The Axonal Arborizations of Lateral Geniculate Neurons in the Striate Cortex of the Cat

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ABSTRACT Horseradish peroxidase (HRP) was injected into the optic radiations of adult cats. With placements close to the lateral geniculate nucleus (LGN), the enzyme diffused retrogradely along the axons of geniculo-cortical relay neurons, entered their cell bodies, and, after reaction with diaminobenzidine, produced a Golgi-like staining of entire neurons. When the injections were made close to the visual cortex, the enzyme diffused anterogradely and filled complete axonal arborizations in area 17.

In the LGN, examples of type 1 and type 2 relay neurons (Guillery, '66) were reconstructed, and their axon diameters measured. The type 1 neurons (thought to correspond to Y-cells — LeVay and Ferster, '77) possessed large diameter axons (2-3.3 μm), while the type 2 neurons (thought to be X-cells) had medium-sized axons (1-1.7 μm). Both types of neuron gave off axon collaterals to the perigeniculate nucleus.

In the cortex, two types of afferent supplied layer IV. One distributed to the upper part of the layer (layer IVab), extending a short distance into layer III. The parent trunks of these axons, measured in the white matter, had diameters matching those of type 1 LGN relay cells. The other type distributed to layer IVc. The diameters of these axons matched those of type 2 LGN relay cells. Most afferents of both types gave off collaterals to layer VI — there were no axons which exclusively innervated this layer.

The axons supplying layer IVab had a wide lateral spread in the cortex (up to 2 mm), and the boutons were grouped into two to five clumps, whose size and arrangement were similar to ocular dominance columns. The axons supplying layer IVc had a much more restricted arborization, usually consisting of a single clump of boutons.

LGN neurons with very fine axons (less than 1 μm) were found in laminae C1-C3. They probably corresponded to Guillery's type 4 neurons. In the cortex, fine-diameter axons arborized in the upper half of layer I. These axons sometimes had collaterals in the lower part of layer III and in layer V.

Taken together, the arborizations of the cortical afferents observed in the present study account fully for the autoradiographic labelling pattern seen after ³H-proline injections into the LGN (LeVay and Gilbert, '76).

The identification of type 1 and type 2 neurons as Y- and X-cells is strengthened by the observed difference in their axon diameters, in agreement with the different axonal conduction velocities reported for Y- and X-cells. The presence of cells with very fine axons in the deeper C laminae is consistent with the reported presence of W-cells (which have slowly conducting axons) in these layers. We conclude that the different classes of geniculate relay neuron have different laminar projections in area 17.
The distribution of afferents from the lateral geniculate nucleus to the visual cortex in the cat has been described with the use of autoradiography, either after the injection of $^3$H-proline into single laminae of the LGN (LeVay and Gilbert, '76) or after injections into one eye, utilizing the phenomenon of transneuronal transport (Shatz et al., '77; LeVay et al., '78). These methods have revealed the laminar and columnar distribution of the afferents, but they say little about the manner in which individual axons arborize. How many terminals, for example, are sustained by a single axon? Are these terminals distributed to one layer, to part of a layer, or to all the cortical layers innervated by a single geniculate lamina? Does one axon ramify within a single ocular dominance column, or does it branch and supply several columns serving the same eye?

These questions are particularly relevant in view of the known heterogeneity of neurons within single geniculate laminae. In Golgi preparations of the A laminae, two relay cell types of very different appearance have been described by Guillery ('66). Physiologically, cells can be distinguished which relay input from X- and Y-type retinal ganglion cells (Hoffmann et al., '72; Cleland et al., '71). A match between the physiological and anatomical cell types (Y-cells corresponding to Guillery's type 1, and X-cells to his type 2) has been proposed on the basis of the similarity of their distributions with respect to visual field eccentricity (LeVay and Ferster, '77). The detailed description of the arborization pattern of the axons of these two cell types may provide some insight into their separate roles in cortical function.

The usual neuroanatomical tracing methods are not capable of distinguishing the projections of different neuronal types if their somata are intermixed and their axons project to the same region. Furthermore, the Golgi method cannot be used to demonstrate the complete terminal arbor of a single axon, at least in the adult animal, because individual terminal-bearing branches are connected by myelinated segments which prevent the spread of impregnation to the entire tree. What impregnations have been obtained with the Golgi method in adult animals (e.g., Lund, '73) probably represent small portions of much larger arborizations. Much more extensive impregnations have been obtained in young kittens before myelination (O'Leary, '41), but it is known that substantial changes in the axonal arborization patterns occur during postnatal life (LeVay et al., '78).

To circumvent these problems we have taken advantage of the recently developed technique by which neurons or terminal arborizations are stained by diffuse filling with horseradish peroxidase (HRP) (Adams and Warr, '76). When the enzyme is applied to a cut in the optic radiation close to the LGN, it diffuses back along the axons of the geniculate neurons and into their cell bodies and dendrites, producing a Golgi-like staining. Similarly, when placed in a cut in the radiation close to the cortex, the enzyme fills entire terminal arborizations, in spite of the presence of myelin.

Several different types of geniculate neurons and cortical arborizations may be seen by this method, but it is not possible to identify individual arborizations with their parent cell bodies on account of the cut in the radiation. We expected, however, from their reported conduction velocities (Cleland et al., '71; Hoffmann et al., '72) that the axons of different classes of geniculate neurons would differ in size. This turned out to be so, and it has permitted us to match each class of geniculate neuron with its characteristic arborization in area 17.

**METHODS**

Six adult cats were used in these experiments. Injections of 30% HRP (Sigma type VI or Boehringer Mannheim) and 2% dimethyl sulfoxide in distilled water were placed stereotactically in the optic radiations with the animals under thiopental anesthesia. For the backfilling of LGN neurons injections were made approximately 1 mm dorsal to the dorsal border of the LGN (the depth of this border was determined by prior recording of visual responses with a tungsten microelectrode). For the filling of the cortical arborizations injections were made into the optic radiation as it passed into the lateral gyrus (5 mm deep to the surface of the brain and 4 mm lateral to the midline, close to the intra-aural plane). Injections were made with a 10-μl Hamilton syringe. In some cases a 20-gauge needle was first lowered to the injection site for the purpose of increasing the number of axons transected, and the needle of the Hamilton syringe was then lowered through the 20-gauge needle. Volumes injected ranged from 0.1-0.5 μl. After survival times between 2 and 18 hours (during which anesthesia was maintained) the animals were perfused with a mixture of...
0.5% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4. Sections were cut at 100 μm on a Vibratome (Oxford Instruments), the cortex being cut coronally and the LGN either coronally or parasagittally. Although good staining was obtained within the entire range of injection volumes and survival times given, best results were obtained with the smaller injection volumes and with a survival of about ten hours.

Sections were incubated at room temperature according to the following schedule (Adams, '77):

- 0.1 M Tris buffer (pH 7.4): 5 minutes (x 2)
- 1% cobalt chloride in Tris buffer: 15 minutes
- Tris buffer: 5 minutes
- 0.1 M phosphate buffer, pH 7.4: 5 minutes
- 0.05% diaminobenzidine HCl in phosphate buffer: 10 minutes
- Previous solution plus 1 drop of 3% H2O2 per 5 ml: 15 minutes
- Phosphate buffer: 5 minutes

Sections were mounted in serial order on subbed slides and, in order to facilitate the serial reconstruction of stained processes, care was taken to mount successive sections as nearly as possible in exact register with each other. Drawings of filled cells and axons were made using a microscope equipped with serial reconstruction of stained processes, care was taken to mount successive sections as nearly as possible in exact register with each other. Drawings of filled cells and axons were made using a microscope equipped with a camera lucida attachment. Because of the accurate alignment of adjacent sections, stained processes could easily be followed from section to section using local landmarks such as blood vessels and stained axons.

Measurements of axon diameters were made using a 100 x oil immersion objective and a 12.5 x eyepiece equipped with an ocular micrometer. The values reported below refer to the diameter of the internodal axon, not including the myelin sheath, and are the means of measurements taken at three to six points along the axon. These points were chosen to be close to the cut surfaces of sections, where the staining of myelinated axons was most intense (RESULTS). Measurements for a single axon varied by 0.2-0.4 μm. This amount of variation may be attributable to the limited resolving power of the light microscope and other technical factors, but there did seem to be, for some axons at least, slight variations in thickness along their course.

RESULTS

Lateral geniculate nucleus

Examples of lateral geniculate neurons backfilled with horseradish peroxidase are illustrated in the photograph of figure 1 and in the camera lucida reconstruction in figure 2. The filled neurons were scattered rather widely throughout the nucleus, allowing them to be seen in their entirety. The intensity of the staining varied from cell to cell: in some it rivalled the best Golgi preparations in showing fine details such as dendritic spines, while in others it was much fainter, hardly revealing the dendrites at all. In general larger cells were filled better than smaller ones. Not surprisingly, the axon was the most intensely stained part of the neuron, and it could be followed from its origin at the cell body until it was lost in the dense reaction product surrounding the injection site. The nodes of Ranvier were easily recognizable, not only by the characteristic narrowing of the axon at these points, but also by a markedly greater density of staining as compared with the internodal regions, as if the myelin sheath hindered the access of the histochemical reagents to the enzyme within the internodal axon. The staining of axons was also more intense where they met the cut surfaces of sections, presumably because reagents could diffuse a few microns into the cut ends. This inhibiting effect of myelin was more pronounced for large-diameter fibers; indeed, in some heavily-myelinated fibers no reaction product could be seen in the internodal regions, even though the nodes of Ranvier and the cell bodies were intensely stained. In these cases it was usually still possible to trace the filled axon, using the stained nodes and cut ends. Also in these large axons the myelin sheath, though unstained, was visible on account of its refractility, and could be followed between stained nodes.

In the A laminae, cells could be recognized as belonging to types 1 or 2 as described by Guillery ('66) in Golgi material. No labeled type 3 neurons (probable interneurons) were seen. The cell in figure 1, and the two largest cells in figure 2, are examples of type 1 neurons. Altogether, ten neurons of this type were reconstructed. Their dendrites radiated symmetrically from a more or less spherical cell body and the dendrites were free of any appendages except an occasional spine. In agreement with previous observations these cells were the largest seen in the A laminae; it was not surprising therefore that their axons were also relatively large, ranging from 2-3.3 μm in diameter (not including the thickness of the myelin sheath). The axons did not achieve their full diameter until several internodes distant from the cell body. Measurements
were therefore made a considerable distance away from the cell bodies.

Also shown in figure 2 are three examples of the smaller, type 2, relay cells. These are characterized by the grape-like dendritic appendages at dendritic branch points close to the cell body. These appendages are the sites of glomerular synapses with incoming optic nerve fibers (Guillery, '69). Eleven cells of this type were reconstructed. Their axon diameters ranged from 1.0-1.7 μm.

As has been shown in previous studies, (Guillery, '66; LeVay and Ferster, '77) some neurons had an appearance intermediate between that of type 1 and type 2 cells. In his Golgi study, Guillery found that these formed approximately 40% of the total population. It was difficult to estimate the exact numbers of these neurons in our material, given the capriciousness of the staining process, but they seemed to form much less than 40% of the total.

Filled cells were also found in the C laminae. Type 1 cells were restricted to the magnocellular portion (lamina C proper). Three cells of this type were reconstructed. Their axons and somata were among the largest of all the type 1 cells in the LGN, their axons measuring 3.3 μm.

The filling of smaller cells in the C laminae was faint and usually did not allow us to identify cells on the basis of their morphology. In lamina C proper, one type 2 cell was positively identified, and its axon diameter was 1.3 μm — i.e., similar to the values for the type 2 cells in the A laminae. Two other cells also had cell body and axon diameters similar to those of type 2 cells in the A laminae, but were too faintly stained to be positively identified. In laminae C1-C3 no good fillings of cells were obtained, but the axon and soma diameters were obtained for four cells. The axon diameters ranged from 0.7-0.9 μm, and thus were the finest of all those measured in the LGN. It is very likely that these cells correspond to the type 4 cells described in the C laminae by Guillery. Although we have only examined four cells, the results are consistent with the
Fig. 2  Five neurons in lamina A of the LGN. This is a camera lucida reconstruction from eight serial parasagittal sections, each 100 μm thick. The inset, top left, shows the position of the reconstructed area and the needle tip shows the injection site. Anterior is to the right. The dotted line in the main drawing indicates the dorsal border of lamina A. The two largest cells (labelled 1) are examples of Guillery's type 1 neurons, and the three others (labelled 2) are type 2 neurons. One cell of each type is drawn at higher magnification in the insets. Note the characteristic clusters of appendages on the dendrites of the type 2 cells.

The axons have been drawn with their myelin sheaths. The axons increase in diameter as they enter the myelin sheath, and narrow down again at each node of Ranvier. There is also a gradual increase in the diameter of the internodal axon over the first 2-3 internodes; thereafter the axons maintain a constant diameter. Of the five neurons in this drawing, all but one were seen to give off axon collaterals in the region antero-dorsal to the LGN (perigeniculate nucleus).
physiological observation (Wilson et al., '76) that the units recorded in laminae C1-C3, the W-cells, have the lowest axonal conduction velocity of all cells in the LGN.

**The relation between cell diameter and axon diameter**

The relationship between cell body and axonal diameters for all cells reconstructed is plotted in figure 3. It may be noted that there is a strong correlation between these two variables ($r = 0.81$). Furthermore, although there is some overlap between cell body sizes of type 1 and type 2 neurons there is no overlap between their axon diameters. There is also a clear separation between the axon diameters of the samples of cells in laminae C1-C3 and those in the rest of the LGN. It may well be that if a larger number of neurons had been examined, some overlap between the classes would have become evident (as is suggested by a partial overlap of conduction velocities (Hoffman et al., '72; Cleland et al., '71), but the observed differences are great enough to allow individual geniculocortical afferents to be assigned with a good probability to particular classes of geniculate neurons solely on the basis of axon diameter.

**Axon collaterals**

Many of the geniculate neurons whose axons were traced displayed axon collaterals which emerged from nodes of Ranvier and gave rise to a small number of very large terminal boutons (up to 4-μm in diameter). In the case of most cells in the A laminae these collaterals were found not in the main body of the lateral geniculate nucleus, but in the perigeniculate nucleus, a thin strip of cells and neuropil that caps the dorsal surface of the LGN. Both type 1 and type 2 cells gave off these collaterals, examples of which are shown in figure 2. One type 2 cell in the lower portion of lamina A1 did send collaterals to the area surrounding its soma, but this seemed to be exceptional. Of the cells in the C laminae, only some smaller cells in lamina C proper (probably type 2 cells) had collaterals, which were found in the plexus between layers C and A1. None of the type 1 cells in lamina C nor the small cells in C1-C3 gave off collaterals within the geniculate itself; it could not be determined whether they did so in the perigeniculate nucleus.

The presence of collaterals in the perigeniculate nucleus, at least from the geniculate Y-cells, had been predicted on the basis
of physiological observations by Dubin and Cleland ('77). It seems from the present anatomical results that both types of relay cell in the A laminae send collaterals to this nucleus.

**Cortex**

After injections into the optic radiation, labelled fibers were seen to enter area 17 and to ramify at various levels. In some regions single axons were visualized without any overlap with other stained processes; in others many fibers were labelled, their arborizations overlapping to form a dense plexus of branches and terminals. In these heavily-labelled regions the terminals could be seen to be distributed exclusively to three horizontal strata. The most superficial band of terminals occupied the upper half of layer I, immediately under the glial limiting membrane. The middle band occupied the whole of layer IV and extended for about 100 \( \mu m \) into the lower part of lamina III. The large pyramidal cells at the base of layer III were within this band. The lowest stratum was in layer VI, almost entirely in its upper half. This trilaminar distribution of peroxidase-filled terminals was identical to the distribution of geniculo-cortical terminals revealed autoradiographically (LeVay and Gilbert, '76) (DISCUSSION).

A total of 30 axons (from 4 different cats) were reconstructed from the serial sections, as described in the METHODS section. It was apparent from these reconstructions that axons fell into three major classes on the basis of the levels at which they terminated. Each of these classes had a characteristic range of axon diameters, which matched one of the three classes of geniculate neurons described above.

**Afferents to layer IVab**

These axons were characterized by the possession of a terminal arborization in the upper part of layer IV (layer IVa + b) extending into the lowest 100 \( \mu m \) of layer III. A photograph of a small portion of one such arborization is shown figure 4. Three ex-
examples of complete camera lucida reconstructions of this type of axon are shown in figures 6-8.

The parent trunks of the afferents, measured deep in the white matter, were the largest of all those labelled, ranging from 2-3.3 μm in diameter (exclusive of myelin). The axons often branched once or twice in the white matter before entering the cortex, and several more times during the ascent through the lower layers. Of nine afferents to layer IVab that were reconstructed, eight gave off collaterals which terminated in the upper half of layer VI. The one cell which did not give off collaterals in this layer was the largest axon examined (the axon illustrated in figure 6).

The axon diameter decreased as the fiber branched repeatedly, but the branches maintained their myelin sheaths through many divisions, even within layer IV. In fact, only the final, terminal-bearing segments appeared to lack myelin. (In the drawings, the representation of the myelin sheaths is somewhat schematic: for clarity, only the thicker, more proximal branches have been shown with their myelin sheaths, and the thickness of the sheaths has been slightly exaggerated.)

The initial branchings of the axons, in the white matter and during their ascent through the lower layers, were usually simple bifurcations, but subsequent divisions were often more complex, with one axonal branch splitting into as many as five daughter branches. Often one of the daughter branches was myelinated, and of a diameter similar to that of the parent branch. It would continue on to give rise to several more branches. The rest of the daughter branches were shorter unmyelinated segments. These terminal branches were extremely fine (approximately 0.5 μm in diameter — it has been necessary to exaggerate their thickness in the drawings). They followed a tortuous path, in comparison to the straighter myelinated segments, and they bore numerous boutons along their course, either of the en passage or terminal type (fig. 5). These varicosities measured from 1-3 μm in diameter, and were presumably the sites of synaptic contact with cortical neurons. We were impressed by the large number of boutons on the branches of a single axon. For example, on one axon there were 1,543 boutons in layer IVab and the lower part of layer III, as well as another 203 boutons in layer VI. The actual number of synapses formed by this axon must have been even greater, since individual boutons, particularly the larger ones, can form synapses with several postsynaptic elements (Garey and Powell, '71).

One peculiarity of the axonal branching pattern that seems worthy of note concerns the course taken by the daughter branches emerging from nodes of Ranvier. In many cases the daughter branch would not set off immediately in its final direction, but for a variable stretch would travel close alongside its sister branches or back along the parent branch, before turning sharply away (see inset to fig. 6). One might speculate that this configuration results from the translocation of the branch point to a node during myelination.

The lateral spread of the axonal arborizations in layer IVab ranged from 1-2 mm in the coronal plane. Their extent in the anteroposterior direction was roughly similar. None of the arborizations were markedly elongated in any one direction. The terminals, however, were not distributed uniformly within this total field, but were arranged into two or more patches separated by areas of comparable size containing few terminals. The width of both the patches and of the intervening gaps was about 0.5 mm, a distance corresponding to the width of single ocular dominance columns in the cat, as determined autoradiographically (Shatz et al., '77). It is therefore likely that the patchy distribution of terminals repre-
Fig. 6 An afferent with cortical arborization restricted to lamina IVab and the lower part of lamina III. This is a camera lucida reconstruction from 16 serial coronal sections, each 100 μm thick. The inset, top left, shows the position of the reconstruction and of the injection site. The main axonal trunk, which measured 3.3 μm in diameter, was followed back in the white matter almost as far as the injection site, without any other branches being found. The axonal arborization is separated into four discrete regions, two of which are superimposed in the lower part of the reconstruction. It has been necessary to exaggerate slightly the thickness of the myelin sheath. Also, in the main part of the figure the myelin sheaths of the more distal axonal segments have been omitted.

In the inset, bottom right, a small part of the arborization is drawn at higher magnification. The lightly-myelinated axon branch, which runs across the field from upper left to lower right, gives off terminal-bearing branches at nodes of Ranvier. Of the four branches which emerge from the first node, one doubles back for about 15 μm alongside the parent trunk, before turning away to the right. This is a very common arrangement, which cannot be clearly illustrated in the low-power drawings (see text). Note the great variety in the size and shape of the boutons.
Fig. 7 Another axon with its main arborization in layer IVab and lower III. Unlike the axon reconstructed in figure 5, it gives off collaterals which arborize in the upper half of layer VI. The parent trunk divides deep in the white matter, but both daughter branches innervate the same region of area 17.
Fig. 8 A third example of an axon arborizing in lamina IVab and lower III. The parent trunk measured 2 µm in diameter. Note the collaterals to layer VI, and the division of the main arborization into two clumps.

...sent the innervation by one axon of more than one ocular dominance column devoted to the same eye.

Since the reconstructions made from coronal sections give a somewhat distorted view of the columnar arrangement of terminals, we have also made reconstructions from tangential sections of the cortex, which allow a surface view of the branching pattern in the fourth layer. One such reconstruction is shown in figure 9 (upper part). A notable feature is the large number of myelinated branches which cross and recross between columns. In making this and other reconstructions we were struck by the apparent inefficiency of this design, in that myelinated branches took long and roundabout routes to supply a few terminals to a region already well supplied by other, shorter branches. It may be that these peculiarities arise during the reorganization of the axonal arborizations that seems likely to accompany the postnatal segregation into ocular dominance columns (LeVay et al., '78).

The columnar pattern is seen most clearly when the terminals are viewed in isolation, as in figure 9 (lower part). We do not have independent information on the position of the ocular dominance columns in the area of the reconstruction, but the figure suggests that a few terminals may be present in the inappropriate columns, particularly near their borders (see also LeVay et al., '78).

For clarity the branches to layer VI have been omitted from the drawing of figure 9. The boutons on these branches were also arranged in patches, in register with the patches in layer IV.

**Afferents to layer IVc**

Examples of afferents to layer IVc are shown in figures 10 and 11. The eight such afferents that we reconstructed had axon diameters ranging between 1.0 and 1.7 µm. There
Fig. 9 Above: An axon whose main arborization is in layer IVab and lower III, reconstructed from serial tangential sections. The drawing is thus a surface view of the arborization; anterior is to the right and dorsal is above. The parent trunk entering the field from the right is still in the white matter. At the point marked by an arrow, a branch is given off which supplies an elaborate arborization in layer VI; this has been omitted from the drawing. The other branches rise through the lower layers and then run tangentially in layer IV, mostly in a posterior direction. The fine, terminal-bearing branches are arranged in several discrete clusters, and the larger, myelinated trunks criss-cross rather aimlessly between them. As in the previous figures, the thickness of the myelin sheaths is exaggerated, and not all the myelin has been drawn in.

Below: The positions of all the boutons in layer IVab and lower III, for the same axon as shown above. The segregation of the boutons into clumps is seen more clearly without the presence of the axon branches which supply them. From the size of the clumps, and of the gaps between them, it seems likely that they correspond to several ocular dominance columns belonging to the same eye. Comparison may be made with the tangential reconstructions of cat ocular dominance columns from autoradiographs (Shatz et al., '77; LeVay et al., '78). The boutons in layer VI were also clustered, in register with those in layer IV.
was therefore no overlap in diameters between the axons projecting to layer IVab and those projecting to layer IVc.

Every axon that projected to layer IVc gave rise to collaterals in layer VI, mainly in the upper half of the layer. As far as we could tell there was no laminar segregation of the two types of collaterals in layer VI, as there was of the principal arborizations in layer IV. (We did not see any axons which arborized exclusively in layer VI.)

The arborizations of the axons in layer IVc were altogether smaller and simpler than those in layer IVab. The lateral extent of the arborizations was around 500 μm with sometimes one or two straggly branches extending beyond this distance. An occasional branch was also seen to extend upwards into layer IVab. The terminals were borne along the course and at the tips of fine, unmyelinated branches. On the whole, the boutons were somewhat smaller than those of axons projecting to layer IVab, though there was great variability in size among the terminals of a single axon. It was evident that there were many fewer terminals on the arborizations of axons supplying layer IVc than on those of axons supplying layer IVab: the axon in figure 10 carried 575 terminal boutons in layer IV and 76 in layer VI.

Very few of the axons projecting to layer IVc had branches in the white matter or in the lower layers, except for the collaterals within layer VI. The single main trunk usually branched just underneath layer VI to give rise to a single patch of terminals, presumably restricted to a single ocular dominance column. A few axons had small numbers of terminals in a region separated from the main patch by several hundred microns, probably in the next column serving the same eye (fig. 11). This is also evident in the reconstruction from serial tangential sections of the axon shown in figure 12.
Fig. 11 Another axon with a main arborization in layer IVc. The collateral arborization in layer VI consists only of a few twigs. Unlike the axon in the previous figure, this one has a branch which supplies a few boutons to a region separated from the main arborization, perhaps in the next column belonging to the same eye.
Fig. 12 Tangential reconstruction of an axon with its main arborization in layer IVc. The total arborization is much smaller in extent than that of the axon supplying layer IVab which is illustrated in figure 9 (note the difference in scale). Most of the boutons are gathered in a single patch, but a few are in a region (at top of figure) about 300 μm distant from the main group.

Afferents to lamina I

A large number of axons terminating in lamina I were seen after injections of HRP into the optic radiation. In contrast to layer IV, however, which receives its principal if not its entire afferent innervation from the dorsal lateral geniculate nucleus, layer I receives fibers from various sources, including the LGN, the pulvinar, the MIN, the posterior nucleus, area 18 and other cortical areas (DISCUSSION). It was therefore not possible to identify particular axons with confidence as originating from cells in the LGN. Since the axons ramifying in layer I formed a rather uniform population in several respects, we believe that the axons we have reconstructed (for example those shown in fig. 13) either are or resemble geniculate afferents. Some reasons for thinking that they are geniculate afferents, originating from laminae C1-C3, will be discussed below.

The axons supplying layer I were the finest of all those seen in our preparations. They never exceeded 1 μm in diameter and could be as fine as 0.5 μm. Most of them were lightly myelinated: the myelin itself could not be seen but the axons displayed the characteristic periodic intensification of the stain which, we believe, indicates the position of the nodes of Ranvier. A curious feature of these axons was the occurrence of a single minute excrescence at each node (not illustrated). A few unmyelinated axons were also seen.

Most axons of this type passed vertically through the lower layers without bifurcating. They sometimes gave off very fine, horizontally running collaterals. These were found in the upper part of layer V, or (in two instances) in the lower part of layer III, but not at any
other level (fig. 13). These horizontal collaterals were usually not well filled, so that their full extent could not be determined.

The terminal arborizations in layer I were remarkable in several ways. Firstly, they were restricted to a very narrow band immediately under the glial limiting membrane and did not occupy the full thickness of layer I. Secondly, the axons branched very sparingly, but these secondary branches travelled great distances in the layer. Again, the full extent of the arborization could usually not be determined because of unsatisfactory filling. One example of a complete arborization, traced from tangential sections, is shown in figure 14. It spanned a total distance of 2.1 mm in one direction and 1.7 mm in the other. In spite of this huge extent — greater than any axon in layer IV — the actual boutons, mostly of the en passage variety, were quite few in number. In the illustrated example there were only 151 boutons on the entire arborization. There is a suggestion of a columnar organization of the boutons, but given their sparseness it is hard to be sure on this point without an independent demonstration of the columnar pattern in the area.

Two of the reconstructed axons which innervated layer I gave off a branch while deep in the white matter (fig. 13). Both of these branches could be traced into the cortex of area 18, to a zone in approximate retinotopic correspondence with the region of termination in area 17. Unfortunately, in neither case could the branch to area 18 be followed to its termination, on account of the poor filling.

Comparison of axon diameters of geniculate neurons and cortical afferents

In figure 15 are plotted in histogram form the axon diameters of all the geniculate neurons and all the cortical afferents that were reconstructed. Within the histogram for the LGN (lower half of figure) three different cell types have been indicated: type 1 cells, type 2 cells, and those cells which were situated in laminae C1-C3. As described earlier, type 1 and type 2 cells were distinguished by their characteristic dendritic morphology. The cells in laminae C1-C3 probably correspond, at least in part, to another morphological type of relay cell (see above). The cortical sample is divided into three groups on the basis of their principal layer of termination as described above: those ending in layer IVab and lower III, those ending in layer IVc, and those ending in layer I. We should stress that our samples do not necessarily reflect the true relative frequencies of the different types of cells and axons.

It was the object of this study to describe the manner in which the axons of different types of geniculate neuron terminate in the visual cortex. The histogram of figure 15 suggests that it is possible to do this by matching the axon diameters of the three classes of geniculate neurons to those of the three types of cortical afferent. The axon diameters of the cortical afferents to layer IVab match those of type 1 geniculate neurons, the axon diameters of the affereits to layer IVc match those of type 2 neurons, and the axons terminating in layer I match those belonging to neurons in laminae C1-C3 in the LGN. This correspondence is strengthened by the lack of overlap between the individual classes in our samples.

DISCUSSION

The main conclusion that we wish to draw from this study is that the different types of relay cells in the lateral geniculate nucleus have axons which terminate at different levels within the visual cortex. The type 1 cells, the largest type of relay cell in the LGN, possess the thickest axons in the optic radiation. These terminate primarily in layer IVab and the lower part of layer III, as well as giving off collaterals, in most cases, to layer VI. The type 2 cells, medium-sized neurons which carry the "grape-like appendages" on their dendrites, send medium-sized axons to the cortex, which terminate in layer IVc and in layer VI. The cells of laminae C1-C3, whose morphology we have not been able to study in detail, but that most likely correspond to Guillery's type 4 cells, possess extremely fine axons which terminate in layer I, with collaterals in the upper part of layer V and sometimes in the lower part of layer III.

Since our approach has been to study geniculate neurons and cortical afferents separately, our conclusion depends on two assumptions. One is that the terminal arborizations that we have studied in the cortex are actually the terminals of geniculate cells. The other is that the fibers maintain a constant diameter throughout their course in the optic radiation.

As to the identity of the afferents which we have reconstructed, the location of the injections is hardly sufficient assurance that they originate from the LGN, for there are doubtless other fibers mingled in the optic radia-
Fig. 13 Very fine axons (less than 1 \( \mu \text{m} \) diameter) with arborizations in layer I. The filling of these axons is not complete, as indicated by the dashed lines at their end. Of the four axons in this field, two (labelled 1 and 3) give off a branch in the white matter which enters area 18. In area 17, axon 1 supplies layer I only, axons 2 and 3 give off collaterals in layer V, and axon 4 gives off collaterals in the lower part of layer III. All four of these axons are myelinated, but the myelin sheaths have not been included in the drawing because this would have grossly exaggerated the overall fiber diameter.
tion. Our belief that they originate in the LGN is based primarily on the observation that they distribute exactly to those levels of the cortex known to receive geniculate afferents as determined by autoradiography (LeVay and Gilbert, '76). Injections of \(^3\)H-proline into either of the A laminae of the LGN produced labelling throughout layer IV and the lowest 100 \(\mu\)m of layer III, and in layer VI. This projection is accounted for fully by the two types of axons found in layer IV, one projecting to layer IV\(_{ab}\) and the bottom of layer III and one to layer IVc. The projection to layer VI is accounted for by collaterals of these two types of axon. Furthermore, the afferents that do not arise from the LGN appear to avoid layer IV. This includes the fibers from the MIN, posterior nucleus and lateral posterior nucleus, which terminate predominantly in layer I (Rosenquist et al., '75), as well as the callosal fibers, which end in all layers except layers IV and V (Shatz, '77). Injections of \(^3\)H-proline into the C laminae of the LGN produced a three-tiered labelling pattern in area 17, with one band in the upper part of layer I, a second in the lower part of layer III, and a third in the upper part of layer V. Our third class of cortical afferents — fine-diameter axons which project to layer I — sometimes had collaterals at these two lower levels, and thus are reasonable candidates to be geniculate afferents. The existence of many non-giniculate afferents to layer I, however, makes this assignment uncertain. (The middle tier of input from the C laminae, situated at the III/IV border, may be contributed in part by the axons of large type 1 neurons in lamina C proper. Most of these neurons, however, project to area 18 and not to area 17 (LeVay and Ferster, '77)).

The second assumption, that fibers do not change in diameter en route to the cortex, is central to our identification of each afferent type with one type of geniculate neuron. We have no direct evidence on this point, but it does seem to be substantiated by the results, inasmuch as we found, both in the cortex and in the LGN, three classes of axons with similar diameters. The simplest interpretation is certainly that they are the same three sets of axons, which have maintained a constant diameter along their course.

The termination of the (large-diameter) type 1 afferents in a tier above the type 2 afferents is in agreement with the current source density analysis of Mitzdorf and Singer ('78), which suggests that fast-conducting LGN afferents synapse in more superficial parts of layer IV than slow-conducting afferents.

**Columnar organization**

A greater lateral extent of the arborization of type 1 afferents within layer IV, as compared with those of the type 2 afferents, had been suggested previously on the basis of retrograde transport studies (LeVay and Ferster, '77). Besides confirming this suggestion, the present results show that the type 1 arborizations are divided into several discrete sub-regions which, from their size and arrangement, we assume correspond to ocular dominance columns. The gaps between these sub-regions, which presumably correspond to columns for the other eye, are joined by myelinated segments that often run tangentially in the fourth layer. The type 2 axons, on the other hand, have arborizations which are confined to a single column, or at most have a few twigs in a neighboring column. This difference lends support to the notion that the greater susceptibility of the type 1 geniculate neurons to the effects of monocular visual deprivation (LeVay and Ferster, '77) results from anatomical differences permitting greater interocular competition among their terminals in the cortex. It remains, however, to examine their arborization pattern during the period of susceptibility to visual deprivation.

**Correlation with physiological results**

In a previous study we presented evidence that the two morphological cell types in the A laminae of the LGN, types 1 and 2, correspond to the two physiological types described there, the Y- and X-cells (LeVay and Ferster, '77). The correspondence is now strengthened by the determinations of their axon diameters. These agree with values predicted from the reported conduction velocities of the two physiological classes. From the data given by Hoffmann et al. ('72) the axonal conduction velocities may be calculated to be 8-20 m/sec for X-cells and 15-40 m/sec for Y-cells. Using the calculations of Waxman and Bennett ('72) on the relationship between conduction velocity and fiber diameter for myelinated nerves, and our own measurements of the ratio between axon diameter and overall fiber diameter in the optic radiation \((g = 0.67 \pm 0.1)\), we would predict diameters of 1-2.5 \(\mu\)m for X-cell axons and 2-5 \(\mu\)m for Y-cell axons (exclusive
Fig. 14. Tangential reconstruction of an axon supplying layer I. Posterior is to the left, and dorsal is below. This axon has no collaterals in lower layers. The point of entry into the gray matter is indicated by the arrow, and the parent trunk is seen almost end on as it ascends to layer I, which it reaches at the first branch point. The extent of the arborization in layer I is greater than any other cortical afferent examined (note scale, and compare with figs. 9 and 12), but the boutons are scattered quite sparsely along the terminal branches.
of the myelin sheath). Our measurements of 1.0-1.7 μm and 2.0-3.3 μm for the axon diameters of morphologically defined type 1 and type 2 neurons are therefore in quite good agreement with the calculated values for Y- and X-cells.

This agreement can also be extended to the axons arising from cells in the deeper C laminae. Physiologically, cells in these laminae are predominantly of the W-type, with axon conduction velocities in the range 2-10 m/sec (Wilson et al., '76). The measured axon diameters (0.5-1.0 μm) are appropriate for these velocities.

Comparison with the monkey

In the monkey, the bulk of the geniculate input also terminates as two tiers, one above the other (Hubel and Wiesel, '72), in sub-laminae that were called IVb and IVc in that study (now referred to as IVcα and IVcβ). These two tiers arise from the magnocellular and parvocellular geniculate laminae. The correspondence between the cat and monkey is even more striking in view of the reports that the receptive fields of neurons in the magnocellular laminae are of the Y-type, and those in the parvocellular laminae of the X-type (Sherman et al., '76; Dreher et al., '76; Malpeli and Schiller, '77). The cat does not separate the two neuronal types so cleanly in the LGN, but the end-result in the cortex appears to be similar.

Nature of target cells in the cortex

The termination of type 1 and type 2 afferents at different levels in the cortex suggests that for the most part they form synaptic con-
conections with separate populations of cortical neurons, that is, with those in layer IVb and lower III, and those in layer IVc. To some extent these two populations differ in morphology. Firstly, the cells in layer IVc, the zone of termination of the type 2 afferents, are distinctly smaller and more closely packed when viewed in Nissl preparations — Otsuka and Hassler ('62) refer to them as granule cells. In our own Golgi material, these cells seem to consist largely of small spiny-dendrite stellate cells (in agreement with O'Leary, '41). The upper zone, which receives the type 1 afferents, contains a much larger proportion of pyramids: layer IVb contains a mixed population of pyramids, spiny stellate cells and transitional forms, and the lower part of layer III contains almost exclusively large pyramidal cells (border pyramids of O'Leary, '41). To what extent this arrangement reflects a real segregation of type 1 and type 2 afferents onto pyramidal and spiny stellate neurons remains somewhat uncertain, given the rather imprecise cytoarchitecture of the cat's cortex.

Physiologically, neurons in layer IVc have predominantly simple receptive fields (Hubel and Wiesel, '62; Kelly and Van Essen, '74; Gilbert, '77). In layer IVb Kelly and Van Essen reported roughly equal numbers of simple and complex cells, while Hubel and Wiesel ('62) and Gilbert ('77) again found a predominance of simple cells. The lowest part of layer III contains mainly complex cells (Gilbert, '77). Thus the type 2 afferents arborize among simple cells, while the type 1 afferents arborize among what appears to be a mixed population of simple and complex cells. The findings are consistent with the report by Hoffmann and Stone ('71) that some complex cells in the cortex receive a direct input from the Y-type geniculate afferents. Although the full significance of the laminar arrangement of the geniculate afferents remains to be explored, it seems warranted to draw the conclusion that the X- and Y-channels remain largely separate, at least up to the level of the first-order cortical neurons.

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