al., '81), attention was focused on the similarity in the distribution of GAD immunoreactivity and the patchy pattern of cytochrome oxidase staining in lamina 3. However, it is not clear from that report whether the increased GAD immunoreactivity in the cytochrome-rich patches consisted of GAD immunoreactive terminals alone or an increase in both labeled cell bodies as well as terminals. Another study using the uptake of $^3$H-GABA as a marker for GABAergic neurons presented evidence for an increased density of GABA-accumulating neurons within the cytochrome-rich patches (Carroll and Wong-Riley, '85). The present report demonstrates that the cytochrome-rich regions of lamina 3 are distinguished by an increased size and, perhaps, density of GAD immunoreactive terminals. However, these regions do not differ from the rest of lamina 3 in their density of GABAergic neurons. These results are in agreement with a previous report of the distribution of GAD immunoreactive neurons in lamina 3 of squirrel monkey striate cortex (Fitzpatrick et al., '83b) and the recent description of GABA immunoreactive neurons in macaque striate cortex (Jones et al., '86). Since the cytochrome-rich patches in lamina 3 are the targets of a direct projection from the LGN (Livingstone and Hubel, '82; Fitzpatrick et al., '83a), it seems reasonable to view the distinctive pattern of GAD immunoreactive terminals as another example of the prominent role that
Fig. 15. a: Distribution of GAD immunoreactive neurons in a tangential section through lamina 3 of macaque striate cortex. Each dot represents a single labeled neuron. Circles represent blood vessels used as landmarks. b: Comparison of the distribution of GAD immunoreactive neurons shown in Figure 15a with the distribution of cytochrome oxidase activity in an adjacent section. Cytochrome-oxidase-rich regions are depicted with dark gray stipple. Scale bar = 250 μm.
GABAergic inhibition plays in regions that receive direct projections from the LGN.

The coincidence in the spatial distribution of large GABAergic axon terminals and terminals derived from the LGN is further emphasized by their correspondence in the honeycomb matrix of lamina 4A. The exact significance of the honeycomb distribution of geniculate terminations in lamina 4A remains unknown. Presumably it reflects some form of segregation between different afferents to this region, such as those from the LGN and from lamina 4C (Fitzpatrick et al., '85), or some specialization in the arrangement of the dendrites that are postynaptic to the geniculate terminations. Whatever the basis for this pattern of terminations, the close spatial correspondence of GABAergic axon terminals and LGN axon terminals raises the question of whether some of the GAD immunoreactive axon terminals might be derived from the LGN. Most lines of evidence do not support the idea that GABAergic neurons in the LGN project to striate cortex. For example, GAD or GABA immunoreactive neurons within the LGN are not labeled following injections of retrograde tracers into cat (Fitzpatrick et al., '84; Montero and Zempel, '85) or macaque (Montero, '86) striate cortex, and GAD immunoreactive terminals within layer 4 are not noticeably diminished following the undercutting of striate cortex (Bear et al., '85). Furthermore, most studies report that LGN axon terminals have round vesicles and establish asymmetric synaptic contacts (Garey and Powell, '71; LeVay and Gilbert, '76; LeVay, '86; Einstein et al., '87) in contrast to GAD immunoreactive profiles that have flat vesicles and make symmetric synaptic contacts (Ribak, '78; Hendry et al., '83; Somogyi et al., '85b). While Einstein et al. ('87) have recently reported that some of the profiles within layer 4 of cat striate cortex that are labeled following injections of 3H-amino acids into the LGN have flattened vesicles and make symmetric synaptic contacts, there is, at present, no evidence that these terminals are immunoreactive for GAD or GABA.

If one accepts the prevailing view that GABAergic terminals within cortex have a local origin, then it is natural to wonder whether the large GABAergic terminals in the geniculate-recipient regions originate from a particular type of neuron. One possible source for the prominent endings in the geniculate recipient regions is a class of small aspiny neurons that has been given a variety of names including "spider cell," "clewed" cell, and more recently, "clutch cell" or "B1 variety" (Ramón y Cajal, 1899; Valverde, '71; Lund '73, '87; Lund et al., '77; Mates and Lund, '83; Kisvarday et al., '85, '86). This suggestion is consistent with the fact that these neurons (1) appear to be restricted to layers 4C and 4A, where they are the most frequently impregnated smooth dendritic neurons; (2) have axons characterized by a dense, highly branched field close to the soma and by the presence of conspicuously large boutons; and (3) are immunoreactive for GABA. Kisvarday et al. ('86) have also argued for a unique association between the axon terminals of this cell class and those from the LGN, citing the similarity in the sizes of the axonal fields of clutch cells and LGN afferents and the correspondence in their postsynaptic targets within lamina 4C. In the macaque the major targets for both clutch cell axon terminals and LGN axon terminals are spines and dendritic shafts, presumed to be from spiny stellate neurons. It would be of some interest to determine the spatial relationship between the synaptic contacts of LGN terminals and clutch cell terminals along the dendrites of individual postsynaptic neurons. Perhaps the matching distribution of GABAergic and LGN terminals in lamina 4A signifies an underlying similarity in the postsynaptic targets of these two populations of boutons. Whether or not the large GABAergic axon terminals are derived from a single cell type, the question remains as to the functional significance of prominent terminal staining in the geniculate-recipient regions. One major difference between neurons in the LGN and those in striate cortex lies in their level of spontaneous activity. Neurons in the LGN have spontaneous activity levels at least three times higher than neurons in the striate cortex (Creutzfeldt and Ito, '68). This difference that can be detected in the cortical termination zones of geniculate afferents (low spontaneous discharge rates characterize the neurons in lamina 4C and lamina 4A in monkey striate cortex; Blasdel and Fitzpatrick, '84). Presumably, this difference reflects the fact that neurons within layer 4 are under the influence of tonic inhibition derived from interneurons within the cortex. Perhaps the larger-caliber swellings of GABAergic neurons in the geniculate-recipient regions are specializations that act to "balance" the strong excitatory drive of the geniculate afferents. For example, large-caliber endings provide a larger surface area that could give rise to a greater number of synaptic contacts. Against this suggestion is the evidence from Beaulieu and Colonnier ('85) that type 2 synaptic contacts are not more numerous in layers 4A and 4B in cat striate cortex—layers also distinguished by large-caliber GAD immunoreactive profiles. Another possibility is that the more frequent release of neurotransmitters which would be expected in a tonically active system might require a larger pool of synaptic vesicles or a larger number of mitochondria—needs that could be met by a larger axon terminal. In this respect it is interesting to note that Kageyama and Wong-Riley ('86) have described a distinct type of large-caliber axon terminal in layer 4 of cat striate cortex that establishes symmetric synaptic contacts and contains a large number of mitochondria that are rich in cytochrome oxidase activity. Also, the recent observation that the axonal swellings of clutch cells contain "large groups of mitochondria" is consistent with this idea (Kisvarday et al., '85).

**Distribution of GABAergic cell bodies**

The results of this study support the idea that GABAergic neurons constitute at least 15% of the neurons in monkey striate cortex. Previous attempts to estimate the percentage of GABAergic neurons in macaque neocortex have produced a wide range of values. For example, Mates and Lund ('83) used the observation that Golgi-impregnated aspiny neurons are distinguished from spiny neurons by the presence of both type 1 and type 2 contacts on their somata (LeVay, '73) to estimate that roughly 6% of the neurons in lamina 4C were aspiny stellate neurons. In contrast, Hendry and Jones ('81) suggested that up to 40% of the neurons in monkey somatosensory and motor cortex might be GABAergic since they appeared to selectively accumulate 3H-GABA. The present results are closer to the values obtained with GABA immunocytochemistry by Gabott and Somogyi ('86) in cat striate cortex (20%) and recently by Jones et al. ('86) in monkey striate cortex (20%).

While GABA immunocytochemistry appears to be a reliable method for identifying GABAergic neurons, it does...
have several limitations that may influence the accuracy of quantitative analysis. For example, even though 15–20-μm vibratome sections were used for cell counts, and alcohol pretreatment was used to increase the depth of penetration, it is still possible that our figures underestimate the total number of GABA immunoreactive neurons because a certain percentage of small cell bodies that lie in the center of the section were not detected. Likewise, it is possible that some GABAergic neurons are not detected because the levels of GABA within their somata fall below the detection threshold of the antisera. Recent evidence that the number of GABA immunoreactive neurons detected in layer 4 of macaque striate cortex is reduced by enucleation or visual deprivation (Hendry and Jones, '86) suggests that GABA levels within neurons can vary and opens up the possibility that at any given time, some percentage of the GABA-containing population may be below the threshold for detection. Given these considerations, it seems prudent to view cell counts as minimal estimates since a variety of factors, physiological and/or methodological, may affect the number of cells labeled in any given animal.

Both the GAD and GABA antisera show consistent laminar differences in the density of labeled neurons. The greatest density of labeled neurons is found in laminae 4C, 4A and in laminae 2-3. The increased density of labeled neurons in the geniculate-recipient zones (4C, and 4A) is consistent with the observations made by Gabbott and Somogyi ('86) in cat visual cortex. In the cat, the greatest number of GABAergic neurons is found in laminae 4 and 3B, and since these regions are the major targets of geniculate afferents, they argued that this distribution reflects the allocation of a large share of cortical GABAergic inhibition to the early stages of visual processing. However, it should be pointed out that laminae 4 and 3B in the cat and lamina 4C, and 4A in the monkey also contain the greatest density of non-GABAergic neurons. As a result, the ratio of GABA immunoreactive neurons to the unlabeled population in these layers is not greater (and may be less) than that found at other levels within the superficial layers. In addition, lamina 4C, and the cytochrome-rich patches of lamina 3 in macaque are prominent geniculate-recipient regions, but they do not have an increased density of GABAergic neurons.

There does seem to be a significant difference in the percentage of GABA immunoreactive neurons in the superficial and deep layers of striate cortex. The present study suggests that almost 20% of the neurons in the upper part of lamina 3 are GABAergic, while roughly 12% of the neurons in laminae 5 and 6 are GABAergic. A difference in the proportion of GABAergic neurons in the superficial and deep layers has been reported also in the visual cortex of the cat (Gabbott and Somogyi, '86). This difference may reflect the fact that there is a greater number of potential synaptic contact sites for the GABAergic neurons within lamina 3 since they have access to the processes of neurons whose somata lie within the layer as well as the apical dendrites of pyramidal neurons whose somata lie in the deeper layers. This seems unlikely to be the whole explanation, however, since at least some GABAergic neurons in the superficial layers project to the deep layers and vice versa (Valverde, '71; Lund, '73; Somogyi et al., '81, '83a).

**Morphology of GABAergic neurons**

The results of this study are consistent with the evidence derived from a variety of sources that GABAergic neurons in macaque striate cortex are non-pyramidal neurons with smooth or beaded dendritic processes. In addition, these results demonstrate conspicuous laminar differences in the soma size and the orientation of the initial dendritic segments of GABAergic neurons. Perhaps the most distinctive neurons observed in this study are the large GABA and GAD immunoreactive neurons found in the upper part of 4C, and in lamina 6. Jones ('75) and DeFelipe et al. ('86) have argued that the largest of the smooth dendritic neurons in somatic and motor cortex of macaque belong to the class of cells referred to as basket cells. Basket cells are distinguished from most other classes of smooth dendritic neurons by axon arbors that form terminal nests around the somata of pyramidal neurons and other cell types (Marin-Padilla, '69; Szentágothai, '73; Jones '75; Somogyi et al., '83c).

If this difference in size applies to the smooth dendritic population in macaque striate cortex, then the large neurons in the upper part of 4C, and in lamina 6 would be good candidates for basket cells. A recent Golgi study (Lund, '87) has described a population of aspiny neurons restricted to the upper part of 4C, that has horizontally spreading dendrites much like the GABA immunoreactive neurons described here. These neurons also have axon arbors that extend horizontally for long distances (over 1 mm) within upper 4C, and 4B and give rise to short vertical collaterals resembling those identified as basket cell terminations by Somogyi et al. ('83c) and DeFelipe et al. ('86). Long-distance lateral connections are also characteristic of basket cells in somatosensory and motor cortex (Marin-Padilla, '69; Jones, '75).

Large aspiny neurons with horizontally displayed dendrites in lamina 6 have received only casual attention in previous studies using the Golgi method. Jones ('75) noted that basket cells in layer 6 of somatosensory cortex could be extended in a horizontal form, and both Lund ('73) and Tömböl ('78) mentioned the presence of large aspiny neurons with horizontally directed dendritic processes in lamina 6 of monkey striate cortex. Horizontally directed axonal processes with basketlike terminations have been observed in lamina 6 of macaque striate cortex, but the source of these axon arbors remains to be determined (Lund, unpublished observations).

If the large GABA immunoreactive neurons in lamina 4C, and lamina 6 are, indeed, basket cells, then these regions may be expected to have long-distance lateral connections that are, at least in part, inhibitory. Long-distance lateral connections are also characteristic of the superficial layers of striate cortex, but the bulk of the evidence suggests that these projections originate in pyramidal neurons and that their effect is excitatory (Gilbert and Wiesel, '83; Rockland and Lund, '83; Rockland, '85; Ts'o et al., '86). Differences in the lateral extent of inhibitory connections could obviously contribute to laminar differences in receptive field properties. In this context it is worth noting that lamina 4C, 4B and lamina 6 contain neurons with directionally selective receptive fields and that neurons in 4B and 6 are the source of projections to the middle temporal area (MT) which contains a high proportion of directionally selective neurons (Dow, '74; Rockland and Pandya, '79; Tigges et al., '81; Livingstone and Hubel, '84; Hawken and Parker, personal communication). Whether long-distance inhibitory connections contribute to the directionally selective responses of these neurons remains to be determined.

Finally, the presence of a population of large GABA immunoreactive neurons in the upper portion of 4C, provides
further evidence for a functional subdivision within the cortical target of the magnocellular layers of the LGN. The original subdivision of lamina 4C into two tiers, $\alpha$ and $\beta$, was based on differences in the dendritic morphology and projections of spiny stellate neurons; those in 4C$_\alpha$ were shown to send their axons into lamina 4B, whereas the spiny stellate neurons in 4C$_\beta$ were shown to send their axons vertically through 4B, without arborization, to terminate in lamina 3B (Lund, '73). This distinction was reinforced by the demonstration that the magnocellular and parvcellular layers of the LGN had largely separate terminal fields within 4C, and 4C$_\alpha$ respectively (Hubel and Wiesel, '72; Hendrickson et al., '78). More recent evidence suggests that 4C and 4C$_\alpha$ may not be uniform throughout their depth, and that both contain sublaminae with different patterns of connections (Blasdel et al., '85; Fitzpatrick et al., '85).

The upper part of 4C$_\alpha$ appears to be distinguished from the lower part by (1) the termination of a distinct type of geniculocortical axon (Blasdel and Lund, '82); (2) the presence of laterally spreading axon arbors that terminate in patches in upper 4C, and in 4B (Rockland and Lund, '83; Blasdel et al., '85; and) (3) the presence of orientation-selective neurons (Blasdel and Fitzpatrick, '84). Our finding of a class of large GABAergic neurons in upper 4C, adds to the evidence for further subdivision of 4C, and complements Lund's observation that upper 4C$_\alpha$ contains aspiny neurons that are distinct from those in lower 4C$_\alpha$ and 4C$_\beta$ (Lund, '87).

ACKNOWLEDGMENTS

This research was supported by grants EY-06661 and EY-05282 from the National Eye Institute, and VA Career Development Award and National Institute on Aging ADRC grant AG-05128. David Fitzpatrick is an Alfred P. Sloan research fellow. We thank Jill Einstein for her helpful comments, Jay Gill for his invaluable help with the quantitative analysis, Carolyn Cox and Tom Harper for their help with the histology and Susan Havrilesky for preparing the manuscript.

LITERATURE CITED


Kisvarday, Z.F., K.A.C. Martin, D. Whitberidge, and P. Somogyi (1985) Synaptic connections of intracellularly filled clutch cells, a type of small
GABAERGIC NEURONS IN STRIATE CORTEX


