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# GLUTAMIC ACID DECARBOXYLASE-IMMUNOREACTIVE NEURONS AND TERMINALS IN THE LATERAL GENICULATE NUCLEUS OF THE CAT[1]

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## Abstract

We have examined the distribution of neurons and terminals that are immunoreactive for glutamic acid decarboxylase (GAD), the synthesizing enzyme for the inhibitory neurotransmitter γ-aminobutyric acid within the lateral geniculate nucleus of the cat. We estimate that GAD-positive neurons constitute approximately one-fourth of the neurons in all layers of the lateral geniculate nucleus and in the medial interlaminar nucleus (MIN). In addition, almost all of the neurons within the perigeniculate nucleus are GAD-positive. The mean size of GAD-positive cell bodies is significantly smaller than the mean size of unlabeled neurons in all subdivisions of the lateral geniculate nucleus. GAD-positive neurons have thick primary dendrites which are associated with thin lightly immunoreactive processes that give rise to clusters of GAD-positive terminals. Clusters of GAD-positive terminals are prominent in lamina A, A1, magnocellular C, and MIN but are rare in the parvocellular C laminae. Within the A laminae, GAD immunoreactivity is found within vesicle-containing profiles of the synaptic glomerulus lying postsynaptic to optic axon terminals and presynaptic to unlabeled dendritic profiles. GAD-positive neurons in the A laminae are distinguished from other small to medium-sized neurons by their failure to label following injections of HRP into visual cortex and by their lack of cytoplasmic laminated body. These results support the idea that GAD-positive neurons constitute a distinct population of neurons in the lateral geniculate nucleus of the cat; a population which has a number of features in common with previous descriptions of presumed local circuit neurons based on Golgi staining.

The idea that there are two fundamental classes of thalamic neurons, projection neurons whose axons terminate in the cortex and smaller size local circuit neurons whose axons remain in the thalamus, has provided the conceptual framework for numerous studies of cell types within the thalamus. The distinction between short axon and long axon thalamic neurons was first established in the Golgi studies of Ramón y Cajal (1909, 1966). More recent studies of Golgi-impregnated material have noted additional differences between these classes of neurons, including the presence of complex appendages on the dendrites of local circuit neurons (Guillery, 1966; Morest, 1971; Famiglietti and Peters, 1972). These dendritic appendages are thought to be the source of the numerous presynaptic dendritic profiles of the synaptic glomerulus: vesicle-containing profiles which lie postsynaptic to axon terminals and are presynaptic to other dendritic profiles (Peters and Palay, 1966; Guillery, 1969; Morest, 1971; Famiglietti and Peters, 1972).

The morphological distinction between projection neurons and local circuit neurons has been accompanied by a proposed functional distinction. Projection neurons are regarded as providing excitatory relays to the cortex, whereas local circuit neurons are viewed as a major source of inhibition within the thalamus. Support for the idea that inhibition within the lateral geniculate nucleus of the cat is mediated by a population of local circuit neurons comes from several sources. First, physiological studies have demonstrated postsynaptic inhibitory potentials within the lateral geniculate nucleus following stimulation of the optic tract, and the cells responsible

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for this inhibition cannot be antidromically activated following stimulation of the visual cortex (Dubin and Cleland, 1977; Lindström, 1982). Second, these inhibitory potentials appear to be at least partially mediated by the neurotransmitter γ-aminobutyric acid (GABA) (Curtis and Tebecis, 1972), and a distinct population of small neurons within the A lamina of the lateral geniculate body has been found to have a high affinity uptake system for [$^3$H]GABA (Sterling and Davis, 1980). Third, the number and size distribution of the neurons which accumulate [$^3$H]GABA are similar to those of a population of cells which remain unlabeled following injections of HRP into visual cortex (LeVay and Ferster, 1979; Geisert, 1980; Weber and Kalil, 1983).

In the present report, we have studied the proposed relationship between local circuit neurons and inhibition by examining the cells and terminals which are immunoreactive for glutamic acid decarboxylase (GAD), the enzyme necessary for the synthesis of GABA. The results of this study show that GAD-immunoreactive neurons constitute a distinct class of small neurons which is present in all subdivisions of the lateral geniculate nucleus of the cat and exhibits many of the morphological features previously associated with local circuit neurons. In addition, we have found that almost all of the neurons in the perigeniculate nucleus are immunoreactive for GAD. Some of these results have been presented previously in abstract form (Fitzpatrick et al., 1982).

## Materials and Methods

For light microscopy, adult cats were deeply anesthetized and perfused through the heart with 0.9% saline solution containing 0.5% sodium nitrite followed by one of the following fixatives: 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4; 0.5% zinc salicylate dissolved in 10% formalin in $H_2O$; or a mixture of paraformaldehyde, lysine, and sodium periodate made according to the protocol of McLean and Nakane (1974). The animals perfused with 4% paraformaldehyde or the zinc-formaldehyde mixture had the fixative flushed out with a solution of 10% sucrose in 0.1 M phosphate buffer, pH 7.4, containing 2% dimethyl sulfoxide. For electron microscopy, cats were perfused with 4% paraformaldehyde, 0.25% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4.

Sections of the lateral geniculate nucleus were cut either coronally or parasagittally at 25 to 30 μm on a freezing microtome or at 15 to 20 μm on an Oxford vibratome and were collected in 0.01 M phosphate-buffered saline, pH 7.6 (PBS). Selected sections were processed for GAD immunocytochemistry using either the unlabeled antibody, peroxidase-antiperoxidase method (PAP) (Sternberger, 1979), or the avidin-biotin method (ABC) (Hsu et al., 1981) (Vector Laboratories).

We used the antiserum to GAD developed by Oertel et al. Details of the purification of GAD and the preparation of the antiserum have been published (Oertel et al., 1981a, b). Sections were incubated in primary antiserum diluted 1:1500 to 1:2000 in PBS containing 0.5 to 1% normal rabbit serum for 2 to 24 hr. Linking antisera (rabbit anti-goat) were used at a dilution of 1:20 for PAP or 1:50 for ABC. The ABC or PAP complexes were localized with 0.1%, 3,3′-diaminobenzidine tetrahydrochloride (DAB) in PBS containing 0.001% $H_2O_2$. For controls, adjacent sections were incubated in normal sheep serum at 1:1000 in PBS and then processed in the same way as the sections incubated in anti-GAD serum. Control sections showed no labeling of cell bodies or terminals.

Some GAD-immunoreacted Vibratome sections from cats processed for light microscopy were dehydrated and flat embedded in Epon between Teflon-coated coverslips. For examination of cytoplasmic laminar bodies, serial 2-μm Epon sections were cut on a Sorval ultramicrotome, floated onto glass slides, and stained with toluidine blue. For electron microscopy, immunoreacted sections were treated for 30 min in 1 or 2% $OsO_4$ prior to dehydration and embedding in Epon. Thin sections from this material were collected onto either Formvar-coated or slotted copper grids. Some grids were stained with uranyl acetate and lead citrate, and others were examined without heavy metal staining.

In two cats injections of horseradish peroxidase (HRP) were made into the visual cortex to test whether the GAD-immunoreactive neurons in the lateral geniculate nucleus project to visual cortex. Multiple injections of 0.1 to 0.2 μl of 20% HRP dissolved in 0.1 M trizma buffer, pH 7.6, or 0.9% saline containing 2% lysophosphatidyl choline (Sigma) were made into the medial bank of the lateral gyrus using a Hamilton syringe attached to a glass micropipette. An additional cat received an injection of HRP into the lateral geniculate nucleus via iontophoresis (25-μm glass micropipette tip, 2 μA, 20 min). Vibratome-cut sections through the lateral geniculate nucleus were processed in the following manner:

1. Tissue sections were incubated with a chromagen, either DAB (brown) or cobalt-DAB (black) (Adams, 1977), and $H_2O_2$ to demonstrate transported HRP.
2. The HRP in the tissue was inactivated by immersing the sections in 5 to 10% formalin for 24 hr.
3. Tissue sections were processed for GAD immunocytochemistry using a chromagen which would contrast with the chromagen used in step one. The chromagen combinations used were: cobalt-DAB and DAB; DAB and 4-chloronapthol (blue); DAB and *o*-dianisidine (green).

All three of the chromagen combinations produced clear examples of transport-labeled and immunolabeled neurons. However, some problems were encountered with the cobalt-DAB and DAB combination since the cobalt-DAB reaction product was accompanied, in some instances, by a light brown background labeling of the cells. Since this potential source of confusion could be detected prior to the immunoreaction, sections displaying this feature were rejected prior to further processing. For the purposes of determining percentages and making cell measurements from the double label material, selected sections were counterstained with cresyl violet.

Cell measurements on counterstained vibratome or frozen sections were made by drawing samples of GAD-labeled or Nissl-stained somata at × 1667 using a × 100 oil objective and a camera lucida. To ensure that fragments of cells were not measured, only neurons with well defined nucleoli were drawn. Cell bodies were drawn in

the focal plane of the nucleolus. From these drawings, somal areas were calculated using an Apple graphics tablet and computer.

In one experiment (#2262) somal areas of GAD-immunoreactive neurons were measured in sections which had not been counterstained, and these were compared with measurements taken from adjacent Nissl-stained sections, not processed for immunocytochemistry. In these cases, GAD-immunoreactive neurons were drawn and measured only if they displayed a clear nucleus.

Cell measurements were also made on the cell bodies of neurons which were reconstructed from Epon-embedded sections. These reconstructions were made by drawing the outlines of cell bodies and blood vessels from serial sections onto tracing paper and using the blood vessels as landmarks to align the sections.

The percentage of GAD-immunoreactive neurons in the lateral geniculate nucleus was estimated by counting the relative numbers of GAD-immunoreactive neurons and unlabeled Nissl-stained neurons in samples taken from counterstained sections. This percentage was calculated according to the formula: percentage of GAD = $100 \times (\text{GAD}/(\text{GAD} + \text{unlabeled Nissl}))$. In counts taken from double label material, the number of HRP-labeled neurons was substituted in place of unlabeled Nissl-stained neurons.

## Results

*Distribution of GAD-positive neurons and terminals in the lateral geniculate nucleus.* The distribution of GAD-immunoreactive cell bodies and terminals in the lateral geniculate nucleus of the cat is shown in Figures 1 and 2. GAD-immunoreactive cell bodies and terminals are found in all subdivisions including the A laminae, the C complex, and the medial interlaminar nucleus (MIN). The interlaminar zones between A and A1 as well as between the various sublaminae of the C complex are distinguished by a paucity of GAD-positive terminals (Figs. 1*a* and 2*a*). However, the interlaminar zones are not without GAD-positive cell bodies, and several examples of GAD-positive cell bodies within the A, A1 interlaminar zone are shown in Figure 7.

The majority of the GAD-positive terminals within the A laminae appear either singly or are aggregated into clusters within the neuropil. GAD-positive terminals are seen surrounding the proximal dendrites of unlabeled neurons but are only rarely found in direct apposition to the somata of either GAD-positive neurons or unlabeled Nissl-stained neurons. Distinct terminal clusters, varying in size from 2 to 10 $\mu$m in diameter, are rather evenly distributed in the neuropil of the A laminae and are also found in the magnocellular C laminae and MIN. In contrast, well defined GAD-positive terminal clusters are rarely encountered within the parvocellular C laminae or in the interlaminar zones (Fig. 2, *b* and *c*).

In addition to the GAD-positive neurons within the lateral geniculate nucleus, a prominent zone of GAD-positive cell bodies and terminals is also found lying immediately dorsal and rostral to lamina A. This scattered array of GAD-positive cell bodies and terminals, separated by nonimmunoreactive fibers of the optic radiation, constitutes the perigeniculate nucleus (Laties and Sprague, 1966; Sanderson, 1971; Kawamura et al., 1974). Virtually all of the cells of the perigeniculate nucleus are GAD-positive since no unlabeled cells are present in sections counterstained with cresyl violet. The proximal dendrites of these cells are, in general, oriented parallel to the laminae of the geniculate and are contacted by numerous GAD-positive terminals. GAD-positive terminals are also found surrounding the cell bodies of GAD-positive neurons in the perigeniculate nucleus (Fig. 1, *c* and *d*).

At rostral and caudal levels, the perigeniculate nucleus is clearly separate and distinct from the reticular nucleus which lies more dorsal and rostral. However, at other levels this distinction is more difficult to make, particularly since the neurons of the reticular nucleus are also GAD-positive. Indeed, it is probably reasonable to consider the perigeniculate nucleus as an extension of the reticular nucleus, given the similarities in their relation to thalamus and cortex, and the fact that virtually all of the neurons in both structures are GAD-positive.

*Size and percentage of GAD-positive neurons.* We compared the size and relative number of the GAD-positive neurons with the rest of the neurons in the lateral geniculate nucleus by examining sections which were counterstained with cresyl violet. As shown in Figure 3*c*, the nucleus of GAD-positive neurons does not contain reaction product so that Nissl-stained nucleoli can be identified in these neurons and can be used as a basis for counting neurons and subsequently measuring somal areas and determining percentages.

The most striking feature of the GAD-immunoreactive neurons in comparison to unlabeled Nissl-stained cells of the same region is their small size (Fig. 3, *b* and *c*). We measured the sizes of GAD-positive neurons and unlabeled Nissl-stained neurons from different subdivisions of the lateral geniculate nucleus, and the results from one cat are displayed in histogram form in Figure 4. In each region, the GAD-positive neurons have a significantly smaller mean somal area than unlabeled-neurons (median test: A laminae, $X^2 = 27.1$; parvocellular C layers, $X^2 = 15.3$; MIN, $X^2 = 25.8$; $p < 0.005$). To show that the difference in size between GAD-positive and unlabeled Nissl-stained neurons is a reliable one, we determined the mean size of GAD-positive and unlabeled neurons in a number of different cats. The ratio of the mean size of GAD-positive neurons to unlabeled neurons (Table I) remains relatively constant across samples, despite some differences in the actual size of the GAD-positive and unlabeled neurons in different animals. In the A laminae, the mean size of GAD-positive neurons is 40 to 50% smaller than the mean size of unlabeled neurons, whereas in the parvocellular C layers, GAD-positive neurons are on the average 30% smaller. Variation in actual cell size between animals could reflect individual differences and/or differences in fixation or tissue processing.

We arrived at an estimate of the percentage of GAD-positive neurons in various subdivisions by counting the number of GAD-positive and unlabeled neurons with nucleoli in samples which had been used for cell measurement. We found that GAD-positive neurons make up 19 to 26% of the cells in the A laminae (Table I) and a

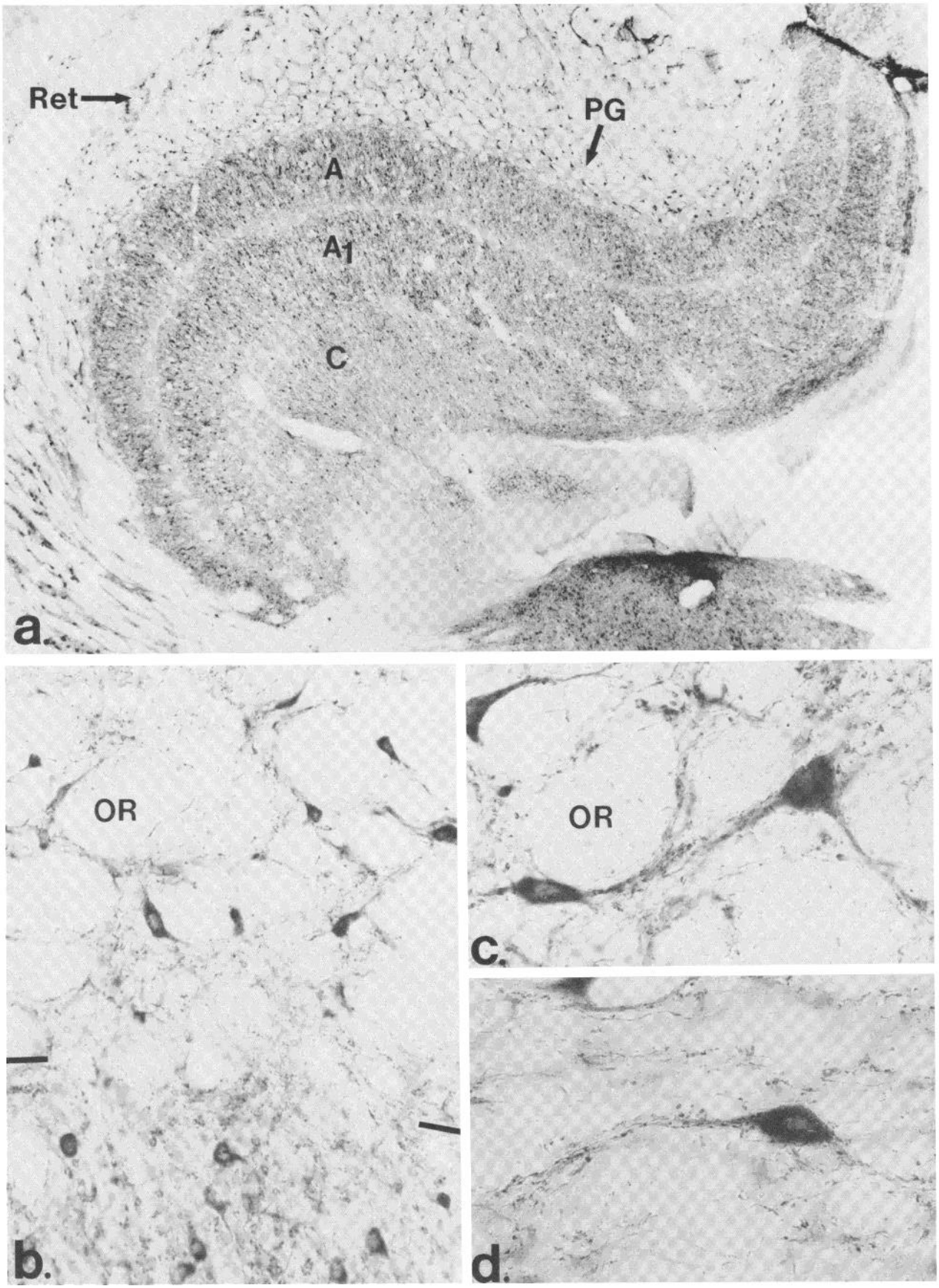


*Figure 1.* *a*, Low power photomicrograph of a parasagittal section through the lateral geniculate nucleus of the cat processed for GAD immunocytochemistry. GAD-positive neurons are found in all layers, while interlaminar zones have fewer GAD-positive cells and terminals. GAD-positive cells and terminals are also found in the perigeniculate nucleus (*PG*) and in the reticular nucleus (*Ret*). The distinction between these two regions is not always sharp. Magnification × 30. *b*, Distribution of GAD-positive cells and terminals at the border of lamina A and the perigeniculate nucleus (*solid black lines* denote this border). GAD-positive cells and terminals in the perigeniculate nucleus form a web-like network through which unlabeled optic radiation (*OR*) fibers pass. Magnification × 350. *c* and *d*, Higher power photomicrographs of the perigeniculate nucleus showing GAD-positive neurons contacted by numerous GAD-positive terminals. Magnification × 600.

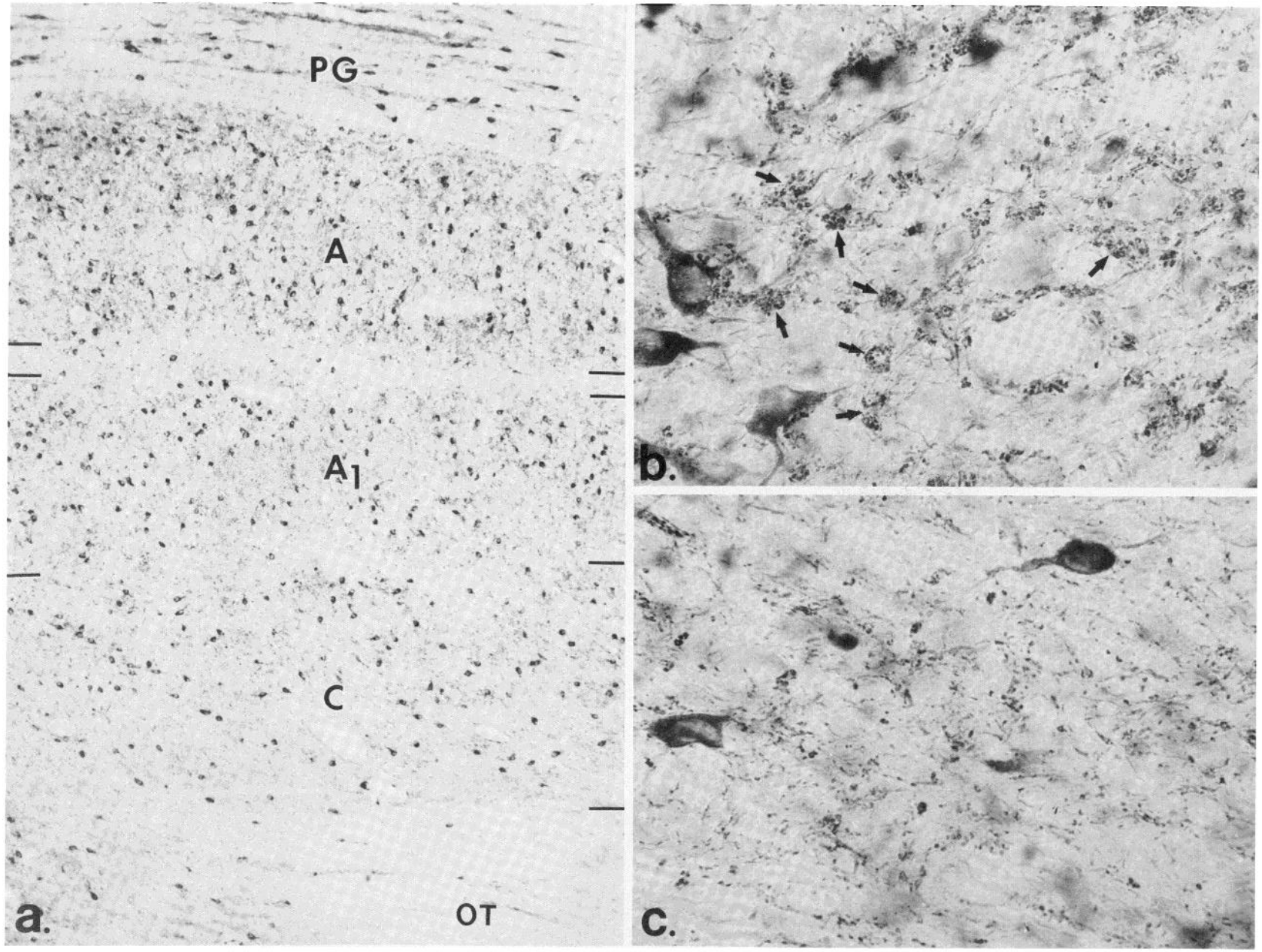


*Figure 2. a*, The distribution of GAD-positive neurons and terminals in the layers of the lateral geniculate nucleus. Cell bodies appear to be randomly distributed across the layers with no apparent preference for the dorsal or ventral portions of the layer. Note the presence of "holes" in the distribution of GAD-positive cell bodies (i.e., regions where few GAD-positive cell bodies are located). These regions have a normal amount of GAD-positive terminals. Magnification × 65. *b*, GAD-positive cell bodies and terminals in lamina A of the lateral geniculate nucleus. Note the presence of fine, lightly immunoreactive fibers and the clumped distribution (*arrows*) of GAD-positive terminals compared with *c*. Magnification × 800. *c*, GAD-positive cell bodies and terminals in the parvocellular C laminae. Light immunoreactive fibers and dark immunoreactive terminals are present but are not aggregated into clumps like those in the A lamina. Magnification × 800.

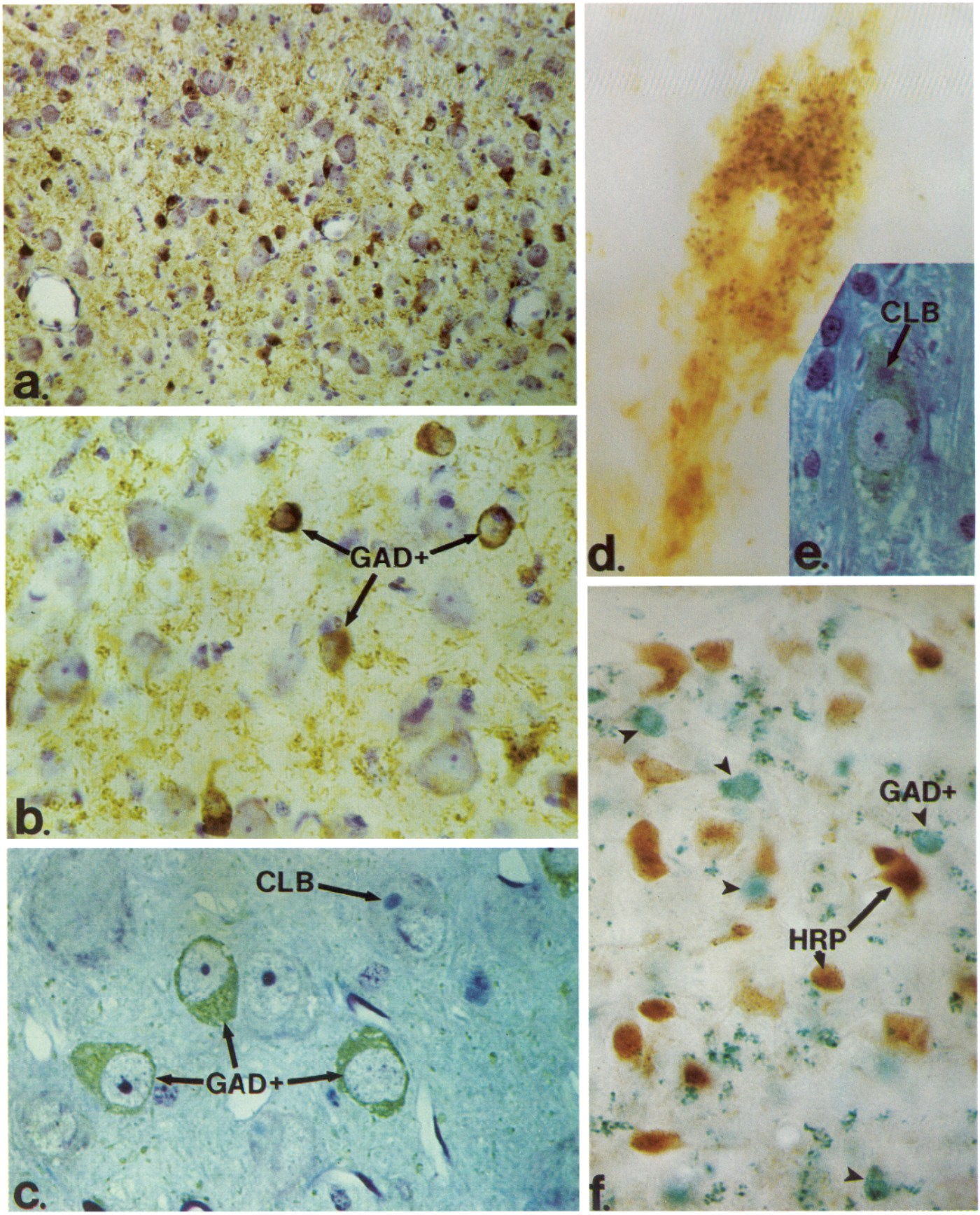


*Figure 3*

similar fraction of the neurons in the parvocellular C layers (20%) and MIN (21%). These percentages should be considered minimal estimates since the GAD antiserum may not penetrate the full width of our Vibratome sections, and it is possible that some neurons which contain GAD went undetected.

We evaluated the depth of antiserum penetration in 2-μm Epon sections of flat-embedded, GAD-reacted sections that were cut perpendicular to the surface of the section. In these sections GAD-positive terminal labeling is restricted to a uniform band in the outer 2 to 3 μm of the tissue section. The depth of penetration for labeled cell bodies is more variable, and GAD-positive cell bodies can be labeled well into the section provided some part of the cell body intersects a cut surface of the section. Still, even with the thinnest sections we used (15 μm), it is possible that some GAD-containing neurons (average diameter 13 μm) might lie in the center of the section and fail to be reached by the antisera. Given the number of variables that could influence detection of GAD-containing cell bodies lying within a section (for example, section thickness, cell size, extent of exposure to a cut surface), it is difficult to know how much this factor might affect the determination of percentages. But as a rough estimate of the magnitude of this effect, we calculated the probability that a cell 13 μm in diameter might lie in the middle of a section and not be transected by the edges. This calculation suggests that in 15- to 20-μm sections our counts might underestimate the actual percentage of GAD-positive neurons by 3 to 8%. There are reasons to think that the actual error is less than this (see section on double label experiments), but we emphasize that the percentages listed in Table I may be slight underestimates of the actual percentages.

*Relation of GAD-positive neurons to neurons which contain cytoplasmic laminar bodies (CLBs).* LeVay and Ferster (1977) identified a group of small cells that they regarded as local circuit neurons on the basis of Golgi morphology and on the basis of failure to label following cortical injections of HRP. They noted that these small neurons could be distinguished from small to medium-sized projection neurons (class II neurons of Guillery, 1966) in the A laminae by the presence in the projection neurons of small cytoplasmic inclusions known as cytoplasmic laminar bodies. Support for this idea was provided by the finding that neurons within the A lamina which accumulate exogenous [$^3$H]GABA do not contain CLBs (Sterling and Davis, 1980). Given these findings, we thought it would be important to determine whether CLBs are present in the population of GAD-positive neurons.

Figure 3*c* is a photomicrograph of a representative 2-μm Epon section through the A lamina of the geniculate reacted for GAD immunocytochemistry and counterstained with toluidine blue. In this photomicrograph, three GAD-positive neurons can be seen as well as several unlabeled neurons. One of the unlabeled neurons contains a CLB in this section (*arrow*). A total of 89 cells were partially reconstructed from the A lamina of one cat from eight adjacent 2-μm Epon sections. Of these cells, 24% were GAD-positive, 38% contained CLBs, and the remainder (38%) contained neither GAD nor CLB markers. None of the GAD-positive neurons contained a CLB. This finding would be trivial if the DAB reaction product in the GAD-positive cells simply obscured the CLB. But this seems unlikely since we occasionally observed CLBs in the GAD-positive neurons of the perigeniculate nucleus (Fig. 3*e*).

Further support for the idea that GAD-positive and CLB-containing neurons represent separate populations comes from measurements of cell size. The results of these measurements are shown in histogram form in Figure 5 (*top* and *middle*). The GAD-positive and CLB-containing neurons have different distributions of somal areas, and the mean of the GAD-labeled population is significantly less than that of the CLB-labeled population.

The distribution of somal areas for the third class of neurons which contained neither CLBs nor GAD immunoreactivity is shown in Figure 5 (*bottom*). Many of the neurons are large (greater than 300 μm), and this finding is consistent with the absence of CLBs in the large Y cell class of relay cell. However, both markers are also absent from other neurons which are in the size range of the GAD-positive and CLB-containing neurons, and this finding is not entirely consistent with the LeVay and Ferster study (1977). But in light of the problem of antibody penetration, some of these cells could have contained GAD which was not detected. Likewise, we could have failed to identify CLBs in some neurons because the CLB was located in an adjacent section which was not used in the reconstruction. Since we

*Figure 3. a*, Low power photomicrograph of the A lamina from a 20-μm Vibratome section which was reacted for GAD immunocytochemistry and counterstained with cresyl violet. GAD-positive cell bodies are *brown*; unlabeled cell bodies are *violet*. Magnification × 125. *b*, Higher power photomicrograph from the section illustrated in *a*, demonstrating the clear difference between GAD-positive neurons (*brown*) and unlabeled Nissl-stained cell bodies. Magnification × 430. *c*, GAD-positive and unlabeled neurons from a 2-μm Epon section counterstained with toluidine blue. The three GAD-positive cells are surrounded by several unlabeled neurons, one of which contains a CLB. Note that the nucleus of GAD-positive cells is devoid of the immunocytochemical reaction product. Magnification × 950. *d*, Double-labeled cell from the perigeniculate nucleus. This cell contains a light brown homogeneous reaction product in the cell body and proximal dendrites (*DAB*) reflecting GAD immunoreactivity. The dark granular reaction product (*Co-DAB*) is found only in the soma and is the result of retrograde transport of HRP from an injection into the lateral geniculate nucleus. Magnification × 2100. *e*, GAD-positive neuron from the perigeniculate nucleus which contains a CLB. This illustrates the fact that CLBs are not obscured by the reaction product in GAD-labeled neurons. Magnification × 1300. *f*, Section through the A lamina of the geniculate from an animal which received multiple injections of HRP into the striate cortex. The tissue was processed for HRP (*brown*) and for GAD immunoreactivity (*green*). Both HRP-labeled and GAD-positive cells were found, but no cells were found to contain both markers. In addition to GAD-positive cell bodies, note also the presence of GAD-positive (*green*) terminal clusters. Magnification × 550.

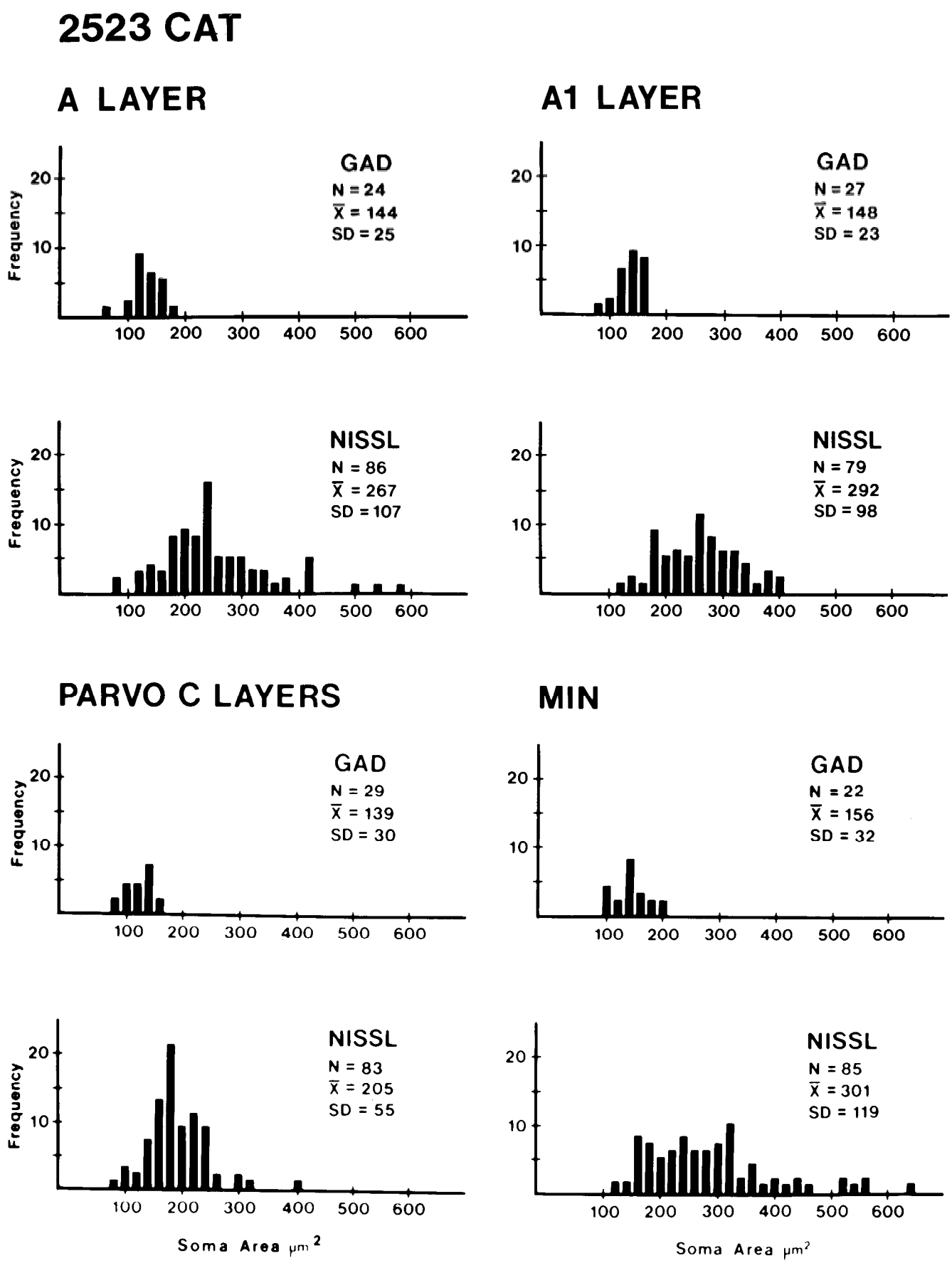


*Figure 4.* Histograms showing a representative distribution of cell body areas of GAD-positive and unlabeled Nissl-stained neurons from different subdivisions of the lateral geniculate nucleus of the cat.

TABLE I

*Cell soma sizes of samples of GAD-immunoreactive, HRP-labeled, and Nissl-stained cells*

| Animal No. | Structure | GAD | | | Nissl | | | HRP | | | GAD/Non-GAD Size Ratio | Percentage of GAD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | X̄ | N | SD | X̄ | N | SD | X̄ | N | SD | | |
| 2262 | A Layer | 142 | 100 | 42 | 273 | 100 | 134 | | | | 0.5 | |
| 2412 | | 129 | 23 | 31 | | | | 273 | 66 | 130 | 0.5 | 26 |
| 2426 | | 144 | 35 | 36 | 246 | 112 | 89 | | | | 0.6 | 24 |
| 2451 | | 133 | 25 | 28 | 213 | 73 | 64 | | | | 0.6 | 26 |
| 2452 | | 150 | 19 | 29 | 250 | 79 | 90 | | | | 0.6 | 19 |
| 2453 | | 102 | 24 | 31 | 179 | 73 | 74 | | | | 0.6 | 25 |
| 2463 | | 100 | 29 | 28 | | | | 171 | 93 | 58 | 0.6 | 24 |
| 2523 | | 144 | 24 | 25 | 267 | 86 | 107 | | | | 0.5 | 22 |
| 2426 | A1 Layer | 158 | 33 | 40 | 264 | 101 | 105 | | | | 0.6 | 25 |
| 2463 | | 105 | 26 | 20 | 393 | 2 | | 215 | 96 | 86 | 0.5 | 21 |
| 2523 | | 148 | 27 | 23 | 292 | 79 | 98 | | | | 0.5 | 25 |
| 2426 | Parvo C Layers | 150 | 26 | 38 | 202 | 102 | 49 | | | | 0.7 | 20 |
| 2523 | | 139 | 21 | 30 | 205 | 83 | 56 | | | | 0.7 | 20 |
| 2523 | MIN | 156 | 22 | 32 | 301 | 85 | 119 | | | | 0.5 | 21 |

cannot rule out either of these possibilities, we can only conclude that CLBs and GAD immunoreactivity are present in separate populations of neurons, and the issue of whether CLBs are present in *all* small unlabeled neurons remains open.

*Double labeling for GAD immunoreactivity and retrograde transport of HRP from visual cortex.* To try to answer the question of whether GAD-immunoreactive neurons project to striate cortex, we injected HRP into visual cortex and prepared tissue sections through the geniculate by using two different chromagens to show both retrogradely transported HRP and GAD immunoreactivity.

Following these injections, a wide zone of HRP-labeled cells was found in laminae A, A1, and the C lamina of the geniculate, but in no instance did we find evidence for the retrograde transport of HRP to the GAD-positive neurons. Examples of HRP (labeled by brown DAB reaction product) and GAD-positive neurons (labeled by green *o*-dianisidine reaction product) from one experiment are shown in the color plate (Fig. 3*f*).

While such a negative result could be due to a number of factors (which are considered under "Discussion"), it is important to point out that this result is *not* due to an incompatibility of the GAD immunocytochemical and HRP histochemical protocols; for example, it is possible to demonstrate both reaction products in the same cell (see Penny et al., 1983). Figure 3*d* shows a GAD-positive cell in the perigeniculate nucleus of the cat which has been labeled with HRP from an injection into the lateral geniculate body. The cell body of this neuron is brown (DAB), indicating the presence of GAD immunoreactivity, and the black grains which are superimposed (Co-DAB) represent transported HRP. In fact, we were also able to double label the GAD-immunoreactive neurons of the lateral geniculate body following injections of HRP directly into the geniculate. But even if there were some sort of negative interference between these two methods, this should be reflected in a decreased percentage of GAD-positive cells in the zone of HRP-labeled cells as well as a change in the cell size distributions of GAD-positive and GAD-negative neuron populations. (The demonstration of HRP-activity in retrogradely labeled cells would not be blocked by the GAD reaction since the HRP procedure always preceded the immunoreaction. See "Materials and Methods.")

To test this idea, we counterstained some of the double-labeled sections and determined the size and percentage of GAD-positive cells. This calculation was made by taking samples from the central region of the projection column, the zone with the densest HRP cell labeling. Within this zone, almost all neurons are labeled with either GAD immunoreaction product or transported HRP reaction product. In one cat, no unlabeled Nissl-stained neurons were detected, while in the other, two large neurons, probably Y cells, were found unlabeled within the center of the projection column in lamina A1. The percentage of GAD-positive neurons in the double label material was similar to what was found in the regular histochemical protocol (see cat 2421 and cat 2463 in Table I).

A histogram of somal area measurements from the A lamina in one double label experiment is shown in Figure 6, and this illustrates that the retrogradely labeled cell bodies and GAD-positive cell bodies have size distributions comparable to their counterparts in the single label experiments. Furthermore, the ratio of the mean size of GAD-positive neurons to the mean size of HRP-labeled neurons is 0.5 to 0.6 (Table I), the same as the ratio of GAD-positive neurons to unlabeled Nissl-stained neurons in single label experiments. These observations taken together support the idea that the two methods are compatible.

Given our previous discussion of the effect of antiserum penetration on the detection of GAD-containing cell bodies and the fact that GAD-positive cell bodies and HRP-labeled cell bodies appear to form mutually exclusive populations, it is somewhat surprising that we failed to find any small unlabeled Nissl-stained cell bodies in the double label tissue. This could reflect either the fact that our estimates of the effect of the penetration problem are too high and that almost all GAD-containing neurons are detected in our sections or that small, pale, unlabeled cell bodies are difficult to detect in the double label material.

*Dendritic morphology.* The GAD immunocytochemical

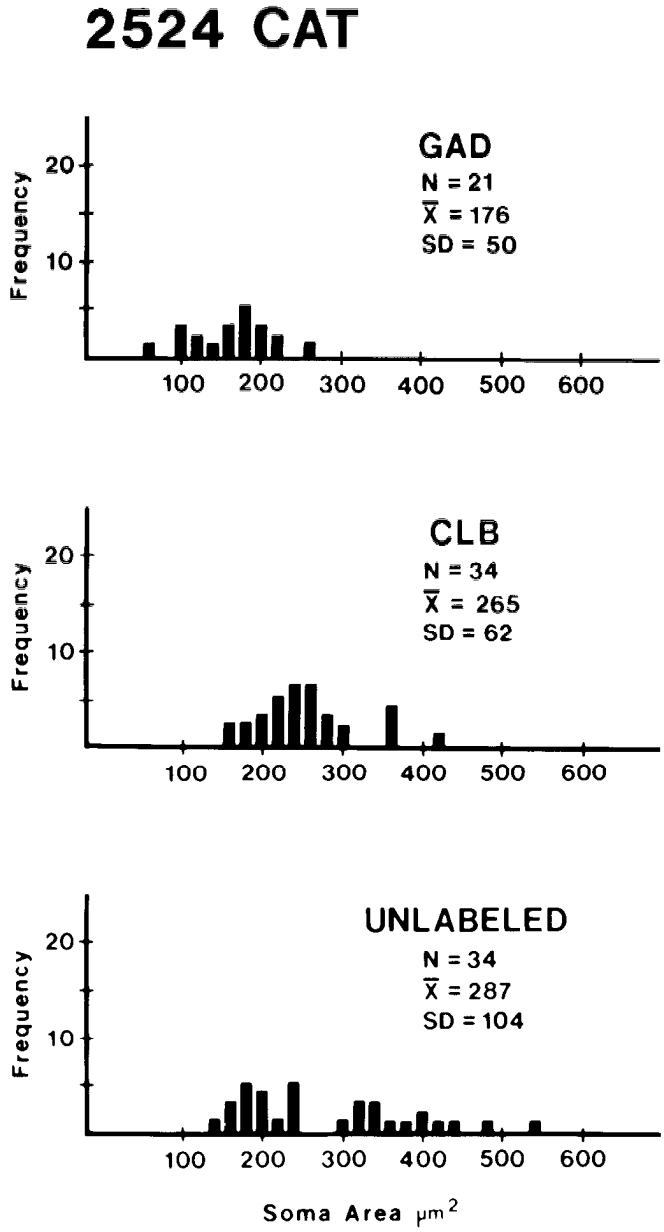


*Figure 5.* Histograms showing the distribution of cell body areas of GAD-positive, CLB-containing, and unlabeled neurons from 2-μm Epon-embedded sections counterstained with toluidine blue.

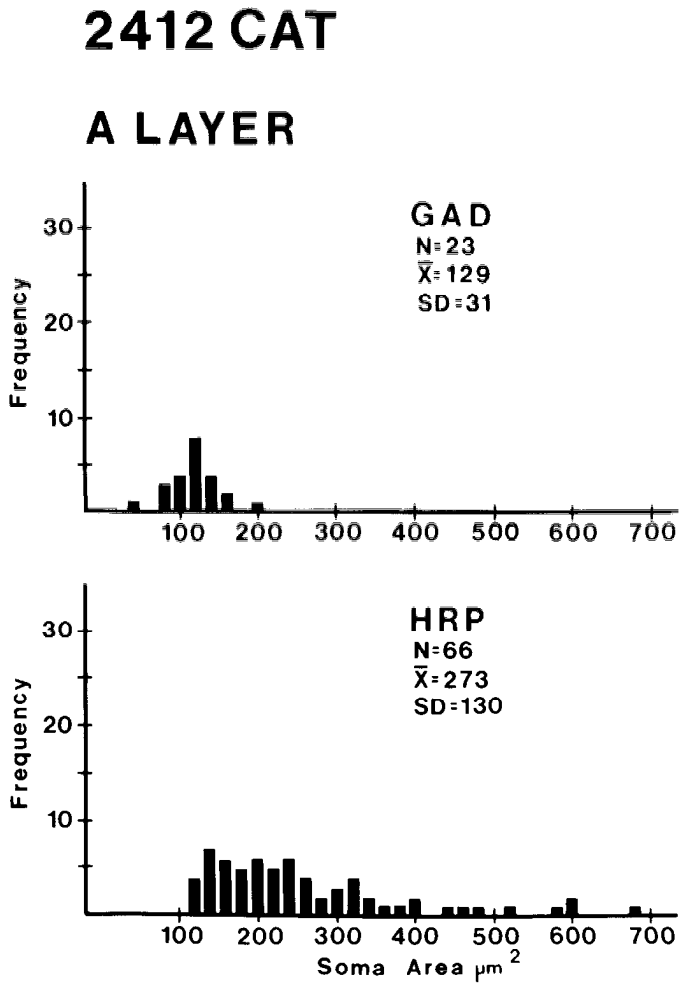


*Figure 6.* Histograms showing the distribution of cell body areas of GAD-positive and HRP-labeled neurons following an injection of HRP into striate cortex.

reaction product densely filled cell bodies and terminals within the lateral geniculate nucleus, but dendritic processes tended to be lightly labeled and were difficult to trace. For this reason, our line drawings and photomicrographs should be taken as illustrations of some features of GAD-positive neurons and not as an indication of the number, extent, or complexity of the processes associated with any one neuron.

GAD-positive neurons display thick primary dendrites which in some instances were seen extending for 150 μm before markedly decreasing in diameter. Some primary dendrites appear to bifurcate into smaller diameter processes (Fig. 7, *a* to *c*), while others end abruptly and give rise to extremely thin processes (Fig. 7*d*). Thin processes are also seen emerging as branches from thicker dendrites (Fig. 7*a*) or from proximal dendrites close to the soma. In general, the orientation of the proximal dendrites of GAD-positive neurons varies according to the layer in which the cell lies. In laminae A and A1, the proximal dendrites are oriented vertically (perpendicular to the layer), while in the parvocellular C laminae and in the interlaminar zone between laminae A and A1, the dendrites are oriented horizontally, parallel to the laminae. This difference in orientation is illustrated in Figure 8, which is a composite drawing of cell bodies displaying some features of their dendritic processes from a number of different sections.

In several cases we have observed fine, lightly immunoreactive processes which appear to connect the dendrites of GAD-positive neurons in the A laminae with clusters of GAD-immunoreactive terminals (Figs. 7, *a* and *d* and 8). The association of terminal clusters with the dendrites of GAD-immunoreactive neurons suggests that these clusters may represent the complex dendritic appendages which have been described as characteristic of local circuit neurons in Golgi material and which have been suggested to be the source of the presynaptic dendritic profiles or "F profiles" of the synaptic glomerulus (Guillery, 1966; Famiglietti and Peters, 1972). These

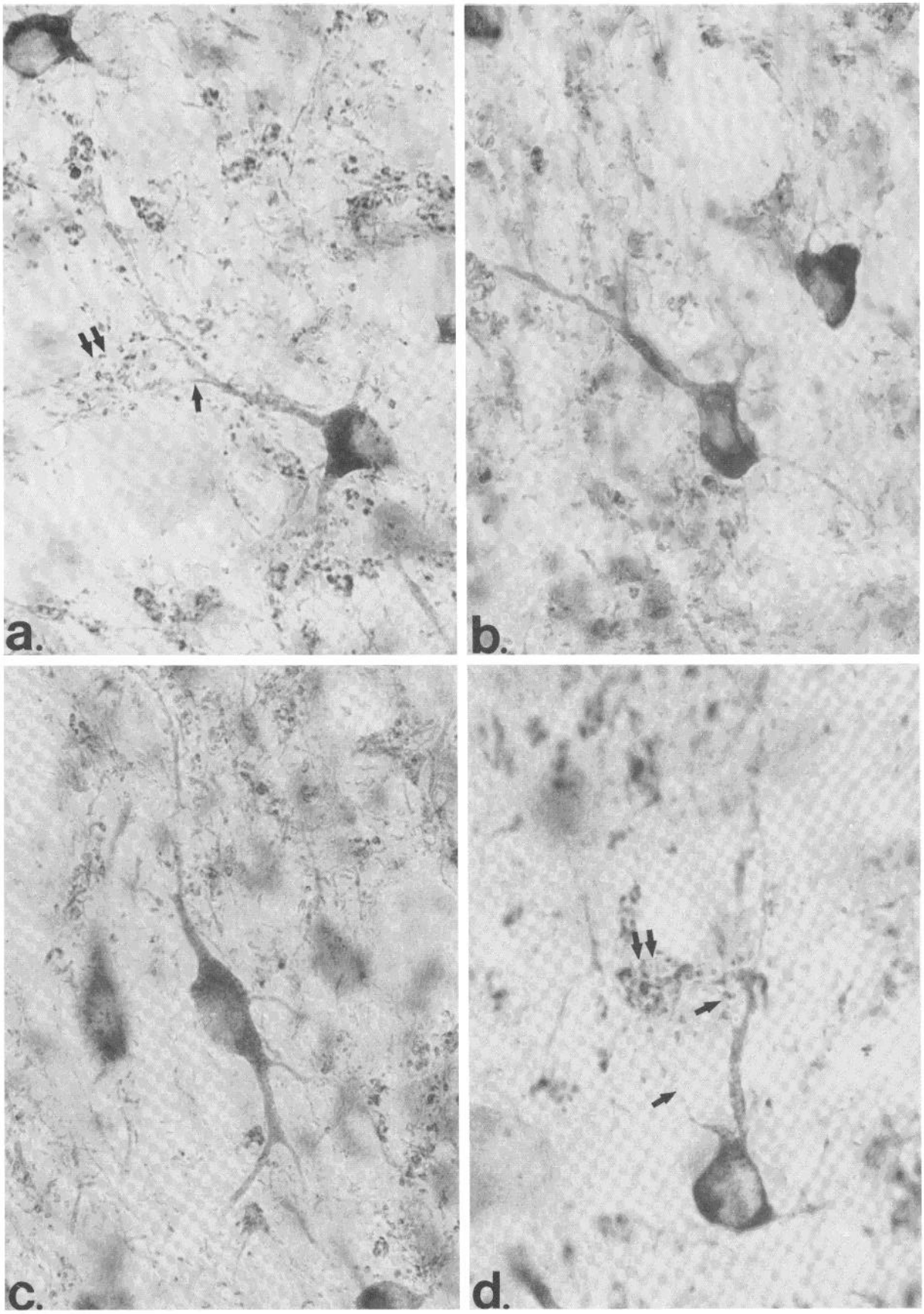


*Figure 7. a* to *d*, Four examples of GAD-positive neurons from the A lamina of the lateral geniculate nucleus. The *single arrows* in *a* and *d* point to thin immunoreactive processes which contribute terminals to the terminal clusters marked with *double arrows*. Magnification × 1000.

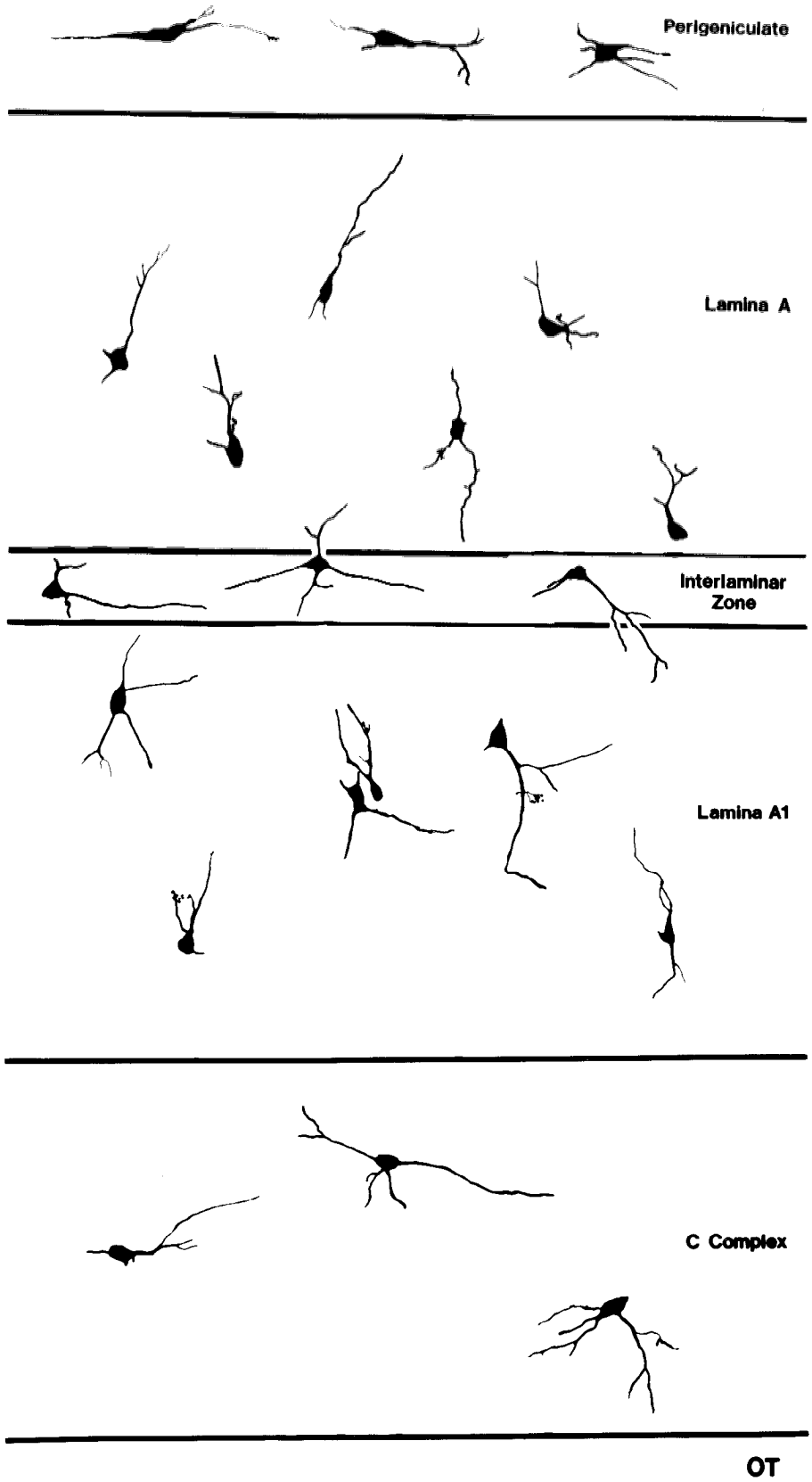


*Figure 8.* Camera lucida drawings of GAD-positive neurons in the lateral geniculate and perigeniculate nuclei. Drawings of individual cells were made from different sections. The orientation of dendrites and their position relative to adjacent layers of interlaminar zones have been maintained.

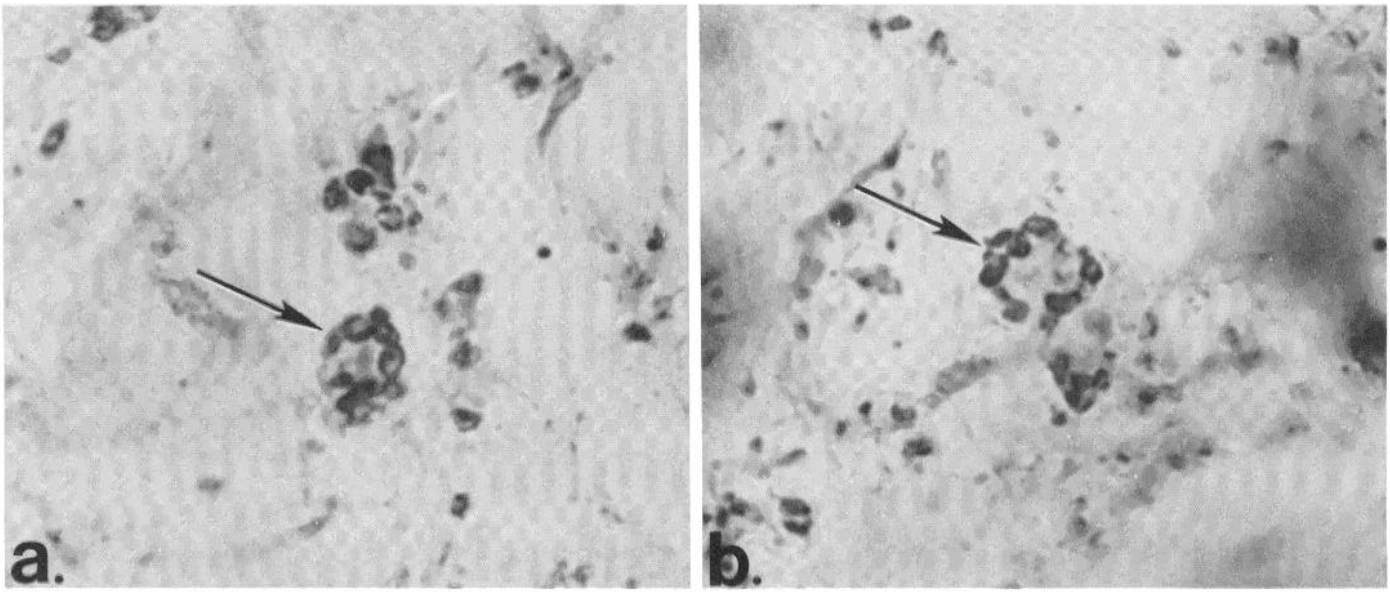


*Figure 9.* Two examples of small clusters of GAD-positive terminals from the A lamina of the lateral geniculate nucleus. Both of these clusters display a central core which lacks GAD immunoreactivity. This arrangement is consistent with the configuration of a synaptic glomerulus (i.e., GAD-positive profiles surrounding an unlabeled central optic axon). Magnification × 3000.

profiles are the most numerous elements of the synaptic glomerulus and surround and lie postsynaptic to large irregularly shaped optic axon terminals. Indeed, when cut in an appropriate plane of section, some of the smaller GAD-immunoreactive terminal clusters appear as circular or ovoidal shells surrounding a nonimmunoreactive core (Fig. 9), precisely the configuration that one would expect for a synaptic glomerulus.

A detailed electron microscopic study of the localization of GAD immunoreactivity is beyond the scope of this paper, but we think it is important to present our observations on the A lamina which support the idea that the GAD-positive terminal clusters seen with the light microscope represent, at least in part, F profiles or presynaptic dendrites of synaptic glomeruli. The identification of vesicle-containing profiles lying postsynaptic to large optic axon terminals and presynaptic to regular dendritic profiles in the cat lateral geniculate nucleus as presynaptic dendrites rests largely on the work of Famiglietti and Peters (1972), and their work should be consulted for a summary of the evidence in support of this view. Because of the rather poor preservation of tissue constituents in the immunocytochemically reacted material, the fact that cues normally associated with dendrites are, in fact, rarely found in the vesicle-containing profiles of the synaptic glomerulus, and the fact that we have not reconstructed from serial sections an immunoreactive dendrite giving rise to immunoreactive profiles in a glomerulus, our identification of these profiles as presynaptic dendrites must remain provisional. However, the similarities of these labeled profiles to profiles regarded as presynaptic dendrites by other investigators (Ralston and Herman, 1969; LeVay, 1971; Ralston, 1971; Morest, 1971; Famiglietti and Peters, 1972; Lieberman and Webster, 1972; Lieberman, 1973; Hamori et al., 1974) and their similarity with the clusters of GAD-positive terminals that have fine processes joining them to dendrites of immunoreactive neurons in light microscopic material provide support for this interpretation.

In electron micrographs, the immunocytochemical reaction product appears as a dark amorphous substance and is localized primarily to the outer membrane of the mitochondria and to the membranes of synaptic vesicles. The contrast between labeled and unlabeled profiles is best seen in sections without heavy metal staining (Fig. 10, *a* to *c*). In Figure 10*a* a hook-shaped group of GAD-positive, vesicle-containing profiles can be seen surrounded by unlabeled axon terminals (*RLP*) and dendritic profiles (*D*). The two large axon terminals are undoubtedly optic axon terminals (RLP terminals of Guillery, 1969) given their size, density of vesicles, and irregular contour. While the reaction product tends to obscure synaptic contact zones (and vesicle morphology), two sites of probable synaptic contact between the RLP profile and vesicle-laden profiles (*G+*) in the *upper left-hand corner* are shown enlarged in Figure 10*c* (*arrows*).

Another example of the morphology of GAD-positive profiles is shown in Figure 10*b*. Two GAD-positive, vesicle-containing profiles lie at the edge of a synaptic glomerulus in contact with an unlabeled central optic axon (*RLP*) and unlabeled dendrites (*D*). RLP profiles were never found labeled by the immunoreaction product, and GAD-positive profiles were often found in proximity to unlabeled dendritic profiles.

The typical configuration of profiles in a synaptic glomerulus from the A lamina is shown in Figure 11. This micrograph is from material which was stained with uranyl acetate-lead citrate, and, therefore, the contrast between labeled and unlabeled profiles is reduced compared to Figure 10. In the center is a large unlabeled optic axon terminal (*RLP*) surrounded by numerous profiles, some displaying reaction product (indicated by *small circles* in Fig. 11*b*) and others without. The RLP terminal makes prominent synaptic contacts with both labeled and unlabeled profiles, whereas the labeled pro-

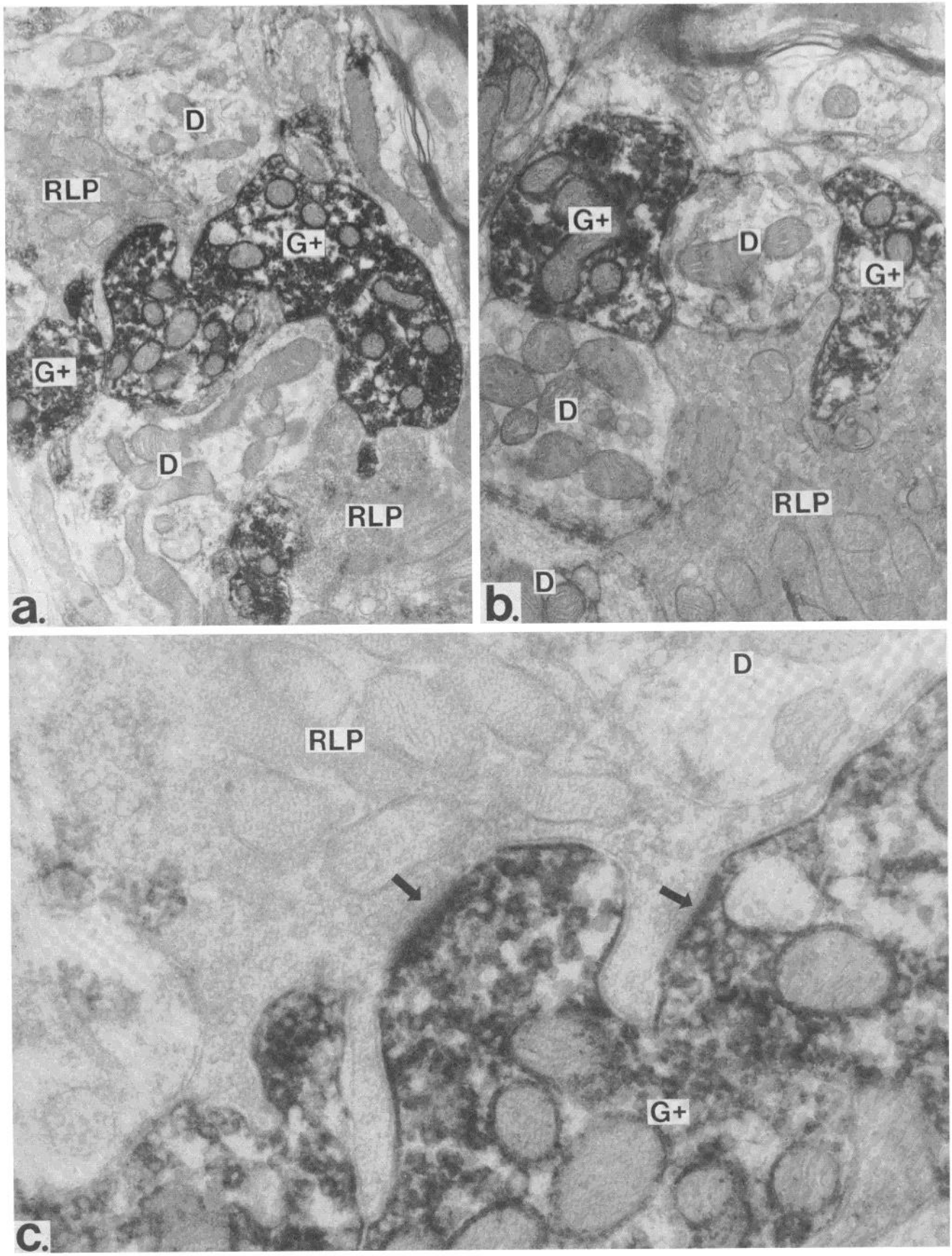


*Figure 10.* Electron micrographs of GAD-immunoreacted material from lamina A of the lateral geniculate nucleus. These sections were not stained with uranyl acetate-lead citrate, and, thus, the contrast between labeled (*black*) and unlabeled profiles (*gray*) is sharp. *a*, Several GAD-positive profiles are seen surrounded by unlabeled optic axon terminals (*RLP*) and unlabeled dendrites (*D*). The RLP terminal in the *upper left-hand* corner makes synaptic contact with the GAD-labeled profiles, and this is shown at higher power in *c*. Magnification × 20,000. *b*, Two GAD-positive, vesicle-containing profiles are seen lying adjacent to unlabeled dendritic profiles (*D*) and a large optic axon terminal (*RLP*). Magnification × 30,000. *c*, Synaptic contacts between a large crenulated optic axon terminal and GAD-positive, vesicle-containing profiles (*arrowheads*). This is a higher power electron micrograph of the RLP terminal in the *upper left-hand corner* of Figure 9*a*. Magnification × 66,000.

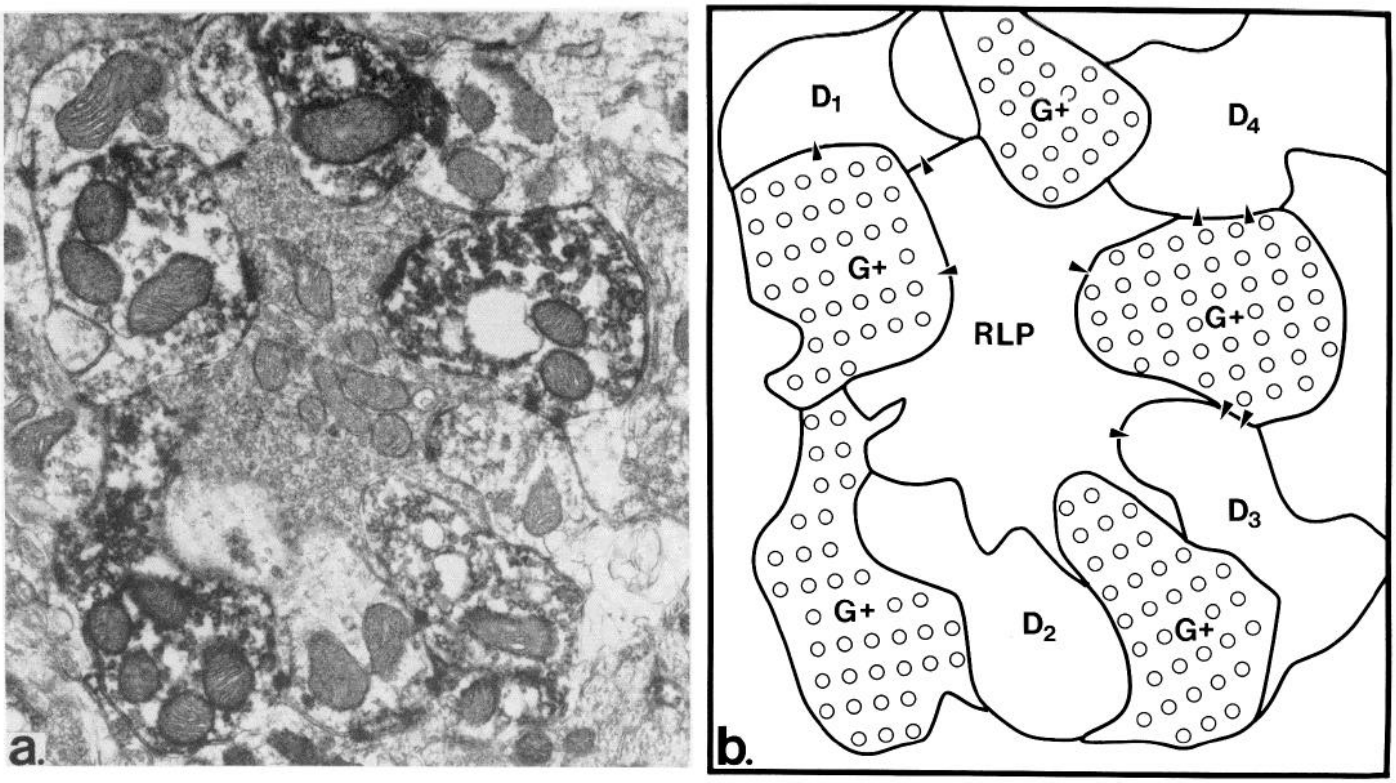


*Figure 11.* Electron micrograph of a synaptic glomerulus from the A lamina of the geniculate. Five GAD-positive, vesicle-containing profiles (*G+*) can be seen, two of which lie postsynaptic to the unlabeled optic axon terminal (*RLP*). Sites of synaptic contact between the various elements of this glomerulus are indicated by *arrows* in *b*. An enlargement of the synaptic triad associated with dendrite *D* is shown in Figure 12. Magnification × 21,000.

files make synaptic contacts with unlabeled dendrites (see *arrowheads* in Fig. 11*b*).

Both the RLP terminal and a GAD-positive profile make synaptic contacts with dendrite $D_1$, and the GAD-positive profile receives a synaptic contact from the RLP terminal. This example of a triadic synaptic relationship is shown at higher power in Figure 12*a*. A serial synaptic array is illustrated in connection with dendrite $D_4$ where the RLP terminal synapses with a GAD-positive profile which, in turn, synapses with dendritic profile $D_4$.

Another example of the relationship between GAD-positive profiles and unlabeled structures is shown in Figure 12, *b* and *c*. In this case, a triangular-shaped optic axon terminal is surrounded on two sides by large unlabeled dendritic profiles (*D*) and smaller GAD-positive profiles. Both the RLP terminal and the GAD-positive profile on the *right* appear to have synaptic contacts with the unlabeled dendrite, and these are shown at higher magnification in Figure 12*c*.

In summary, GAD-positive profiles at the ultrastructural level are found to contain synaptic vesicles and to lie postsynaptic to unlabeled optic axon terminals and presynaptic to unlabeled dendritic profiles, and they have the morphology and configuration of F terminals described by other investigators. However, we emphasize that these are not the only synaptic relationships entered into by GAD-positive profiles, and we found numerous examples of GAD-positive profiles that were not postsynaptic to optic axon terminals or contained within synaptic glomeruli. Without serial reconstructions, we do not know if these processes are postsynaptic to axon terminal profiles at other levels and, thus, whether they should be considered as presynaptic dendrites or axon terminals.

## Discussion

The results of this study provide evidence that GAD-immunoreactive neurons constitute a distinct population of neurons within the lateral geniculate nucleus of the cat, a population which has a number of features in common with previous descriptions of local circuit neurons based on Golgi staining (Guillery, 1969; Famiglietti and Peters, 1972; LeVay and Ferster, 1977). The most obvious distinguishing feature of these neurons is the small size of their cell body. In all subdivisions of the lateral geniculate nucleus, the mean soma area of GAD-positive neurons is significantly less than that of unlabeled neurons. These neurons are also characterized by having thick primary dendrites which, at least in some cases, are associated with very thin, lightly immunoreactive processes that, in turn, give rise to clusters of GAD-positive terminals. At the light microscopic level, the clusters of GAD-immunoreactive terminals have a size and shape consistent with the appearance of synaptic glomeruli, and at the ultrastructural level GAD immunoreactivity is found within certain vesicle-containing profiles of the synaptic glomerulus lying postsynaptic to optic axon terminals and presynaptic to unlabeled dendritic profiles. Finally, GAD-immunoreactive neurons

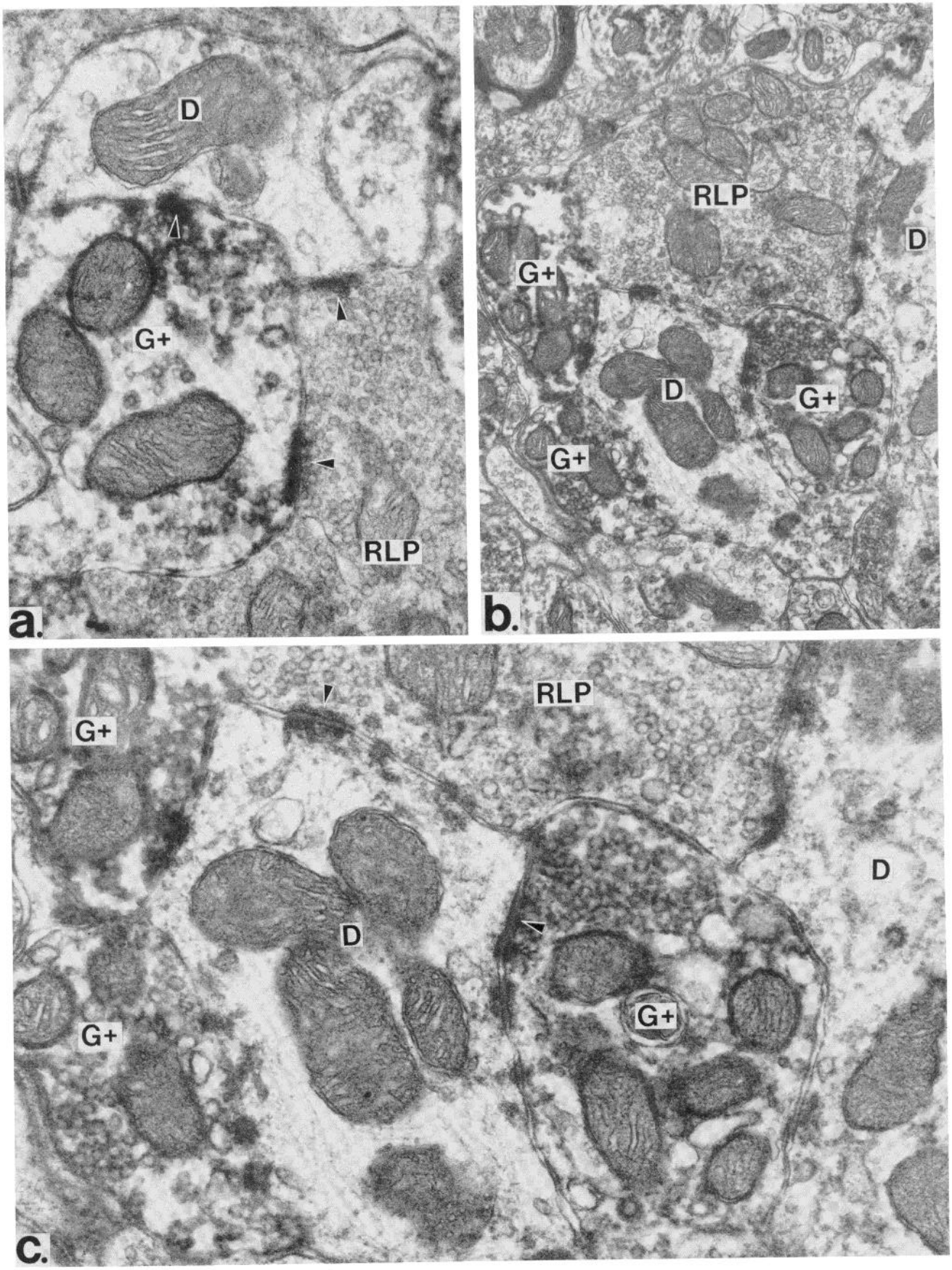


*Figure 12. a*, A triadic synaptic arrangement from the A lamina of the lateral geniculate nucleus. An unlabeled optic axon terminal (*RLP*) has synaptic contacts with a GAD-positive profile (*G+*) and with an unlabeled dendrite (*D*). The GAD-positive profile also makes synaptic contact with the unlabeled dendrite. Magnification × 47,000. *b*, An unlabeled dendritic profile (*D*) contacted by a large unlabeled optic axon terminal and several GAD-positive profiles. Magnification × 25,000. *c*, Higher power electron micrograph from the same region shown in *b* to show the nature of the synaptic contacts between the RLP profile, the G+ profile, and the dendritic profile (see *arrowheads*). Magnification × 54,000.

are distinguished from other neurons in the lateral geniculate nucleus by their failure to label following injections of horseradish peroxidase into visual cortex.

*GAD-immunoreactive neurons and GABAergic inhibition within the lateral geniculate nucleus.* Finding a distinct population of GAD-positive neurons and terminals in the lateral geniculate nucleus provides support for the idea that inhibition within the lateral geniculate nucleus is mediated, at least partially, by neurons utilizing the neurotransmitter GABA. Other lines of evidence which support this conclusion include: (*1*) biochemical assays which show that GABA and GAD are present in abundance within the lateral geniculate nucleus (Lund et al., 1976; McDonald et al., 1981); (*2*) evidence from other regions of the nervous system which indicates that GAD and GABA are associated with inhibitory neurotransmission (Kuriyama et al., 1966; Roberts and Kuriyama, 1968; Fonnum and Walberg, 1973; McLaughlin et al., 1974; Fahn, 1976); (*3*) the blockage of inhibitory interactions within the lateral geniculate nucleus by the administration of the GABA antagonist bicuculline (Curtis and Tebecis, 1972); (*4*) the presence of small cell bodies and F terminals in the A laminae of the lateral geniculate body of the cat which selectively accumulate [$^3$H]GABA (Sterling and Davis, 1980), a characteristic shared by other presumed GABAergic neurons in other regions of the brain (Hokfelt and Ljundahl, 1972). The neurons shown to accumulate [$^3$H]GABA are like those labeled by the GAD antiserum in size and frequency and in their lack of a CLB.

In addition to the GAD-immunoreactive cell bodies and processes within the laminae of the geniculate, almost all of the cell bodies within the perigeniculate nucleus are immunoreactive for GAD. Previous studies have shown that the neurons in the perigeniculate nucleus receive a projection from the neurons of the lateral geniculate nucleus, apparently as a collateral of relay cell axons on their way to the cortex (Ahlsen and Linström, 1978; Friedlander et al., 1981). There is also evidence that the neurons of the perigeniculate nucleus project upon the laminae of the lateral geniculate nucleus (O'Leary, 1940; Ahlsen and Lindström, 1978; including our own control experiments). Therefore, it seems reasonable to suggest that the perigeniculate nucleus may be an additional source of GABAergic inhibition within the lateral geniculate nucleus.

A number of physiological studies support the idea that the perigeniculate nucleus has an inhibitory influence upon the neurons of the lateral geniculate nucleus. Indeed, the physiological studies of Dubin and Cleland (1977), Singer (1977), and Lindström (1982) all support the concept of two distinct forms of inhibition within the lateral geniculate nucleus: (*1*) feed-forward inhibition which affects relay cells with a short delay time following optic nerve stimulation and (*2*) recurrent inhibition, a longer latency effect. Furthermore, the same studies provide evidence that these effects are mediated by separate classes of inhibitory neurons with local circuit neurons providing inhibition of the feed-forward type and perigeniculate neurons providing the recurrent type.

How the perigeniculate contributes to the neuropil of the lateral geniculate nucleus remains an open question, but it is reasonable to suggest that the relation between the perigeniculate nucleus and the lateral geniculate nucleus in the cat may be like the relation between the reticular nucleus and the lateral geniculate nucleus in the rat. In this species the reticular nucleus is a source of recurrent inhibition to the lateral geniculate nucleus (Sumitomo et al., 1976; Hale et al., 1982) and contributes terminals with pleomorphic vesicles and symmetric synaptic contacts onto the dendrites of geniculate neurons outside glomeruli and occasionally onto cell bodies (Ohara et al., 1980; Montero and Scott, 1981). Since almost all of the neurons of the rat reticular nucleus are GAD-positive (Houser et al., 1980), it seems likely that some of the GAD-positive terminals within the extraglomerular neuropil of the lateral geniculate nucleus of the rat originate from the reticular nucleus (Ohara et al., 1983). In the lateral geniculate nucleus of the cat, we have found examples of GAD-positive terminals in locations similar to those described for reticular afferents in the rat, but we need to use transport methods to establish whether the perigeniculate nucleus is the source of these terminals.

*GAD-immunoreactive terminals in the lateral geniculate nucleus.* The present study has shown that GAD-positive terminals are present in all subdivisions of the lateral geniculate in the cat and that they correspond in location and synaptic relations to the class of profiles called "F-profiles" or presynaptic dendrites by other investigators (Guillery, 1969; Famiglietti and Peters, 1972). This finding is consistent with the pattern of GAD immunoreactivity found in the lateral geniculate nucleus of the rat (Ohara et al., 1983) and monkey (Hendrickson et al., 1983) and with the evidence that F terminals in the lateral geniculate nucleus of cat accumulate [$^3$H] GABA (Sterling and Davis, 1980).

GAD-positive terminals are abundant in the cell-rich layers of the lateral geniculate nucleus but are notably sparse in the interlaminar zones. This finding provides support for Guillery's (1969) observation that there are fewer F terminals in the interlaminar zones. The difference in GAD-positive terminal density between laminar and interlaminar regions appears to be a general feature of geniculate organization since it also occurs in the lateral geniculate nucleus of monkey, *Galago*, and tree shrew (Fitzpatrick et al., 1982; Hendrickson et al., 1983). The cellular layers of the lateral geniculate nucleus are the major site of termination of retinal axons, whereas cortical and (in some species) subcortical inputs predominate in the interlaminar zones. Presumably, the differential distribution of GAD-positive terminals is largely due to the intimate association between GAD-positive profiles and optic axon terminations.

Although GAD-positive terminals are found in all layers, there is a difference in the distribution pattern of GAD-positive terminals between layers. GAD-positive terminals are aggregated into conspicuous clusters in laminae A, A1, magnocellular C, and MIN, but well defined clusters are rare in the parvocellular C layers. We have argued, on the basis of size and configuration, that the clusters of GAD-positive terminals seen at the light microscopic level represent GAD-positive F profiles associated with synaptic glomeruli. This interpretation

is strengthened by Guillery's observation that "encapsulated zones" or glomeruli are abundant within the A layers but are scarce within the C layers (Guillery, 1969; Guillery and Scott, 1971).

The absence of well defined GAD-positive terminal clusters in the parvocellular C layers suggests that the inhibitory interactions related to the so-called "W cell" pathway may differ from those related to the X and Y cell pathways. Evidence that the morphological substrate for inhibition within the geniculate may be specialized for different pathways is also found in the monkey lateral geniculate nucleus, where a conspicuously greater number of GAD-positive terminals and cell bodies is found in the magnocellular layers than in the parvocellular layers (Hendrickson et al., 1983; D. Fitzpatrick, unpublished observation). How these different distributions of GAD-positive elements are related to the physiological properties of distinct geniculocortical pathways remains to be determined.

*GAD-immunoreactive neurons within the geniculate, the concept of local circuit neurons, and the definition of cell class.* In the present report, we found that GAD-positive neurons in the A lamina of the lateral geniculate nucleus were not labeled following injections of HRP into the striate cortex, and from this we infer that the GAD-positive neurons do not project to the cortex. The finding that 20 to 25% of the neurons in the A lamina do not project to the cortex is consistent with the results of several different retrograde transport studies (LeVay and Ferster, 1979; Geisert, 1980; Weber and Kalil, 1983; but see Lin et al., 1977). Furthermore, our finding that GAD-positive neurons do not contain CLBs and are not labeled following HRP injections into striate cortex is consistent with the finding of LeVay and Ferster (1977) that small neurons without CLBs do not project to striate cortex.

While this finding supports the view that GAD-positive neurons do not project to striate cortex, it is based on negative evidence (i.e., failure to label neurons) and is, therefore, open to other possible interpretations. For example, we cannot rule out the possibility that these neurons have a major axonal arborization within the geniculate and only a minor projection to cortex which the HRP method is not sufficiently sensitive to demonstrate, or that these neurons project with wide-spreading collaterals to several areas of cortex, making them difficult to label retrogradely. Furthermore, GAD-positive neurons could still have axons which leave the geniculate but project to targets other than striate cortex (for example, the perigeniculate nucleus).

Recent evidence that some autoradiographically labeled geniculate terminals in layer 4 of cat striate cortex have symmetric synaptic contacts and pleomorphic vesicles has been used to suggest that "local circuit neurons" of the lateral geniculate nucleus might actually have an axon which projects to the cortex (Einstein et al., 1983). But we do not know whether these labeled geniculate terminals are GABAergic or are, in fact, derived from the GAD-positive or GABA-accumulating cells of the lateral geniculate nucleus. To be sure, we have found that there are a good number of GAD-positive terminals in layer 4 of cat striate cortex (as well as GAD-positive cell bodies) (Fitzpatrick et al., 1983), but more experiments are necessary to establish whether any of these terminals are derived from sources outside of the cortex.

We consider our evidence for the lack of a projecting axon as only *one feature* of GAD-positive thalamic neurons and not the single, most important, defining characteristic of this class of neurons. It is unfortunate, in a way, that one of the major criteria for defining cell classes in the thalamus has come to depend on assessing whether a cell has a local or a projecting axon. While there is no doubt that this is an important consideration, it is one which is difficult to address unequivocally with either anatomical or physiological methods. Furthermore, as recent intracellular studies in the lateral geniculate nucleus and in the neostriatum have shown, local and projecting axons are *not* mutally exclusive categories since axons which project out of a structure can also have collaterals which give rise to local terminal fields (Preston et al., 1980; Friedlander et al., 1981; Stanford et al., 1983).

Thus, while we acknowledge the limitations of the HRP transport method in addressing this issue, we would emphasize: (*1*) that GAD-positive neurons must be at least qualitatively different from the rest of the neurons in the lateral geniculate nucleus neurons which readily transport HRP following injections into striate cortex, and (*2*) that the presence of a projecting axon would not diminish the evidence that the dendrites of GAD-positive neurons, by themselves, have the capacity to act as a local circuit and are in a strategic position to influence the transmission of neural information from ganglion cell axons to cortical projection neurons.

This then brings us to the issue of how the class of GAD-positive neurons identified in this study relates to definitions of cell class based strictly on Golgi morphology and on the combination of physiology and Golgi-like morphology derived from intracellular recording and injections. In his original Golgi studies of the cat lateral geniculate nucleus, Guillery (1966) defined three classes of neurons in laminae A and A1 on the basis of cell size, dendritic features, and, to a lesser extent, axonal characteristics. Class I consisted of large multipolar neurons with thick, radiating dendrites; class II consisted of medium-sized neurons with thinner dendrites and grape-like clusters at dendritic branch points; and class III neurons were the smallest neurons and had very fine dendrites with complex, stalked appendages. Guillery regarded classes I and II as projection neurons and suggested with appropriate reservations that the class III neurons were local circuit neurons. Famiglietti and Peters (1972) further reinforced the association of delicate and complicated dendritic appendages with a class of small local circuit neurons and implicated the dendritic appendage as the source of the vesicle-containing profile lying postsynaptic to optic axon terminals in the synaptic glomerulus.

By making intracellular injections, Friedlander et al. (1981) attempted to relate physiologically defined X and Y cell types to these morphological classes by using conduction velocity, linearity, and response to moving stimuli to distinguish X and Y cell classes. While they found that X and Y cell types as a population differ from one another, the differences were not strictly along the lines of the cell classes defined by Golgi morphology. For

example, grape-like dendritic clusters (associated with class II neurons) were found on both X and Y cells, and complex dendritic appendages (associated with class III neurons) were found on some X cell neurons that had an axon which left the geniculate. The association of complex dendritic appendages with some projection neurons caused these investigators to question the existence of a distinct class of local circuit neurons.

In light of the present results, it seems important to point out that finding complex dendritic appendages on neurons which have axons that leave the lateral geniculate nucleus could also mean that complex dendritic appendages are not an exclusive feature of "local circuit neurons," much like grape-like dendritic clusters are not an exclusive feature of physiologically defined X cells. In fact, the available evidence supports the idea that dendritic specializations, by themselves, are not diagnostic for any of the physiologically defined cell classes since they are found on the dendrites of X, Y, and W cells (Stanford et al., 1983). X, Y, and W cell types do form morphologically distinct cell classes, but it is only by examining the morphology of a number of cells of each physiological type that the "cluster" of morphological features which distinguish them can be specified (Friedlander et al., 1981).

We suspect that a similar principle applies to the morphological identification of biochemically distinct cell types. For example, our evidence supports the existence of a distinct class of GAD-positive neurons in the lateral geniculate nucleus which do not project to cortex and which have small cell bodies and dendrites which give rise to complex dendritic appendages. But we would not be surprised if there were some degree of morphological heterogeneity among small neurons in the lateral geniculate nucleus such that "complex dendritic appendages" are not an *exclusive* feature of GAD-positive neurons. We should point out, in addition, that it remains to be determined whether all of the structures designated as "complex dendritic appendages" in Golgi studies or in intracellular studies are really the same (whether they all act as presynaptic elements in a synaptic glomerulus, like those associated with GAD-positive neurons).

It is also reasonable to expect some morphological diversity *within* the class of GAD-positive geniculate neurons (like that found within the X and Y cell classes). Perhaps complex dendritic appendages are not found on all GAD-positive neurons or these appendages may vary in size and shape. This seems particularly likely in the case of the parvocellular C layers where GAD-positive terminals are rarely gathered into clusters like those in the A laminae.

Thus, while we have provided evidence that GAD-positive neurons are a morphologically distinct population of neurons within the lateral geniculate nucleus, this is only a first step. A more complete description of this class will require examining the morphology of a number of its members, presumably through a combination of intracellular injection and immunocytochemistry.

*GAD-immunoreactive neurons are a basic feature of the organization of the mammalian thalamus.* Previous reports of GAD-immunoreactive neurons within the lateral geniculate nucleus of the rat (Ohara et al., 1983) and macaque monkey (Hendrickson et al., 1983), along with our own studies in the rabbit, opossum, *Galago*, and squirrel monkey (Fitzpatrick et al., 1982; G. R. Penny, M. Conley, I. T. Diamond, and D. E. Schmechel, submitted for publication), support the idea that GAD-immunoreactive neurons are a basic feature of the organization of the lateral geniculate nucleus in all mammals. This finding of a biochemically distinct population of neurons in the lateral geniculate nuclei of such diverse species of mammals offers a unique opportunity to study a functionally similar cell class in a nucleus which appears to have undergone considerable specialization in different lines of descent.

In addition to the lateral geniculate nucleus, other thalamic nuclei in cat and monkey contain GAD-immunoreactive neurons with the exception of the center median and parafascicular nuclei (Penny et al., 1983; Spreafico et al., 1983). This suggests that these neurons are a basic feature of the organization of thalamus and that they serve a generalized function common to different sensory pathways.

Finally, the results of this study and those in other species should serve to emphasize that the thalamus does not simply serve a relay function, but is a site of interaction of a number of distinct systems, both subcortical and cortical, which can influence and shape the nature of the signal which is transmitted to the cortex. How these subcortical and cortical inputs interface with the inhibitory network described here remains to be determined. Likewise how GAD-positive neurons relate to different classes of projection neurons and to different classes of retinal ganglion cells is an open question. An examination of these issues should provide insights into the ways in which the lateral geniculate nucleus and the thalamus in general contribute to the modulation of neural activity within sensory pathways.

## References


Adams, J. C. (1977) Technical considerations on the use of horseradish peroxidase as a neuronal marker. Neuroscience *2:* 141–145.

Ahlsen, G., and S. Lindström (1978) Axonal branching of functionally identified neurons in the lateral geniculate body of the cat. Neurosci. Lett. (Suppl.) *1:* 156.

Curtis, D. R., and A. K. Tebecis (1972) Bicuculline and thalamic inhibition. Exp. Brain Res. *16:* 210–218.

Dubin, M. W., and B. G. Cleland (1977) Organization of visual inputs to interneurons of the lateral geniculate nucleus of the cat. J. Neurophysiol. *40:* 410–427.

Einstein, G., T. L. Davis, and P. Sterling (1983) Convergence on neurons in layer IV (cat area 17) of lateral geniculate terminals containing round or pleomorphic vesicles. Soc. Neurosci. Abstr. *9:* 820.

Fahn, S. (1976) Regional distribution studies of GABA and other putative neurotransmitters and their enzymes. In *GABA in Nervous System Function,* E. Roberts, T. N. Chase, and D. B. Tower, eds., pp. 169–186, Raven Press, New York.

Famiglietti, E. V., Jr., and A. Peters (1972) The synaptic glomerulus and the intrinsic neuron in the dorsal lateral geniculate nucleus of the cat. J. Comp. Neurol. *144:* 285–334.

Fitzpatrick, D., G. R. Penny, D. E. Schmechel, and I. T. Diamond (1982) GAD immunoreactive neurons in the lateral geniculate nucleus of the cat and *Galago.* Soc. Neurosci. Abstr. *8:* 261.

Fitzpatrick, D., J. S. Lund, and D. E. Schmechel (1983) Glutamic acid decarboxylase immunoreactive neurons and ter-

minals in the striate cortex of monkey and cat. Soc. Neurosci. Abstr. *9:* 616.

Fonnum, F., and F. Wallberg (1973) An estimation of the concentration of γ-aminobutyric acid and glutamate decarboxylase in the inhibitory Purkinje axon terminals in the cat. Brain Res. *54:* 115–127.

Friedlander, M. J., C. S. Lin, L. R. Stanford, and S. M. Sherman (1981) Morphology of functionally identified neurons in the lateral geniculate nucleus of the cat. J. Neurophysiol. *46:* 80–129.

Geisert, E. E. (1980) Cortical projections of the lateral geniculate nucleus in the cat. J. Comp. Neurol. *190:* 793–812.

Guillery, R. W. (1966) A study of Golgi preparations from the dorsal lateral geniculate nucleus of the adult cat. J. Comp. Neurol. *238:* 21–50.

Guillery, R. W. (1969) The organization of synaptic interconnections in the laminae of the dorsal lateral geniculate nucleus of the cat. Z. Zellforsch. *96:* 1–38.

Guillery, R. W., and G. L. Scott (1971) Observations on synaptic patterns in the dorsal lateral geniculate nucleus of the cat: The C laminae and perikaryal synapses. Exp. Brain Res. *12:* 184–203.

Hale, P. T., A. V. Sefton, L. A. Baur, and L. J. Coffee (1982) Interrelations of the rat's thalamic reticular and dorsal lateral geniculate nuclei. Exp. Brain Res. *45:* 217–229.

Hamori, J., T. Pasic, P. Pasik, and J. Szentagothai (1974) Triadic synaptic arrangements and their possible significance in the lateral geniculate nucleus of the monkey. Brain Res. *80:* 379–393.

Hendrickson, A. E., M. P. Ogren, J. E. Vaughn, R. P. Barber, and J. -Y. Wu (1983) Light and electromicroscopic immunocytochemical localization of glutamic acid decarboxylase in monkey geniculate complex: Evidence for GABAergic neurons and synapses. J. Neurosci. *3:* 1245–1262.

Hokfelt, T., and A. Ljundahl (1972) Autoradiographic identification of cerebral and cerebellar cortical neurons accumulating labeled gamma-amino-butyric acid ($^3$H-GABA). Exp. Brain Res. *14:* 354–362.

Houser, C. R., J. E. Vaughn, R. P. Barber, and E. Roberts (1980) GABA neurons are the major cell type of the nucleus reticularis thalami. Brain Res. *200:* 341–354.

Hsu, S. M., L. Raine, and H. Ganger (1981) The use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. J. Histochem. Cytochem. *29:* 577–580.

Kawamura, S., J. M. Sprague, and K. Niimi (1974) Corticofugal projections from the visual cortices to the thalamus, pretectum and superior colliculus in the cat. J. Comp. Neurol. *158:* 339–362.

Kuriyama, K., L. B. Haber, B. Sisken, and E. Roberts (1966) The γ-aminobutyric acid system in rabbit cerebellum. Proc. Natl. Acad. Sci. U. S. A. *55:* 846–852.

Laties, A. M., and J. M. Sprague (1966) The projection of optic fibers to the visual centers in the cat. J. Comp. Neurol. *127:* 35–70.

LeVay, S. (1971) On the neurons and synapses of the lateral geniculate nucleus of the monkey and the effects of eye enucleation. Z. Zellforsch. *113:* 396–419.

LeVay, S., and D. Ferster (1977) Relay cell classes in the lateral geniculate nucleus of the cat and the effects of visual deprivation. J. Comp. Neurol. *172:* 563–584.

LeVay, S., and D. Ferster (1979) Proportions of interneurons in the cat's lateral geniculate nucleus. Brain Res. *164:* 304–308.

Lieberman, A. R. (1973) Neurons with presynaptic perikarya and presynaptic dendrites in the rat lateral geniculate nucleus. Brain Res. *59:* 35–59.

Lieberman, A. R., and K. E. Webster (1972) Aspects of the synaptic organization of intrinsic neurons in the dorsal lateral geniculate nucleus. An ultrastructural study of the normal and the experimentally deafferented nucleus in the rat. J. Neurocytol. *3:* 677–710.

Lin, C. -S., K. E. Dratz, and S. M. Sherman (1977) Percentage of relay cells in the cat's lateral geniculate nucleus. Brain Res. *131:* 167–173.

Lindström, S. (1982) Synaptic organization of inhibitory pathways to principal cells in the lateral geniculate nucleus of the cat. Brain Res. *234:* 447–453.

Lund, J. S., R. Karlsen, and F. Fonnum (1976) The toxic effect of sodium glutamate on rat retina: Changes in putative transmitters and their corresponding enzymes. J. Neurochem. *27:* 1437–1441.

McDonald, J. K., S. G. Speciale, and J. G. Parnavelas (1981) The development of glutamic acid decarboxylase in the visual cortex and the dorsal lateral geniculate nucleus of the rat. Brain Res. *217:* 364–367.

McLaughlin, B. J., J. G. Wood, K. Saito, R. Barber, J. E. Vaughn, E. Roberts, and J. -Y. Wu (1974) The fine structural localization of glutamate decarboxylase in synaptic terminals of rodent cerebellum. Brain Res. *76:* 377–391.

McLean, I. W., and P. K. Nakane (1974) Periodate-lysine-paraformaldehyde fixative. A new fixative for immunoelectron microscopy. J. Histochem. Cytochem. *22:* 1077.

Montero, J. M., and G. L. Scott (1981) Synaptic terminals in the dorsal lateral geniculate nucleus from neurons of the thalamic reticular nucleus: A light and electron microscopic study. Neuroscience *6:* 2561–2577.

Morest, D. (1971) Dendrodendritic synapses of cells that have axons: The fine structure of the Golgi type II cell in the medial geniculate body of the cat. Z. Anat. Entwicklungsgesch. *133:* 216–246.

Oertel, W. H., D. E. Schmechel, M. L. Tappaz, and I. J. Kopin (1981a) Production of a specific antiserum to rat brain glutamic acid decarboxylase by injection of an antigen-antibody complex. Neuroscience *6:* 2689–2700.

Oertel, W. H., D. E. Schmechel, E. Mugnaini, M. L. Tappaz, and I. J. Kopin (1981b) Immunocytochemical localization of glutamate decarboxylase in rat cerebellum with a new antiserum. Neuroscience *6:* 2715–2735.

Ohara, P. T., A. J. Sefton, and A. R. Lieberman (1980) Mode of termination of afferents from the thalamic reticular nucleus of the rat. Brain Res. *197:* 503–506.

Ohara, P. T., A. R. Lieberman, S. P. Hunt, and J. -Y. Wu (1983) Neural elements containing glutamic acid decarboxylase (GAD) in the dorsal lateral geniculate nucleus of the rat: Immunohistochemical studies by light and electron microscopy. Neuroscience *8:* 189–212.

O'Leary, J. (1940) A structural analysis of the lateral geniculate nucleus of the cat. J. Comp. Neurol. *73:* 405–430.

Penny, G. R., D. Fitzpatrick, D. E. Schmechel, and I. T. Diamond (1983) Glutamic acid decarboxylase-immunoreactive neurons and horseradish peroxidase-labeled projection neurons in the ventral posterior nucleus of the cat and *Galago senegalensis*. J. Neurosci. *3:* 1868–1887.

Peters, A., and S. Palay (1966) The morphology of lamina A and A1 of the dorsal nucleus of the lateral geniculate body of the cat. J. Anat. (Lond.) *100:* 451–486.

Preston, R. J., G. A. Bishop, and S. T. Kitai (1980) Medium spiny neuron projection from the rat striatum: An intracellular horseradish peroxidase study. Brain Res. *183:* 253–262.

Ralston, H. J. (1971) Evidence for presynaptic dendrites and a proposal for their mechanism of action. Nature *230:* 585–587.

Ralston, H. J., and M. M. Herman (1969) The fine structure of neurons and synapses in the ventrobasal thalamus of the cat. Brain Res. *14:* 77–97.

Ramón y Cajal, S. (1909) *Histologie du Systeme Nerveux de l'Homme et des Vertebrés*, Maloine, Paris.

Ramón y Cajal, S. (1966) *Studies on the Diencephalon*, Charles C Thomas, Springfield, IL.

Roberts, E., and K. Kuriyama (1968) Biochemical-physiological correlations in studies of the γ-aminobutyric acid system. Brain Res. *8:* 1–35.

Sanderson, K. J. (1971) The projection of the visual field to the lateral geniculate and medial interlaminar nuclei in the cat. J. Comp. Neurol. *143:* 101–118.

Singer, W. (1977) Control of thalamic transmission by corticofugal and ascending reticular pathways in the visual system. Physiol. Rev. *57:* 386–420.

Spreafico, R., D. E. Schmechel, L. C. Ellis, and A. Rustioni (1983) Cortical relay neurons and interneurons in the n. ventralis posterolateralis of cats: An HRP, EM, Golgi, and immunocytochemical study. Neuroscience *9:* 491–509.

Stanford, L. R., M. J. Friedlander, and S. M. Sherman (1983) Morphological and physiological properties of geniculate W-cells of the cat: A comparison with X- and Y-cells. J. Neurophysiol. *50:* 528–608.

Sterling, P., and T. L. Davis (1980) Neurons in cat lateral geniculate nucleus that accumulate exogenous [$^3$H]-γ-aminobutyric acid (GABA). J. Comp. Neurol. *192:* 737–749.

Sternberger, L. A. (1979) *Immunocytochemistry*, Ed. 2, John Wiley & Sons, New York.

Sumitomo, I., M. Nakamura, and K. Iwana (1976) Location and function of the scattered interneurons of rat lateral geniculate body. Exp. Neurol. *51:* 110–123.

Weber, A. J., and R. E. Kalil (1983) The percentage of interneurons in the dorsal lateral geniculate nucleus of the cat and observations on several variables that affect the sensitivity of horseradish peroxidase as a retrograde marker. J. Comp. Neurol. *220:* 336–346.