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Organization of Pyramidal Neurons in Area 17 of Monkey Visual Cortex

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ABSTRACT

In sections of area 17 of monkey visual cortex treated with an antibody to MAP2 the disposition of the cell bodies and dendrites of the neurons is readily visible. In such preparations it is evident that the apical dendrites of the pyramidal cells of layer VI form fascicles that pass into layer IV, where most of them gradually taper and form their terminal tufts. In contrast, the apical dendrites of the smaller layer V pyramidal cells come together in a more regular fashion. They form clusters that pass through layer IV and into layer II/III where the apical dendrites of many of the pyramidal cells in that layer add to the clusters. In horizontal sections taken through the middle of layer IV, these clusters of apical dendrites are found to have an average center-to-center spacing of about 30 μm, and it is proposed that each cluster of apical dendrites represents the axis of a module of pyramidal cells that has a diameter of about 30 μm and contains about 142 neurons.

The MAP2 antibody reaction also reveals that some pyramidal cells in layers IVA and IVB have their cell bodies arranged into cones. There are about 118 such cones beneath 1 mm^2 of cortical surface and the apical dendrites of the pyramidal cells within them bundle together at the apex of each cone to pass into layer III. Surrounding the cones of neurons there are horizontally aligned, thin dendrites. The location of these dendrites coincides with the dark walls of the honeycomb pattern seen in layer IVA after cytochrome oxidase reactions, or after the parvocellular input from the lateral geniculate nucleus has been labeled. Thus the cones of pyramidal cells within upper layer IV fit into the pockets of the honeycomb pattern. Below the cones of pyramidal cells are the outer Meynert cells within layer IVB, and the cell bodies of these large neurons are disposed so that they preferentially lie beneath the neuropil between the cones of pyramids.

It is suggested that pyramidal cell modules are a basic feature of the cerebral cortex, and that these are combined together by afferent inputs to the cortex to generate the systems of functional columns.

Key words: MAP2, *Macaca mulatta*, pyramidal cells, modules, cytochrome oxidase

During the past 20 years the primary visual cortex of the monkey has been the focus of numerous studies, and much of the impetus for these studies has been provided by the work of Hubel and Wiesel (e.g., '68; '72; '77), who clearly showed this cortex to contain vertically oriented columns in which the neurons all respond to the same visual stimulus. Thus, some neurons encountered in a single electrode penetration tend to be driven best by one eye or the other, to prefer the same orientation of a bar of light, and to prefer the same monochromatic color (e.g., see Michael, '85). These preferences exist because neurons in primary visual cortex are organized into functional columns. In the monkey the eye preference, or ocular dominance columns, are about 300–400 μm wide (e.g., LeVay et al., '75, '85; Tootell et al., '88a; Wiesel et al., '74), and if an orientation column is defined as the distance over which there is a response to a 10° shift in image orientation, then the orientation columns are 25 to 30 μm wide (Hubel and Wiesel, '74, '77). The color columns extend throughout the entire thickness of the cortex and they are shaped like slabs 100 to 250 μm wide (Michael, '81). How boundaries of these systems of columns are related is not yet known, but it is clear that they do occupy the same cortical space. This suggests that there must be a system of neuronal units, each at least as small as the smallest functional column. To generate the various types of functional columns these units would be recruited in various combinations, depending upon the afferent inputs to which they are responding at a given time.

The existence of small vertically oriented units of neurons extending through the depth of the cerebral cortex was

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postulated by Mountcastle ('57, '78), who has postulated that the neocortex is composed of basic, irreducible units of neurons, which he calls "minicolumns." However, thus far vertical groupings of neurons that might correspond to such units have not been found, although the search for a fundamental neuronal organization that might underlie and explain the response properties of neurons has produced excellent accounts of the kinds of neurons that exist in the various layers of the monkey visual cortex (e.g., Lund, '73; Lund and Boothe, '75; Valverde, '85). Other studies have been made of the synaptic relationships of examples of the various kinds of neurons in monkey visual cortex (e.g., Saint Marie and Peters, '85; Somogyi et al., '82; Kisvarday et al., '86). But since these studies have mainly relied upon Golgi impregnations, they have been of individual neurons and so they have not revealed the existence of neuronal groupings that would correspond to narrow vertical units or modules from which the functional columns might be generated.

However, vertical groups of neurons have been demonstrated in a number of other cortices. These groups are centered around the layer V pyramidal cells, the apical dendrites of which "cluster" or "bundle" as they ascend through the cerebral cortex. This was first demonstrated by von Bonin and Mehler ('71) in primate cortex, by Peters and Walsh ('72) in rat somatosensory cortex, and by Fleischhauer et al. ('72) in the rabbit and cat sensory motor cortex. Since then, similar vertically aligned and clustered apical dendrites have been encountered in a number of the neocortical areas (see Feldman and Peters, '74; Fleischhauer, '74; Fleischhauer and Detzer, '75; Winkelmann et al., '75; Feldman, '84; Shmolke, '87). In rat visual cortex, for example, the pyramidal cells are arranged in vertically aligned modules with a center-to-center spacing of 55–60 μm (Peters and Kara, '87). There the apical dendrites of the layer V pyramidal cells cluster together and as these apical dendrites pass into layer III, the apical dendrites of the layer III pyramidal cells, and subsequently those of the layer II pyramidal cells, are added to the clusters. The apical dendrites of the layer VI and the layer IV pyramidal cell dendrites do not appear to participate in these clusters. Instead the apical dendrites of the layer VI pyramidal cells form fascicles and contiguous sheets that pass between the layer V pyramidal cells to ascend to layer IV where most of their apical dendrites terminate. The apical dendrites of the layer IV pyramidal cells in the rat also ascend through the cortex in a rather random fashion (Peters and Kara, '87).

When the studies cited above were carried out, it was only possible to visualize the dendritic clusters generated by these pyramidal cell modules by examination of either semithin or thin sections of plastic embedded material examined by light or electron microscopy, but our attempts to apply this same approach to determine if the primary visual cortex of the monkey contains pyramidal cell modules similar to those in the rat visual cortex met with little success. The problem is that there are about twice as many neurons per unit volume in primate visual cortex than in any other cortical area (see Peters, '87), and the small neurons have very thin apical dendrites. However, as shown by de Camilli et al. ('84), Bernhardt and Matus ('84), and by Escobar et al. ('86), arrangements of neurons and their dendritic trees can be studied very nicely in material incubated with antibodies to microtubule-associated protein 2 (MAP2). MAP2 is associated with the microtubules within the perikarya and dendrites of neurons (de Camilli et al., '84), and its application to the primary visual cortex of the rhesus monkey demonstrates that pyramidal cell modules do exist within this cortex. In addition it is revealed that unusual aggregations and arrangements of neurons occur in layers IVA and IVB of that cortex.

MATERIALS AND METHODS

Fixation of tissue

The cortical tissue used in this study was obtained from four female monkeys (*Macaca mulatta*), used primarily for other investigations not involving the visual system. One monkey was 2 years old and the others were 8 years old. In three cases the monkeys were perfused intracardially with 4% paraformaldehyde in 0.1 M cacodylate buffer at pH 7.2. Following this primary fixation some pieces of the opercular surface of the occipital lobe (area 17) of these monkeys were postfixed in a fixative consisting of 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3), containing lysine and sodium metaperiodate (PLP fixative; McLean and Nakane, '74), while other pieces were vibratomed to produce serial sections that were immersed individually in the PLP fixative. Pieces of the brain of the other monkey were immersed directly in the PLP fixative.

Reaction with microtubule-associated protein 2 monoclonal antibody 5F9

The monoclonal antibody to microtubule-associated protein 2 (MAP2:5F9) was supplied by Dr. K.S. Kosik (Department of Neurology, Harvard Medical School and Massachusetts General Hospital, Boston, MA). The preparation and characterization of this antibody to MAP2 has been reported previously (Kosik et al., '84). The antibody was raised in BALB/c mice by using rat microtubules. Essentially the antibody is a selective marker for microtubules in dendrites and in the perikarya of neurons (see Escobar et al., '86).

To perform the antibody reaction, serial vibratome sections, cut at a thickness of about 30 μm, were taken in both the vertical and horizontal planes with respect to the pial surface of area 17. The sections were kept in sequence and rinsed first in Tris-buffered saline (TBS). The sections were then transferred to solutions of the primary antibody (anti-MAP2: 5F9) diluted to either 1:50, 1:100, or 1:200 with TBS to which 1% normal horse serum and 0.3% Triton-X had been added. The Triton-X improved the penetration of the antibody into the tissue. The sections were incubated in these solutions for 24 to 48 hours at 4°C.

Following incubation in the antibody solutions, the treated sections were rinsed in TBS solution and transferred to anti-mouse biotinylated secondary antibody (Vector ABC kit) for 60 minutes at 25°C. After rinsing in TBS solution, the sections were incubated with Avidin biotinylated HRP for 60 minutes at 25°C.

Finally, the antibody binding sites were visualized with a solution of TBS containing 0.5% diaminobenzidine and 0.01% hydrogen peroxide at 25°C. This reaction was terminated after about 20 minutes, and, following a rinse in TBS, the density of the reaction product was enhanced by rinsing the sections in a 0.5% solution of osmium tetroxide in TBS. The sections were then dehydrated in a series of glycerine solutions and mounted on glass slides in pure glycerine for light microscopic analysis.

Cytochrome oxidase reaction

Pieces of area 17 were removed from the opercular portion of the occipital pole and sectioned at 50 μm, using a

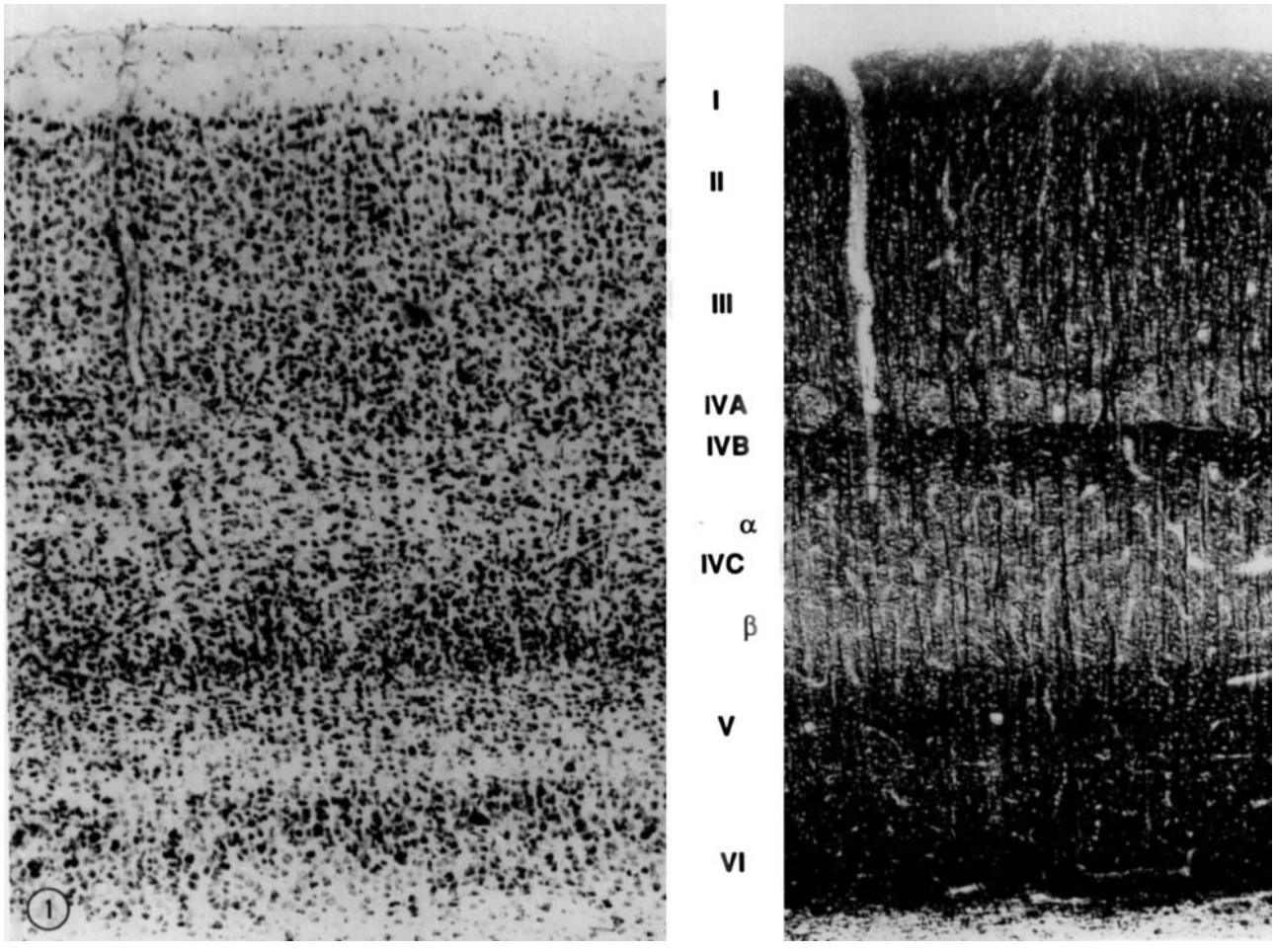


Fig. 1. Portions of adjacent sections of area 17. The section on the left was Nissl stained and the one on the right was reacted with antibody to MAP2. The photomicrographs have been aligned to show how the various layers of the cortex appear after using MAP2 antibody staining. × 100.

vibratome, in either the horizontal or vertical planes. The sections were collected in a series in 0.1 M phosphate buffer (pH 7.3). The sections were then rinsed in a solution of 1 ml of 1% cobalt chloride in 100 ml of 0.1 M phosphate buffer and immersed for 24 hours at 37°C in solution containing 0.05% diaminobenzidine, 0.02% cytochrome C, and 1.2% sucrose (see Seligman et al., '68; Wong-Riley et al., '78). The density of the reaction product was sometimes enhanced by adding cobalt chloride to the incubating solution (Adams, '81) and in all cases the sections were briefly rinsed in 0.5% osmium tetroxide, after which they were dehydrated in a series of glycerine solutions and mounted on glass slides in pure glycerine.

In some instances successive sections in a series were treated with the MAP2 antibody, with cytochrome C, or stained with thionin, so that the resulting images could be compared and correlated.

RESULTS

Lamination

To better understand the significance and disposition of the dendritic configurations displayed by use of the MAP2 antibody, it is appropriate to give a brief description of the lamination of the monkey striate cortex as visualized in Nissl stained preparations, (see Fig. 1) and to mention the kinds of neurons present in each of the layers. For more complete details of the various kinds of neurons present, the accounts given by Lund ('73; '81), Lund and Boothe ('75), and Valverde ('85) can be consulted, while details of the synaptic connections between the laminae of the lateral geniculate nucleus and visual cortex can be found in the articles by Hendrickson et al. ('78) and Fitzpatrick et al. ('83).

In this account, the numbers assigned to the layers of the cortex follow the description given by Lund ('73), which essentially conforms to nomenclature used by Brodmann ('05).

As in other cortical areas, layer I of monkey visual cortex is a cell sparse layer. It is very pale in Nissl-stained material and consequently its border with layer II is clearly defined, because in the upper part of layer II there is a condensation of small pyramidal cells. No clear-cut boundary separates layer II from layer III, for they both contain pyramidal cells that often seem to be arranged in vertical stacks. For the most part, these pyramidal cells are of relatively uniform size, but there is a scattering of larger pyramidal cells throughout layer II/III, especially within the deeper portion

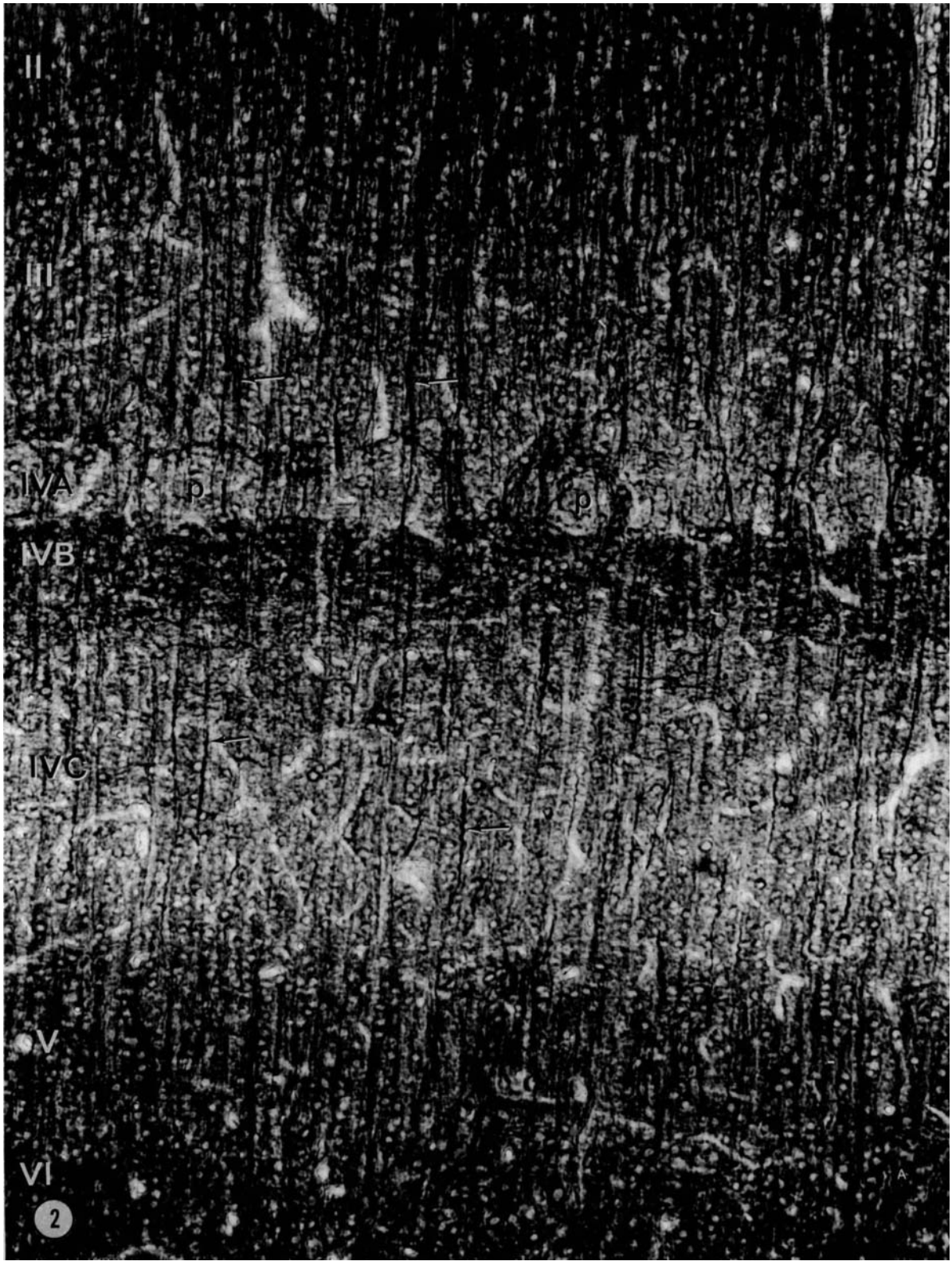
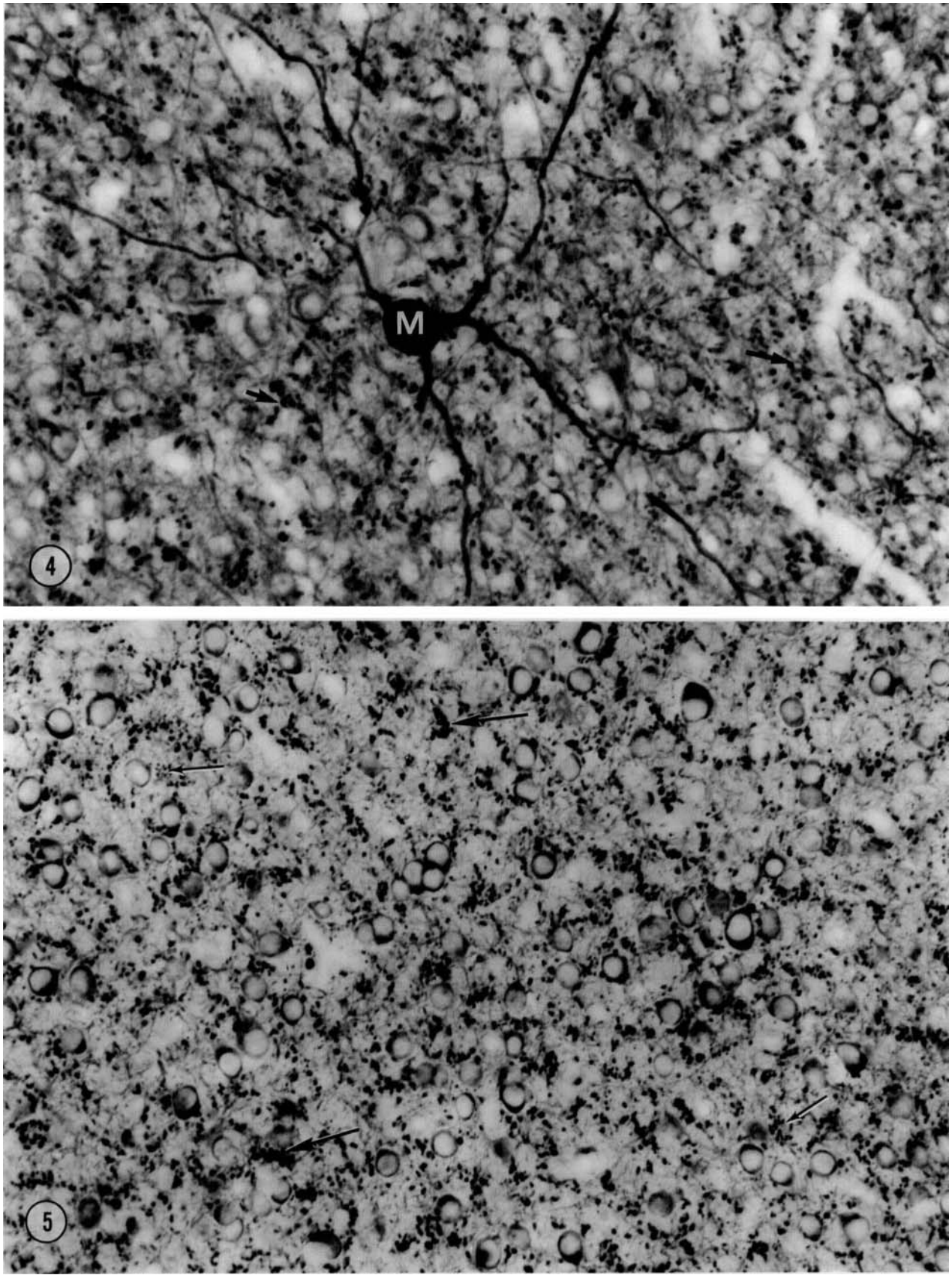


Fig. 2. A higher magnification micrograph of area 17 reacted with MAP2 antibody. The cortical layers are indicated on the left. Note the thin dense band of reaction that coincides with layer IVB, and the reticulated appearance of layer IVA, in which thick bundles of dendrites surround pale staining pockets (p). Note the thin, vertically oriented bundles of dendrites extending through the cortex (arrows). × 185.

Fig. 3. Vertical section of the deep layers reacted with MAP2 antibody. The pyramidal cells of layer VI have darkly reacted cell bodies and their apical dendrites often pass obliquely to form thick bundles with other apical dendrites (arrowheads). These dendritic bundles taper within layer IVC. The layer V pyramidal cells are only faintly visible, but their apical dendrites aggregrate to form thin clusters (arrows) that pass vertically through layer IVC in which the densely packed neurons lie between these dendritic clusters. × 300.



Figures 4–5

of layer III. It is also difficult to precisely define the border between layer III and the narrow layer IVA, but the neurons in layer IVA are smaller than those in layer III and they are more closely packed. Layer IVA mainly contains small spiny stellate cells, but some pyramidal cells are also present and in this layer a thalamic input from the parvocellular layers of the lateral geniculate nucleus terminates.

Below layer IVA is layer IVB in which the neurons are more loosely packed, and the upper and lower limits of layer IVB can be basically defined because of the large cell bodies it contains. These are the cell bodies of the outer solitary cells of Meynert, the majority of which are large spiny stellate cells with horizontally oriented dendrites, although some of the large cells are pyramidal neurons that also have horizontally arranged dendritic trees (Valverde, '85). Layer IVB essentially coincides in position with the line, or stria, of Gennari, and it receives no thalamic afferents.

Layer IVC contains two laminae. The one most easily recognized is the lower one, lamina IVCβ, because this contains very densely packed neurons, most of which are small spiny stellate cells. Like layer IVA, lamina IVCβ also receives thalamic input from parvocellular layers of the lateral geniculate nucleus. Interposed between layer IVB and lamina IVCβ is lamina IVCα in which the neurons are more widely separated from each other. Lamina IVCα also contains spiny stellate cells, and the position of this lamina is best defined by the fact that it is the site of termination of thalamic afferents from the magnocellular layers of the lateral geniculate nucleus.

The border between lamina IVCβ and layer V is well marked by the change in cell packing density. Layer V contains rather loosely packed neurons. Most of them are small pyramidal cells, and their cell bodies are frequently seen to be arranged into vertical stacks, separated laterally by spaces. The apical dendrites of most of the pyramidal cells in layer V extend as far as layer I.

Layer VI also contains pyramidal cells, but their cell bodies are larger and more randomly arranged than those of layer V and their apical dendrites rarely reach layer I. Most of the apical dendrites of the neurons in the upper part of layer VI reach only as far as layer IVA, while the apical dendrites of the deeper pyramids usually ascend to layer IVC before terminating (Lund, '81).

Finally, it needs to be mentioned that at the border between layers V and VI, the cell bodies of the large, deep Meynert cells are located. These large and solitary pyramidal neurons are sparse. They are not randomly disposed, but they are arranged so that their cell bodies are in a sheet within which there are holes, or gaps (Payne and Peters, '89).

Fig. 4. Horizontal section of area 17 reacted with MAP2 antibody. The section is at the level of the border between layers VI and V, and the large neuron with horizontally spread dendrites in the middle of the field is a Meynert cell (M). At this depth, the apical dendrites of the layer VI pyramidal cells are visible as dark dots (arrows). They form irregular fascicles and sheets as they pass towards layer V. × 420.

Fig. 5. Horizontal section of area 17 reacted with MAP2 antibody and taken at the level of upper layer V. The transversely sectioned apical dendrites of both layer VI and layer V pyramidal cells are present at this level, and they appear as aggregates of dark dots. It is presumed that the thinner apical dendrites (small arrows) belong to the layer V pyramidal cells, and the thicker apical dendrites (large arrows) to the layer VI pyramids. × 420.

MAP2 reacted material

Vertically oriented sections. In MAP2 antibody reacted material in which the antibody binding sites are visualized using the DAB reaction, dendrites and some neuronal cell bodies are apparent, and when sections cut vertical to the pial surface are examined at low magnification several horizontal bands of staining are apparent. By comparing the positions of these bands with the cell layers seen in Nissl-stained preparations, the following correlations can be made (see Figs. 1 and 2).

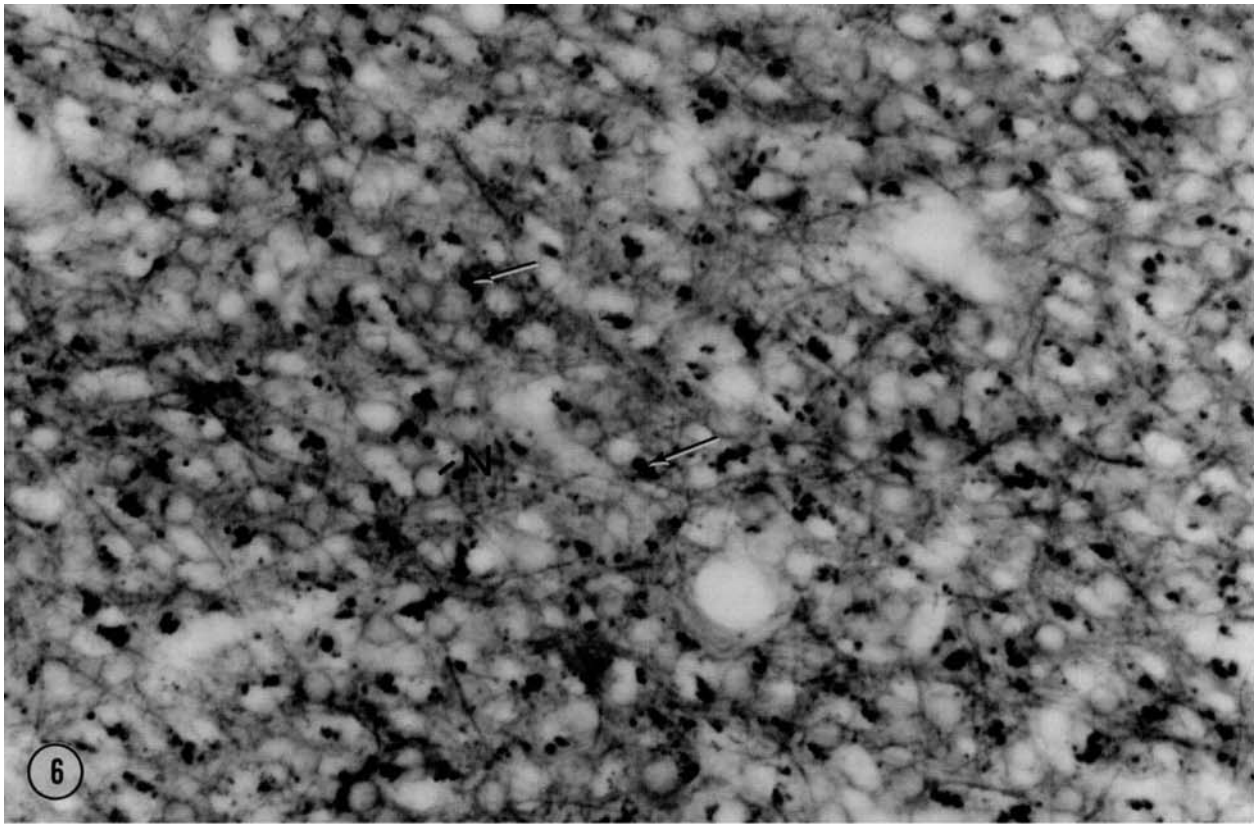
In the MAP2 reacted material, a good landmark for orientation is a band of dense staining that is located about half-way through the depth of the cortex. This dense band is about 0.1 mm thick and coincides with the location of layer IVB. Below it is a paler staining zone, which corresponds to layer IVC, and beneath that is another dense band of staining. The upper edge of this deepest dense band occurs approximately at the border between layer IVC and layer V. This band becomes progressively darker as it approaches the level of layer VI, and it ends abruptly where layer VI meets the underlying white matter. Below that border, and in the white matter itself, a few isolated neurons can be seen. These have a basically horizontal orientation and are the neurons of layer VIB.

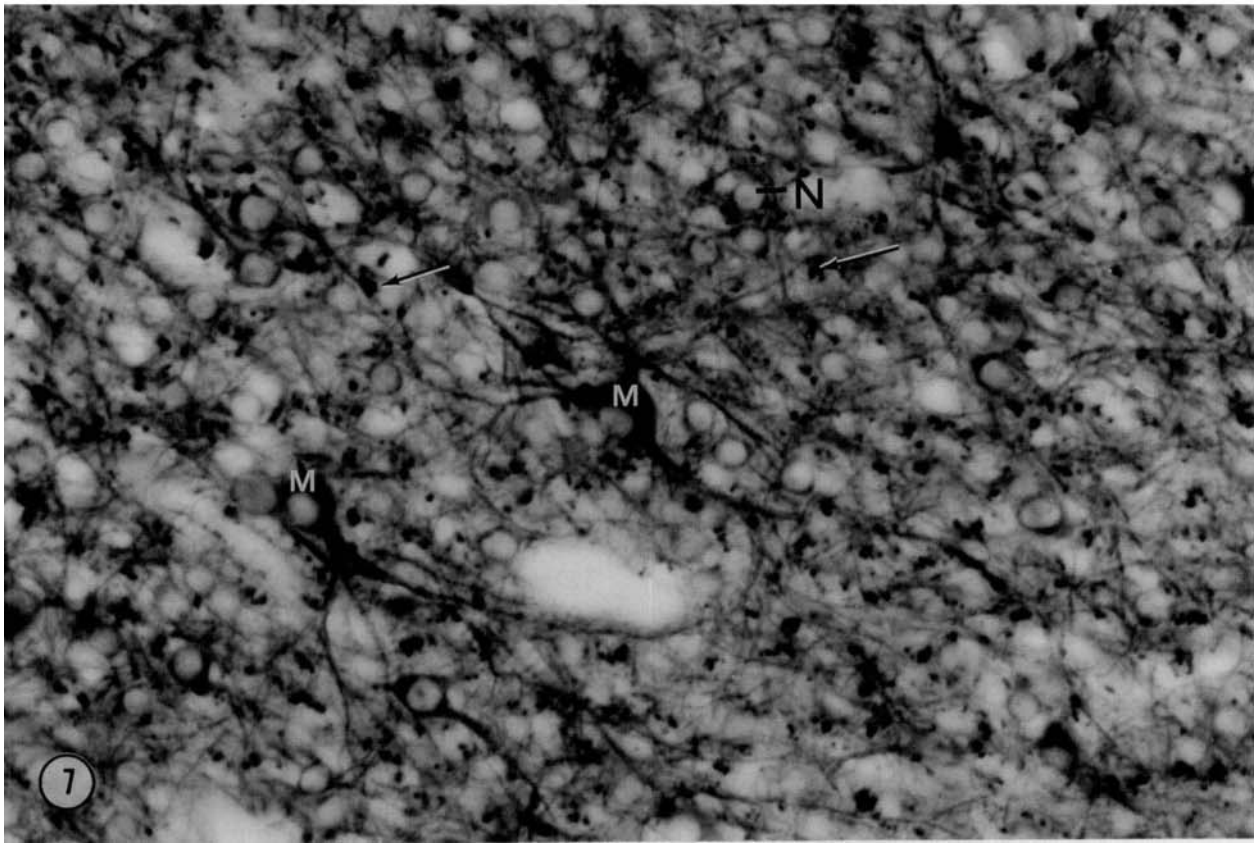
On the outside of the cortex where layer I is located, there is a dense zone of staining that is produced by the presence of the numerous dendritic branches contained within the apical tufts of the pyramidal neurons. Beneath this outer zone there is a gradual lightening of the staining reaction with increased depth as the section passes through layer II/III, and this continues until a pale reticulated band is reached. The reticulated band coincides with layer IVA.

In addition to this horizontal banding, vertical bundles of stained dendrites are visible as thin, dark strands that extend through much of the depth of the cortex (Fig. 2; arrows). The bundles are thickest in layer V and VI and in layers II and III. But they are also obvious in layer IVC because of the pale staining of the other elements in that layer. However, the vertical pattern of staining appears to be somewhat disrupted in the upper reaches of layer IV. In layer IVB the integrity of the dendritic bundles becomes masked by the dark staining of this entire sublayer, and, in addition, as the bundles emerge from the top of layer IVB, many of them appear to be disrupted or made less obvious because of thick bundles of dendrites that traverse layer IVA. Some of the dark and thick bundles of dendrites ascending through layer IVA seem to bend and even form arches that enclose pale staining patches (Fig. 2; p) in layer IVA, and it is this combination that results in layer IVA having a reticulated appearance in MAP2 reacted material.

Interpretation of the MAP2 staining pattern

The staining pattern observed in vertically oriented sections examined at low magnification can be interpreted by examining the preparations at higher magnification, and by correlating these images with those displayed by other MAP2 reacted sections cut in the horizontal plane, that is, parallel to the pial surface. The interpretation of the image is also aided by our knowledge of the types of neurons present in the various layers. Because most of the vertical bundles of MAP2 reacted elements contain the ascending apical dendrites of pyramidal cells, it is easiest to begin the account with the deepest layers.





Figures 6–7

Layer VI. In deep layer VI, the neurons are loosely arranged. Their dendrites are often oriented horizontally and they are intermixed with the myelinated axons of the white matter. Because the MAP2 antibody does not bind to axons, the neurons of deep layer VI are clearly visible and the antibody reaction makes them appear as though they had been Golgi impregnated, because they stand out against the clear background of the unreacted myelinated axons (e.g., see Chun and Shatz, '89).

In upper layer VI the neurons have darkly reacted cell bodies, which at high magnification appear as dark circles surrounding a clear space in which the unstained nucleus lies (Fig. 3). Most of these neurons are pyramidal cells with squat cell bodies and their stout apical dendrites often pass obliquely to join similar apical dendrites, so that they become aggregated together to form thick fascicles (Fig. 3; arrowheads). These thick fascicles extend through layer V and enter the pale layer IVC, where they gradually taper and disappear. Since it is known from studies of Golgi impregnated that many layer VI pyramidal neurons have apical dendrites which form their apical tufts in layer IV (Lund and Boothe, '75; Lund, '81), it is assumed that this accounts for the gradually tapering of the fascicles of layer VI apical dendrites as they enter layer IVC.

In horizontal sections through layer VIA, the cell bodies of the neurons are well stained with the MAP2 antibody and between them are dark, dot-like profiles that represent transverse sections of their rather thick apical dendrites. These are interlaced with the more horizontally oriented basal dendrites of these same neurons. Towards the top of layer VI, where most of the apical dendrites of the layer VI pyramidal cells should be present, the dendrites are indeed aggregated, as the vertically oriented sections show (Fig. 3), but the aggregations are not discrete (Fig. 4) and they are not arranged in any discernible pattern. Instead, some of the profiles of the apical dendrites are in clumps, and others are in rows, with the whole blending together so that the apical dendrites seem to occupy much of the space between the cell bodies.

At the border between layer VI and layer V, the large deep Meynert cells are present. In tangentially oriented sections reacted with the MAP2 antibody it is apparent that the basal dendritic trees of these neurons are spread horizontally and extend for long distances (see Fig. 4). As described in a previous communication (Payne and Peters, '89), the cell bodies of these neurons are not distributed randomly, but they are arranged to form a sheet in which there are holes or gaps. These gaps lie beneath the cytochrome oxidase "patches" or "blobs" that can be visualized in layer II/III, and on average there are some 20 deep Meynert cells beneath each 1 mm^2 of cortical surface.

Fig. 6. Horizontal MAP2 stained section at the level of upper layer IVC. At this level discrete and thin clusters of apical dendrites of layer V pyramidal cells are evident as black dotted profiles (arrows). Between these clusters of apical dendrites are the pale staining cell bodies (N) of the layer IVC stellate cells together with their interlacing dendrites. × 420.

Fig. 7. Horizontal section at the level of layer IVB. The MAP2 staining shows the large outer Meynert cells (M) with their horizontally spread dendrites. Other components visible are small neurons with little perikaryal cytoplasm (N), and a network of thin dendrites, through which the clusters of apical dendrites (arrows) pass. × 420.

Layer V. The layer VI apical dendrites continue into layer V and pass between the layer V neurons, which have rather smaller, rounder, and paler staining cell bodies than those in layer VI (Fig. 3). In Nissl-stained vertical sections the cell bodies of the layer V neurons are often seen to be arranged in stacks with clear spaces between them (Fig. 1), and a similar arrangement is apparent in the MAP2-stained sections, which show some of the spaces to be occupied by ascending bundles of dendrites (Fig. 3). These bundles of dendrites are also obvious in horizontal sections through upper layer V, where the apical dendrites of both layer V and of layer VI pyramidal cells are present (Fig. 5). Careful examination of the horizontal sections suggests that bundles of both thin and thicker profiles of apical dendrites exist and it is supposed that the thin apical dendrites belong to layer V pyramids, and the thicker ones to layer VI pyramids. However, it is also evident that not all of the cross-sectioned dendrites are contained within bundles.

Layer IVC. As the ascending clusters, or bundles, of apical dendrites of the layer V pyramidal cells pass into layer IVC they become more compact, so that in tangential sections they are visible as quite discrete, darkly staining groups of small dendrites. This pattern becomes even more accentuated towards the middle of layer IVC (Fig. 6). At this level the vertical clusters basically contain only the apical dendrites of the small pyramidal cells in layer V because most of the apical dendrites of layer VI pyramidal cells are beginning to form their apical tufts. But again, some of the apical dendrites remain free of the clusters. The small stellate cells, which are the main neuronal component of upper layer IVC, stain only lightly with the MAP2 antibody, and their dendrites are only visible as thin strands criss-crossing in the neuropil (Fig. 6).

Because of the clarity of staining of the clusters of layer V apical dendrites at the level of upper layer IVC it is easy to count them in tangential sections, and to so determine their packing density. Data of counts from three different animals are presented in Table 1, in which it will be seen that there are about 1,270 clusters of apical dendrites per mm^2 of tangential sections taken at the level of upper layer IVC. This means that on average, each cluster occupies a vertically oriented volume of cortex some 31 μm in diameter. This point will be returned to later, when the modular organization of neurons in the cortex is considered.

Layer IVB. Occasional large and darkly stained neurons are encountered in layer IVC, but, as pointed out, most of the neurons are very small and so give little reaction to the MAP2 antibody. However, the staining of layer IVB, which coincides with the line of Gennari, is quite different. There the vertically ascending clusters of layer V apical dendrites enter, and pass through, a dark layer in which most of the other darkly staining dendrites are arranged more or less horizontally (Fig. 2). Layer IVB contains many medium sized spiny stellate cells and a few large spiny neurons (Fig. 7; M). As pointed out, these large neurons are both stellate cells and pyramidal cells, and together they are referred to as the outer solitary cells of Meynert (e.g., Valverde, '85). Other than being obviously multipolar, the shapes of the outer Meynert cells are not readily seen in vertical sections, but horizontal sections of the MAP2 reacted material show their thick dendrites to be spread out in rather flat sheet (Fig. 7), similar to the basal dendrites of the deep Meynert cells (Fig. 4).

The outer Meynert cells are easy to identify by virtue of their large cell bodies and thick radiating dendrites. When

TABLE 1. Packing Density of Layer V Apical Dendritic Clusters (Counted in Horizontal Sections Through Layer IVC)

Monkey 1	Count 1	1,280 per mm^2
	Count 2	1,192 per mm^2
	Mean	1,236 per mm^2
Monkey 2	Count 1	1,275 per mm^2
	Count 2	1,310 per mm^2
	Mean	1,293 per mm^2
Monkey 3	Count 1	1,300 per mm^2
	Mean of all counts	1,271 per mm^2

their positions are plotted in a series of horizontal sections, it is found that they tend to occur in groups (Fig. 12). In these groups the cell bodies of three or four large neurons lie quite close to each other, and there is some tendency for the dendrites of neighboring large neurons to have a similar orientation.

Layer IVA. In vertically oriented sections stained with the MAP2 antibody and examined at low magnification, the border between layer IVB and IVA is well marked because the staining of layer IVA is much paler than that of layer IVB (see Fig. 2). However, the border between the two layers appears quite irregular and almost scalloped. At low magnification it is evident that there are groups of pyramidal cells at the layer IVB/IVA transition zone. The perikarya of these pyramidal cells are basically organized into cones and their apical dendrites emerge from the top of each cone to form the thick bundles that pass vertically across layer IVA to enter layer III. And in effect it is the arrangement of these pyramidal cells into cones that gives rise to the scalloped appearance of the border between layer IVB and layer IVA.

This arrangement of some pyramidal cells into cones is illustrated in Figure 8, in which the pyramidal cells in the cones are seen to have their basal dendrites passing obliquely into layers IVA and IVB. Between the tops of the cones of pyramidal cells and the bundles of their apical dendrites are paler staining areas that contain a few small cell bodies (Fig. 8; asterisks). It will also be noticed that some pale streaks pass through layers IVB and IVC. These are caused by the fascicles of myelinated axons that traverse the cortex, but are not visualized by the MAP2 antibody (Fig. 8; Ax).

The cones of pyramidal cells at the border between layers IVB/IVA are also evident in horizontal sections (see Figs. 9 and 10). In such sections the cells of the cones form groups, within which are profiles of their apical dendrites, and the grouping of the neurons is accentuated by their surrounds of a few small pale neurons intermixed with thin and pale horizontally oriented bundles of dendrites (see Fig. 9 and 10; D). When the groups of darkly staining neurons are followed in horizontal serial sections it again becomes evident that they represent sections through the cones of pyramidal neurons. As the sections approach the tops of the cones the number of cell bodies decreases and the number of profiles of their apical dendrites increases, until at the upper levels of layer IVA each cone is represented by only a bundle of darkly stained dendrites that extend upwards into layer III (Figs. 8 and 9, arrows; see Fig. 13, b).

When the locations of the cones of dark staining cells in layer IVA are plotted in the horizontal plane (Figs. 11 and 12), they are found to be relatively evenly spaced with centers about 0.1 mm apart. Counts made from three sets of horizontal sections from different monkeys give values of 127, 108, and 120 cones of pyramidal neurons per 1 mm^2. The mean value is 118 per mm^2. When reconstructions are made of the positions of the cones of neurons in layer IVB/IVA and of the locations of the cell bodies of the large outer cells of Meynert beneath them in layer IVB, it is found that the Meynert cells tend to lie either beneath the edges of the cones of layer IVA cells or beneath the spaces between the cones. This relationship is shown in the reconstruction presented in Figure 12, for the cones shown in Figure 11.

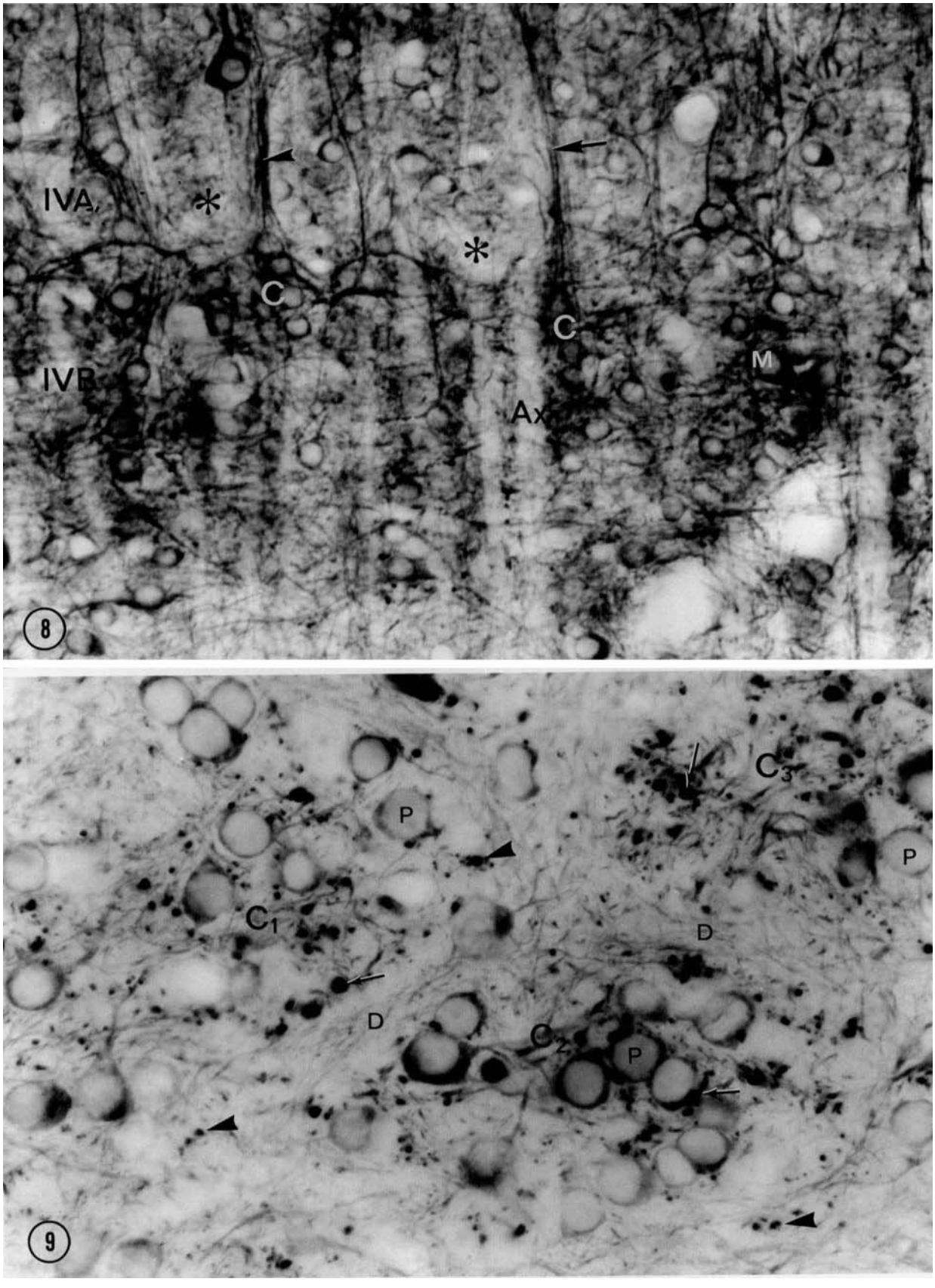
Additional studies will be necessary to determine the differences between those neurons that have their cell bodies aggregated into the cones, and other paler reacting neurons that lie around them (see Fig. 10). However, since the neurons in the cones have ascending dendrites that aggregate to pass in bundles into layer III, it seems obvious that they are small pyramidal cells, and in all likelihood the pale neurons lying between the cones are some of the small spiny stellate cells that abound in layer IVA.

Layers II and III. As the ascending dendrites from the layer V pyramidal cells pass through layer IVA to reach layer III, they are still clustered together, but their integrity is partially masked by the ascending dendrites derived from the medium-sized pyramidal cells in layer IVB, and from the bundles of dendrites derived from the cones of pyramidal cells in upper layer IV (Fig. 13). All of these contribute to the groups of ascending dendrites that pass into and through layer III/II. In that layer the apical dendrites of its own pyramidal cells are added to the ascending dendrites from below, so that in vertically oriented sections, MAP2 staining shows ascending clusters of dendrites that become thicker as they pass towards layer I (Figs. 1 and 2), and at the same time the overall MAP2 staining becomes gradually darker. The basal dendrites of layers II/III pyramidal cells are not very prominent, but at the layer III/layer IVA border, basal dendrites of some of the deepest cells in layer III can be seen to descend obliquely into layer IVA, so that they sometimes seem to arch over the pale staining patches in layer IVA (see Fig. 2).

In horizontal sections through the middle of layer II/III, MAP2 staining reveals groups of ascending dendrites in which there are both thin and thick apical dendrites (Fig. 15; arrows).On the basis of the vertical sections, it is assumed that the thinner dendrites are largely derived from the pyramidal cells of layer V, and that the thicker dendrites in the groups originate from the pyramidal cells in layer II/III (Fig. 14). Horizontal sections taken through layer II show increasing numbers of apical dendrites when compared to sections taken at lower levels. Towards layer I the field becomes filled by apical dendrites that completely surround the cell bodies of pyramidal cells, while in layer I

Fig. 8. A vertical section through layers IVA and IVB. Layer IVB is rather darkly reacted and at its top are some cones of pyramidal cells (C). The apical dendrites of these pyramidal cells form bundles (arrows) that pass into layer III. Between the bundles of apical dendrites the neuropil of layer IVA shows only a pale staining (asterisks). Note the large outer Meynert cell (M) in layer IVB and the pale vertical streaks where fascicles of myelinated axons (Ax) are located. ×400.

Fig. 9. Horizontal section at the level of layer IVA to show the cones of pyramidal cells (C_1–C_3). The cones contain groups of pyramidal cells (P) and some of their apical dendrites (arrows). One cone (C_3) is sectioned near its apex so that the apical dendrites are in bundles. Passing between the cones of neurons are sheaves of thin and horizontally aligned dendrites (D). The clusters of transversely sectioned dendrites passing through them (arrowheads) are presumed to belong to layer V pyramidal cells. × 800.



Figures 8–9

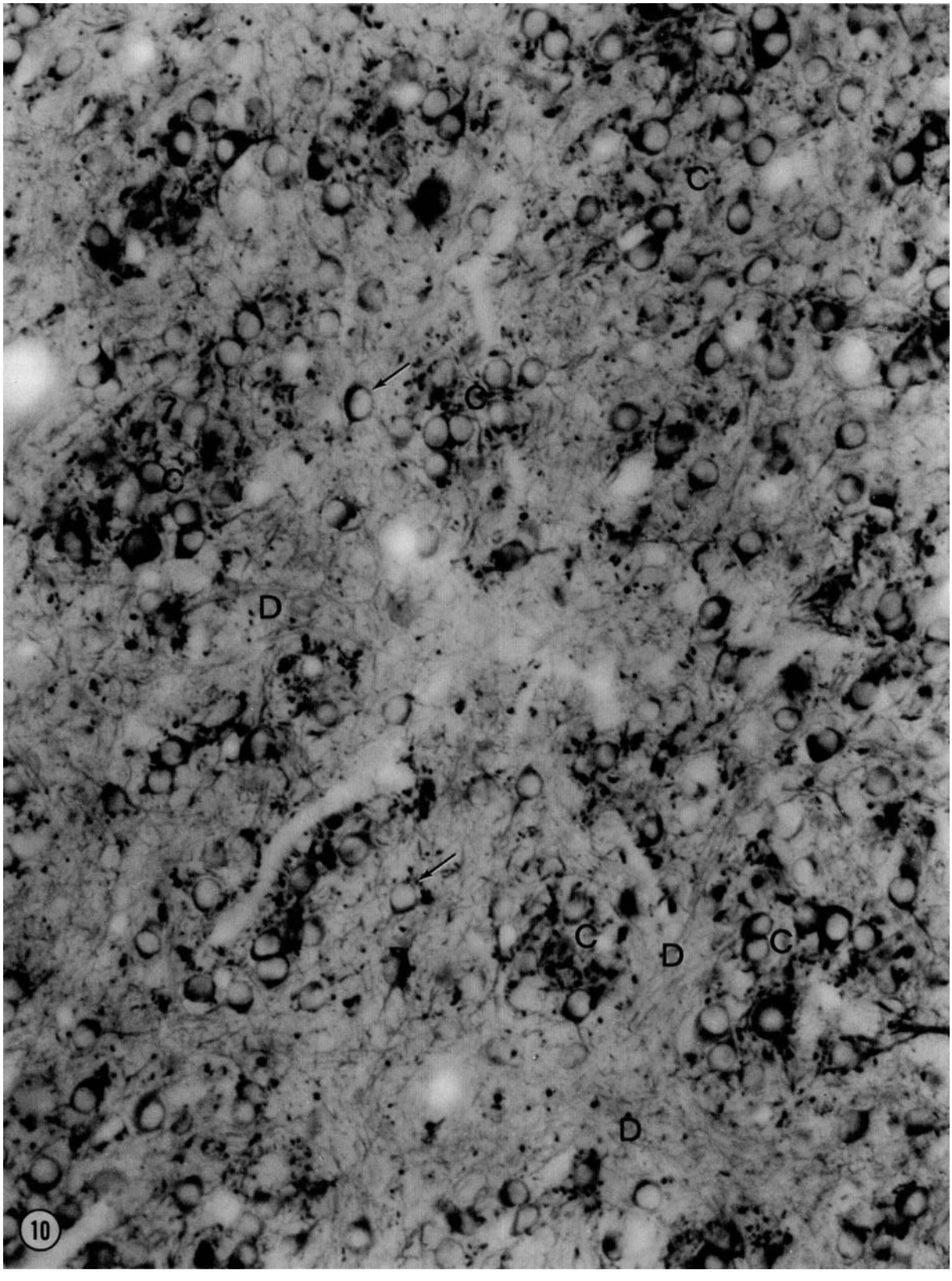


Fig. 10. A horizontal section at the level of the IVA/IVB border. The MAP2 antibody shows the cones of pyramidal cells (c) present at this depth. In addition to the neurons contained in the cones, there are other ones (arrows) embedded in the feltwork of thin processes (D) between the cones. × 450.

itself the MAP2 antibody shows the dense plexus of dendritic staining produced by the formation of the apical tufts of the ascending pyramidal cells. In vertical sections these tufts seem to largely emerge from the tops of the clusters of apical dendrites, so that the image is one of looking at a forest, with the trunks of closely packed trees giving rise to intermingling branches that form the forest canopy (Fig. 1).

Cytochrome oxidase staining

It is of interest to examine how the MAP2 staining of monkey area 17 correlates with the image produced by histochemical stains that reveal the locations of cytochrome activity. Detailed accounts of the appearance produced by staining for cytochrome oxidase activity have been given previously (e.g., Humphrey and Hendrikson, '80; Hendrikson et al., '81; Horton, '84; Carroll and Wong-Riley, '84; Fitzpatrick et al., '85; Payne and Peters, '89) and so only those aspects pertinent to the present account of the organization of monkey visual cortex will be considered here.

As shown in Figure 16, vertical sections through area 17 show blobs or patches of high cytochrome oxidase activity in layer II/III. Each patch (Fig. 16, p) is about 200 μm wide, and beneath the blobs is a thin, horizontal, and interrupted band of activity (Fig. 16, arrows) that coincides with layer IVA. In horizontal sections this band of activity in layer IVA is seen to be produced by sectioning through a reticulated or honeycomb pattern in which the dark walls of the honeycomb surround pockets or islands of low activity (Fig. 17). This honeycomb pattern is not completely regular because the pockets in the pattern are of various sizes, but the center-to-center spacing between the larger pockets in the pattern is about 100 μm.

When the cytochrome oxidase pattern seen in horizontal sections of layer IVA is examined closely, it becomes evident that groups of neurons lie within the spaces of the honeycomb pattern. This can be discerned even at low magnification (Fig. 17), but it becomes particularly obvious when horizontal sections are incubated in solutions containing cobalt salts (Adams, '81) to darken the cytochrome oxidase reaction product. When such sections are subsequently osmicated the reaction product appears as black granules (Fig. 18) that are most concentrated within the darkened walls of the honeycomb pattern, and interference microscopy reveals that the walls surround groups of neurons (Fig. 18, N). These obviously correspond to the cones of pyramidal cells that MAP2 staining reveals in layer IVA, so that the cones shown by MAP2 staining (Figs. 9 through 10), and the honeycomb pattern revealed by cytochrome oxidase reactions (Fig. 17) are complementary images.

It may also be noticed that the dark band of cytochrome oxidase activity in layer IVC corresponds to the pale staining band present in MAP2 reacted material.

DISCUSSION

Vertical organization

In addition to the horizontal bands of staining, the MAP2 antibody clearly reveals the vertical arrangements of neurons and their dendrites in monkey visual cortex. This is represented diagrammatically in Figure 20. The staining pattern shows that some of the pyramidal cells are arranged into modules, as they are in rat visual cortex (Peters and Kara, '87). In both species the pyramidal cell modules can be considered to be based upon the layer V pyramidal cells, the apical dendrites of which form discrete clusters as they ascend through layer IV. In the monkey visual cortex, these clusters of layer V apical dendrites are quite slender, but they are readily visible in horizontal sections, in which the mean center-to-center spacing of the clusters is about 30 μm, a value deduced from the number of such bundles within 1 mm^2 of horizontal sections passing through upper layer IVC. Examination of sections stained with the MAP2 antibody and cut in the horizontal and vertical planes suggests that, as in the rat visual cortex, the apical dendrites of layer III and of layer II pyramidal cells are added to clusters of layer V pyramidal cell apical dendrites. The details of how these supragranular cell apical dendrites are added is difficult to see in the monkey visual cortex because of the close packing of the apical dendritic clusters, and of the neurons. But as in the rat (Peters and Kara, '87), vertically oriented sections through the supragranular layers of monkey visual cortex show the clusters of apical dendrites to gradually become thicker as they ascend towards the outside of the cortex. Consequently, in this respect the arrangement of apical dendrites is assumed to be the same in the two cortices, and just beneath layer I, adjacent thickened clusters meet each other. The dendrites within them branch to form their apical tufts, so that at their tops the dendritic clusters arborize to generate the very dark MAP2 antibody reaction that characterizes layer I.

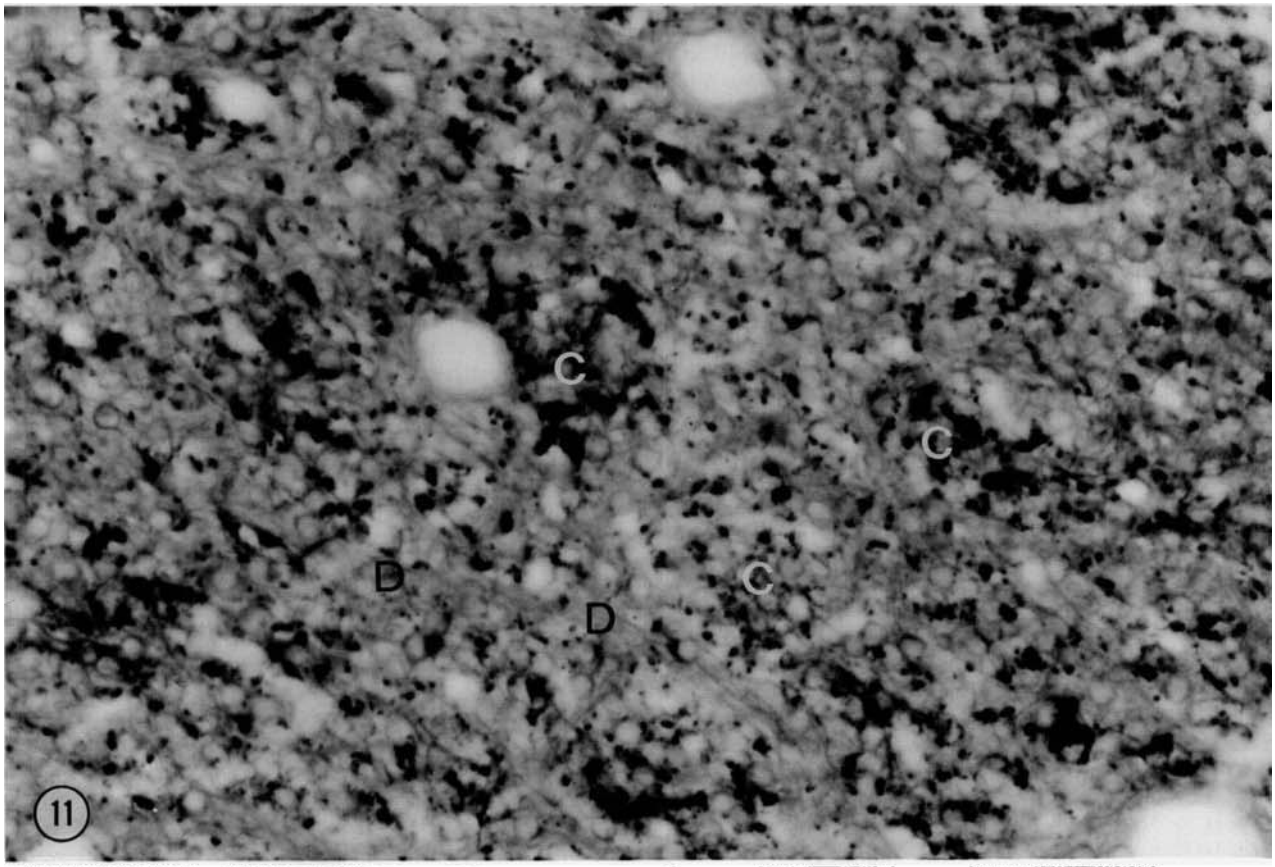
In the rat visual cortex, it was shown that the apical dendrites of the layer VI and the layer IV pyramidal cells do not add to the ascending clusters of layer V apical dendrites (Peters and Kara, '87). The apical dendrites of the layer VI pyramids of the rat do aggregate into fascicles and sheets, but these lack the order of the clusters of layer V apical dendrites. Indeed their aggregation seems to be imposed upon them by the necessity for them to pass through the gaps between layer V neurons to reach layer IV where most of the layer VI pyramidal cells form their apical tufts. The same is true of the monkey visual cortex. That is, apical dendrites of layer VI pyramids aggregate, but their disposition is very irregular, and the fascicles and sheets that they form in monkey visual cortex become quite slender as they reach layer IV and branch into their apical tufts (Lund and Boothe, '75).

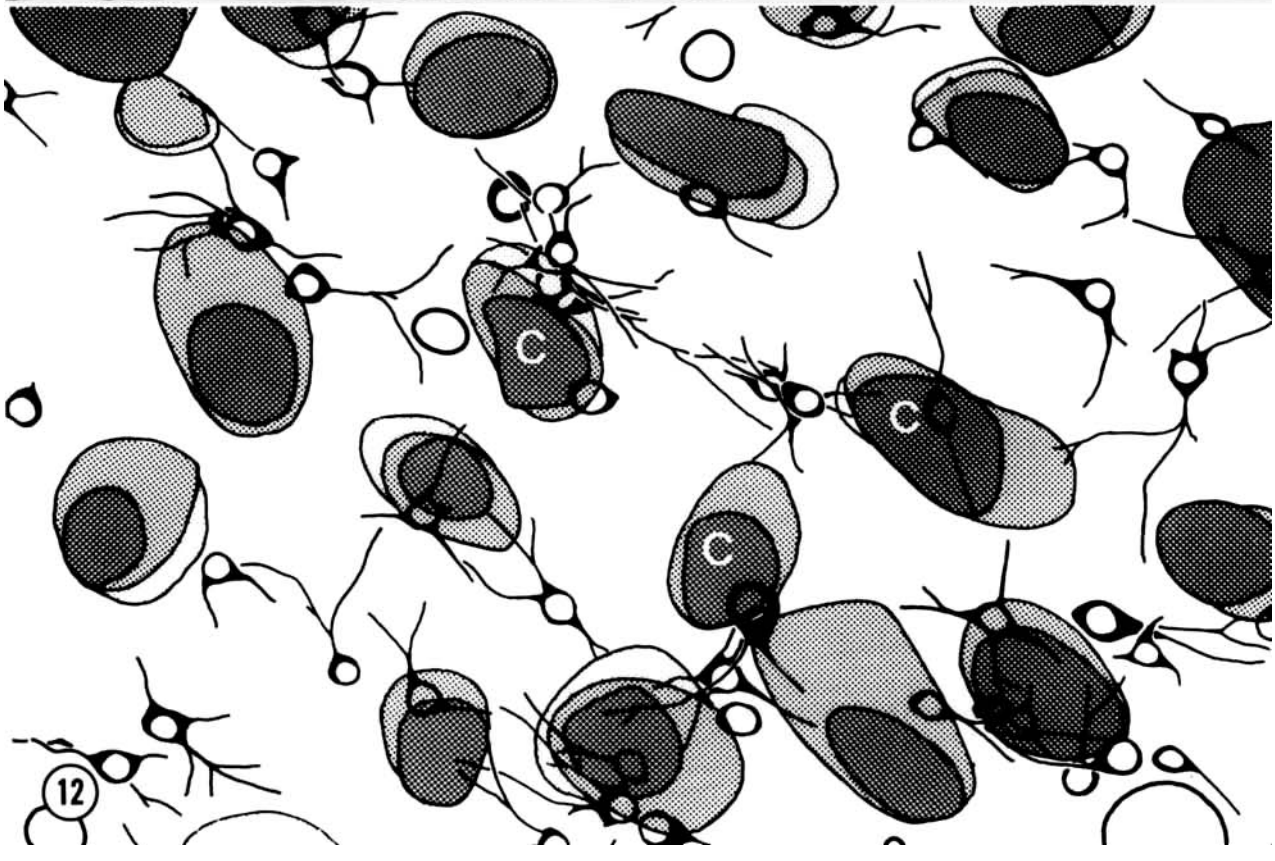
In rat visual cortex most of the neurons in layer IV are pyramidal cells (Peters and Kara, '85), whereas in monkey visual cortex most of the neurons in layer IV are spiny stellate cells (e.g., Valverde, '71; Lund, '73; Saint Marie and Peters, '85). As such, these layer IV neurons of monkey visual cortex can make little, if any, contribution to the clusters of layer V apical dendrites as they pass through layer IV.

Pyramidal cell modules

On the basis of the foregoing, it is proposed that the pyramidal cells in monkey visual cortex are arranged in vertically oriented modules, which are centered around the layer V pyramidal cells. Their apical dendrites form discrete clusters that pass through the center of each module, and based on the counts of clusters of layer V, apical dendrites contained within 1 mm^2 of horizontally sectioned visual cortex (Table 1), each such module would have a cross-sectional area of 786 μm^2, and consequently a diameter of about 31 μm, and they would extend through the depth of the cortex.

Values for the number of neurons per mm^3 and for the thickness of the monkey visual cortex have been deter-





Figures 11–12

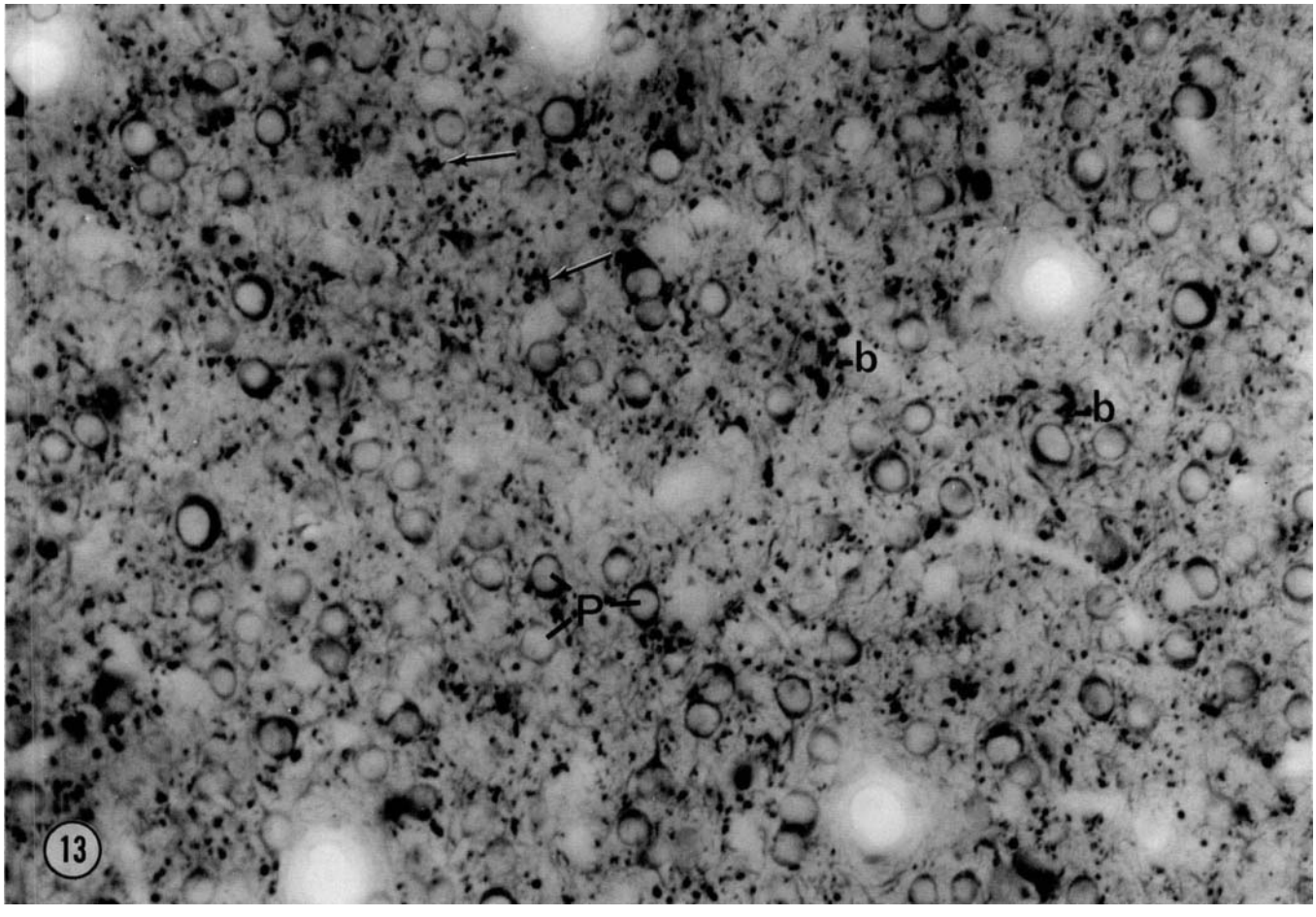


Fig. 13. Horizontal section through lower layer III. Between the cell bodies of the pyramidal cells (P), the MAP2 staining shows groups of apical dendrites. Some of the compact bundles of thicker dendrites (b) are derived from the cones of pyramidal cells in upper layer IV, while the clusters of thinner apical dendrites (arrows) are largely derived from the layer V pyramidal cells. × 420.

mined by O'Kusky and Colonnier ('82) and by Vincent et al. ('89) and we have also measured the thickness of area 17 in the monkeys used in this study. These values are given in Table 2, which shows that an average vertical module of neurons 31 μm in diameter would contain between 138 and 146 neurons, the mean value of the two sets of data being 142 neurons. Fortunately, O'Kusky and Colonnier ('82) have also determined the concentrations of neurons contained within the various layers of monkey visual cortex, and they also give the thickness of those layers, so that it is possible to determine how 142 neurons would be distributed among the layers within a module of 31 μm diameter. These data are shown diagrammatically in Figure 19, which also shows how the apical dendrites of the pyramidal cells are arranged. It should be pointed out, however, that in the construction of this model, all of the neurons are shown as either pyramidal cells or spiny stellate cells and all of the apical dendrites of layers V, III and II pyramidal cells are shown to be clustered, whereas some ascend individually. Also, to be more accurate the number of pyramidal and spiny stellate cells should be reduced to take into account those neurons that are inhibitory and GABAergic, but it is not yet certain what proportion of the cortical neurons is inhibitory. Hendry et al. ('87) estimate that 20% of the neurons in monkey visual cortex are labeled by GABA antibodies. They find that nearly all of the neurons in layer I are inhibitory and that in layer IVA inhibitory neurons account for 25% of the neuronal population, so that in all other layers about 15% of the neurons are GABAergic. Other investigators (e.g., Fitzpatrick et al., '83; Houser et

Fig. 11. A horizontal section at the level of layer IVA. The MAP2 antibody shows the cones of pyramidal cells (C) in which the cell bodies are intermingled with groups of thick apical dendrites. Surrounding the patches of neurons are bundles of thin horizontally oriented dendrites (D) that include a few pale staining neurons. × 330.

Fig. 12. A reconstruction, taken from serial vibratome sections stained with MAP2 antibody of the cones of pyramidal cells at the layer IVB/IVA border. One of the sections used in the reconstruction is shown in Figure 11. The cones of neurons are shown as the shaded areas in which the lighter shading is the deepest level of section through each cone of neurons, and the darkest shading is the uppermost plane of section. Consequently, the conical forms of the pyramidal cell groups can be appreciated. Also shown in the reconstruction are the positions of the outer Meynert cells in layer IVB. It will be noticed that the Meynert cells tend to form groups and that their cell bodies preferentially lie beneath the edges of the cones of pyramidal cells and the spaces between them. The cones of neurons labeled (C), are the same ones labeled in Figure 11.

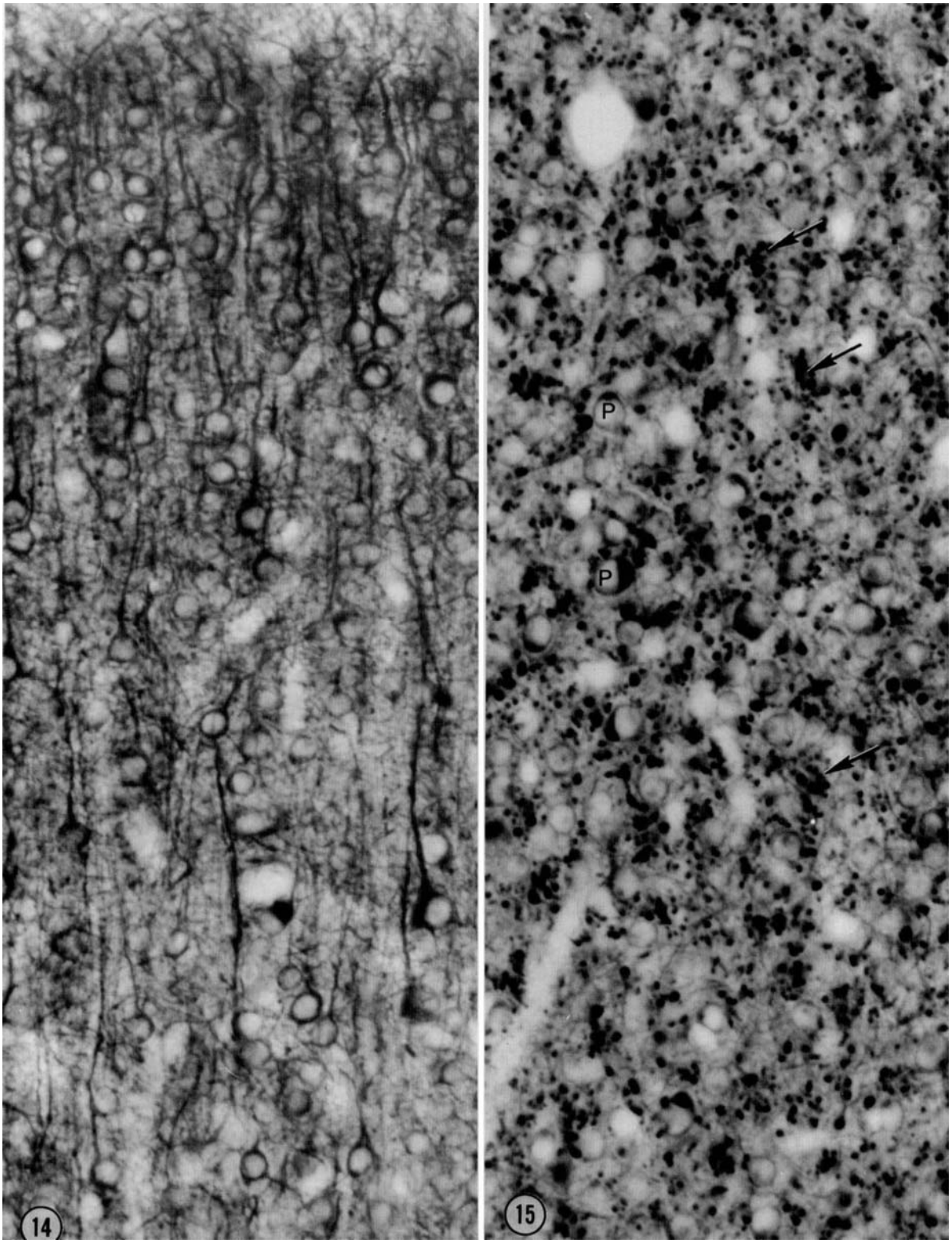


Fig. 14. A vertical section through layer II/III to show the various types of pyramidal cells that send their apical clusters towards layer I, where they form their apical tufts. × 400.

Fig. 15. A horizontal section taken through the middle of layer II/III. Intermixed with the cell bodies of the pyramidal neurons (P) are numerous cross-sectioned apical dendrites, which tend to be grouped (arrows), although there is a scattering of dendrites throughout the field. × 500.

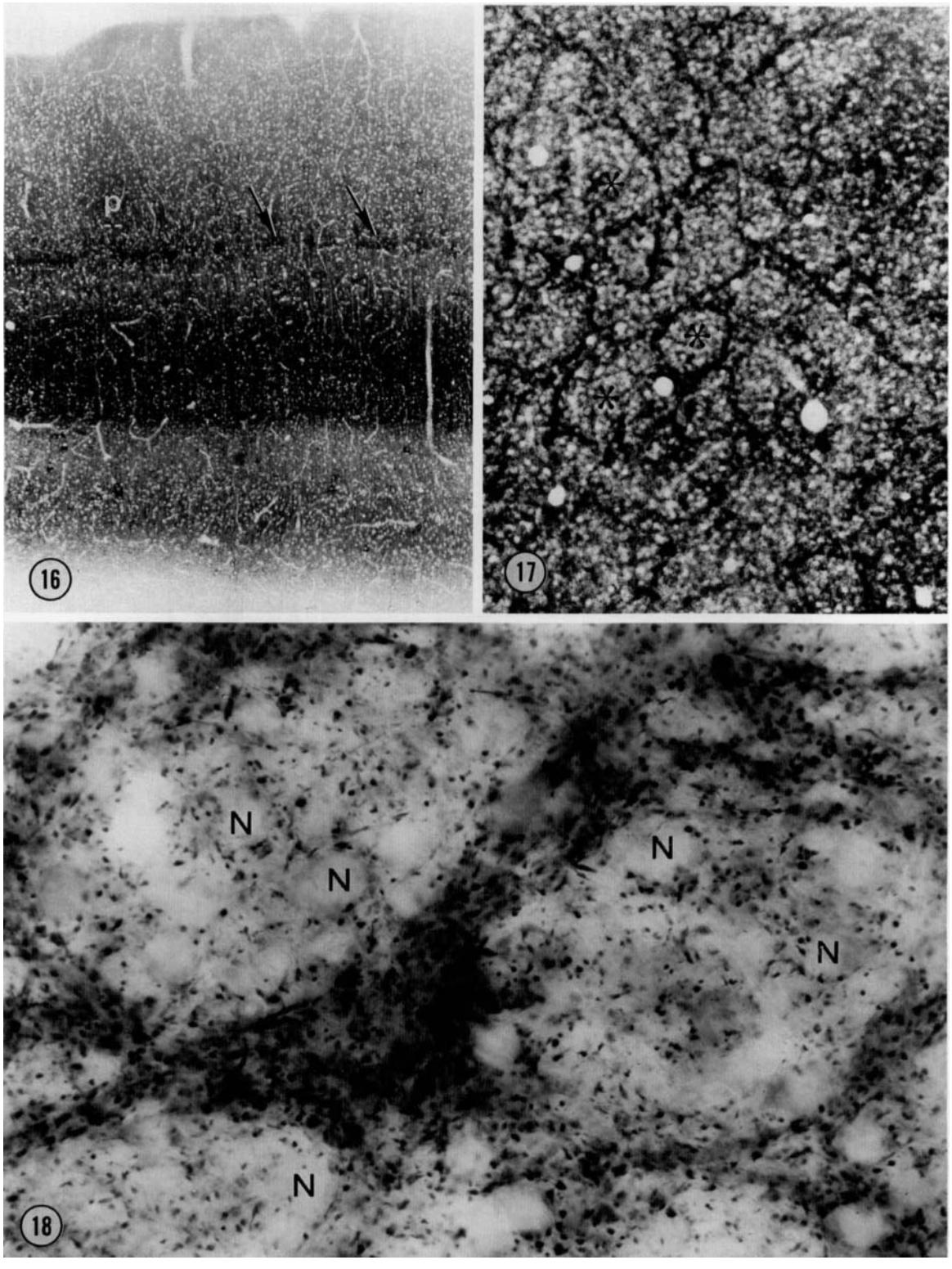


Fig. 16. A cytochrome oxidase reacted section through area 17. There are patches (p) of cytochrome oxidase activity in layer II/III, and below them is an interrupted band of activity at the level of layer IVA (arrows). The deeper thick band of intense staining is at the level of layer IVC. × 80.

Fig. 17. A horizontal section showing the cytochrome oxidase activity in layer IVA. The reaction shows a honeycomb pattern, with the darkly reactive walls surrounding pale areas (asterisks). × 130.

Fig. 18. A horizontal section at the level of layer IVA processed to show the cytochrome activity and photographed using interference microscopy. Cobalt chloride was added to the incubating solution so that the darkly reacted walls of the honeycomb pattern can be seen to surround the cones of neurons (N) present at this depth. × 800.

TABLE 2. Number of Neurons per Vertical Module of 31 μm Diameter

	Neurons per mm^3	Cortical depth	Neurons beneath 1 mm^2 of surface	Neurons in a 31 μm diameter module
O'Kusky and Colonnier (1982)	119×10^3	1.59 mm	189×10^3	146
Vincent et al. (1989)	117×10^3	1.53 mm	180×10^3	138
This study	—	1.60 mm	—	—
Mean				142

al., '83), using antibodies to glutamic acid decarboxylase, have determined that only about 10% of the neurons in monkey cerebral cortex are inhibitory ones. In view of this uncertainty about the frequency of GABAergic neurons, they have not been distinguished from other neurons in the model of the pyramidal cell module shown in Figure 19.

It is proposed that such pyramidal cell modules are the basic units of organization of monkey visual cortex, and that functional columns or systems are generated through the various afferent inputs to the cortex exciting different combinations of modules. Thus, a given module could be part of more than one functional column, as proposed in the Introduction. Furthermore, since the pyramidal neurons are the projection neurons of the cerebral cortex, the neurons contained within a particular module, and present within the various cortical layers, may carry the same basic output information to other cortical areas and to brain stem structures. It may also be considered that several neurons within one module project to the same structure, so that transfer of a specific output does not depend upon a single neuron. This replication may be important to assure against unpredictable response properties. Such unpredictability might be expected from the fact that the average neuron in monkey visual cortex is subject to the influence of several thousand synapses. For example, O'Kusky and Colonnier ('82) estimate that the average neuron in monkey area 17 receives about 2,300 synapsing axon terminals.

To continue with numbers one stage further, O'Kusky and Colonnier ('82) calculate that the total surface area of the primary visual cortex in one hemisphere of the macaque brain occupies about 840 mm^2 of cortical surface. Given the cross-sectional area of a pyramidal cell module to be about 786 μm^2, this means that in each hemisphere the primary visual cortex would contain some 1.07×10^6 modules.

Vertical modules of pyramidal cells have been also studied in detail in rat primary visual cortex. In this cortex the modules are wider than in the monkey visual cortex, for they have a center-to-center spacing of 55–60 μm, and if the modules are considered to span the entire depth of the cortex, they would each contain about 335 neurons (see Peters and Kara, '87). In both rat and monkey visual cortex, the pyramidal modules, as defined here, are centered around the clustered apical dendrites of layer V pyramidal cells, and the apical dendrites of the layer II and IV pyramidal cells are added to these clusters. This same pattern of organization has been also encountered in other cortices, such as rat somatosensory cortex in which the center-to-center spacing of the apical dendritic clusters is about 50 μm (Peters and Walsh, '72), rabbit visual cortex in

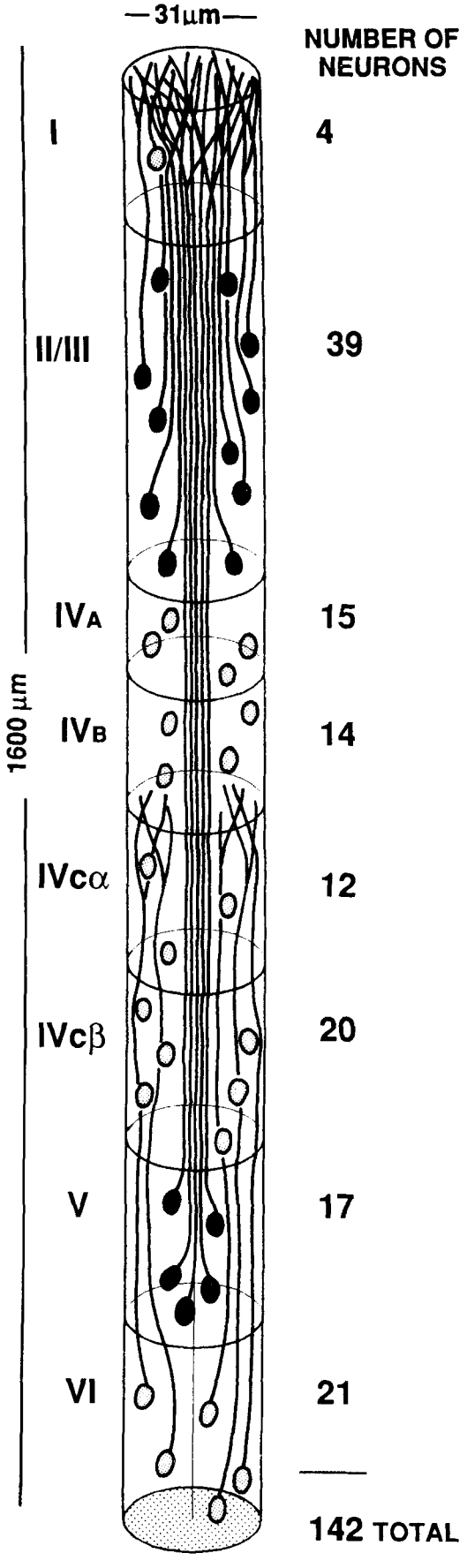


Fig. 19. Diagrammatic representation of the basic pyramidal cell model proposed to exist in area 17 of monkey. For further explanation see the text.

which the spacing is 40–45 μm (e.g., Fleischhauer et al., '72; Schmolke and Fleischhauer, '84; Schmolke, '87), and cat visual, auditory, and somatosensory cortices, in which the dendritic clusters are 50–70 μm apart (Feldman and Peters, '74).

Thus, the existence of pyramidal cell modules in which apical dendrites form clusters seems to be a uniform feature of neocortex, and while it is not entirely clear how the modules form, it seems likely that they are each derived from the neurons that have migrated into the cortex, from the germinal epithelium, along the same radial glial fiber (see Rakic, '72, '88a,b; Peters and Feldman, '73). In most cortical areas examined, the data given above suggest the spacing between clusters is usually 40 to 70 μm, but in monkey area 17, the spacing is only about 30 μm. This small diameter of the modules may be related to the fact that monkey area 17 contains about twice as many neurons per unit volume than other cortical areas (e.g., Peters, '87). Rakic ('88b) points out that neurogenesis is extended in primary visual cortex as compared to other cortical areas in the monkey, and he refers to the neurons that migrate along the same radial glial fiber as forming an "ontogenetic" or "embryonic" column. Interestingly, Rakic ('88b) estimates that in monkey area 17 such an ontogenetic column contains 120 or more neurons, which is close to the 142 neurons that we estimate to be contained within a pyramidal cell module.

It might be mentioned that apical dendritic bundles have recently been described in the retrosplenial cortex of the rat (Wyss et al., '90), but these are somewhat different from those in neocortex. In retrosplenial cortex the bundles are produced by the apical dendrites of the layer II commisural neurons and they are large. They are 30 to 100 μm wide and between 30 to 200 μm apart.

As an aside, it might be mentioned that the passage of the clusters of apical dendrites (and also the passage of bundles of myelinated axons) through the cerebral cortex might produce the vertical strings of cell bodies frequently encountered in Nissl preparations, because the cell bodies would have to fit into the spaces between the dendritic clusters. If so, then the vertical strings of cell bodies would be a reflection of the organization of the neurons into vertical modules. In this context, it is interesting that in one of their earlier experiments on orientation columns in monkey striate cortex Hubel and Wiesel ('74) found a noticeable shift in orientation to occur for almost every 25–30 μm advance of the electrode in a direction parallel to the pial surface. They found this to be of a similar order of magnitude to the intervals between radial bands of Nissl-stained cell bodies.

Layers IVB and IVA

In addition to the visualization of the pyramidal cell modules, another previously undescribed morphological feature revealed by the MAP2 antibody reaction is the arrangement of some pyramidal cells in upper layer IV. The cell bodies of these neurons are arranged in cones that have their bases at the interface between layers IVB and IVA. The mounds of cell bodies extend into layer IVA and their apical dendrites aggregate to form bundles that pass into layer III. There are some 120 cones of these cells beneath 1 mm^2 of cortical surface, so that the average center-to-center spacing is about 90 μm and in the MAP2-labeled material they are surrounded by fascicles of horizontally oriented dendrites. The dendrites in these fascicles are probably derived in part from the pyramidal cells in the cones, and they form a meshwork that coincides with the locations of the dark walls of the honeycomb pattern that appears in horizontal sections after cytochrome oxidase staining (e.g., Horton, '84; Fitzpatrick et al., '85). In effect, the cones of neurons fit into the holes in the honeycomb pattern and in all likelihood their dendrites receive geniculocortical input, because a honeycomb pattern similar to the cytochrome oxidase one is evident by autoradiography after tritiated amino acids are injected into the lateral geniculate nucleus of macaques (Hendrickson et al., '78). The investigation by Fitzpatrick et al. ('83) shows that this input to layer IVA is from the parvocellular layers of the lateral geniculate nucleus. A similar honeycomb pattern is also visible in material reacted with antibodies to glutamic acid decarboxylase (GAD), which shows the locations of inhibitory axon terminals (Fitzpatrick et al., '87), as well as in material used to localize the $GABA_A$ receptors by immunocytochemistry (Hendry et al., '90).

These data suggest that interesting synaptic relationships can be expected to occur in the neuropil around the cones of pyramidal cells if the dendrites of the pyramidal cells receive excitatory inputs from the geniculate body, as well as inhibitory inputs from local circuit neurons.

It should also be emphasized that the arrangement of the cones of pyramidal cells in layers IVA and IVB is lain over the pyramidal cell modules. The apical dendrites of the neurons in the modules pass uninterrupted through layer IVA and the cones of pyramidal cells generate their own separate bundles of ascending apical dendrites. But like the neurons in the cones through which they pass, the apical dendrities of the layer V pyramidal cells that traverse layer IVA might also receive the parvocellular input from the lateral geniculate body. This would serve to unite all of these neurons into a basic functional unit.

The Meynert cells

In a previous publication (Payne and Peters, '89) it was shown that the deep Meynert cells at the border between layers V and VI have their cell bodies arranged on a sheet, in which they outline gaps or holes. These gaps lie beneath the spaces between the regions of high cytochrome oxidase activity in layer II/III (also see Fries, '86 and Shipp and Zeki, '89). In other words, the dark Meynert cells lie beneath the interblob, or interpatch areas. As emphasized in this report, there is another sheet of so-called Meynert cells in layer IVB, and interestingly, these outer cells tend to lie in groups beneath the edges of the cones of pyramidal cells in layers IVA and IVB and beneath the spaces between them (see Fig. 12). The meaning of these distributions of the cell bodies of the two sets of Meynert cells is not apparent, but it is of interest that these large cells have some features in common. For example, layers IVB and VI are the two layers in which directional sensitivity is most commonly encountered among neurons in primary visual cortex (e.g., Dow, '74; Livingstone and Hubel, '84; Orban et al., '86); the large neurons in both these layers project to area V5, or MT, of primate extrastriate cortex, an area that is specialized for analysis of motion (see Shipp and Zeki, '89). The aggregations of neurons that they avoid, namely, those in the cytochrome oxidase blods in layers II/III, and those in the cones in layers IVA/IVB receive input from the parvocellular and intercalated layers of the lateral geniculate nucleus, which suggest that they are involved in color or form perception, in contrast to the movement and direction sensitivity of the Meynert cells.

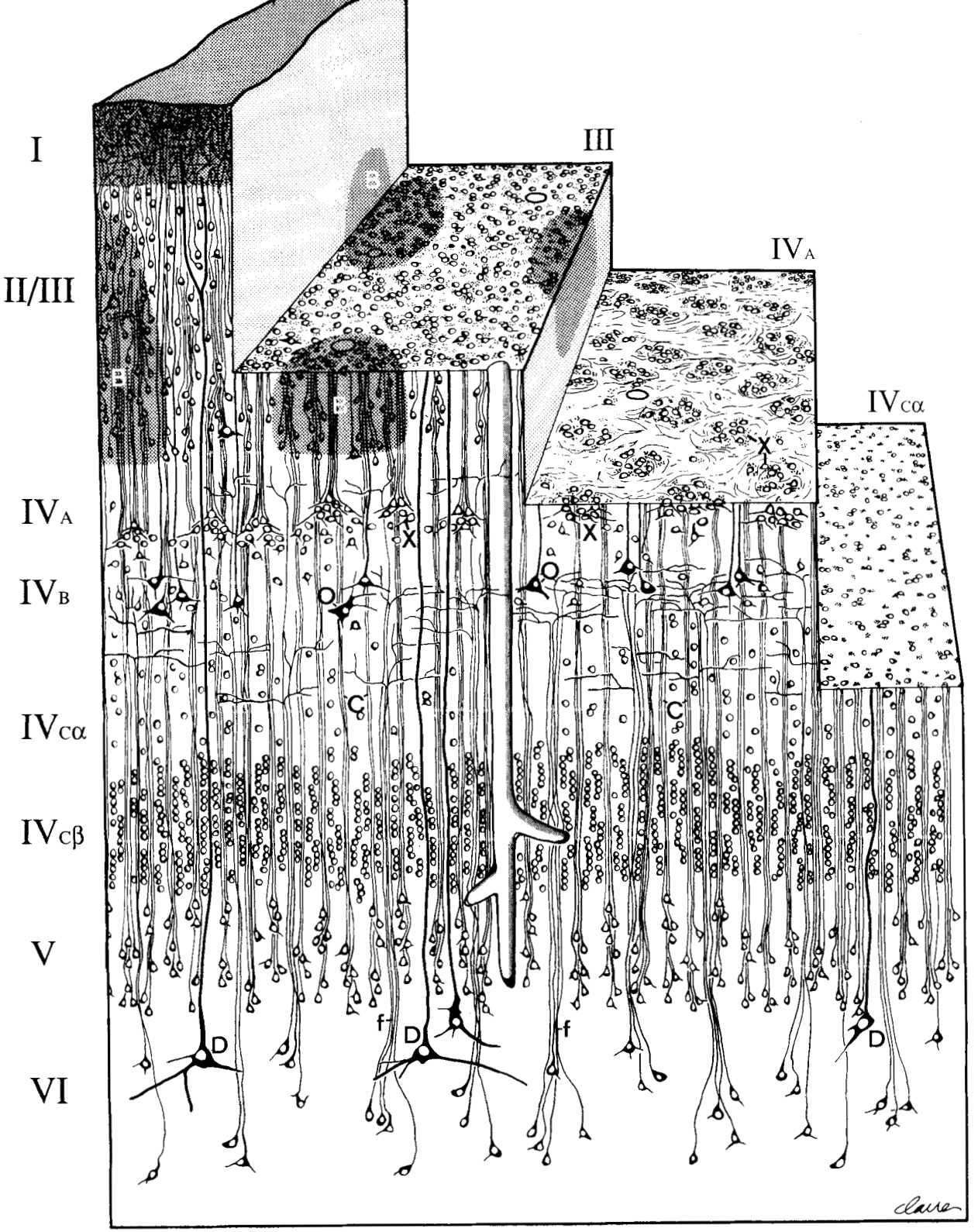


Fig. 20. Diagrammatic representation of the main features of area 17 of monkey visual cortex as

CONCLUSION

The organization of the pyramidal cells in monkey area 17 that begins to emerge from this study is depicted in Figure 20 and it can be summarized as follows. The deepest pyramidal cells of visual cortex are those contained in layer VI. The rather thick apical dendrites of these neurons become grouped into fascicles (f) and sheets as they pass through layer V and these groups of apical dendrites gradually taper as they enter layer IV, where many of them form their terminal tufts. The apical dendrites of the smaller layer V pyramids form clusters (C) that are relatively discrete, are spaced about 30 μm apart, and represent the centers of pyramidal cell modules. These clusters pass between the stellate cells of layer IV and when they enter layer III the pyramidal cells of that layer, and subsequently the pyramidal cells of layer II, add their apical dendrites to the clusters. Consequently, the clusters become thicker as they approach layer I, where the apical dendrites within them form their tufts.

Superimposed upon this basic pattern of vertical pyramidal cell modules are other pyramidal cell arrangements. Thus, within layers IVA and IVB there are some pyramidal cells whose cell bodies are arranged in cones (X), and their apical dendrites emerge from the tops of the cones in bundles that also pass into the supragranular layers. There are about 118 cones of pyramidal cells in upper layer IV beneath each 1 mm^2 of cortical surface so their centers are about 100 μm apart, and the spaces between the cones coincide with the walls of the honeycomb pattern of reactivity revealed by cytochrome oxidase staining in layer IVA. Consequently, the cones of pyramidal cells occupy the spaces in the honeycomb pattern. In all probability their basal dendrites contribute to the bundles of dendrites that extend horizontally around the cones of neurons, where they probably receive much of the parvocellular input from the lateral geniculate nucleus. This input is revealed by anterograde transport of horseradish peroxidase (Blasdel and Lund, '83), and of radioactive aminoacids (e.g., Hendrikson et al., '78), and its disposition coincides with the labeling seen in layer IVA with both GAD antibodies (Fitzpatrick et al., '87) and antibodies to $GABA_A$ receptors (Hendry et al., '90). Although no records seem to have been taken of the response properties of the neurons in layer IVA, since these neurons receive input from the parvocellular layers of the lateral geniculate nucleus, they are probably involved in color and form perception. The cytochrome oxidase blobs in layer II/III are also color sensitive, and they represent yet another basic modular pattern (Zeki, '83; Livingston and Hubel, '84; Tootell et al., '88b), because a single row of blobs can be demonstrated to lie within each ocular dominance column in monkey primary visual cortex (e.g., Horton, '84; Tootell et al., '88).

Other patterns of organization involve the Meynert cells. The deep Meynert cells (D), which have their cell bodies at the border between layers V and VI, are arranged so that their apical dendrites ascend to the superficial layers through the spaces between the cytochrome oxidase blobs (B) in layer II/III. The outer Meynert cells (O) of layer IVB, on the other hand, are arranged so that their cell bodies lies beneath the edges and between the cones of pyramidal cells in layer IVA. Both sets of Meynert cells share the property of being direction sensitive and projecting to area V5, or MT, of the extrastriate cortex, an area specialized for analysis of motion.

Thus, the primate visual cortex is conceived of being composed of a basic set of pyramidal cell modules that are probably derived from those neurons which migrate along individual radial glial fibers during development. In effect, these are output modules since many pyramidal cells project out of the cerebral cortex, but how they are related to the bundles of myelinated axons that extend vertically through the cortex has not yet been determined. Since the neurons in each module are so closely related they probably have a common physiological response. But that response would be unique for the neurons in a particular module and depend, for example, upon the part of the visual field from which the module is receiving input, the eye that dominates that input, and the color and orientation of the image. The neurons in the module would convey their responses to other structures, and in all likelihood the output from each module to a particular structure involves several of its neurons, making that output reliable and not subject to the unpredictability that may be expected from an individual neuron with several thousand synapses. In this sense, the neurons contained within a pyramidal cell module can be regarded as a consortium, which responds in a limited number of ways, and which functions as part of several physiologically functional systems, depending upon the particular physiological property being tested.

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LITERATURE CITED

Adam, S.J.C. (1981) Heavy metal intensification of DAB-based HRP reaction product. J. Histochem. Cytochem. *29:*775.

Bernhardt, R., and A. Matus (1984) Light and electron microscopic studies of the distribution of microtubule-associated protein 2 in rat brain: A difference between dendritic and axonal cytoskeletons. J. Comp. Neurol. *226:*207–222.

Blasdel, G.G., and J.S. Lund (1983) Termination of afferent axons in macaque striate cortex. J. Neurosci. *3:*1389–1413.

Brodmann, K. (1905) Beitrage fur histologische lokalization der Grosshirnrinde: Dritte Mitteilung, Die Rindenfelden der niederen Affen. J. Psychol. Neurol. *4:*177–226.

Carroll, E.W., and M.T.T. Wong-Riley (1984) Quantitative light and electron microscopic analysis of cytochrome oxidase-rich zones in the striate cortex of the squirrel monkey. J. Comp. Neurol. *222:*1–17.

Chun, J.J.M., and C.J. Shatz (1989) Interstitial cells of the adult neocortical white matter are the remnants of the early generated subplate neuron population. J. Comp. Neurol. *282:*555–569.

De Camilli, P., P.E. Miller, F. Navone, W.E. Theurkauf, and R.B. Vallee (1984) Distribution of microtuble-associated protein 2 in the nervous system of the rat studied by immunofluorescence. Neuroscience *11:*819–846.

Dow, B.M. (1974) Functional classes of cells and their laminar distribution in monkey visual cortex. J. Neurophysiol. *37:*927–946.

Escobar, M.I., H. Pimienta, V.S. Caviness, M. Jacobson, J.E. Crandall, and K.S. Kosik (1986) Architecture of apical dendrites in the murine neocortex: Dual apical dendritic systems. Neuroscience *17:*975–989.

Feldman, M.L. (1984) Morphology of the neocortical pyramidal neuron. In A.

Peters and E.G. Jones (eds): Cerebral Cortex, Vol. 1. New York: Plenum Press, pp. 123–200.

Feldman, M.L., and A. Peters (1974) A study of barrels and pyramidal dendritic clusters in the cerebral cortex. Brain Res. *77:55–76.*

Fitzpatrick, D., K. Itoh, and I.T. Diamond (1983) The laminar organization of the lateral geniculate body and the striate cortex in the squirrel monkey (*Saimiri sciureus*). J. Neurosci. *3:*673–702.

Fitzpatrick, D., J.S. Lund, and G.G. Blasdel (1985) Intrinsic connections of macaque striate cortex. Afferent and efferent connections of lamina 4C. J. Neurosci. *5:*3329–3349.

Fitzpatrick, D., J.S. Lund, and D.E. Schmechel (1983) Glutamic acid decarboxylase immunoreactive neurons and terminals in the visual cortex of monkey and cat. Soc. Neurosci. Abstr. *9:*616.

Fitzpatrick, D., J.S. Lund, D.E. Schmechel, and A.C. Towles (1987) Distribution of GABAergic neurons and axon terminals in the macaque striate cortex. J. Comp. Neurol. *264:*73–91.

Fleischhauer, K. (1974) On different patterns of dendritic bundling in the cerebral cortex of the cat. Z. Anat. Entwickl. Gesch. *143:*115–126.

Fleischhauer, K., and K. Detzer (1975) Dendritic bundling in the cerebral cortex. In G.W. Kreutzberg (ed): Physiology and Pathology of Dendrites: Advances in Neurology, Vol. 12. New York: Raven Press, pp. 71–78.

Fleischhauer, K., H. Petsche, and W. Wittkowski (1972) Vertical bundles of dendrites in the neocortex. Z. Anat. Entwickl. Gesch. *136:213–223.*

Fries, W. (1986) Distribution of Meynert cells in primate striate cortex. Naturwissenschaften *73:*557–558.

Hendrickson, A.E., J.R. Wilson, and M.P. Ogren (1978) The neuroanatomical organization of pathways between the dorsal lateral geniculate nucleus and visual cortex in old world and new world primates. J. Comp. Neurol. *182:*123–136.

Hendrickson, A.E., S.P. Hunt, and J.Y. Wu (1981) Immunocytochemical localization of glutamic acid decarboxylase in monkey striate cortex. Nature *292:*605–607.

Hendry, S.H.C., H.D. Schwark, E.G. Jones, and J. Yan (1987) Numbers and proportions of GABA-immunoreactive neurons in different areas of monkey cerebral cortex. J. Neurosci. *7:*1503–1519.

Hendry, S.H.C., J. Fuchs, A.L. deBlas, and E.G. Jones (1990) Distribution and plasticity of immunocytochemically localized $GABA_A$ receptors in adult monkey visual cortex. J. Neurosci. *10:*2438–2450.

Horton, J.C. (1984) Cytochrome oxidase patches: A new cytoarchitectural feature of monkey visual cortex. Phil. Trans. R. Soc. Lond. [Biol.] *304:*199–253.

Houser, C.R., S.H.C. Hendry, E.G. Jones, and J.E. Vaughn (1983) Morphological diversity of immunocytochemically identified GABA neurons in the monkey sensory-motor cortex. J. Neurocytol. *12:*617–638.

Hubel, D.H., and T.N. Wiesel (1968) Receptive fields and functional architecture of monkey striate cortex. J. Physiol. Lond. *195:*215–243.

Hubel, D.H., and T.N. Wiesel (1972) Laminar and columnar distribution of geniculo-cortical fibers in the macaque monkey. J. Comp. Neurol. *146:*421–450.

Hubel, D.H., and T.N. Wiesel (1974) Sequence regularity and geometry of orientation columns in the monkey striate cortex. J. Comp. Neurol. *158:*267–294.

Hubel, D.H., and T.N. Wiesel (1977) Functional architecture of macaque monkey visual cortex. Proc. R. Soc. Lond. [Biol.] *198:*1–59.

Humphrey, A.L., and A.E. Hendrickson (1980) Radial zones of high metabolic activity in squirrel monkey striate cortex. Soc. Neurosci. *6:*133.6.

Kisvarday, Z.F., A. Cowey, and P. Somogyi (1986) Synaptic relationships of a type of GABA-immunoreactive neuron (clutch cell), spiny stellate cells and lateral geniculate afferents in layer IVC of the monkey striate cortex. Neuroscience *19:*741–761.

Kosik, K.S., L.K. Duffy, M.M. Dowling, C. Abraham, A. McClusky, and D.J. Selkoe (1984) Monoclonal antibody to microtubule-associated protein 2 (MAP2) labels Alzheimer neurofibrillary tangles. Proc. Natl. Acad. Sci. U.S.A. *81:*7941–7945.

LeVay, S., M. Connolly, J. Houde, and D.C. Van Essen (1985) The complete pattern of ocular dominance stripes in the striate cortex and visual field of the macaque monkey. J. Neurosci. *5:*486–501.

LeVay, S., D.H. Hubel, and T.N. Wiesel (1975) The pattern of ocular dominance columns in macaque visual cortex revealed by a reduced silver stain. J. Comp. Neurol. *159:*559–576.

Livingstone, M.S., and D.H. Hubel (1984) Anatomy and physiology of a color system in the primate visual cortex. J. Neurosci. *4:*309–356.

Lund, J.S. (1973) Organization of neurons in the visual cortex, area 17, of the monkey (*Macaca mulatta*). J. Comp. Neurol. *147:*455–496.

Lund, J.S. (1981) Intrinsic organization of the primate visual cortex, area 17, as seen in Golgi preparations. In F.O. Schmitt, F.G. Worden, G. Adelman and S.G. Dennis (eds): The Organization of the Cerebral Cortex. Cambridge, MA: MIT Press, pp. 105–124.

Lund, J.S., and R.G. Boothe (1975) Interlaminar connections and pyramidal neuron organisation in the visual cortex, area 17, of the macaque monkey. J. Comp. Neurol. *159:*305–334.

McLean, I.W., and P.K. Nakane (1974) Periodate-lysine-paraformaldehyde fixative: A new fixative for immunoelectron microscopy. J. Histochem. Cytochem. *22:*1077–1083.

Michael, C.R. (1981) Columnar organization of color cells in monkey's striate cortex. J. Neurophysiol. *46:*587–604.

Michael, C.R. (1985) Serial processing of color in the monkey's striate cortex. In D. Rose and V.G. Dobson (eds): Models of the Visual Cortex. Chichester, Great Britain: John Wiley and Sons Ltd., pp. 301–309.

Mountcastle, V.B. (1957) Modality and topographic properties of single neurons in cat's somatic sensory cortex. J. Neurophysiol. *20:*408–434.

Mountcastle, V.B. (1978) An organizing principle for cerebral function: The unit module and the distributed system. In G.M. Edelman and V.B. Mountcastle (eds): The Mindful Brain. Cambridge, MA: MIT Press, pp. 7–50.

O'Kusky, J. and M. Colonnier (1982) A laminar analysis of the number of neurons, glia, and synapses in the visual cortex (area 17) of adult macaque monkeys. J. Comp. Neurol. *210:*278–290.

Orban, G.A., H. Kennedy, and J. Bullier (1986) Velocity sensitivity and direction selectivity of neurons in areas V1 and V2 of the monkey: Influence of eccentricity. J. Neurophysiol. *56:*462–480.

Payne, B.R., and A. Peters (1989) Cytochrome oxidase patches and Meynert cells in monkey visual cortex. Neuroscience *28:*353–363.

Peters, A. (1987) Number of neurons and synapses in primary visual cortex. In E.G. Jones and A. Peters (eds): Cerebral Cortex, Vol. 6. New York: Plenum Press, pp. 267–294.

Peters, A., and M.L. Feldman (1973) The cortical plate and molecular layer of the late rat fetus. Z. Anat. Entwickl. Gesch. *141:*3–37.

Peters, A., and D.A. Kara (1985) The neuronal composition of area 17 of rat visual cortex. I. The pyramidal cells. J. Comp. Neurol. *234:*218–241.

Peters, A., and D.A. Kara (1987) The neuronal composition of area 17 of rat visual cortex IV: The organization of pyramidal cells. J. Comp. Neurol. *260:*573–590.

Peters, A., and T.M. Walsh (1972) A study of the organization of apical dendrites in the somatic sensory cortex of the rat. J. Comp. Neurol. *144:*253–268.

Rakic, P. (1972) Mode of cell migration to the superficial layer of fetal monkey cortex. J. Comp. Neurol. *145:*61–84.

Rakic, P. (1988a) Defects of neuronal migration and the pathogenesis of cortical malformations. Prog. Brain Res. *73:*15–37.

Rakic, P. (1988b) Specification of cerebral cortical areas. Science *241:*170–176.

Saint Marie, R.L., and A. Peters (1985) The morphology and synaptic connections of spiny stellate neurons in monkey visual cortex (Area 17): A Golgi-electron microscopic study. J. Comp. Neurol. *233:*213–235.

Schmolke, C. (1987) Morphological organization of the neuropil in laminae II–V of rabbit visual cortex. Anat. Embryol. *176:*203–212.

Schmolke, C., and K. Fleishhauer (1984) Morphological characteristics of neocortical laminae when studied in tangential semithin sections through the visual cortex of the rabbit. Anat. Embryol. *169:*125–132.

Seligman, A.M., M.J. Karnovsky, H.L. Wasserkrug, and J.S. Hanker (1968) Nondroplet ultrastructural demonstration of cytochrome oxidase activity with a polymerizing osmiophilic reagent, diaminobenzidine (DAB). J. Cell. Biol. *38:*1–14.

Shipp, S., and S. Zeki (1989) The organization of connections between areas V5 and V1 in macaque monkey visual cortex. Eur. J. Neurosci. *1:*304–332.

Somogyi, P., T.F. Freund, and A. Cowey (1982) The axo-axonic interneuron in the cerebral cortex of the rat, cat and monkey. Neuroscience *7:*2577–2607.

Tootell, R.B.H., S.L. Hamilton, M.S. Silverman, and E. Switkes (1988a) Functional anatomy of macaque striate cortex. I. Ocular dominance, binocular interactions, and baseline conditions. J. Neurosci. *8*(5):1500–1530.

Tootell, R.B.H., M.S. Silverman, S.L. Hamilton, R.L. De Valois, and E. Switkes (1988b) *Functional anatomy of macaque striate cortex. III.* Color. J. Neurosci. *8*(5):1569–1593.

Valverde, F. (1971) Short axon neuronal subsystems in the visual cortex of the monkey. Intern. J. Neurosci. *1:*181–197.

Valverde, F. (1985) The organizing principles of the primary visual cortex in

the monkey. In A. Peters and E.G. Jones (eds): Cerebral Cortex: Visual Cortex, Vol. 3. New York: Plenum Press, pp. 207–257.

Vincent, S.L., A. Peters, and J. Tigges (1989) Effects of aging on the neurons within area 17 of rhesus monkey cerebral cortex. Anat. Rec. *223:*329–341.

von Bonin, G., and W.R. Mehler (1971) On columnar arrangement of nerve cells in cerebral cortex. Brain Res. *27:*1–10.

Winkelmann, E., K. Brauer, and U. Berger (1975) Zur columnaren Organisation von Pyramidenzellen im visuellen Cortex der Albinoratte. Z. Mikrosk. Anat. Forsch. *89:*239–256.

Wiesel, T.N., D.H. Hubel, and D.M.K. Lam (1974) Autoradiographic demonstration of ocular dominance columns in monkey striate cortex by means of transneural transport. Brain Res. *79:*273–279.

Wong-Riley, M.T.T., M.M. Merzenich, and P.A. Leake (1978) Changes in endogenous enzymatic reactivity to DAB induced by neuronal inactivity. Brain Res. *141:*185–192.

Wyss, J.M., T. Van Groen, and K. Sripanidkulchai (1990) Dendritic bundling in layer I of granular retrosplenial cortex: Intracellular labeling and selectivity of innervation. J. Comp. Neurol. *295:*33–42.

Zeki, S.M. (1983) The distribution of wavelength and orientation selectivity in different areas of monkey visual cortex. Proc. R. Soc. Lond. [Biol.] *207:*449–470.