A Study of the Organization of Apical Dendrites in the Somatic Sensory Cortex of the Rat

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ABSTRACT

In the parietal cortex of the rat, sections cut tangentially show that profiles of medium and large apical dendrites are grouped into clusters. The number of apical dendrites in each cluster is variable and the usual separation between individual clusters is about 50 μ. Despite these variations the pattern does not appear to be random. Reconstructions from one micron serial sections show that neurons giving rise to the ascending dendrites forming clusters are located at different levels in layer V. The cell bodies of these neurons are arranged vertically below their dendrites and show a tendency to form groups. All of the neurons have apical dendrites that ascend through the cortex with a few secondary branches occurring close to the base. The principal secondary branching begins in layer III and spreads obliquely up through layer I. Furthermore, beginning in the inferior region of layer III apical dendrites are added to the clusters at their peripheries. These are from layer III pyramids. It is clear that the superior aspects of the cluster arrangements must intermingle with those of the neurons in adjacent clusters. The neuropil surrounding the dendrites forming clusters appears to contain a few smaller dendrites. Small unmyelinated axons are the most frequent component of the surrounding neuropil and these form terminals which synapse on the spines and trunks of the clustered dendrites. There is no obvious function that can be ascribed to the clusters other than they may form a component of the columnar organization in cortex described in part by physiological techniques.

The concept that the cerebral cortex is organized by interconnected cells forming vertical columns was presented by Lorente de Nó ('48) in his comprehensive studies of the neuronal architecture of the mammalian cerebral cortex. This hypothesis was given considerable credibility by Mountcastle ('57) on the basis of micro-electrode studies of S1 cortex in the cat. He showed that if an electrode is passed in a direction normal to the cortical surface, the successive neurons along the path of penetration are excited by the same stimulus at the body surface. Further, not only do the neurons in a vertical column respond to the same modality type, they are also related to nearly identical peripheral receptive fields (Mountcastle and Darian-Smith, '68). With regard to any possible anatomical substrate, Mountcastle was unable to determine the exact widths of the vertical columns but stated that they are at least one and probably several cells wide. From the results of slanting electrode penetrations, it appeared that the columns were not wider than 500 μ. This varied in different areas. Since then other sensory cortices have been examined and a functional columnar organization appears to occur in each of them (see Chow and Leiman, '70). There are, however, differences in the form and dimensions of the columns in different parts of the cortex. In the auditory cortex of the cat, Abeles and Goldstein ('70) found the columns to be about 100 μ wide with centers separated by a distance of about 150 μ. In the visual cortex of the monkey, Hubel and Weisel ('69) found eye preference columns to have the form of vertical slabs or stripes about 350 μ wide.

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Abeles and Goldstein ('70) pointed out that from Nissl stained preparations used to examine the paths of their electrode preparations, neurons are arranged in radial chains, and that in these chains the neurons have nearly equal best tuning frequencies. von Bonin and Mehler ('71) also observed radial chains or columns of neurons using blocks of tissue from human and the macaque cortex stained either by cresylecht violet or by the Kluver-Barrera ('53) technique. The Kluver-Barrera technique in addition, showed radial bundles of processes which apparently represent the apical dendrites of pyramidal neurons. According to their account von Bonin and Mehler ('71) state that the widths of the columns vary, in some places being five to six cells wide and elsewhere only two to three cells. The greatest distance between columns is given as 80 μ.

In a somewhat different investigation dealing with the vertical organization of elements in the cerebral cortex, Woolsey and Van der Loos ('70) have described "barrels" in layer IV of the somato sensory cortex of the mouse. In the head and face-limb region of S1, they found cylindrical structures, whose limits are defined by concentrations of neurons, which are of two types. Large ones whose diameter is between 150 and 380 μ that seem to correspond to the receptive fields of the large vibrissae on the snout and smaller ones that have diameters of about 100 μ. Similar barrels have also been found in the somato sensory cortex of the rat (Welker, '71) and the brush-tailed possum (Weller, '71). Since the diameters of the barrels are within the size range given for the functional columns, Woolsey and Van der Loos ('70) argue that the barrels may represent their morphological counterpart within layer IV.

Colonnier ('66) also considered which anatomical features of the organization of the cerebral cortex might account for the physiological columns, and proposed that the fusiform stellate cells are involved. He suggested that these stellate cells which have vertically oriented axons extending through the entire depth of the cortex, make "climbing" contacts with the apical dendrites of pyramidal cells and activate them. Colonnier ('66) further suggests that the activation by those same axons, of other fusiform stellate cells would presumably potentiate the vertical spread of activity and that simultaneously the basket-like cells in layers II and IV might inhibit cells to the sides of the vertical unit through their pericellular terminals and so give rise to an inhibitory periphery.

In our laboratory during an electron microscopic study of the parietal cortex of the rat, sections cut tangentially or parallel to the surface of the cortex, were examined. In this plane, and particularly in those sections passing through layers III and IV of the cortex, it was found that profiles of medium and large dendrites were grouped into clusters. The number of such dendrites in each cluster was variable and the separation between individual clusters was usually about 50 μ. This observation led us to carry out a preliminary electron and light microscopic study of the clusters to determine both their distribution within a particular topographical area and their composition.

So far we have examined the somato sensory cortex, the primary auditory cortex and the visual area in the cat and rat. Clusters of dendrites are present in each of these areas, although their size and distribution is variable. In the present account we will describe only the clusters as they appear in Area 3 (Krieg, '46) of the rat cerebral cortex, since this is the area we have studied most completely. A comparison between this region and other primary sensory cortices in the rat and cat will form the subject of a later article.

MATERIALS AND METHODS

For the preparation of tissue to be examined with the electron microscope, and with the light microscope in the form of 1 μ sections, the brains of young adult rats were fixed by vascular perfusions with the two aldehyde mixtures suggested by Reese and Karnovsky ('67). The first mixture contained 1.25% glutaraldehyde and 1% paraformaldehyde in a 0.08 M cacodylate buffer or pH 7.2. The perfusion was initiated with this solution and then completed with a stronger one containing 5% glutaraldehyde and 4% paraformaldehyde in the same buffer. Both solutions were warmed to 40°C before use and the rats were re-
spired with a 95% oxygen-5% carbon dioxide mixture until the perfusion was commenced.

After fixation, the brains were left in the skull overnight. Next day blocks of the somatic cerebral cortex containing Area 3 as defined by Krieg ('46), were removed and post-fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer. The specimens were then dehydrated in ethanol and embedded in Araldite. These sections were stained with uranyl acetate and lead citrate (Venable and Coggeshall, '65) before examination in an AEI EM6B electron microscope.

For examination in the light microscope, 1 μ sections from these same blocks were cut and stained either with 1% toluidine blue in 1% borax or with a mixture of 1% toluidine blue in 1% borax and 1% pyronin B in water, in equal parts. After staining, the sections were differentiated in absolute methyl alcohol and cover slips mounted with unpolymerized araldite. In a number of cases, sections parallel to the lengths of the apical dendrites were taken and then the blocks turned through 90° to produce serial 1 μ sections oriented tangential to the surface of the cortex. By this procedure we were able to determine the exact depth at which a tangential section had been taken.

For additional light microscopic analysis, paraffin embedded rat brains were serially sectioned and stained with cresyl-echt violet. Other brains were stained by the Van der Loos ('56) modification of the Golgi-Cox procedure.

RESULTS

Figure 1 shows a relatively high magnification micrograph of a typical cluster of large and medium sized dendrites as seen in a tangential section of layer III in Area 3 of the rat cortex. The neuropil surrounding the dendrites forming the cluster also contains a few smaller dendrites, some in cross section and others obliquely oriented. However, the most common component of the surrounding neuropil is the small unmyelinated axon. These form axon terminals that synapse predominantly upon the spines of the dendrites and occasionally upon the dendritic trunks. In the lower magnification micrograph of figure 2, a more complete picture of the surrounding environment of a cluster is shown as it appears in layer IV. The cluster occupying the lower portion of the micrograph contains nine medium to large dendrites and at the upper edge of the cluster is the cell body of a neuron. This is the typical location of neuronal cell bodies relative to the clusters, for they are almost never imposed between the dendrites.

In Nissl preparations, Area 3 of the rat cerebral cortex (fig. 3) is characterized by a cytoarchitectural organization which is clearly of the parietal type: Cell bodies in layers. In the lissencephalic brain, areas layer II above and IV below. The deeper pyramids in III do not approach the size of the very large pyramids in layer Vb and layer IV consists of closely packed, small granular cells which form a distinct lamination distinguishing it from all other layers. In the lissencephalic brain, areas not characterized by a bending of the cortex are very seldom seen to have their neurons arranged in vertical strings. However, close examination of layer V does show arrangements which could be interpreted as small vertical groups of neurons stacked one on top of another.

In the rat cortex, Area 3 is the most laminated of all cortical regions. That is, the cellular layering may be readily discerned in its vertical orientation and because of this it is easily distinguished from the surrounding regions. Most prominently, Area 3 is singled out from Area 4 medially by its very granular fourth layer and the presence of a greater number of large pyramidal cells. Laterally, layer V cells in Area 2 are less numerous and somewhat smaller than in Area 3. Also layer VI is more sparsely populated. Area 3 is situated between Area 4 and 7, and we found that in young adult rats, measuring from front pole of the hemisphere, Area 3 is located in a position that is 0.48–0.51 of the entire anteroposterior length of the hemisphere. This is consistent with other findings that in the adult lissencephalic brain, the positions of the Areas of cortex are proportionally constant (Walsh and Ebner, '70). Area 3 is cytologically and cytoarchitecturally distinguished quite readily in both Nissl preparations (fig. 3A) and in 1 μ plastic sections (fig. 3B).
Fig. 1 An electron micrograph of a tangential section through layer III of the cerebral cortex. In the field is a cluster of transversely sectioned dendrites (D). These dendrites have spines (s) that protrude into the sounding neuropil to receive axon terminals (t) which synapse upon them. Note that a number of smaller dendrites (d) are also present within the cluster. × 13,000.
Fig. 2. A low power electron micrographic montage of a tangential section passing through layer IV. At the bottom of the field is a cluster of dendrites (D) and at the edge of the cluster is a small neuron (N). Note the fixed distribution of large dendritic profiles in the field. × 6,000.
Fig. 3 Light micrographs of vertically oriented sections of Area 3. Figure 3A on the left, is a Nissl stained preparation which stains the cell bodies. On the right, figure 3B, is the same region as it appears in a 1 μ plastic section. The neuronal cell bodies are also visible in the plastic section which in addition clearly shows the clusters of apical dendrites apparently emanating from layer V.
Fig. 4 A tangentially oriented plastic section of Area 3 taken through layer IV. For clarity, the apical dendrites forming the clusters have been outlined. At this level, most of the neurons present are stellate cells.
One-micron plastic sections have a different appearance from Nissl preparations (figs. 3A, B) because all of the cells and their processes, both neuronal and neuroglial, are visible. Principally important is the fact that the medium to large dendrites forming the clusters may be seen. The basic features described above for characterizing Area 3 in Nissl preparations are readily discernible in these plastic sections and have been used to identify this area. In practice, once the limits of Area 3 have been defined in vertical sections such as those shown in figure 3A and 3B, the plastic blocks are turned through 90° and the tangentially oriented sections obtained.

A tangentially oriented thick plastic section of Area 3 taken at a depth within layer IV is illustrated in figure 4. At this level, the clusters of apical dendrites recognized in the electron microscope preparations are readily apparent. To display their distribution more effectively, outlines of the individual dendrites have been traced with India Ink. At this level in cortex the clustered dendrites have diameters of between 8 and 3 μ. It is also apparent that the numbers of dendrites within a cluster is variable. Commonly four to six dendrites make up a cluster, but other clusters may contain as many as 14 dendrites and occasionally a single dendrite is isolated. Despite these variations, the pattern does not appear to be a random arrangement of single or small groups of dendrites. The most frequent distance separating the clusters is about 50 μ, but between large clusters it may be as great as 150 μ.

The tangential appearance of Area 3 at different depths is shown in figures 5–8. These sections are taken from a set of serial 1 μ sections and the fields may be superimposed, in the sense that they are along the same vertical axis, so that the cluster indicated by arrows is the same one at each depth. Figure 5 is a section taken at the level of mid layer V; figure 6 at the level of layer IVb; figure 7 at the lower level of layer III and figure 8 at the upper level of layer III.

In figure 6 the field is at a similar depth to that shown in figure 4. In each, the clusters are interspersed between the perikarya of the layer IV stellate cells, whose dendrites pass both into and between the clusters of apical dendrites which, as will be shown later, originate from layer V pyramidal neurons. At this level in cortex, most of the medium sized randomly oriented dendrites in the neuropil appear to belong to the stellate cells. Many of the finer caliber dendrites which appear in cross section interposed around and between the clusters of the layer V apical dendrites, seem to originate in layer VI.

Examination of tangential sections taken in layer V (fig. 5) gives the clue as to the origin of the large dendrites forming the clusters, since the numbers of such dendrites are greatly reduced at this level. Here, the most prominent elements are the cell bodies of large pyramidal neurons and scattered between them are many small, vertically oriented dendrites originating in layer VI (fig. 3). These layer VI dendrites extend upwards so that they are also apparent in layer IV as small diameter elements present in the neuropil around and between the clusters (fig. 6). Nearer to the surface of the cortex, however, these layer VI dendrites can no longer be so clearly distinguished.

At those levels above layer IV the clusters of layer V apical dendrites are still apparent, although in layer III (fig. 7) the dendrites are less massive, since they become more slender as they ascend through the cortex (see also figs. 3, 10). Furthermore, beginning at about the level of layer III (fig. 7), other apical dendrites of somewhat small caliber are added to the clusters, in particular at their peripheries. These are the apical dendrites of the layer III pyramidal neurons (fig. 7). The continuous addition of these layer III...
dendrites as the clusters pass towards the outside of the cortex, and the beginning of the bifurcations of the larger layer V apical dendrites into small branches (fig. 12), leads to a quite different appearance of the clusters when they are examined in the upper part of layer III (fig. 8). Instead of well delineated clusters of mainly large diameter dendrites, fields of small diameter dendrites occur and it is no longer possible to distinguish the apical dendrites of layer V pyramids from those derived from layer III pyramids.

Thus far, it has been assumed that the large diameter profiles that form the cores of the clusters are all apical dendrites of layer V pyramidal neurons. This is in fact true. To establish the point, however, and to determine that none of the ascending dendrites take origin from layer III or IV neurons, involved making reconstructions of clusters and their neurons of origin from serial sections. These serial sections were taken both in vertical and tangential planes and the results are shown in figures 9 and 10. In both cases, the reconstructions extend only as far as layer III, since, as pointed out above, nearer to the outside of the cortex the large apical dendrites begin to branch and to become intermingled with the apical dendrites of layer III pyramids. This makes the further tracing of the apical dendrites of layer V cells an impossible task.

In figure 9, the reconstruction of the cluster indicated by arrows in figures 5-8 is shown. This cluster contains thirteen apical dendrites and their interrelations as they pass upwards toward the outside of the cortex is shown in this serial illustration. For the most part, the apical dendrites follow an almost straight path towards the surface of the cortex and maintain their positions relative to one another; although a few somewhat bend and twist. This is also shown in figure 10, which is the reconstruction based on a set of vertical serial sections and shows a small cluster containing four apical dendrites. In this illustration, the positions of surrounding neurons are also shown so that the layering can be compared with that of the Nissl preparation in figure 3A.

Both of these reconstructions show that the neurons giving rise to the ascending dendrites within any one cluster are located at different levels in layer V. None appear to originate in layer VI. Hence, one might conceive of a column of cell bodies giving origin to a cluster and indeed such an arrangement of layer V cell bodies is sometimes perceived in classical Nissl preparations of Area 3. To determine whether this interpretation is correct, a reconstruction of the positions of layer V pyramidal cell bodies, relative to the clusters they form, has been made. The result is shown in figure 11. In this figure, the positions of apical dendrites composing clusters in layer IV are shown as blackened dots and the layer V pyramidal cell bodies giving rise to them are stippled. Hence, the illustration must be viewed as though the observer is looking vertically downwards through the cortex from layer IV to layer V. It will be seen that the cell bodies are arranged vertically below their dendrites and that the cell bodies show a tendency to form groups. There is some overlap between the groups of cell bodies, however, and many of the cell bodies are displaced laterally away from the center of the cluster to which they contribute dendrites. Appearances compatible with this arrangement may be seen in both 1 μ plastic sections and Golgi preparations oriented vertically to the surface of the cortex. For the perikaryon of some layer V pyramidal neurons are tilted away from the vertical axis and apical dendrites often bend slightly at their base before assuming a more vertical trajectory.

In Golgi preparations, clusters are not readily apparent. This is not surprising, since only a small percentage of the neurons present are impregnated by the Golgi technique. Groupings of some two to four cells from layer V have been seen, however, and a drawing of such a group is shown in figure 12. Again it is apparent that the cell bodies of the neurons are at different levels in layer V. All of the neurons have apical dendrites that ascend through the cortex, and that of the deepest neuron bifurcates in layer IV. A few secondary branches occur close to the bases of these apical dendrites, but no others arise until the dendrites branch at the beginnings of their apical tufts in layer III, and so spread out obliquely. Since the apical tufts and
Fig. 9 Reconstruction of a cluster. The reconstruction was made from a series of tangential 1 μm sections and is segmented to show the arrangements of dendrites as they approach the surface of the cortex. This cluster is the one indicated by arrows in figures 5–8.
the basal dendrites spread out laterally for a distance of some 100 \( \mu \) it is clear that they must intermingle with those of the neurons in adjacent clusters, for the center to center spacing between clusters is usually of the order of 50 \( \mu \). The same is also true of the pyramidal neurons in layer III. They contribute their apical dendrites to the clusters formed by the layer V apical dendrites, and indeed in electron micrographs of layer III the basal dendrites of these pyramidal neurons have been seen to pass both around and between the dendrites of the clusters.

**DISCUSSION**

This account has shown that the apical dendrites of layer V neurons in Area 3 of the cerebral cortex of the rat are not arranged in a random manner. On the contrary, they come together to form groups, or clusters as we have chosen to call them. However, while this clustering does occur, the pattern the clusters make is not a perfect one. The numbers of dendrites contributing to the individual clusters is variable and so is the spacing between clusters. The average center to center spacing between clusters is about 50 \( \mu \), although between large clusters the distance is greater. Furthermore as the layer V apical dendrites ascend towards the surface of the cortex, the apical dendrites of at least layer III pyramids are added to them, usually in a peripheral position.

In retrospect, this arrangement seems to be the columnar arrangement being referred to by von Bonin and Mehler ('71). They describe “tufts of radial bundles” and columns of cells five to six cells wide in some areas of the cortex of the human and macaque, or two to three cells wide in others, and state that the greatest distance between columns of cells is about 80 \( \mu \).

Fifkova ('70) also describes bundles of dendrites originating from layer V pyramids in Area 17 of the rat visual cortex. Fifkova ('70) uses these bundles as landmarks and a means of orientation in an

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*Fig. 10 Reconstruction of a cluster of layer V pyramidal cell apical dendrites. The reconstruction was made from a series of vertically oriented 1 \( \mu \) sections. The positions of other cell bodies in the section are given to indicate the lamination of the cortex.*
Fig. 11 A reconstruction based upon 1 μ serial sections cut in the tangential plane. The apical dendrites forming clusters at the level of layer IVa are indicated by blackened dots. These are superimposed upon the positions of the cell bodies of layer V pyramids, which are stippled, so that in effect the observer is looking from layer IV downwards towards layer VI. Note that the cell bodies are arranged directly beneath the apical dendrites and tend themselves to be clustered. The origins of a few of the apical dendrites could not be determined with certainty and consequently their cell bodies have not been included in this diagram.

electron microscopic assessment of the effect of visual deprivation on the density and size of synaptic contacts. There is little doubt from the illustration in this article that Fifkova ('70) is making use of the entities that we are describing as clusters. These observations on the visual cortex of the rat (Fifkova, '70) and on Areas 4, 17, 7 and 40 of the human and Macaque cortex (von Bonin and Mehler, '71), as well as our own preliminary observations on the primary somato sensory, visual and auditory cortices of the rat and cat, indicate that the existence of the dendritic clustering we describe in Area 3 of the rat is a relatively common occurrence in mammals.

In our thinking about the arrangements of apical dendrites in Area 3, we initially coined the terms "clusters" to describe the grouping of apical dendrites. Fortuitously, however, the term can also be extended to clusters of pyramidal neurons arranged in a racemose manner; somewhat like a bunch of chives or onions held together at their stems of various lengths, so that the
bulbous portions of the plants (representing the pyramidal cell bodies) are arranged at the periphery of the bunch. And indeed, it must be borne in mind that the formation of the clusters, although most apparent as an arrangement of the apical dendrites, is a unit involving a number of complete neurons.

The question is how to interpret this arrangement functionally. Clearly, one possibility is that the dendritic groupings have a metabolic significance and that they are clustered near to a vertically oriented blood vessel. Blood vessels are often in the locale of a cluster, but there is no constancy in this relationship. An alternate interpretation is that the apical dendrites and the neurons giving rise to them share a common afferent input. This seems likely if only for the reason that our electron microscope observations indicate a concentration of small unmyelinated axons and their terminals surrounding the dendrites. It is not likely, however, that the clusters are simply the anatomical correlate of the columns defined by neurophysiologists.

The notion of functional columns is based on the physiological observation (Mountcastle, '57) that a vertically oriented group of neurons in the cerebral cortex receives a stimulus from the same specific afferent input. The widths given for these functional columns varies from about 100 μ in the auditory cortex of the cat (Abeles and Goldstein, '70) to an upper limit of about 500 μ in the somatic sensory cortex of the cat (Mountcastle, '57). These dimensions correlate rather readily with the extents of the spread of individual specific afferent fibers entering the cerebral cortex. Thus Scheibell and Scheibell ('70) suggest that the spread is between 250–500 μ and Ruiz-Marcos and Valverde ('70) in the mouse, calculate that the geniculo-cortical afferents spread for a width of 300–400 μ.

This spread is much greater than that of the clusters which commonly are separated by a distance of only 50 to 100 μ. Indeed, the spread, as Woolsey and Van der Loos ('70) point out, is more commensurate with the size of the barrel arrangements of neurons that they have described in layer IV of the somatic-sensory cortex of the mouse and which Welker...
has described in the somatosensory cortex of the rat. Arguments in favor of this correlation are presented by Woolsey and Van der Loos ('70), who argue primarily on the basis of size, and the fact that the specific thalamo-cortical afferents terminate in layer IV, where the barrels are located. Unfortunately, in Area 3 in the rat, which according to Welker's ('71) study seems to correspond to the receptive area for the afferents to the hind limb and trunk, we have found no obvious signs of barrels and we are not aware that these layer IV configurations have been described in other primary sensory regions where columns have been found.

Consequently, at the present time, it is fair to state that there is no obvious simple physiological function that can be ascribed to the clusters. It may be that a group of them form a physiological column and that there is some correlation between the barrels and the clusters. This will provide the theme for our continuing investigations.

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LITERATURE CITED


