

# CLUSTERED INTRINSIC CONNECTIONS IN CAT VISUAL CORTEX<sup>1</sup>

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## Abstract

The intrinsic connections of the cortex have long been known to run vertically, across the cortical layers. In the present study we have found that individual neurons in the cat primary visual cortex can communicate over surprisingly long distances horizontally (up to 4 mm), in directions parallel to the cortical surface. For all of the cells having widespread projections, the collaterals within their axonal fields were distributed in repeating clusters, with an average periodicity of 1 mm. This pattern of extensive clustered projections has been revealed by combining the techniques of intracellular recording and injection of horseradish peroxidase with three-dimensional computer graphic reconstructions. The clustering pattern was most apparent when the cells were rotated to present a view parallel to the cortical surface. The pattern was observed in more than half of the pyramidal and spiny stellate cells in the cortex and was seen in all cortical layers. In our sample, cells made distant connections within their own layer and/or within another layer. The axon of one cell had clusters covering the same area in two layers, and the clusters in the deeper layer were located under those in the upper layer, suggesting a relationship between the clustering phenomenon and columnar cortical architecture. Some pyramidal cells did not project into the white matter, forming intrinsic connections exclusively. Finally, the axonal fields of all our injected cells were asymmetric, extending for greater distances along one cortical axis than along the orthogonal axis. The axons appeared to cover areas of cortex representing a larger part of the visual field than that covered by the excitatory portion of the cell's own receptive field. These connections may be used to generate larger receptive fields or to produce the inhibitory flanks in other cells' receptive fields.

The classical view of cortical connectivity, derived from Golgi studies, is that axons run predominantly in a direction perpendicular to the cortical surface, from layer to layer, with relatively little spread in the direction parallel to the cortical surface (Lorente de No, 1933). Physiological studies have supported this view by demonstrating the existence of the cortical column. Within a column running from the pia to the white matter, all of the cells have common functional properties, such as orientation specificity and ocular dominance (Hubel and Wiesel, 1962). This finding was consistent with the Golgi studies, since one would expect cells with common properties to be interconnected, whereas cells with different properties should be relatively independent.

There is evidence, however, that there may be considerably more horizontal interaction within a given cortical area than is suggested by Golgi studies. The first work to

show this relied on degeneration techniques. From small lesions within the cortex it was possible to trace degenerating fibers over surprisingly long distances, up to 6 mm in certain layers. (Fisken et al., 1975; Creutzfeldt et al., 1977). However, the degeneration studies could not differentiate between fibers arising from cells in distant sites, running within the cortex before giving off terminals, and fibers representing intrinsic cortical connections. Studies based on tracing techniques using anterograde and retrograde transport, which do not suffer from these limitations, have lent additional support to the existence of long-range intrinsic connections. Furthermore, the distribution of label resulting from focal injections within a given cortical area is not uniform but is patchy in appearance (Kunzle, 1976; Jones et al., 1978; Rockland and Pandya, 1979; Gilbert and Wiesel, 1981; Rockland and Lund, 1982).

It is now possible to examine this phenomenon at the single-cell level with the techniques of intracellular injection of horseradish peroxidase (HRP), which provides a much more complete view of a cell's axonal arbor than do Golgi impregnations. These studies have revealed that afferent fibers from the lateral geniculate nucleus give off collaterals covering wide areas of cortex, innervating

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several ocular dominance columns (Ferster and LeVay, 1978; Gilbert and Wiesel, 1979). The cortical cells themselves also project to points quite distant from their own dendritic fields, but still within the same cortical area (Gilbert and Wiesel, 1979). An intriguing feature of these widespread cortical connections, which is consistent with the results from the studies based on axonal transport, is that there is not a uniform distribution of collaterals within a cell's axonal field. Rather, the collaterals are clustered (Gilbert and Wiesel, 1979), as are the collaterals of the thalamic afferents (Ferster and LeVay, 1978; Gilbert and Wiesel, 1979). While the clustering of the thalamic afferents is responsible for ocular dominance columns, the functional correlate of the clusters of intrinsic cortical connections is not known.

In the present work we have attempted to examine this clustering phenomenon in greater detail, making use of three-dimensional computer graphics to reconstruct cortical cells that have been injected intracellularly with HRP. This work has been presented in preliminary form (Gilbert and Wiesel, 1982).

### Materials and Methods

Intracellular injections were made in cat striate cortex in order to analyze the patchiness of intrinsic cortical connections at the single-cell level. To do these injections, beveled micropipettes (with a tip diameter of 0.5  $\mu\text{m}$ ) filled with 6% HRP in 0.2 M potassium acetate, pH 7.6, were advanced through the cortex. Cells were penetrated, either during the advancing step (advancer by Transvertex, Sweden) or by passage of current pulses. Their receptive field properties were then characterized. Once a complete functional description of a cell was obtained, the cell was injected with the HRP by the passage of pulses of positive current, 1 to 1.5 nA in a 50-msec on/50-msec off duty cycle, for a period ranging from 3 to 5 min. At the end of the experiment the animal was perfused with 2% glutaraldehyde and its brain was sectioned into 100- $\mu\text{m}$  sections. The sections were treated with a combination of the 3,3'-diaminobenzidine reaction at acid pH (Malmgren and Olsen, 1978) and cobalt intensification (Adams, 1977).

The injected cells were reconstructed in a standard two-dimensional representation using a drawing tube and a microscope with a  $\times 40$  oil planapochromatic objective. At this power it is possible to see the finest axonal processes, and boutons can be seen as distinct swellings along the course of the collaterals. Three-dimensional computer graphic reconstructions were done with a system that included a digitizing tablet (Summagraphics) and a vector graphics scope with an array processor (Megatek) in conjunction with a PDP 11/34 computer. In addition, we used a microscope equipped with a stepping motor-driven focusing attachment and X/Y stage (Zeiss). The drawing tube of the microscope was pointed at the graphics scope, and lines were drawn on the scope with the aid of the digitizing tablet. This enabled us to do the reconstructions in much the same fashion as a two-dimensional reconstruction, except that the depth information was retained by recording the focal position.

The sections shrink during the dehydration procedure, and there is considerably more shrinkage along the axis

perpendicular to the section surface than in the plane of the section surface. It is therefore necessary to scale the computer reconstructions to compensate for this differential shrinkage. Sections were measured before and after dehydration. The section thickness was measured using the scale on the fine focus of the microscope, and dimensions along the plane of section were measured with the graticule on the eyepiece of the microscope. Since the sections were mounted on gelatinized slides, there was very little shrinkage in the plane of sections (under 2%), but substantial shrinkage in the orthogonal axis (averaging about 65%). The computer reconstructions presented in this paper are all scaled by the appropriate factors.

The reconstructions are rotated to provide views tangential to the pial surface. In one instance (Fig. 2) the cortex was curved, so the reconstruction was flattened by breaking it at one point and rotating one half to lie in line with the other half. For another cell (Fig. 10) a stereoscopic view is presented. For the other reconstructions, the cortex was sufficiently flat in the region of the labeled axon not to require any special flattening procedure.

### Results

In our previous paper (Gilbert and Wiesel, 1979) relating physiology to the anatomical structure of HRP-injected cells, we emphasized the vertical, interlaminar connections. In the present work we focus on the horizontal distribution of intrinsic connections of cortical cells and afferents. We will present cells from different layers of the cat visual cortex. From a sample of approximately 175 cells and afferents that we have characterized physiologically and injected in the cat striate cortex, we have reconstructed 55; 47 of these were cortical cells. The very long-ranging connections (2 mm or more) tend to be formed by pyramidal and spiny stellate cells, of which we have reconstructed 44. A very striking characteristic of these extensive axonal fields was that the axon collaterals, rather than being uniformly distributed throughout the field, were distributed in discrete, repeating clusters. We divided the reconstructed spiny cells into three categories: cells forming long-range clustered connections, cells with unclustered connections, and cells that were insufficiently filled to categorize them as clustered or unclustered (Table I). It should be noted, however, that even for complete fills, if the plane of section is not appropriate, it is quite possible to miss the axon collateral clustering. Such was the case for the cell in Figures 3 to 5. Only three-dimensional reconstructions can reliably provide information on the clustering of the axonal arbor. Nonetheless, of the 44 spiny cells reconstructed in two dimensions, 17 showed widespread clustered connections,

TABLE I  
Summary of reconstructed spiny cells

Layer	Clustered	Not Clustered	Incomplete Fill
2+3	5	4	5
4	2	2	2
5	6	2	7
6	4	3	2
Total	17	11	16

11 were not clustered, and 16 were not filled sufficiently to see the longest collaterals. From the three-dimensional reconstructions it was apparent that cells forming widespread axonal projections invariably had their axonal collaterals distributed in clusters. As shown in Table I, this phenomenon was exhibited by cells in all layers of cortex and, taken together, at least half of the pyramidal and spiny stellate cortical cells form connections of this type. Of the 11 well filled spiny cells reconstructed three-dimensionally, all had long-range clustered projections.

The first three cells, shown in Figures 1 to 6, were found in layer 2+3. A layer 2 pyramidal cell in area 17 is shown in Figure 1. This cell had a complex receptive field,  $1^\circ \times 1^\circ$  in size. From the coronal view, several discrete clusters of axon collaterals could be observed. When the axon was flattened (Fig. 2A) and rotated to obtain a view of the axon projected onto the cortical surface (Fig. 2B), the pattern was more striking. The axon had four long arms, each giving off one or more discrete collateral clusters along their length. Because some clusters were more clearly separated from their neighbors than others, it is difficult to be precise about the number of clusters or the spacing between them. For this cell approximately six clusters could be seen, and the average center-to-center distance between neighboring clusters was  $800 \mu\text{m}$ . When the axonal boutons were viewed in isolation, the clustering pattern was somewhat more evident (Fig. 2C). The axons did not extend equally in all directions but instead projected for greater distances along the dorsoventral axis than along the anteroposterior axis. It is interesting to note that despite the excellent labeling of this cell, covering a linear distance of nearly 4 mm, we were unable to find any processes projecting any deeper than layer 3. This indicates that the cell, which was located in the layer from which pyramidal cells project to other cortical areas, did not itself participate in corticocortical connections.

A second superficial layer pyramidal cell is shown in Figures 3 to 5. It had a  $3^\circ \times 2^\circ$  complex receptive field, with a 3:00 orientation. This cell had a rich collateral arborization in layer 5, extending into layer 6, as well as a set of collaterals in layer 2+3 (Fig. 3). It appears from the reconstruction that there were a number of collaterals in layer 4, but this was an artifact due to the plane of sectioning, and these collaterals actually lay in layer 2+3. After giving off a number of collaterals in layer 5, the axon entered the white matter and could be followed for several millimeters within it.

When reconstructed in the coronal plane, no obvious clustering pattern could be seen, although from the number of sections involved it was clear that the axon extended several millimeters along the anteroposterior axis. However, once the three-dimensional reconstruction of the axon was rotated about an axis perpendicular to the cortical surface, it showed clear clustering of collaterals (Fig. 4b). In this view, one could also see that the collaterals in layer 5 covered the same area as those in layer 2+3, that they were also clustered, and that the layer 5 clusters were situated nearly directly under those in layer 2+3. The three-dimensional reconstruction was then separated into layer 2+3 and layers 5 and 6 projections (the dividing line (*dashed line*) is indicated in Fig. 4b), and

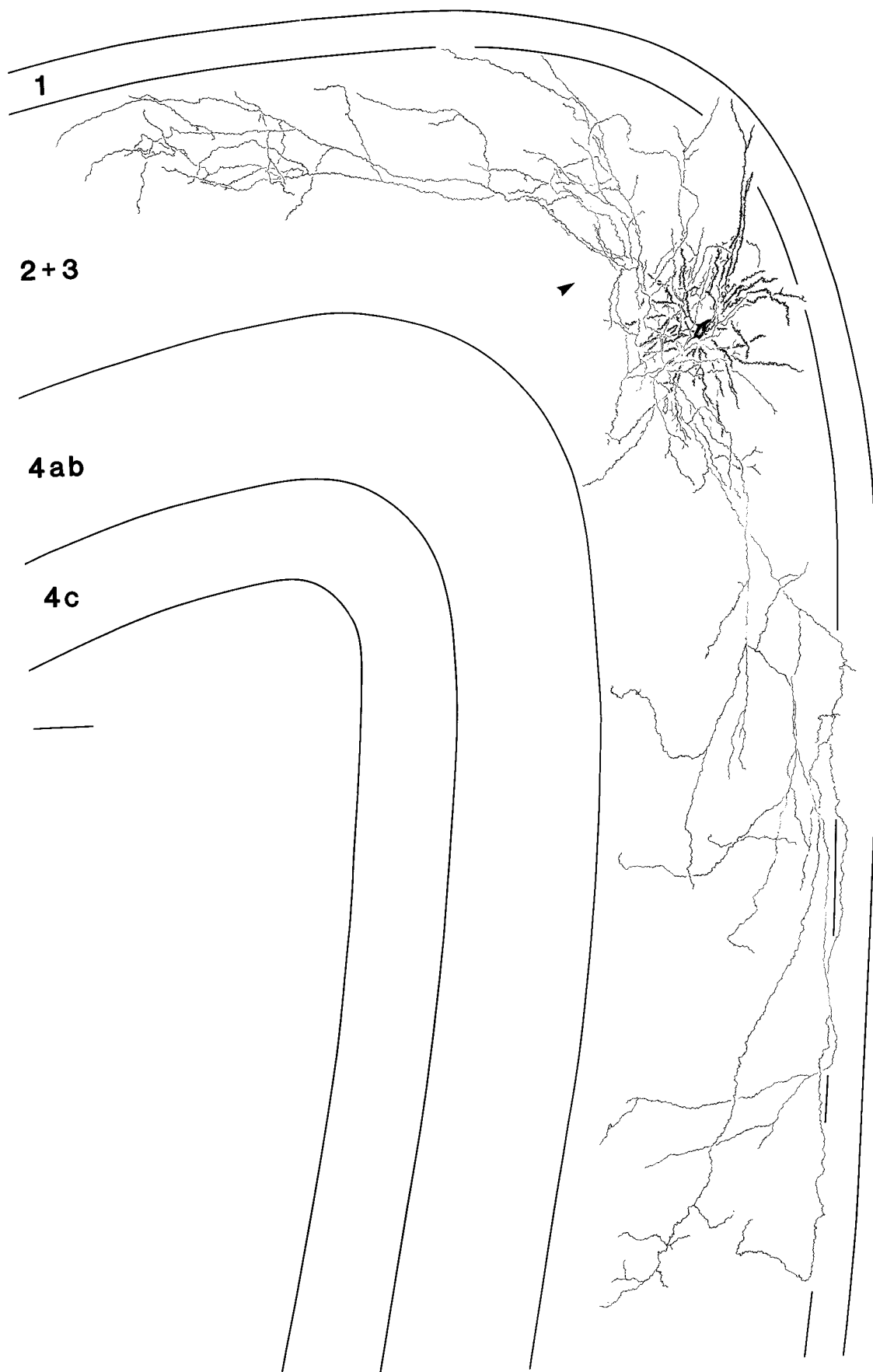
each set was rotated to provide a view projected onto the cortical surface (Fig. 5a is a surface view of the layer 2+3 axon; Fig. 5b is a surface view of the layer 5 axon). Reconstructions of the axonal boutons (Fig. 5c, layer 2+3 boutons; Fig. 5d, layer 5 boutons) are also presented. All of these views show the clustering pattern and demonstrate the similarity between the branching and clustering pattern in layers 5 and 6 and in layer 2+3. The spacing between clusters for this cell was approximately 1.3 mm, nearly twice that of the cell shown in Figures 3 and 4.

A third superficial layer pyramidal cell is shown in Figure 6. Although we have no three-dimensional reconstruction of this cell, the clustering pattern is apparent in the coronal view. It was a complex cell, with a receptive field  $1.5^\circ \times 1.5^\circ$  in size and having an 11:30 orientation. Its axon projected to layer 5 and to layer 2+3. In addition to the dense collateral arbor near the dendritic field, the axon formed two additional discrete clusters at some distance from the cell body. The total horizontal extent of the axon was 2 mm.

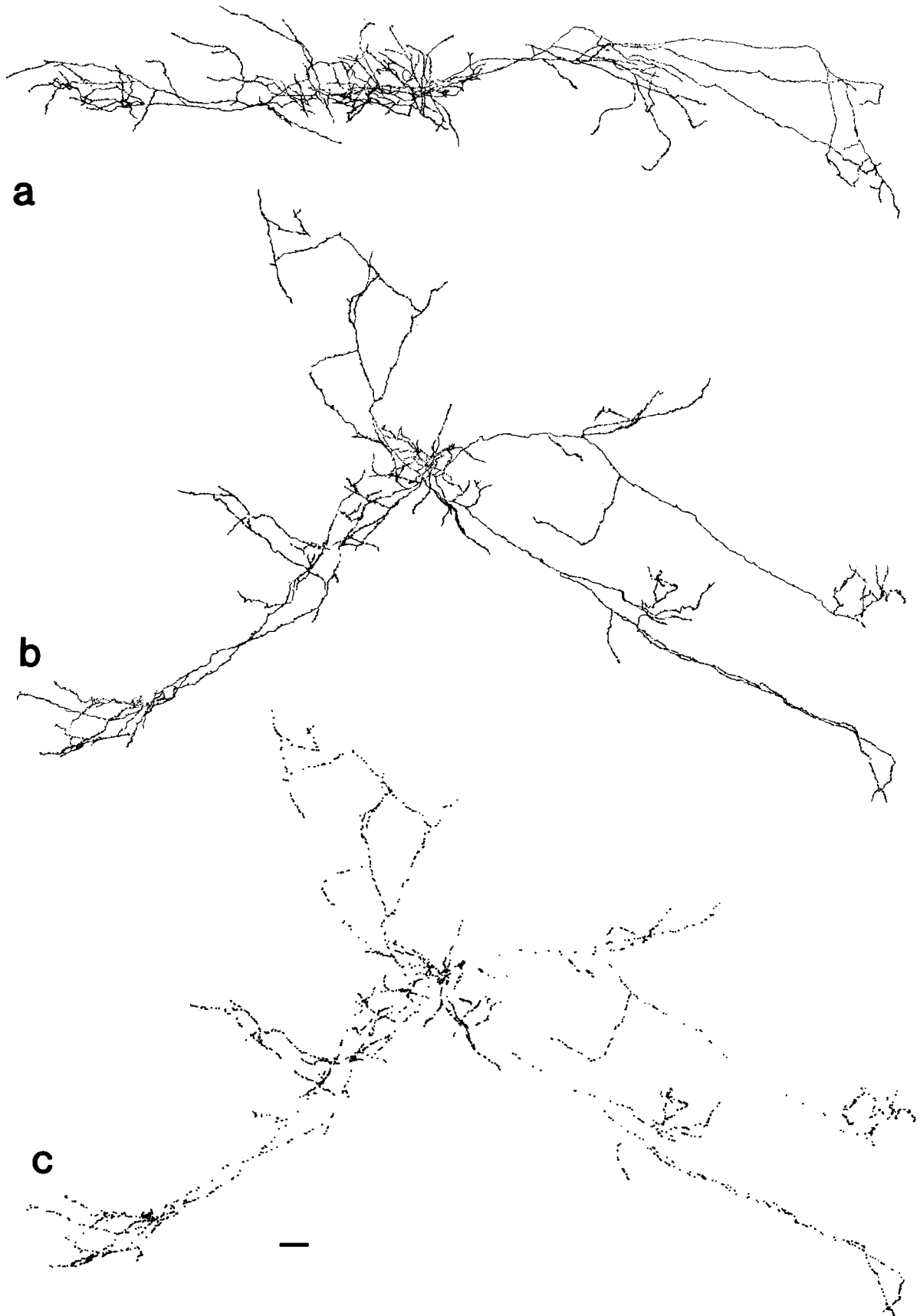
The clustering pattern was not restricted to superficial layer cells. An example of a layer 4c spiny stellate cell is shown in Figure 7. The cell was taken from a monocularly deprived animal but in many ways, it resembled cells from normal animals. The field was  $1^\circ \times 1^\circ$  in size, oriented at 11:30, and responded only to movement of the stimulus toward the peripheral visual field. The receptive field was centered at an elevation of  $-3.5^\circ$  and an azimuth of  $4^\circ$ . Its axon projected to layer 2+3 and extended for  $1\frac{1}{2}$  mm in the direction parallel to the cortical surface. This constitutes the first concrete evidence of a projection from layer 4c to layer 2+3. When rotated to obtain a surface view (Fig. 8), the axon exhibits several clusters of collaterals. In distinction to the ones shown previously, these clusters were arranged radially from the cell body, in three groups. Interestingly, the three clusters were found at 4:00, 7:00, and 10:00, with only a rudimentary projection into the remaining quadrant.

We reconstructed one layer 5 pyramidal cell that projected for nearly 4 mm within layer 6. The cell was complex, with a  $2\frac{3}{4}^\circ \times 1\frac{3}{4}^\circ$  receptive field, oriented at 10:00, centered near the area centralis. It was presented in a standard two-dimensional reconstruction in a previous paper (Gilbert and Wiesel, 1979, Fig. 4). It is illustrated here to show its three-dimensional structure (Figure 9). As seen from the rotated surface view, the axon extended for 4 mm along the medial bank of the gyrus and gave off several discrete and tightly bunched clusters of axon collaterals along its path.

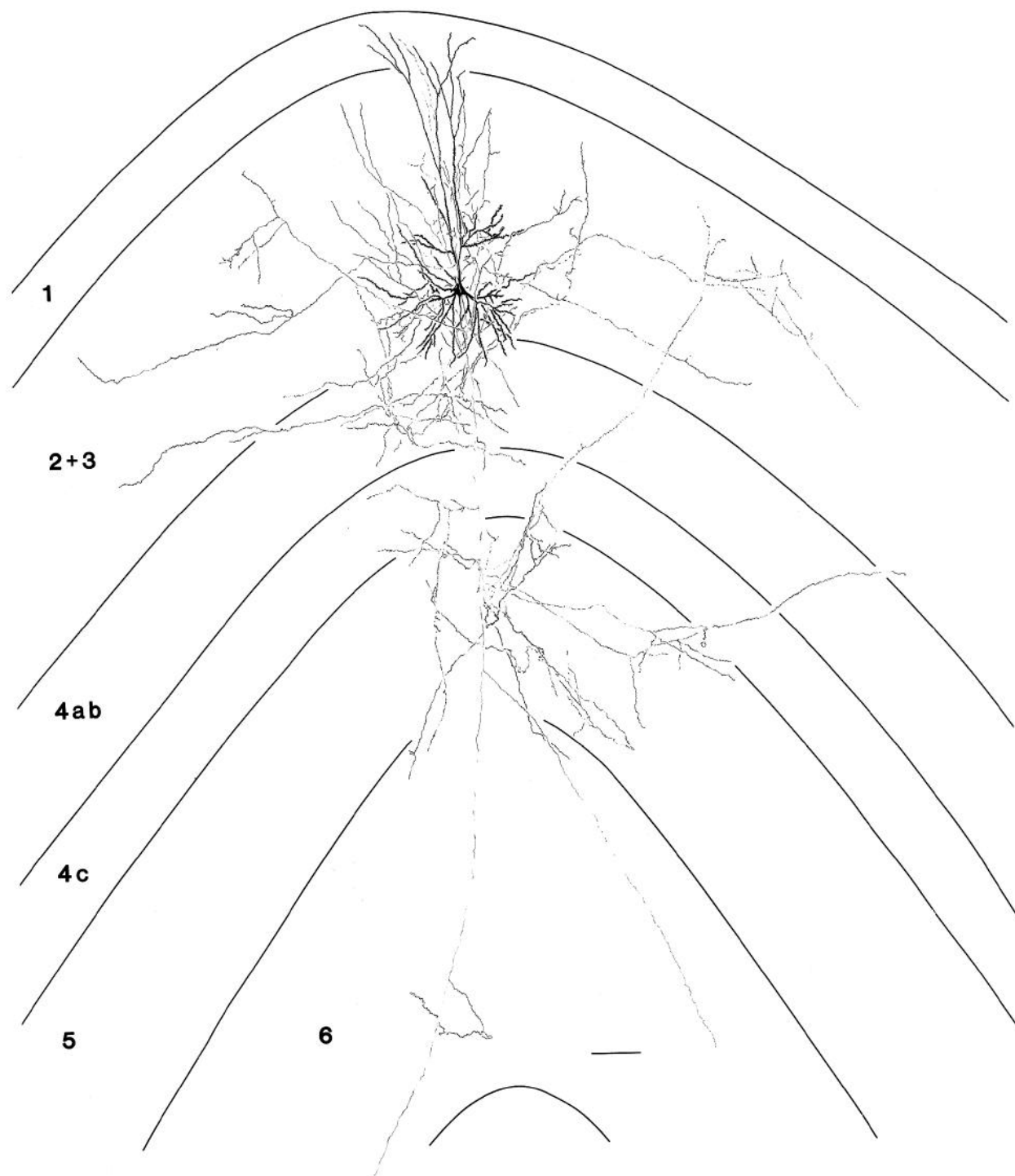
Deep layer cells can have ascending projections as well, and these projections also have a widespread clustered appearance. A reconstruction of a layer 5 cell with both ascending and descending projections is shown in Figure 10. This cell had a complex receptive field, orientation 8:30, directional for stimuli moving downward. The field was  $3^\circ \times 2^\circ$  and was located  $5^\circ$  below and  $3^\circ$  out from the area centralis. The axon projected both to layer 6 and to layer 2+3. The portion within layer 6 extended for nearly 3 mm along the medial bank. The projections to the superficial layers were distributed in



*Figure 1.* A camera lucida drawing of a pyramidal cell in layer 2 of the cat's striate cortex which was injected intracellularly with HRP, after its receptive field properties were determined. This cell was a complex cell, with a  $1^\circ \times 1^\circ$  receptive field. Scale marker = 100  $\mu\text{m}$ .



*Figure 2.* Computer reconstruction of the cell shown in Figure 1. *A*, Transverse view of the axon, which was straightened by extending it at the point indicated by the *arrowhead* in Figure 1. *B* shows the cell rotated  $90^\circ$  in order to view it projected onto a plane parallel to the cortical surface. This illustrates more clearly the clustered nature of its connections within area 17. *C* presents the axonal boutons in the same view as in *B*, further emphasizing the clustering pattern. *Scale marker* =  $100\ \mu\text{m}$ .



**Figure 3.** Reconstruction of a layer 3 pyramidal cell. The cell had a complex receptive field,  $3^\circ \times 2^\circ$  in size and with a 3:00 orientation. Its receptive field was centered at an elevation of  $-16^\circ$  and an azimuth of  $2^\circ$ . The axon had extensive arborizations both in layer 2+3 and in layer 5, and then entered the white matter. Scale marker = 100  $\mu\text{m}$ .

four distinct clumps, distributed around the crown of the gyrus. Because of the curvature of the cortex in the region of the labeled processes, it is difficult to give a surface view for all processes in a single rotated view. One can therefore get a better feeling for the three-dimensional structure of the cell in a stereoscopic presentation (Fig. 10, *b* and *c*). The rotated view (Fig. 10*d*) gives an approximate surface projection of the processes in layer 6 but not of the processes in layer 2+3, because

most of the layer 6 processes are found in the flat part of the gyrus. This view shows that the overall distribution of processes in layer 6 extends for a greater distance in the dorsoventral direction than in the anteroposterior direction. From the different views of the cell one can see that there is clustering of collaterals in both layers and that the layer 6 projection covers a larger part of the cortex than the projection to the superficial layers.

The most substantial ascending projection in the cor-

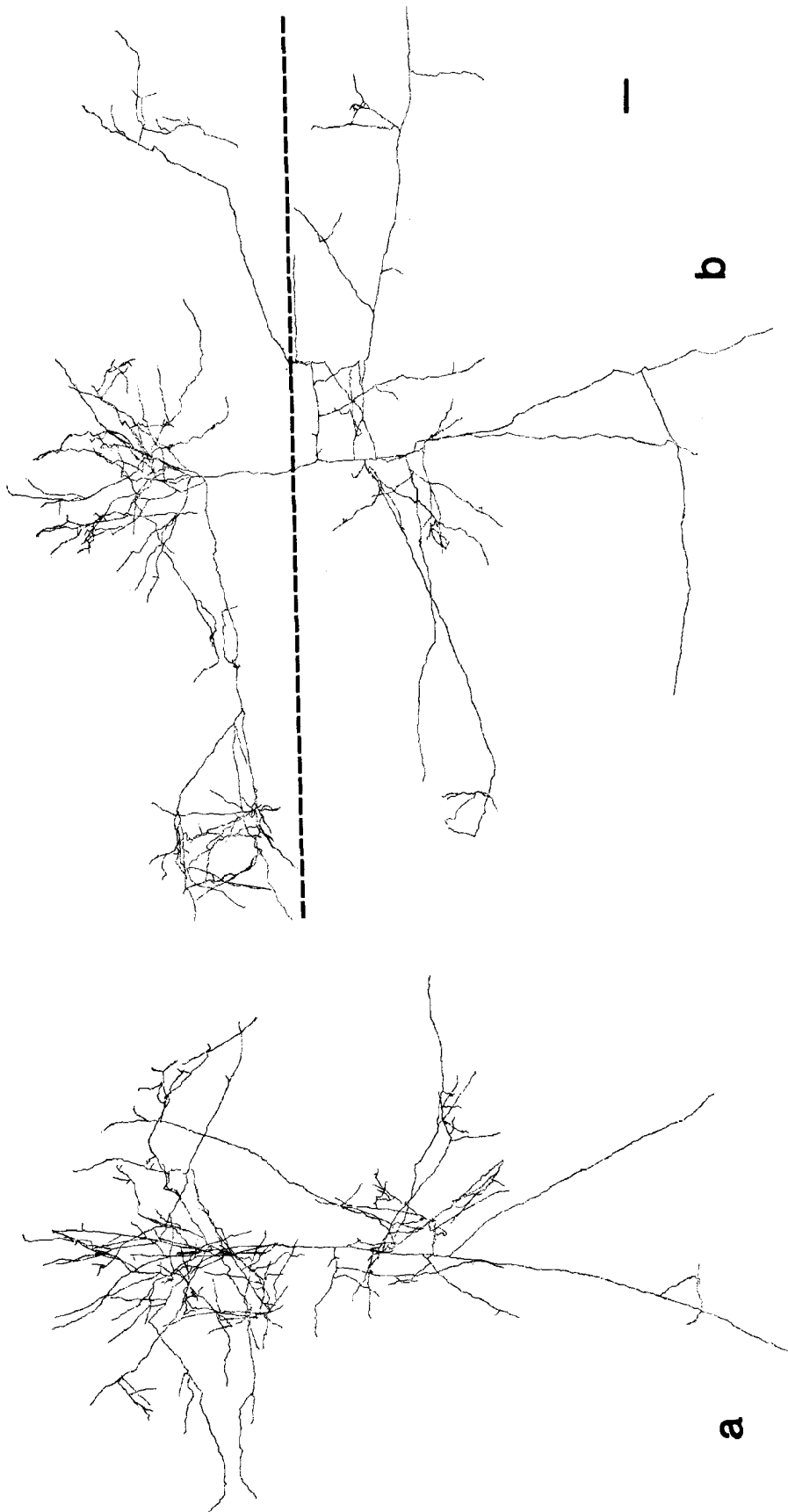
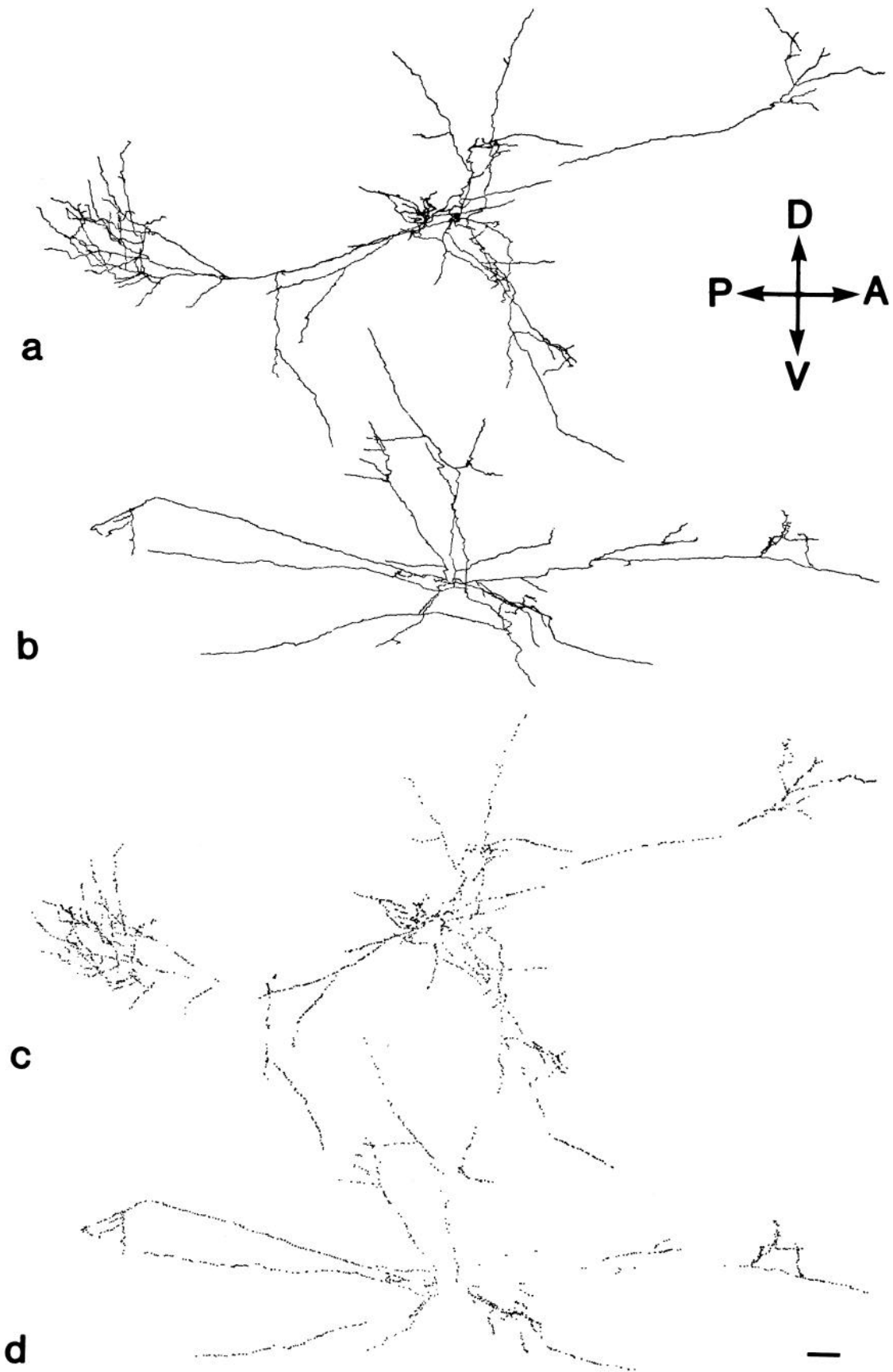


Figure 4. Computer reconstruction of the cell shown in Figure 3. *a*, The axon shown in the same view as the reconstruction in Figure 3. *b*, The axon rotated about an axis running perpendicular to the cortical surface, showing clustering of the collaterals in layer 2+3 and clusters in layer 5 lying directly underneath them. The *dashed line* indicates where the axon was divided for the illustrations in Figure 5. *Scale marker* = 100  $\mu\text{m}$ .



*Figure 5.* Surface views of the axon shown in Figure 4. The axon has been divided into the portion innervating layer 2+3 and that innervating layer 5; then each part has been rotated to present a view projected onto a plane tangential to the cortical surface. *a*, Layer 2+3 axon; *b*, layer 5 axon; *c*, layer 2+3 boutons; *d*, layer 5 boutons. Both parts of the axons produce clusters of collaterals and are similar in the overall distribution and branching pattern. Cortical axes are indicated and apply to *a* through *d*. Scale marker = 100  $\mu\text{m}$ .



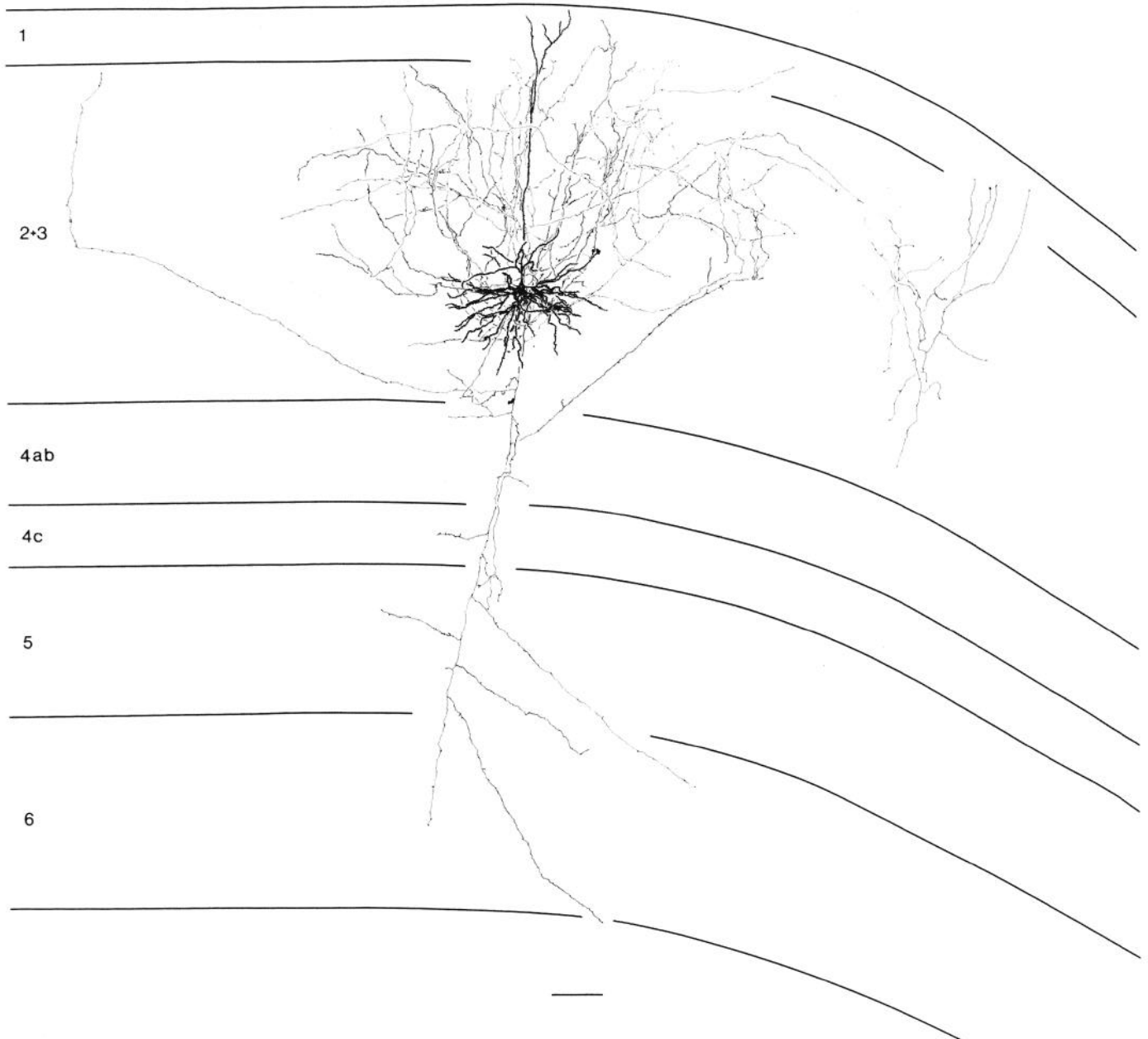


Figure 6. Layer 3 pyramidal cell, in transverse view. Its receptive field was complex, with an 11:30 orientation. The field was  $1.5^\circ \times 1.5^\circ$  in size and was centered at an eccentricity of  $6^\circ$  along the horizontal meridian. Scale marker =  $100 \mu\text{m}$ .

tex is that from layer 6 to layer 4. An example of a layer 6 pyramidal cell is shown in Figure 11. It was a simple cell with the long receptive field that is characteristic of cells in layer 6. The field was  $4^\circ$  long and  $1.5^\circ$  wide and its orientation was 11:30. The cell's axon projected predominantly to layer 4, which is characteristic of cells in this layer (Lund and Boothe, 1975; Gilbert and Wiesel, 1979; Lund et al., 1979). The horizontal extent of the axon in layer 4 was  $2\frac{1}{4}$  mm. Although no three-dimensional reconstruction of the cell is available, in this coronal view one can discern clustering of the collateral arbor.

The afferents to the cortex from the lateral geniculate nucleus have a clear clustering of collaterals with a periodicity of  $800 \mu\text{m}$ . Presumably, these constitute the

morphological substrate of ocular dominance columns (Ferster and LeVay, 1978; Gilbert and Wiesel, 1979). One such afferent is shown in Figure 12. It had a Y-type field, with a  $\frac{3}{4}^\circ$  center, located  $12^\circ$  below and  $7^\circ$  out from the area centralis. The axon arborized in layer 4ab (Fig. 12a). Viewed in the plane of sectioning (Fig. 12a), the axon seemed to give off collaterals over a distance of 2 mm without the accustomed gaps for ocular dominance columns serving the opposite eye. However, when it was rotated  $9^\circ$  about an axis running perpendicular to the cortical surface, the presumed ocular dominance pattern became apparent. This pattern was also visible in a surface view (Fig. 12c), with two large zones of innervation separated by a gap of approximately equal size. Within each of the innervated zones, particularly the one

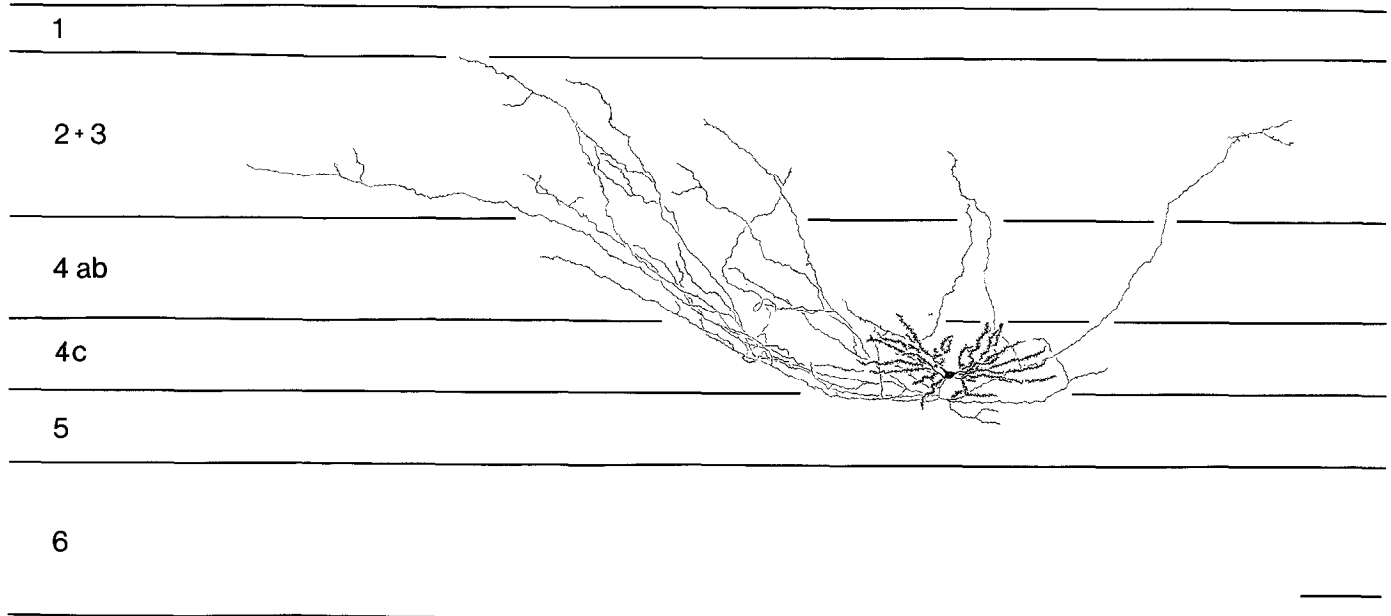


Figure 7. Layer 4c spiny stellate cell, in transverse view. Its receptive field had an 11:30 orientation and it was very directional, preferring movement to the right. The field was  $1^\circ \times 1^\circ$  in size and was centered at an elevation of  $-3.5^\circ$  and an azimuth of  $4^\circ$ . Scale marker =  $100 \mu\text{m}$ .

on the left in Figure 12c, one could see another pattern of clustering at a smaller periodicity, with dense islands of innervation alternating with areas that were relatively free of innervation. In the figure (12c) the arrowheads point along the axes of some of the collateral clusters, with the collateral-sparse areas lying between the arrowheads. The spacing between adjacent clusters averaged  $90 \mu\text{m}$ . This pattern was also evident when examining the axonal boutons (Fig. 12d). Although the pattern is not evident throughout the axonal field of this afferent, we have seen it in the axonal fields of three other three-dimensionally reconstructed axons as well.

### Discussion

Intrinsic cortical connections cover large regions within the cortical area from which they originate. Our findings demonstrate that individual cortical cells are capable of forming projections of extraordinary richness and extent, and that whenever an axon covers large areas of cortex, its collaterals are grouped into a number of discrete, repeating clusters. Furthermore, we find that the overall distribution of an axonal field tends to be asymmetric, extending for greater distances along one cortical axis than another. In area 17 of the cat, intrinsic cortical connections extended up to 4 mm in directions parallel to the cortical surface. At first sight this seems to constitute a violation of the principles of topography. It is worth considering whether these projections are consistent with what is known about magnification factor, receptive field scatter, and receptive field size. In other words, do cells receive input from an area of cortex whose topographic representation is contained within their receptive fields or do they receive input from more distant points? There are two ways of answering this question. One is to refer to visual field maps and figures for magnification factor for the striate cortex (Tusa et al.,

1978) to determine the visuotopic representation of the part of cortex covered by a given axon. Another is to employ a principle established by Hubel and Wiesel (1974) concerning the relationship between magnification factor, receptive field size, and receptive field scatter. They found in the monkey that, because of the scatter in receptive field positions in area 17, one has to move a minimum of 2 mm across the cortex (two "hypercolumns") to get to a position where cells' receptive fields do not overlap with those at the original position. Using visual field maps or estimating the numbers of hypercolumns traversed, we have found that many of the axons we have reconstructed overreach the cortical territory that represents the receptive field area of the cells from which the axons originate. Individual axons commonly cover an area of cortex representing a portion of visual field that is 2 to 3 times the length of the cell's own receptive field. For example, the axon of the cell shown in Figure 3 covered an area of cortex corresponding to  $6^\circ$  of visual angle in its longest dimension, which was approximately twice the length of the cell's receptive field and was larger than receptive fields of other cells in that layer.

For the layer 5 cell, the widespread connections formed by its axon might make sense in view of the receptive field properties of the layer 6 cells to which it projects. Layer 6 cells can have very long receptive fields and might reasonably be expected to collect input from large areas of cortex in order to form them. With the layer 2+3 cells the explanation is not so obvious. They project to other cells in the same layer, with comparable receptive field sizes. These cells, however, possess inhibitory regions flanking the excitatory portion of their receptive fields on their orientation axes as well as on the axes perpendicular to the orientation axes. The first set of inhibitory flanks produces the property of end inhibition,

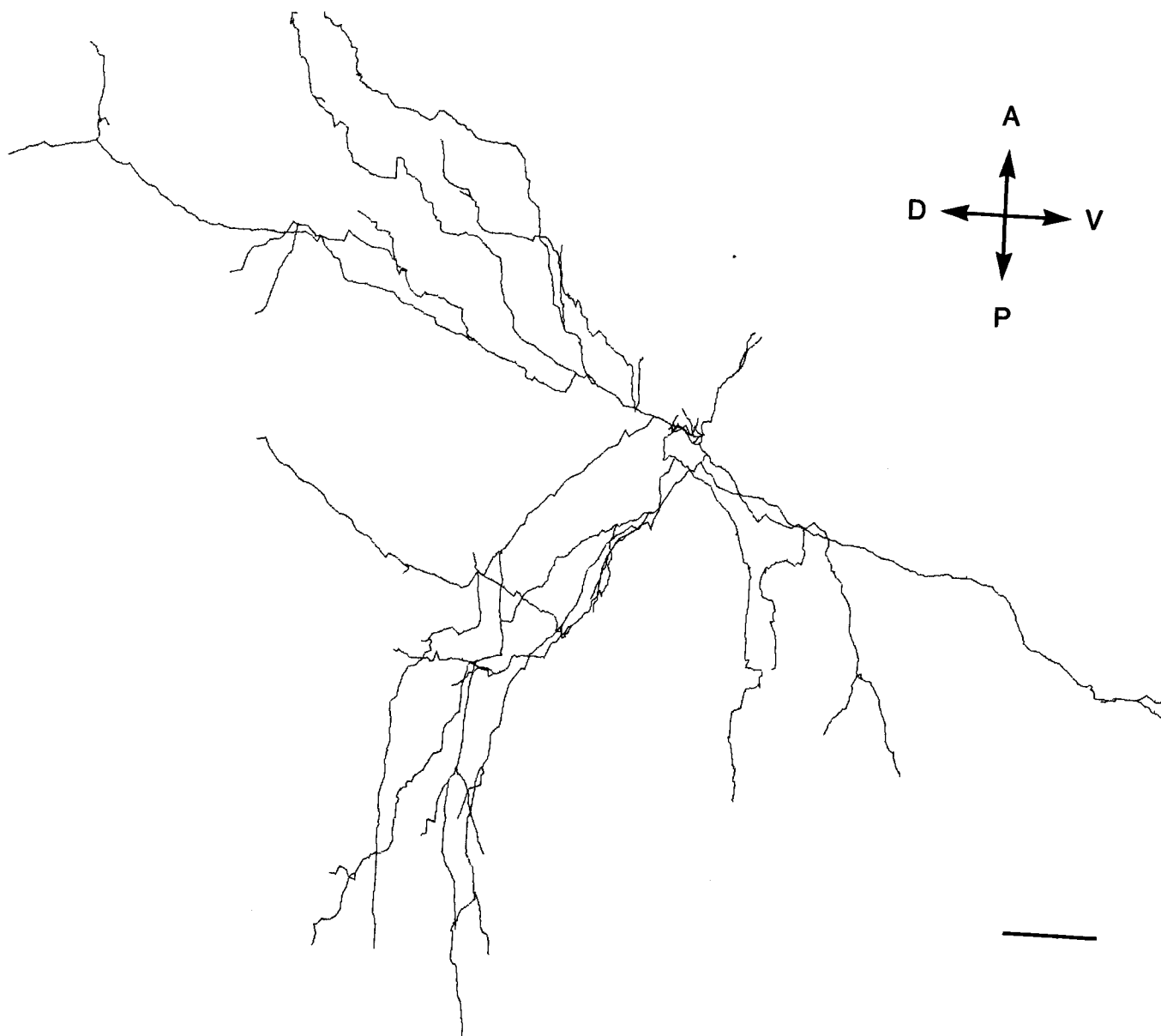
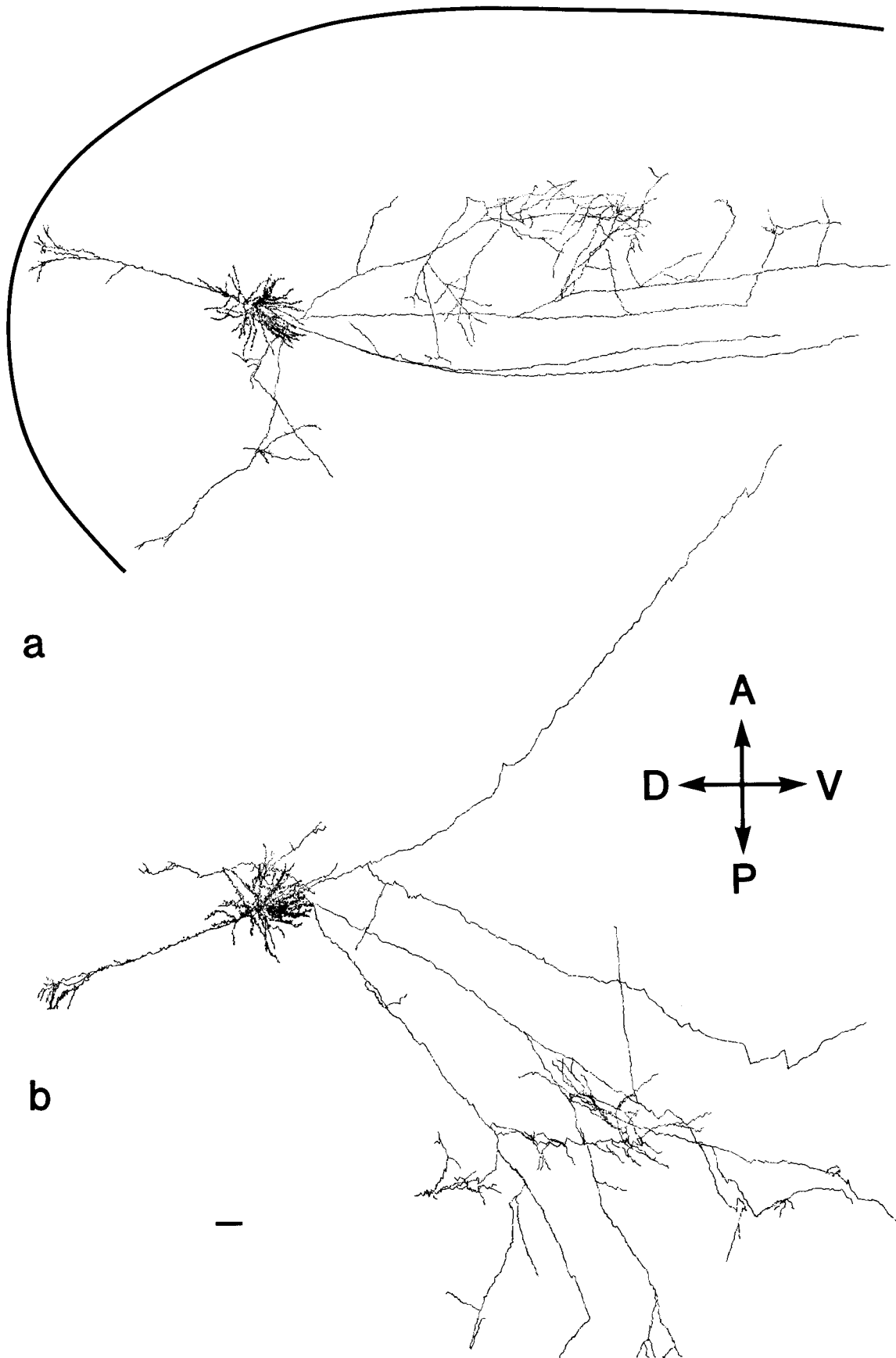


Figure 8. Surface view of the axon of the cell shown in Figure 7. Cortical axes are indicated. Scale marker = 100  $\mu$ m.

and the second produces the property of side-band inhibition (Bishop et al., 1971) or disparity sensitivity (Fischer and Kruger, 1979; Ferster, 1981). By including the inhibitory regions as part of the total receptive field rather than the excitatory region alone, the size of the receptive field becomes more consistent with the existence of widespread connections. Thus, even though a pair of layer 2+3 layer cells can be separated by a considerable expanse of cortex, the distances involved could still be consistent with the overlap in their receptive fields when all parts of the fields are taken into consideration. One may therefore postulate that for some projections, such as that from layer 5 to layer 6, the effect is excitatory and results in an increase in the length of the excitatory portion of the recipient cell's receptive field. For other projections, such as that between superficial layer cells, the net effect could be inhibitory (by

using inhibitory neurons as intermediates), producing inhibitory flanks in the recipient cell's receptive field. Thus the collateral clusters distant from the cell body might have a net excitatory or inhibitory effect on the region of cortex that they innervate, depending on the cell type contacted, and may be useful for generating a wide variety of receptive field properties.

In addition to demonstrating that intrinsic cortical connections can extend for considerable distances, we have shown that they are asymmetric, extending for greater distances along one cortical axis than along the orthogonal axis. Cells' axonal fields are much more strikingly elongated than their dendritic fields. An obvious question to ask is whether the orientation of a cell's axonal field is related to the orientation of its receptive field. One might expect that, for the supposed excitatory projection from layer 5 to layer 6, the axonal field should



*Figure 9.* Layer 5 pyramidal cell with extensive projection to layer 6. Its receptive field was complex, oriented at 10:00, and  $2\frac{3}{4}^{\circ} \times 1\frac{1}{4}^{\circ}$  in size. The field was centered near the area centralis. *a* shows that the axon can be followed for a considerable distance in layer 6. When the axon is rotated to show a view tangential to the cortical surface (*b*), one can see that the axon's collaterals are distributed in a number of densely packed collaterals. Because of the curvature of the cortex, the dendrite is not seen in surface view at this axis of rotation, and the apical dendrite appears in oblique view. The marker for cortical axes applies to *b*. Scale marker = 100  $\mu\text{m}$ .

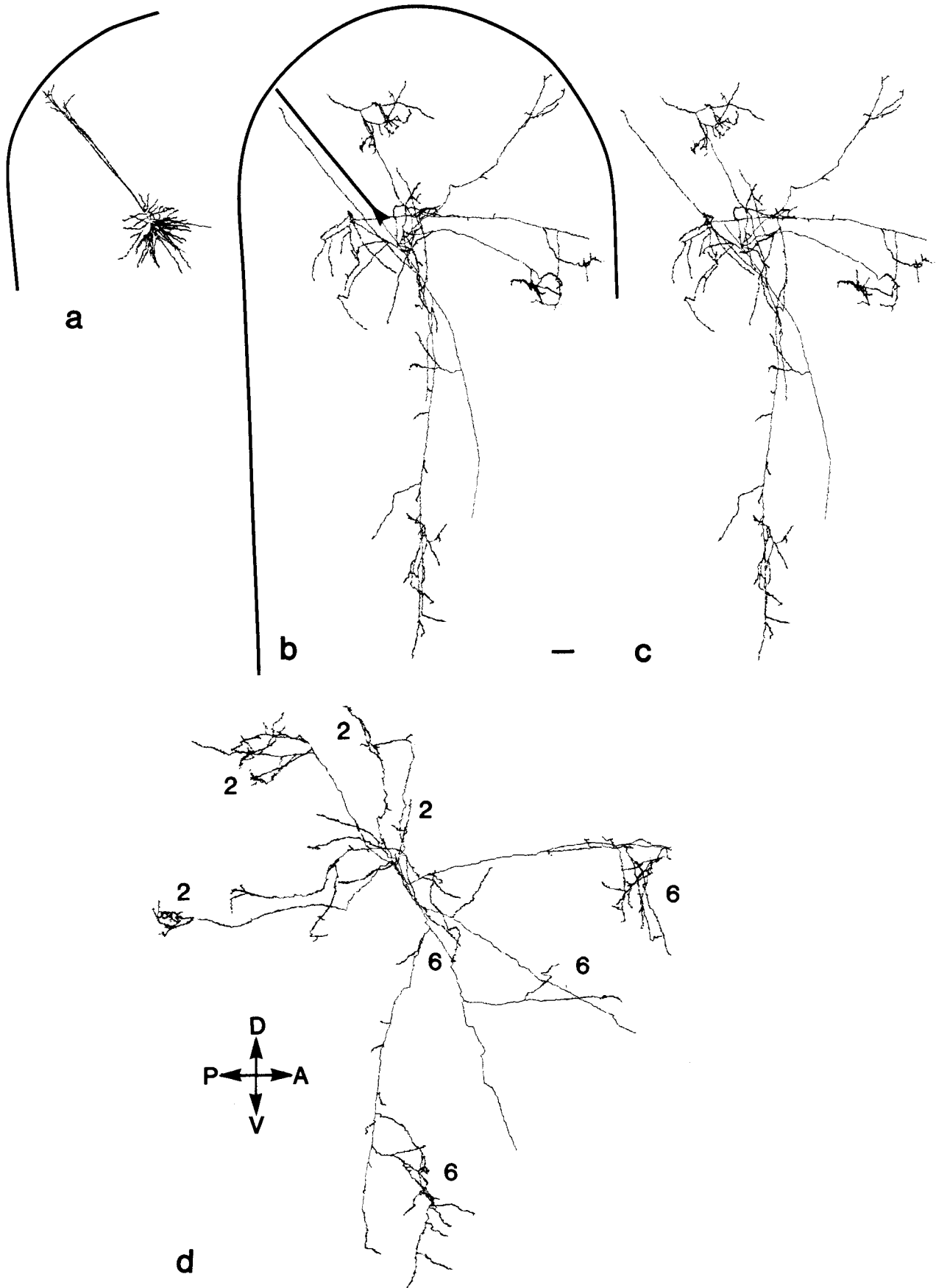
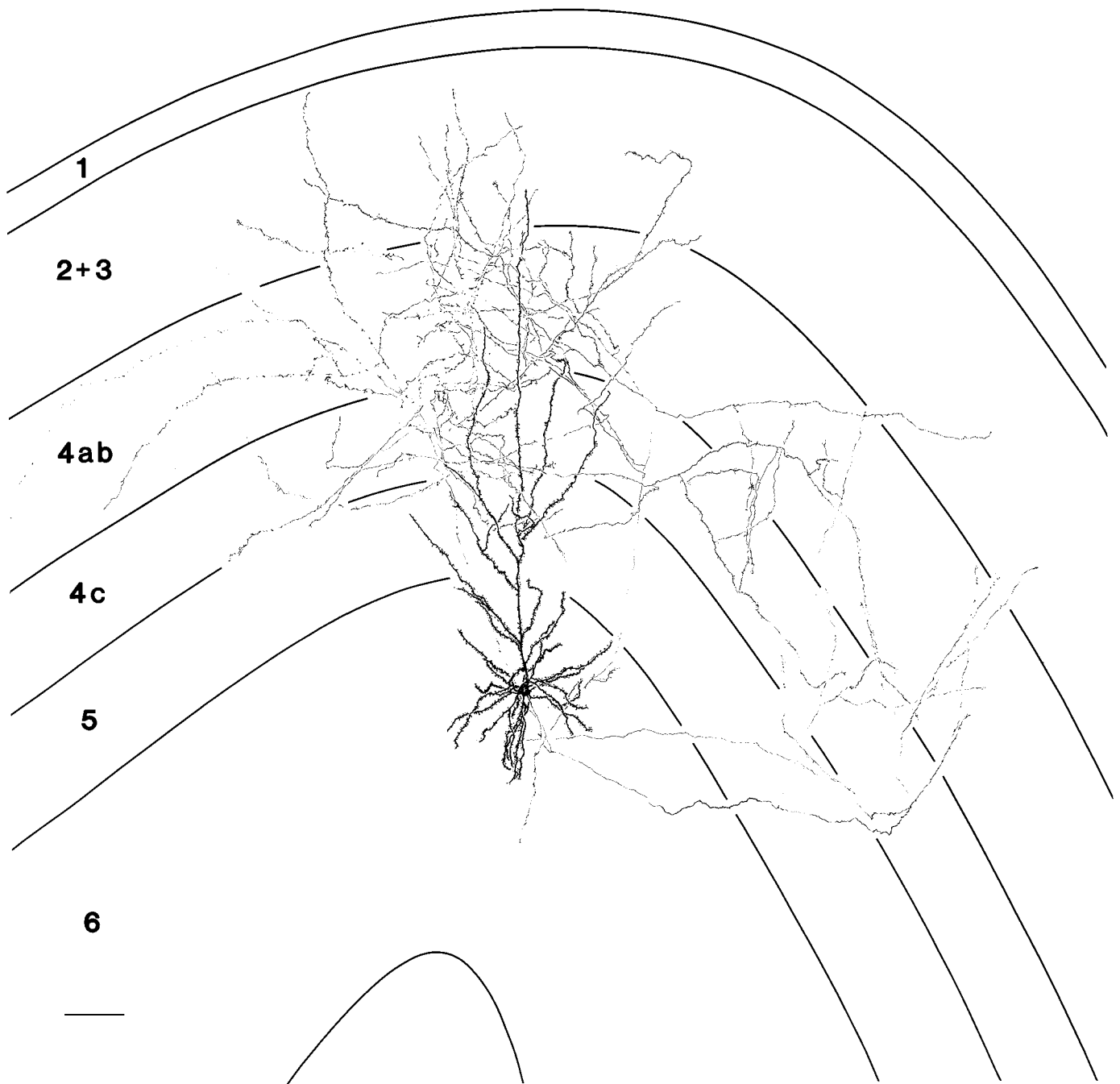


Figure 10. Computer reconstruction of a layer 5 pyramidal cell. It had a complex receptive field with a 2:30 orientation, directional for stimuli moving downward. The field was  $3^\circ \times 2^\circ$  in size and was centered at an elevation of  $-5^\circ$  and an azimuth of  $4^\circ$ . *a* is the reconstruction of the dendrite, which is represented schematically in *b*. *b* and *c* are a stereo pair of the cell's axon.



**Figure 11.** Layer 6 pyramidal cell with a recurrent projection to layer 4. It had a simple receptive field, oriented at 11:30, and was  $4^\circ \times 1.5^\circ$  in size. The field was centered at an elevation of  $-7^\circ$  and an azimuth of  $3^\circ$ . The cell forms an extensive terminal arbor in layer 4, covering 2 mm of cortex, and within that arbor one can discern clustering of the collaterals. *Scale marker* = 100  $\mu\text{m}$ .

be oriented along a visuotopic axis that matches the orientation of the receptive field of the cell forming the projection. Although the sample is small, the two layer 5 cells presented seem to follow this pattern. Both cells have nearly horizontal receptive fields. Their axonal

fields are oriented predominantly along the dorsoventral axis of the medial bank of the lateral gyrus, which corresponds to a horizontal line in the visual field. Furthermore, the axon of the cell in Figure 10 (with a receptive field oriented at 8:30), moves slightly anteriorly as one

The pial surface is indicated by the *bold curved line*. *Scale marker* = 100  $\mu\text{m}$ . The axon formed a recurrent projection to layer 2+3 as well as an extensive projection to layer 6. There are several axon collaterals ascending to layer 2+3, each giving off a cluster of collaterals in that layer, and the clusters are separated from one another by a distance of approximately 850  $\mu\text{m}$ . *d* is a view rotated about the vertical axis of the view in *b*. Because of the curvature of the cortex, it is a surface view only for the processes lying along the medial bank of the gyrus, which are primarily those in layer 6. The clusters in layer 6 are indicated by the number 6, and those in layer 2+3 are indicated by the number 2. The cortical axes are indicated.

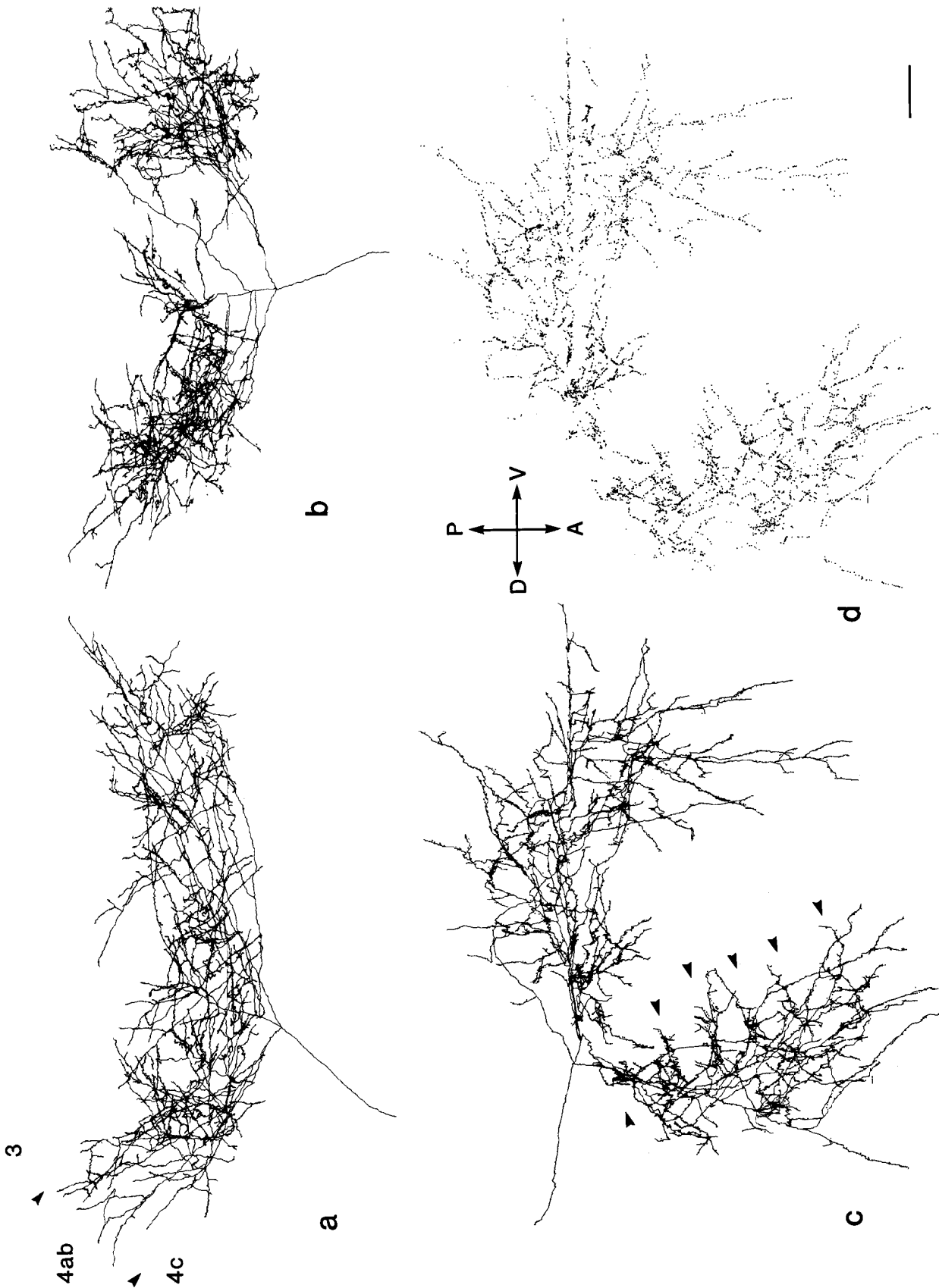


Figure 12. Afferent axons from the lateral geniculate nucleus. It had a Y-type receptive field with a  $3/4^\circ$  center. *a*, Transverse view in the plane of section, showing laminar distribution, with collaterals limited to layer 4ab. *b*, The axon rotated about the vertical axis, revealing a distribution of the collaterals into two major clumps separated by a terminal-sparse zone; these presumably correspond to ocular dominance columns. *c*, The axon rotated about the horizontal axis, giving a surface view. In addition to seeing the coarse clustering of the collaterals into ocular dominance columns, one can see a clustering pattern at a smaller repeat distance. This clustering is particularly evident in the ocular dominance patch on the left and is indicated by the arrowheads. The clustering is somewhat more evident when one displays the boutons alone, as shown in *d*. The bouton-dense and bouton-sparse regions alternate with a spacing of  $90 \mu\text{m}$ . The cortical axis marker applies to *c* and *d*. Scale marker =  $100 \mu\text{m}$ .

scans down the medial bank, while the axon of the cell in Figure 9 (with a receptive field oriented at 10:00) moves posteriorly, which is what one would expect from the visuotopic map. However, as will be discussed below, there are other circumstances in which the visuotopic representation of the axonal field orientation is orthogonal to the receptive field orientation.

While it is quite possible for these long-ranging connections to produce large receptive fields or inhibitory flanks, one must be aware of an alternative possibility. It is possible that there are irregularities in magnification factor or in the visuotopic map, such that the portion of the visual field covered by an axon is smaller than predicted by relatively coarse mapping experiments. Also, there may be anisotropies in magnification factor, so that one cortical axis would have a much smaller magnification factor than does the orthogonal axis. This has been established for ocular dominance columns, where the magnification factor along a column is half of that across the column (Hubel et al., 1974). Since the axons extend farther in one direction than in another, they could be oriented along the axis with the smallest magnification factor (expressed as degrees per millimeter) and consequently could cover an equivalent part of the visual field along both axes.

To this point we have only considered the overall distance covered by individual axons. A second issue is the reason for the clustering of the axon collaterals. Why would a cell skip over (or make relatively few terminals in) a nearby region of cortex to communicate with cells that are more distant? This is probably related to the fact that cells in the superficial layers are not homogeneous in their functional properties or in their extracortical projections. Although they are complex in their receptive field type, there are sufficient differences in receptive field size, degree of end inhibition, and so on to suggest further functional categories. Also, different populations of layer 2+3 cells project to different cortical areas (Gilbert and Wiesel, 1981). It is therefore likely that interactions occur preferentially between cells sharing some functional property and/or projection target. If the purpose of the connection were to provide an inhibitory side band to a cell's receptive field, it would be appropriate that the projection be to cells with the same orientation specificity, but with adjacent receptive fields. This would correspond morphologically to an axonal field having skips (to pass over cells having the wrong orientation) and projections a hypercolumn or more away (to get to a new visual field position). The cell shown in Figure 5 has an axon that is oriented along the anteroposterior cortical axis, which is along an isoazimuth line (i.e., parallel to the vertical meridian). It had a horizontally oriented receptive field and, consequently, could provide inhibitory side bands for other horizontally oriented cells. Similarly, the cell in Figure 6 appeared to be oriented along the dorsoventral axis and had a vertically oriented receptive field, suggesting that it too could provide side bands to other vertically oriented cells with receptive fields displaced horizontally from its own. Interestingly, the layer 2+3 pyramidal cell shown in Figure 5 had clustered projections both to layer 2+3 and to layer 5, and the clusters in layer 5 had the same periodicity

and lay directly under those in layer 2+3. This is suggestive of a relationship between the clustered intrinsic connections and the cortical columns. Based on the extracellular tracing studies in the tree shrew (Rockland et al., 1982), Mitchison and Crick (1982) proposed that the clustering phenomenon may be related to a tendency for cells to communicate with other cells having the same orientation specificity, and they postulated the existence of one population of cells forming clustered connections running perpendicular to the orientation columns, and another population of cells forming continuous connections running along the orientation columns. While our results suggest that any cell that makes long-range intrinsic connections has clustered axon collaterals, this may be related to the tortuous course of the orientation columns in the cat.

The geniculate afferent shown in Figure 12 formed large clusters with a repeat of 700  $\mu\text{m}$ , but within those clusters one could discern a clustering pattern with a finer spacing of 90  $\mu\text{m}$ . The larger clustering pattern is most likely related to the ocular dominance columns, but the functional correlate of the clustering with finer spacing is a mystery. At antecedent stations in the visual pathway, the on and off pathways are segregated (Famiglietti et al., 1977; Nelson et al., 1978; Schiller and Malpeli, 1978), leading one to ask whether the segregation is maintained in the cortex. Since the on- and off-center afferents of a given type (either X or Y) have similar laminar distributions, it is possible that the finer clustering represents an alternation between on-center and off-center afferents in the plane that is parallel to the cortical surface. This would enable a stellate cell lying in layer 4ab or 4c to receive on-input on one set of dendrites and off-input on another, producing a simple receptive field with its characteristic on and off subfields. Thus a single geniculate afferent has terminal clusters at a low periodicity to produce ocular dominance columns and could have a superimposed high periodicity clustering to produce on and off regions. Another clustering pattern in the geniculocortical projection that may be related to the one observed here has been described in the monkey. This projection, from the parvocellular geniculate layers to layer 4A of the cortex, is distributed in a "honeycomb" pattern (Hendrickson et al., 1978). Like the one described here, its functional significance has not yet been revealed.

From the intracellular HRP injections we have found pyramidal cells with no projection into the white matter despite extensive intrinsic axonal connections. The cell shown in Figures 1 and 2 is one example, and we have seen others. The absence of an axon projecting into the white matter is probably not due to a lack of filling with the injected HRP. Although no process went deeper than layer 3, some could be traced for several millimeters from their origin within the superficial layers. The classical view is that pyramidal cells are projection neurons (Ramon y Cajal, 1911), though subsequent Golgi studies maintained that there are some classes of pyramidal cells without axons projecting into the white matter (O'Leary, 1941; Lund, 1973). From these studies it was not clear whether the absence of axons entering the white matter was due to failure of impregnation. Our results lend



support to the idea that some pyramidal cells participate in local circuits exclusively.

That intrinsic cortical connections are widespread is not unexpected in view of the findings of degeneration studies (Fisken et al., 1975; Creutzfeldt et al., 1977) and of studies using axonal transport techniques (Kunzle, 1976; Jones et al., 1978; Rockland and Pandya, 1979; Gilbert and Wiesel, 1981; Rockland and Lund, 1982; Rockland et al., 1982). The axonal transport studies have also shown, on the basis of the projections of large groups of cells, that the projections have a patchy appearance. The results from the extracellular tracing experiments and our intracellular injection are probably demonstrating the same phenomenon, although there may be some discrepancies between the two sets of findings, particularly in regard to the horizontal extent and laminar distribution of the clustering.

Finally, it is worth pointing out the similarity between the pattern of intrinsic cortical connections and the pattern of connections between different cortical areas. The pattern was first observed in examining the distribution of cells that give rise to corticocortical connections (Gilbert and Kelly, 1975). Using retrograde tracing techniques, that study showed the cells projecting from one cortical area to another are distributed in numerous discrete clusters, in addition to being predominantly located in the superficial cortical layers. The clustering pattern has been seen in the monkey as well as in the cat (Gilbert and Wiesel, 1980; Maunsell et al., 1980; Tigges et al., 1981). Other studies, which use techniques that trace connections in the anterograde direction, have shown that a particular site in one cortical area gives rise to patches of terminals in other cortical areas (Zeki, 1976; Tigges et al., 1977; Wong-Riley, 1979; Montero, 1980). The clustered or patchy intercortical connections have been demonstrated in areas other than visual cortex (Kunzle, 1976; Goldman and Nauta, 1977; Imig and Brugge, 1978; Jones et al., 1979). The relationship between the intrinsic and extrinsic projection clusters has not been established, but it would not be surprising if the phenomena were related.

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