SHORT AXON NEURONAL SUBSYSTEMS IN THE VISUAL CORTEX OF THE MONKEY

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In seeking for a functional schema of the organization of the visual centers, we have studied with the Golgi method the varieties and connections of short-axon cells and other related neurons in the middle layers of the striate area in the adult monkey. Short-axon cells in layer IV have been classified into stellate cells with recurrent ascending axons ending in layers III and IVA, and clewed cells with characteristic axonal nets localized in their own dendritic field. Short-axon cells have been found in clusters or glomeruli upon which terminal branches of cortical afferents have been observed. It is suggested that periglomerular stellate cells with ascending axons connect clusters of clewed cells with small, medium and large stellate and pyramidal cells of layers III and IVA, which in turn would activate the giant solitary cells of layer V through synaptic contacts along vertical descending axons. Two levels of cortical afferent ramifications in layers III and IVC have been identified. Their morphological characteristics have been described.

INTRODUCTION

The striate area of primates exhibits a characteristic structure. It was recognized a long time ago by the presence in the middle of the thickness of a white band visible with the naked eye in fresh and fixed material: the stria of Gennari and Vicq-d’Azur or the outer stripe of Baillarger (Bonin, 1960).

Microscopic examination reveals that the striate area of primates has a particular structure and noticeably uniformity. The majority of their short-axon cells possess ascending axons reaching superficial levels, which leads to a tremendous increase in neuronal intracortical connections. It is probably the cortical level with the highest neuronal density.

The present work, using adult monkey material stained with the Golgi method, describes for the first time the varieties of short-axon cells with ascending axons and their connections in layers III to V. It was not capricious to restrict our description to these layers, it is due to the fact that in our present material the staining of these layers has been particularly successful at expenses of incomplete and poor staining of the superficial layers and myelinated systems. We believe however it would be of interest to understand the organization of many short-axon neuronal subsystems which build up the internal granular layer and its most adjacent and related parts of layers III and V.

MATERIAL AND METHODS

Thirteen adult monkeys were used in this study: eight Macaca rhesus and five Erythrocebus patas (red monkey). The animals were anesthetized and sacrificed by bleeding through the carotid vessel. The brains were rapidly removed and 6–8 selected pieces 4–5 mm in thickness from each occipital lobe were immersed in the osmium-dichromate solution for Golgi staining. All pieces were subjected to the triple impregnation schedule as described elsewhere (Valverde, 1970).

Sections 150–200 μ in thickness were cut perpendicular to the pial surface and mounted serially. A total of 1,187 sections have been examined from which relevant details repeatedly observed have been drawn. Complete impregnations were obtained from pieces taken along the dorsal lip of the two posterior prongs of the medial calcarine sulcus. Additional observations were obtained from pieces taken along the lateral calcarine sulcus, superior lip of the medial calcarine sulcus

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and dorsal lip of the lunate sulcus on the medial side.

The observations to be described are camera lucida drawings in which every detail has been carefully checked. In each drawing the cells were lettered; the main axonal branch of each cell was always labelled I followed by the letter of its parent cell; the collaterals of these axons were numbered consecutively addressed with the letter of its parent cell. Other structures were numbered or initialled. The depth from surface of a random cell in each drawing has been plotted. Since the mean linear shrinkage factor of the gray substance in Golgi sections is of the order of 0.95 in relation to fresh material, these plots (figures between brackets) and the drawing scale can be used to know quite approximately the real depth of different structures. The stratification of Brodmann (1905) has been adopted.

DESCRIPTION

Layers III and IVa

Brodmann's (1905) plan of stratification considers the internal granular layer divided into the three sublayers IVa, IVb and IVc. This nomenclature has not been followed by other students who consider sublayer IVa as a part of the layer of medium sized pyramidal cells or layer III.

In his first description of the human visual cortex Cajal (1899) gave a very complete account of the pyramidal and short-axon cells of layer III or layer of medium pyramids. In this description he mentioned diverse types of pyramidal cells and short-axon cells and stated that the deepest large pyramidal cells were here completely surrounded by many small cell bodies similar to those elements described in his layer V. Later, in his book, Cajal (1911) gave only a very short description of layer III and did not mention the existence of short-axon cells.

The lower part of the layer of medium pyramids corresponds to Brodmann (1905) and Vogt (1920) lamina IVa or superficial internal granular lamina and to Henschin (1926) stratum supragennari. The presence of densely arranged short-axon cells, as first described by Cajal (1899), gives the characteristic appearance of a distinct sublayer in Nissl preparations.

Brodmann's (1905) stratification was followed by Poljak (1957), but Bonin (1942) preferred to divide layer III into the two sublayers IIIa and IIIb in reason of different cell density and to the orientation of dendrites in IIIb which are not in contact with fibers of the stria of Gennari. Thus sublayer IIIb of Bonin (1942) is homologous to IVa of Brodmann (1905).

The classification of Cajal (1911) was adopted by Campbell (1905) and has been the basis of many studies.

Recent data obtained from the study of anterograde degeneration in the monkey visual cortex after placing lesions in the lateral geniculate bodies, confirm the existence of degenerating terminals in at least two levels. Wilson and Cragg (1967) have found preterminal fibers in layer IV and some in layer III. Hubel and Wiesel (1969) placed small lesions on single layers of the lateral geniculate bodies and found dense strips of terminal degeneration in the upper part of the lower third of the visual cortex (coincident with Brodmann's IVc) and a less dense, more superficial band of terminal degeneration in about the lower part of layer II. Still Polley (1970) has found terminal degeneration after placing lesions in the lateral geniculate body in the cortical layers IV, lower part of layer III and in layer I.

It is interesting to note the presence of a distinct terminal degeneration in the lower part of layer II in the monkey visual cortex and the non-existence of a similar layer II degeneration in the cat visual cortex, in which Brodmann's IVa is lacking. The existence of at least two different levels of degeneration separated by a terminal free part indicates the existence of major anatomical differences in the intrinsic organization of the internal granular layer in different species.

Layers III and IVa of Brodmann (1905) altogether constitute Cajal's (1899) layer of medium sized pyramidal cells. Layer IVa contains small and medium sized pyramidal cells with bodies 10–15μ on their major axis. They possess a slender apical dendrite reaching undivided layer I with collateral branches distributed in layer IVa (Figure 1, a, c, d, e). The basal dendrites spread downwards in
fantail fashion. Dendritic spines are numerous and thin.

The lower part of layer III is occupied by medium sized (12–18 μ body axis) pyramidal cells. They possess apical dendrites reaching layer I with a moderate number of apical collaterals and abundant basal dendrites which, as was also observed by Bonin (1942), characteristically dive into the subjacent layer (Figure 1, f, g).

The axons of both small and medium sized pyramidal cells of the lower part of layer III and IVa descend to the white matter (Figure 1, 6a, 4f, 3d, 4g, 5e) but they emit always numerous long ascending retrograde and horizontal collaterals. Retrograde collaterals may reach layer I (Figure 1, 5a, 4a, 3f, 3c, 4e) but the horizontal collaterals predominate (Figure 1, 2a, 3a, 2f, 2d, 2g, 3g, 2e, 3e, 4e). These horizontal collaterals form a plexus of fine horizontally running fibers occupying layers IVa and the upper part of IVb to contact presumably dendrites of their own parent cells and the most superficial dendrites of the large stellate and pyramidal cells of layer IVb. This plexus of fine horizontal fibers interweaving with other thick horizontal myelinated fibers, not stained in our material, constitute the stria of Gennari. The descending main axonal branches do not emit collaterals through the rest of layer IVb and IVc but may send a few simple, short collaterals in layer V.

Cajal (1899) described in the human visual cortex, several types of short-axon cells, in his layer III, which have not been observed in our monkey material.

Layer IVb or Layer of Large Stellate Cells

A thin slab of fresh visual cortex placed on a slide on the stage of the microscope permits to recognize the existence of an opaque band, the stria of Gennari, about 220 μ in thickness. Its upper limit roughly coincides with the boundary between the upper and lower half of the cortex. The band is more opaque in its upper zone coincident with layer IVb and with the superficial part of IVc. The opacity to transmitted light mainly is due to the presence of thick myelinated axons and collaterals of large stellate and pyramidal cells of layer IVb.

From the data presented by Cajal (1899), who used very young human material, it is known that the large stellate cells send their axons into the white matter and that their very long horizontal collaterals run throughout the same and underlying levels. Above and below the layer of large stellate cells or layer IVb there are different types of small cells with intracortical axons whose collateral branches and terminal ramifications enter the level occupied by large stellate cells.

In our Golgi sections the bodies of large stellate cells are ovoid, triangular or pyramidal in shape with a major axis of 18–30 μ. The dendrites radiate mainly in a horizontal direction (Figure 4, b, e; Figure 5, a, d) but most of these cells have a characteristic pyramidal shape with well developed apical dendrite reaching layer I (Figure 2, j; Figure 4, b, e, f; Figure 5, b, c). Apical collaterals are distributed in layer IVa and III. The dendrites are densely covered by long stalked spines.

The axon was stained on its initial part except in Figure 4, cell f, which shows a number of horizontal and ascending retrograde collaterals branched out from the two main collaterals 2f and 3f.

Among large stellate and pyramidal cells of layer IVb there are medium sized stellate and pyramidal cells (Figure 1, b; Figure 2, b, e, f, h, k) with dendrites also covered by numerous thin dendritic spines. The bodies of these cells have a major axis in the range of 10–17 μ. There are ovoid cells with two or three branches of horizontal dendrites attached at the poles of the body (Figure 2, b, e), others are more or less pyramidal in shape with basal dendrites distributed within the same layer (Figure 1, b; Figure 2, f, h) and still others are multipolar cells with dendrites irradiating in all directions (Figure 2, k).

The axon originates at the base of the cell body and it is soon resolved into several, relatively straight horizontal and recurrent collaterals. Frequently the main axonal branch after a variable descending course bends upwards to reach layer IVb to run horizontally for long distances (Figure 1, 4b; Figure 2, 2e). In other cases a thin collateral descends to layer V (Figure 2, 3e, 6k). The main axon might enter the white matter (Figure 2, 1b).

A most striking feature is the presence of up to three directly ascending collaterals of the same cell
which could be followed up to layer I (Figure 2, 2k, 3k, 4k).

Layer IVc

This layer shows the most complicated texture of the monkey visual cortex. It corresponds to the layer of small stellate cells or layer V of Cajal (1899) homologous to Brodmann's (1905) IVc or lamina granularis interna profunda and to layer IVb of Bonin (1942). In Nissl preparations it appears filled with small round bodies with scanty cytoplasm and 6–10 μ in diameter. Although this layer is rather homogeneous there is no clear boundary with the overlying IVb. The layer shows however greater cell density towards layer V and the limits separating these two layers are quite sharp. The greatest condensation of this layer corresponds to the IVc subdivision of Polyałk (1957).

Layer IVc is 250–300 μ in thickness, and the lower limit is situated between 950–1,200 μ from the pial surface according to the region studied. A columnar arrangement in lower levels is most patent in Nissl sections. The cell bodies appear piled up forming vertical columns of eight to ten elements, or are grouped into elongated clusters (glomeruli) with major axis in radial direction and separated by clear vertical narrow passages, which in Golgi preparations are occupied by small radial bundles of ascending and descending axons.

We have studied in this layer two types of short-axon cells: stellate cells with spiny dendrites and recurrent ascending axons, and clewed cells with smooth or beaded dendrites and axons which do not leave the dendritic field.

(a) Stellate cells. They can be subdivided into two subgroups according to their size and length of their dendrites. Medium sized stellate cells display round, ovoid or polyhedral bodies with large number of long dendrites irradiating in all directions. The dendrites are covered with numerous long-stemmed spines (Figure 2, c, g, i; Figure 3, b, m, r, s, t; Figure 6, c). These elements predominate in the upper, less dense zone of layer IVc. Many of these cells are identical to the stellate cells described in layer IVb.

The axons of medium sized stellate cells develop descending, horizontal and recurrent collateral systems with different predominances. In cells c and g (Figure 2) horizontal (2c, 3g) and vigorous ascending collaterals (3c, 2g) predominate over thin descending collaterals (4c, 4g). These have been followed, in fortunately oriented sections, to the white matter after leaving small twisted collaterals in layer V. The axon 1i of cell i (Figure 2) sends two thick ascending collaterals (2i, 3i), but a thick descending fiber (4i) reaches layer V where it terminates giving off several collaterals. The axons of cells b, m, r and s in Figure 3 appear to show predominance for ascending collaterals (e.g. 1r, 2s, 3s) over horizontal and thin descending (e.g. 4s) collaterals. The axon 1c of cell c in Figure 6 forms a loop turning into an ascending fiber without emitting collaterals.

Some medium sized stellate cells may appear as pyramidal cells. They have been found rarely (Figure 3, g). Their basal dendrites are slender and extended. A thin, often beaded, apical dendrite reaches unbranched layers IVa and III. The axon (Figure 3, 1g) emits ascending and horizontal collaterals and descends to the white matter (2g, 3g).

The second type of stellate cells or small stellate cells form part of the classical 'granular cells'. Their spherical bodies are 6–8 μ in diameter. A moderate number of thin, thorny dendrites radiate in all directions (Figure 3, a, e, f, i, p, q; Figure 4, d, j; Figure 5, f, l, m; Figure 6, d). Many of these cells appear incrustated, or with several of their dendrites penetrating into densely stained, conspicuous aggregates of bodies, dendrites and fibers of other granular cells forming glomerulus like formations (Figure 3, c, d, h, i, j, k, n, o).

The thin axons of these small stellate cells first descend a variable but relatively short length and then turns upwards forming characteristic loops (e.g. Figure 3, 1o). The axon of each cell may form a single loop or may emit two, three and even four collaterals forming the same number of loops all turning into ascending fibers. These arciform fibers form a series of small ascending bundles (Figure 3) passing between adjacent glomeruli (gl.) to reach layer III and IVa. A group of these small bundles can be seen in Figure 1, 7. They traverse the zone of the stria of Gennaró without emitting collaterals to finally reach layer III where each
axon or fiber branches off into three to five short, beaded terminal fibers (Figure 1, 8). They may contact with dendrites of medium sized pyramidal cells of layer III.

Further examples of small 'granular' stellate cells with axons completely impregnated are given in Figures 3 and 5. The axon If of cell f in Figure 3 branches off forming three loops continued into the three ascending fibers 2j, 3j, and 4f reaching layer IVa. A descending collateral (5f) enters layer V. The axon If turns into the two ascending fibers 2j and 3j. Likewise the axons of cells l and m in Figure 5 divide into two ascending fibers each one (lh and Im respectively) to reach layer IVa where they end.

A number of short collateral branches are given off frequently by many of these axons at the level of their loops (Figure 3, axons of cells f and j). These collaterals enter the adjacent neuropil to contact dendrites of their own parent cells and neighbour cell clusters or glomeruli. Other collateral branches have been followed descending to layer V (Figure 3, 3e) where it has been impossible to trace them further downward on account of the extreme fineness of these fibers. We believe they do not enter the white matter.

(b) Clewed cells These cells are a little bit larger than small stellate cells since they may be 10 μ in diameter. They have been found specially abundant in the lower, cell-dense part of layer IVc. Their bodies are slightly ovoid in shape. Their beaded dendrites are almost devoid of spines and the axons and their collaterals remain closely interwoven in their own dendritic field. These features give the cell a characteristic appearance of a ball of thread. The Golgi image provides an easy identification and contrast with the small stellate cells with recurrent axons described above.

Clewed cells can also be considered as 'granular cells'. They have been called spider-cells (Cajal, 1899), cells with glomerular axons (Lorente de Nó, 1922), sem-like cells (Beritoff, 1965) or class II small stellates (Globus and Scheibel, 1967). The number of dendrites per cell is low. Dendrites are relatively smooth and uniform in certain lengths but usually they appear beaded and complexly twisted around branches of their own axons. This made it very difficult to draw their complete axonal trajectories and for this reason our examples are few (Figure 5, e; Figure 6, a, b).

The axon, always difficult to recognize, has the same caliber as their smooth dendrites and may issue from any point around the body. It is soon resolved into numerous collaterals which cross repeatedly the domain of their own and neighbour dendrites occupying spherical or elongated volumes of 80–150 μ in diameter in which 300–500 cell bodies can be included. Clewed cells are apparently as numerous as small stellate cells.

The glomeruli The New Latin word 'glomerulus' diminutive from the Latin word 'glomerus' (a cluster of outgrowths) was used originally by Meynert (1871) to describe circumscribed small and round granular masses of nuclei and fibers in the olfactory bulb: the olfactory glomeruli. The term glomeruli cerebellares was coined by Held (1897) as 'lokalisirte Vereinigungsstelle von Axencylinderend-protoplasma und Dendritenend-zweigen bedeuten'. Lorente de Nó (1922) described as cortical glomeruli certain clusters of short-axon cells in which the dendrites and axonal branches remain within them, separated by clear vertical bands from adjacent clusters. The term glomerulus has been applied later in electron microscopy to other well known specific points, where closely packed dendritic processes and axons occur, as in the lateral geniculate nuclei (Szentágothai, 1963).

Under the same point of view as Lorente de Nó (1922) we have retained here the term glomerulus to designate those conspicuous clusters of cell bodies, dendrites and fibers which occur in the lower zone of layer IVc (Figure 3, gl.).

As mentioned before the size of each glomerulus may correspond with the axonal spread of a single clewed cell, which is the cell type most frequently found within the glomeruli, i.e. 80–150 μ in diameter. Glomeruli appear as round or elongated masses of complexly twisted fibers, axons of intraglomerular cells, and cell bodies enmeshed in dendrites and other axonal collaterals. The formation is often surrounded by deeply stained glial formations. The periphery of the glomerulus and the spaces between them appear much more clear to the observer. They are occupied by the first type
of granular cells, the stellate cells, whose dendrites penetrate within the glomeruli and whose axons turn, after forming their characteristic loops, into ascending fibers to reach layers III and IVA. Inside the glomeruli small stellate cells with spiny dendrites can also be found.

Figure 3 shows four glomeruli (g). In this Figure c, h, i, n and o are intraglomerular cells of the small stellate type. Cells d, j, k are other small stellate periglomerular cells with dendrites penetrating the glomeruli and whose axons ascend to higher layers.

In Figure 6 two intraglomerular (clewed type) cells could be clearly traced (a and b). The axonal spread of their axons (ia and ib) encompassed one glomerulus in which cortical afferent fibers (in red) were seen to arborize. At the periphery, cells c, d and e (a large and two small stellate cells) send their axons 1c, 5d and 3e into an ascending direction.

Glomeruli and their host of surrounding stellate cells may represent series of functional units devoted to receive and integrate cortical afferent inputs and address the resulting information to layers III and IVA through the fascicles of ascending fibers issued from the stellate cells whose dendrites have penetrated the axonal volumes encompassed by intra-glomerular cells.

Layer V

This layer corresponds to the inner rarefied zone observed in Nissl preparations between the cell-dense Brodmann's layers IVc and VI. It contains pyramidal and other fusiform or round stellate cells with ascending axons, and the giant solitary cells of Meynert (1871).

In his first description of the human visual cortex Cajal (1899) described a separate layer underneath the layer of small stellate cells, layer VI or layer of small pyramids with ascending axons, that he had retained in his book (Cajal, 1911). Cajal (1899) had mentioned however that it was difficult to delimit this layer from the subjacent layer of giant solitary pyramidal cells, what might explain its inclusion, in his latter study of the cat's visual cortex (Cajal, 1922), as the inner substratum of the layer of large stellate cells.

The giant solitary cells of Meynert (1871) in man form a conspicuous stratum in the central part of layer V. For this reason this layer has been subdivided into three sublayers, being the giant solitary cells located in the middle, or sublayer Vb, according to Bonin (1942). This author mentioned however that in lower monkeys the giant solitary cells are situated in the lower dark band, or Vb + Vc subdivisions of layer V.

According to our observations in the monkey, the pyramidal cells with ascending axons predominate in the upper part of Brodmann's layer V, corresponding to sublayers Va of O'Leary (1941), Bonin (1942) and Lorente de Nó (1949). They should not be placed into a separate stratum since they exist also below the stripe of the giant solitary cells of Meynert and even in the upper part of layer VI. In fact Cajal (1899) observed also pyramidal cells with ascending axons underneath giant solitary cells. O'Leary (1941) mentioned that in the cat Va pyramids with arciform axons occur at the same level as the large solitary cells. Campbell (1905) mentioned their existence in the upper relatively giant-cell free part of his layer VI.

The giant solitary cells of Meynert (1871) appeared stained frequently in our preparations occupying according to Bonin (1942) the lower part of layer V. Their axons were never stained in our present material and nothing could be added to the descriptions given by Cajal (1899) and Le Gros Clark (1942) except to mention that their apical dendrites may reach layer I, although they usually end at different levels (Figure 4, 8, 9, 10) and that the horizontal spread of their basal dendrites may be 300 μ in length at each side of the body.

Pyramidal cells with ascending axons have been reproduced in Figure 5 (cells g, h, j, k). Most of them lack a well defined apical dendrite. Cell j has a typical pyramidal shape with apical dendrite reaching layer I but cells g, h and k display round or fusiform bodies with slender apical shafts not exceeding above layer IVc. On the other side cells o and p cannot be recognized as pyramidal

FIGURE 5 Section perpendicular through the medial calcarine sulcus, superior lip. Medium and large stellate and pyramidal cells have been reproduced in sublayers IVa and IVb. Their axons appear stained in the initial segment. Stellate cell f possesses an axon ramifying into ascending, descending and horizontal collaterals. Other stellate cells of sublayer IVc are cells l and m with axons clearly visible turning into ascending fibers (II and Im) to reach IVa and IVb where they may contact dendrites of cells like a through d. Clewed cell e isolatedly stained, whose axon was impossible to trace, shows characteristic beaded, spine-free dendrites. Below the limits separating IVc from V a number of pyramidal cells with recurrent ascending axons have been reproduced. The bridge between the V–IVA level and higher levels (IVa and superficial layers) is established through their ascending axons like fiber I and the collaterals 3j, 2p, 1o. The depth from surface of cell j is indicated. Golgi method. Camera lucida drawing. Adult Erythrocebus patas.
cells, they rather remember stellate cells of layer IVc (Figure 5, l, m, n).

Curiously enough it will be noted that although the cells of layer V (excluding the giant solitary cells) have been grouped under the term of layer of pyramidal cells with ascending axons, true pyramidal shaped bodies are not precisely pre-dominant. Cajal (1899) was aware of this fact and have described three types of cells in which true pyramidal cells were quoted as forming but a small fraction.

The axons of these cells descend for a variable distance. They may enter layer VI, but always turning into an ascending course. Either the main axon itself bends ascending vertically, or a number of directly ascending collaterals are given at the loop. The axon of cell g (Figure 5, lg) could be followed to layer I. A relatively thick axon, from a cell body not stained, follows a similar course (labelled l in Figure 5) ascending to layer I. There is a thin, horizontal collateral (labelled 2) coursing through the dendrites of large stellate cells (a through d) in layer IIV. The axon of cell k develops into several loops from which three long ascending collaterals can be followed. The main descending axon (jj) of cell j also loops into a number of ascending branches among which 2j and 3j can be followed for relatively long ascending distance. Short side branches and small twigs appear to contact dendritic spines of the same cell at s, s.

The axonal loops of cells k and p (located in layer VIa) can be clearly observed, they continue into ascending fibers Ik and 2p respectively. The axon of cell o forms a loop and ascends divided into two unequally thick branches (lo) ending in layers IVA and IVB.

All axonal loops that we have mentioned give off a variable number of relatively short collateral branches distributed below their corresponding cell bodies and coursing through their basal dendrites. Some very thin collaterals descend toward the white matter but their entrance into it is questionable.

The collateral branches given off by the axonal loops in layer V intermingle with other collaterals that we have mentioned earlier, i.e., collaterals of the descending axons of some medium sized pyramidal and stellate cells of layers IVA, IVB and IVc which presumably contact the dendrites of cells of layer V. In some preparations a delicate tangle of thin fibers throughout layer V is patent. It is composed by the interlacing of both types of collaterals.

Cortical Afferent Fibers. General Discussion

In 1883 Monakow, after experimental section of the posterior limb of the internal capsule, reported the existence of marked cell atrophy in Meynert's (1871) granular and multipolar layers in the homolateral visual cortex of the rabbit.

Monakow's (1883) statement that the lateral geniculate bodies are directly and specifically connected with certain cells in the visual cortex was confirmed within the next years through many experimental and pathological observations. In 1896 Leonowka introduced in his paper a diagram with clear indication of the termination of geniculo-cortical axons upon Monakow's 'Schaltzellen' (Golgi type II cells) in the granular layer of the visual cortex. The schema was made to explain the pathway for pupillary reflexes but it was surprisingly advanced for that time.

A few years before Cajal (1891) had described for the first time in Golgi preparations of young mice coarse ascending fibers coming from the white matter and ramifying among small and medium sized pyramidal cells in the upper half of the cortex. He recognized these fibers as the thickest ones in the cortical gray substance. They were described following oblique, horizontal or zig-zag courses and subdividing frequently into numerous free terminal endings. At that time Cajal (1891) did not commit himself as to indicate their origin but had mentioned that they must play a very important role in the cortex. They were termed Ramon'sche Fasern by Kölliker (1893).

It was not until 1899 in which Cajal based on his
own pathological and Golgi observations in newborn babies and on Cramer’s (1898) report, supported his view of the formation of the stripe of Gennari by afferent optic fibers. Cajal’s (1899) reasoning was fully substantiated by Polyak (1927) who traced degenerating Marchi geniculo-cortical fibers in the cat to end in Cajal’s (1899) layers 4 and 5 corresponding to layers IVb and IVc of Brodmann (1905).

In 1922 Cajal and Lorente de Nó gave independently detailed descriptions of the terminations of specific afferent fibers in the visual cortex of the cat and in the acoustic cortex of the mouse respectively. Later O’Leary (1941), also in Golgi preparations, showed cortical afferent fibers and their end-arborizations in layers Iva and IVb of the cat’s visual cortex. As it was shown by Lorente de Nó (1922) the cortical afferent fibers develop into denseplexuses which, in the case of the acoustic cortical area of the mouse, remain confined within the glomeruli he had described in this cortex, sending but a few ascending fibers to reach layer III. Cajal (1891, 1899) had mentioned also the existence of some ascending fibers reaching layer I. We have shown with the Golgi method, cortical afferent fibers at the levels of layers III and IV in the visual cortex of the mouse, ending upon short-axon cells and on the basal dendrites of pyramidal cells in the lower part of layer III (Valverde and Ruiz-Marcos, 1969; Ruiz-Marcos and Valverde, 1970).

It was Polyak (1932, 1957) who first suggested that visual afferent fibers in the stria of Gennari are only one of several contributing factors and that not all visual fibers end in that stria. Le Gros Clark and Sunderland (1939) in their studies of isolated visual cortex of Macaca concluded that the stria of Gennari is predominantly composed of endogenous fibers.

With the advancement of actual refined techniques of anterograde degeneration it has been possible to confirm Golgi observations demonstrating the exact level of termination of geniculo-cortical fibers in layer IV. Sustaining terminations of thalamic origin, ending above layer IV, have been reported by Nauta (1954), by Wilson and Cragg (1967) in the cat and monkey, and by Ebner (1967) in the opossum. In mouse we have shown that about 65% of all degenerating particles recognizable as degenerating axon terminals were localized in layer IV of the visual cortex after lateral geniculate coagulation, while about 20% of these particles were observed above it (Valverde, 1968).

In the monkey, the cortical distribution of specific afferents shows interesting differences with respect to other species. In this animal, terminal degeneration as the result of lateral geniculate destruction, appears to be entirely confined to area 17. Very little terminal degeneration is present in layer I. There is a thin lamina of degeneration in layer III remarkably well separated by a terminal-free zone of about 100–200 µ thick from a much thicker zone of terminal degeneration in layers IVb and IVc (Hubel and Wiesel, 1969; Polley, 1970).

Our present study is in agreement with the last mentioned observations. First, we have identified two different levels in which afferent cortical fibers arborize: the deep zone of layer III on one side, and the lower half of IVb and the entire IVc on the other side. Second, there is a zone between both levels IVa and upper half of IVb altogether coinciding with the opaque (Gennari’s) band in which terminal arborizations of cortical afferents have never been observed. It corresponds quite clearly with the terminal free degenerating zone observed by Hubel and Wiesel (1969) and Polley (1970). Therefore, concurring with Le Gros Clark and Sunderland (1939) and with Wilson and Cragg (1967) we believe that in the monkey the stria of Gennari has no significant terminal ramifications of specific cortical afferents.

Figure 4 shows seven fibers (labelled 1 through 7) arborizing in the lower half of layer IVc which we have identified as cortical afferent fibers. They are the thickest fibers observed in the visual cortex. Fibers 1, 5, 6 and 7 could not be traced below layer V, but 2, 3 and 4 could be traced to layer VI. With the condenser front lens swung out, some of these fibers were seen to abort, deep in layer VI, into faintly contrasted tubelike segments which we interpret as their myelinated envelope. Some of these fibers were traced directly to the white matter. They are probably, in this case, collaterals branched out within the white matter.

The cortical afferent fibers were seen to branch off in layer IVc. Some divide into oblique ascending collaterals but most branches are given out at right angles from the parent fiber. These secondary branches follow parallel horizontal courses (Figure 4, collaterals of 1 and 2). They are characteristically provided with very numerous short clubbed side branches forming in certain points dense grape-like aggregates of terminal endings (Figure 4, 1, 2).

In particular zones intermixing of dendrites of short axon cells, terminals of cortical afferents and terminals of other axons occur. They form such
dense clusters of synaptic complexes (Figure 4, s.c.) and dendritic appendages that it is very difficult to detail each constituent.

Figure 4 shows several such clusters of synaptic complexes on the neighborhood of cells d and i in which their dendrites intervene. Two synaptic complexes close to cell l have been detailed within the amplified circle in A. Grape-like terminals (t) of the afferent fiber 6 appear stained isolatedly. Two clusters s.c. show similar terminals riding upon dendrites d of the short-axon cell l with intervention of terminal small branches of the intracortical axon 11.

It seems apparent that these synaptic complexes might represent important sites of synaptic interaction of special interest for electron microscopical studies.

Figure 1 shows another group of cortical afferent fibers arborizing in the upper part of layer IVc and in the lower part of IVb (fibers 1, 2, 3, 5 and 6). Their collaterals show the same characteristics as those described in Figure 4. Only fiber 3 could be followed downward to the white matter. In Figure 1 the cortical afferents 4 and 9 are two examples of those fibers arborizing in the lower part of layer III. They are probably the same as those degenerating fibers traced by Hubel and Wiesel (1969) and Polley (1970) in layer III. Compare the different type of terminal ramifications of intracortical ascending recurrent axons (8) with the terminals of cortical afferents. Single fibers have been observed emitting collaterals in the lower part of IVc ascending later to higher levels (see fiber labelled 3 in Figure 1). We could not trace cortical afferents in layer I.

The Golgi technique appears to avoid the staining of adjacent structures. For this reason it has been always difficult for the identification of the element post-synaptic to cortical afferent terminals. In the present study we have seen cortical afferents to contact upon clewed cells as well as on dendrites of stellate cells with ascending axons in layer IVc (Figure 6). Contacts also may be established with the apical shafts of the giant solitary cells of Meynert (Figure 4, 8, 9, 10). Also it may be possible that the basal dendrites of large stellate and pyramidal cells of layer IVb receive direct contacts from cortical afferents.

Terminal ramifications of cortical afferents in layer III may establish contacts with the basal dendrites of the small pyramidal cells found there. The location of dendritic spines along the basal dendrites which tend to run in a horizontal course as the collaterals of cortical afferents greatly increases the possibility of such contacts.

Colonnier and Rossignol (1969) have found in electron microscopy degenerating endings mainly on dendritic spines, although some were found also on stellate cell bodies and dendrites at the level of layer IV in the cat's visual cortex (Colonnier, 1968).

In Figure 4 cells d, h, i and l, which seems most directly related to various synaptic complexes of afferent fibers, are short-axon cells with spiny dendrites (cell d) or with smooth dendrites (cells h, i, l). Synthetic contacts of cortical afferents on the last type of cells have been drawn in Figure 6 in which characteristic grape-like terminals of cortical afferents (in red) can be seen in contact with dendrites and bodies of the clewed cells a and b. There are also contacts on cell e which displays dendrites with few spines, and contacts upon cell c, a medium sized stellate cell with long dendrites densely covered by spines. Cells e and c are two periglomerular cells whose axons ascend to layer III.

We believe that cortical afferent fibers do not select any specific dendrite or cell type. It seems more reasonable to think that afferent fibers may contact potentially all kinds of neurons and dendrites they encounter but, it might well be that many of these synapses become inoperative while others would contact active units of specific intracortical circuits. These intracortical short-axon neuronal subsystems appear to be highly complex in the monkey visual cortex. The data presented in this work indicate that clewed cells in the glomeruli receive direct input from cortical afferents. From here periglomerular stellate cells with their spiny dendrites penetrating the glomeruli relate an increasing number of other cells through their radially ascending and descending axons, to reach finally the large stellate cells of layer IVb and the solitary giant pyramidal cells of layer V, i.e. the two apparent major projecting neuron types of the monkey visual cortex. Sholl (1955) has pointed out that in the cat the presence of a large group of neurons with recurrent axon collaterals beneath the Gennari zone leads to secondary influences (through reverberating circuits) at the higher level. The existence of reverberating circuits in the monkey visual cortex appears to be greatly emphasized through the complex systems of ascending axons of short-axon cells.

It should be finally suggested that the cortical circuitry relating sequentially intraglomerular cells with stellate periglomerular cells which send all
their ascending axons to reach layers III and IVa, and the many cells of these layers sending their axonal collaterals to higher layers, horizontally or descending again to layer V, may represent the substrate of the functional columns described by Hubel and Wiesel (1968). The organization of the visual cortex into such vertically interconnected systems, which might coincide in part with particular anatomical models, does not imply however the existence of anatomically determined compartments, it simply indicates that there is an aggregation of cells according to certain physiological properties. This problem has recently been discussed in a Work Session (Chow and Leiman, 1970) where different points of view have been expressed.

REFERENCES


Note added in proof: Since this report went to press, Spatz, Tigges and Tigges have published a paper (Spatz, W. B., Tigges, J., and Tigges, M., 1970, Subcortical projections, cortical associations, and some intrinsic interlaminar connections of the striate cortex in the squirrel monkey (Saimiri), J. Comp. Neurol., 140: 155–174) reporting the existence of a strong projection of interlaminar fibers upon layer V in the striate area of Saimiri. Their findings, using the Fink-Heimer technique, concerning intracortical neuronal chains are in agreement with our present observations.