The Morphology of Minicolumns

Alan Peters

Minicolumns

The cerebral neocortex is a thin sheet of gray matter covering the cerebral hemispheres, and it is composed of six horizontally dispersed layers of neurons. Throughout the cerebral hemispheres these layers vary in thickness, sizes of their neurons and packing density, and these differences have been used to subdivide the cerebral cortex into regions and areas (e.g., Brodmann, 1909). However, despite the horizontal layering, functionally it is the vertical connections between neurons that are of prime importance, because as first expounded by Lorente de Nó (1938), the vertical connections between neurons in the cortex are stronger than the horizontal ones. Subsequently Mountcastle (1978) proposed that cortical neurons are organized into narrow, vertically interconnected, units that extend through layers 2–6, and he suggested that these units should be called "minicolumns," although they are also often referred to as "microcolumns." As recently pointed out by Jones (2000), the minicolumn hypothesis "requires that neurons in the middle layers of the cortex, in which thalamic afferents terminate, should be joined by narrow vertical connections to cells lying superficial and deep to them, so that all cells in the column are excited by incoming stimuli with only small latency differences."

The minicolumns can be demonstrated physiologically by experiments in which a microelectrode is inserted into the cortex in an essentially horizontal direction. When this is done, it is found that there are changes in the receptive properties of the cortical neurons every 50 μm or so, as the electrode passes from one minicolumn into the next one, and that groups of these microcolumns are activated by peripheral stimuli to generate larger units, the macrocolumns, or functional columns. The question that has produced debate is what is the anatomical equivalent

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of the physiologically defined microcolumns? Basically there are two organizational arrays of neuronal components that have been suggested as possible candidates and, as will be shown, morphologically these arrays are related to each other. One array is three-dimensional, while the other is two-dimensional, and the sizes of both of these arrays are commensurate with those of the physiologically defined minicolumns. The three-dimensional vertical arrays have at their centers, or axes, apical dendrites of pyramidal cells that come together to form "clusters," and the pyramidal cells associated with these arrays form "pyramidal cell modules.". The axons of the neurons in these modules also aggregate to form vertically oriented bundles that pass through the deeper layers of the cortex to enter the white matter. Studies of these pyramidal cell modules have generally focused on their organization, development, and physiological functioning in the cortex.

The two-dimensional arrays of neurons that are visible in Nissl stained sections are vertical stacks or strings of neuronal cell bodies that are most obvious in the upper layers of the cortex. These strings of neurons are better defined in some cortices, such as the primate temporal lobe, than in others. Studies of these entities have largely relied upon digitized images and have focused on ascertaining if there are changes in the spacing of neurons in these arrays in various disease states and in cortices of patients with behavioral problems. In this review we will consider how these two anatomically defined entities are related to each other. For recent reviews that have considered the composition, function, and significance of minicolumns those of Jones (2000), Buxhoeveden and Casanova(2002a and 2002b), and Rockland and Ichinohe (2004), and the book edited by Casanova (2005), should be consulted.

Physiologically Defined Units

A more complete account of the physiologically defined minicolumns can be found in the review by Mountcastle (1997) and in his book on cerebral cortex (Mountcastle, 1998). The sizes of the physiologically defined minicolumns are derived from experiments such as those of Favorov and Whitsel (1988), and Favorov and Diamond (1990), who showed that when electrode penetrations of the somatic sensory cortex of the cat are made slightly off vertical, and the skin stimulated, there is a abrupt shift in receptive field properties of the cortical neurons about midway through the depth of the cortex. Such shifts occur with lateral movements of the recording electrode every 40–50 μm, which indicates that the electrode is moving from one minicolumn to the next one.

Another example of an experiment showing the sizes of the physiologically defined minicolumns is the nerve regeneration study carried out by Kaas et al. (1981). These experimenters made an initial electrode penetration of the somatic sensory hand area in a monkey's neocortex in a direction more or less parallel to the surface of the cortex and showed that over a considerable distance the same modality type is observed. They then sectioned the median nerve and allowed time for the nerve to regenerate and re-innervate of the skin. The recording experiment was then

repeated, and it was found that instead of the smooth progression of overlapping receptive fields, sharp shifts in the receptive fields occurred every 40–60 μm. It was proposed that the reason for this change is that during re-innervation of the skin, the nerve fibers of the medial nerve become misguided and are not able to find their way back to their original postsynaptic loci. Consequently a new receptive field distribution is imposed on the entire system of minicolumns. Other examples showing the sizes of the physiologically defined minicolumns can be found in the reviews by Mountcastle (1997; 1998) and Buxhoeveden and Casanova (2002a).

Pyramidal Cell Modules: Anatomically Defined Units Based on Apical Dendrite Clustering

Although the early work of Lorente de Nó (1938) had pointed out that the connectivity between neurons in the cerebral cortex is essentially vertical, it was not until 1972 that vertically organized morphological units of cortical neurons were shown to exist in cerebral cortex. Using light and electron microscopy of plastic-embedded material, Peters and Walsh (1972) demonstrated that in the somatosensory cortex of the rat, groups of apical dendrites of layer 5 pyramidal cells become clustered together, and as they ascend through the cortex and enter layer 3, the apical dendrites of pyramidal cells in that layer are added to the peripheries of the layer 5 clusters. Simultaneously Fleischhauer et al. (1972), using a number of light microscopic stains in combination with electron microscopy showed a similar clustering of apical dendrites in the sensory–motor cortex of the rabbit and cat. Peters and Walsh (1972) found that the center-to-center spacing of the apical dendritic clusters was about 50 μm and Fleischhauer et al. (1972) gave the sizes of the clusters as about 40–50 μm. Both groups of investigators proposed that the vertically arranged groups of neurons with clustered apical dendrites are the anatomical equivalents of the physiological defined minicolumns, the clustered apical dendrites being the centers, or axes, of the minicolumns.

Since the initial descriptions of the existence of clusters of apical dendrites, other anatomical studies have been carried out, and it is now evident that such clustering of apical dendrites occurs throughout the neocortex (see Table 1). Thus clusters of apical dendrites have now been directly visualized and described in such locations as primary visual cortex of the rat (Winkelmann et al., 1975; Peters and Kara, 1987); mouse posteromedial barrel field (White and Peters, 1993) and primary motor cortex (Lev and White, 1997); the somatosensory cortex (Massig and Fleischhauer, 1973) and visual and motor cortex of the rabbit (Schmolke and Fleischhauer, 1984; Schmolke and Viebahn, 1986; Schmolke, 1987); cortical areas 2, 3, 4, and 41 of the rat (Feldman and Peters, 1974); the primary auditory, primary visual, and postcruciate cortex of the cat (Feldman and Peters, 1974; Peters and Yilmaz, 1993); primary visual cortex of the monkey (Peters and Sethares, 1991; 1996); and the cerebral cortex of the lesser Madagascan hedgehog and red-eared pond turtle (Schmolke and Künzle, 1997).

Table 1 Center-to-center spacing of dendritic clusters in neocortex

Species	Area studied	Center-to-center spacing (μm)	Authors
Rat	Somatic sensory cortex	50	Peters and Walsh (1972)
Rat	Somatic sensory cortex	49	Skoglund et al. (2004)
Rat	Area 17	30–40	Feldman and Peters (1974)
Rat	Area 17	76	Winkelmann et al. (1975)
Rat	Area 17	55–60	Peters and Kara (1987)
Rat	Visual cortex	53	Lohmann and Koppen (1995)
Rat	Visual cortex	27	Vercelli et al. (2004)
Rat	Area 3	50	Feldman and Peters (1974)
Rat	Area 41	50–70	Feldman and Peters (1974)
Rat	Prelimbic cortex (area 32)	44	Gabbott and Bacon (1996)
Mouse	Parietal region	50–100	Escobar et al. (1986)
Mouse	Sm1 barrel subfield	22–25	White and Peters (1993)
Mouse	MsI	31	Lev and White (1997)
Hedgehog tenrec	Areas A2, A3, and A4	32	Schmolke and Kunzle (1997)
Rabbit	Sensory motor	40–50	Fleischhauer et al. (1972)
Cat	Area 41	50–70	Feldman and Peters (1974)
Cat	Area 17	56	Peters and Yilmaz (1993)
Monkey	Area 17	31	Peters and Sethares (1991)
Monkey	Area 17	23	Peters and Sethares (1996)
Monkey	Area 18	21	Peters et al. (1997)
Human	Medial prefrontal cortex	52–59	Gabbott (2003)

The Basic Structure of the Pyramidal Cell Modules

Perhaps the best-understood and clearest example of dendritic clustering is found on the rodent primary visual neocortex, in which neurons are stratified into six obvious layers. In this cortex, pyramidal cells occur in all layers except layers 1 and 6b, and they account for 85–90% of all neurons (Peters and Kara, 1985; Gabbott and Stewart, 1987). The majority of apical dendrites of the pyramidal cells in layer 5 are aggregated into clusters as they ascend through the cortex to form their apical tufts in layer 1. As these clusters of layer 5 apical dendrites ascend, the apical dendrites of the pyramidal cells in layer 2/3 are added to them. This information was first obtained by tracing apical dendrites using serial semi-thick plastic sections, but subsequently a less painstaking way to demonstrate this arrangement became available when antibodies were produced against microtubule-associated protein 2 (MAP2), which occurs in the cell bodies and dendrites of neurons (de Camilli et al., 1984). Use of this antibody clearly reveals the bundles of pyramidal cell apical dendrites when they are sectioned either along their length (Fig. 1) or sectioned transversely (Fig. 2). The mean center-to-center spacing of the apical dendritic clusters in rat visual cortex is 55–60 μm, and on average each cluster contains the apical dendrites of 12 large- and 38 medium-sized layer 5 pyramidal cells (Fig. 3; Peters and Kara, 1987; Peters, 1993). However, the apical dendrites of some layer 5 pyramidal cells appear not to contribute to the clusters; instead they ascend either singly or in pairs

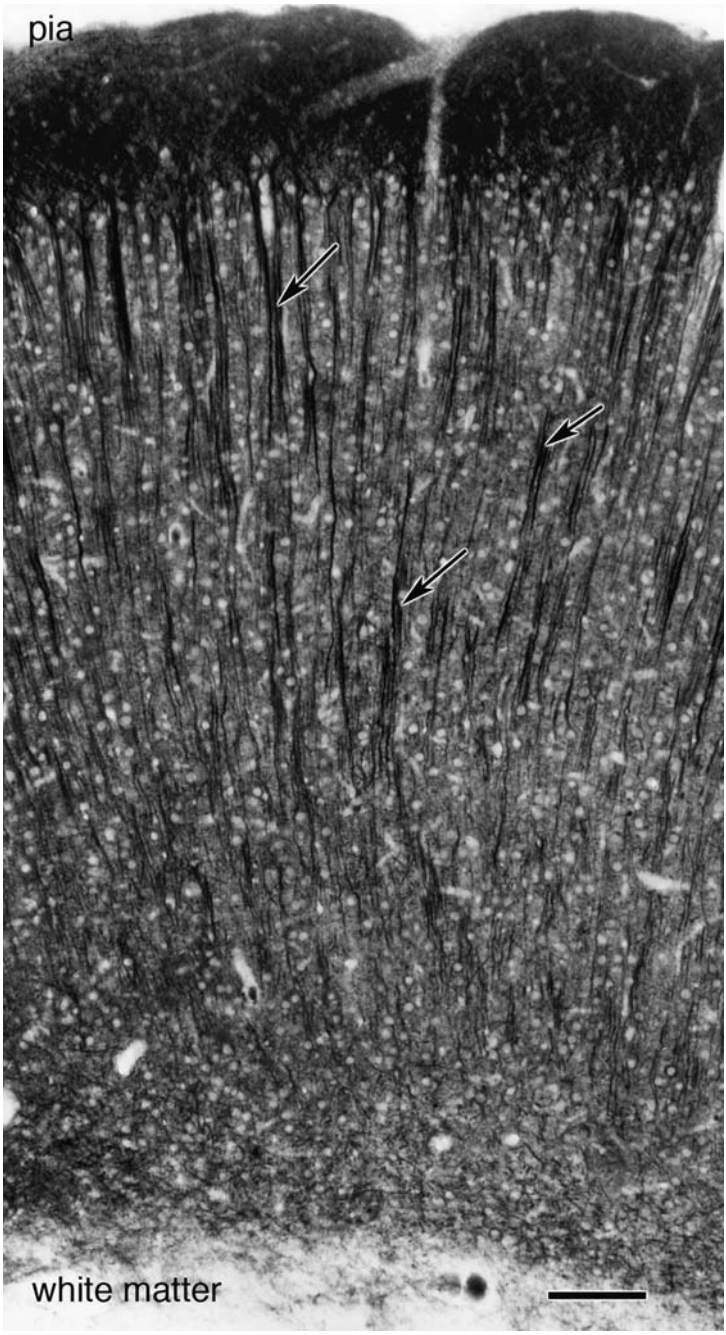

Fig. 1 Rat visual cortex sectioned in the vertical plane and labeled with an antibody to MAP2 to show the apical dendritic clusters (*arrows*). Note the densely stained layer 1 beneath the pia, and the pale staining of the white matter. Scale bar = 100 μm

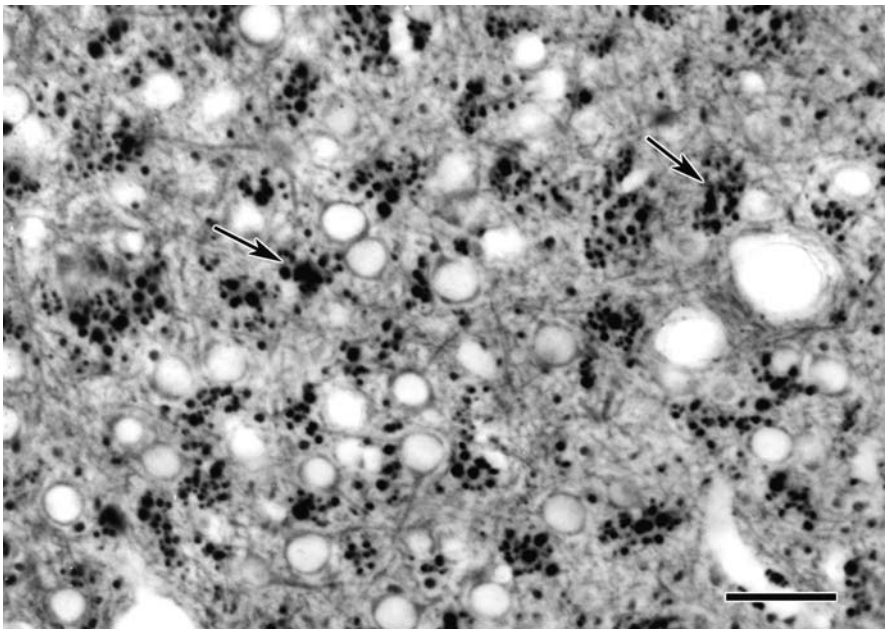

Fig. 2 Transverse section of rat visual cortex at the level of layer 4. The section is labeled with MAP2 antibody to show the darkly stained apical dendrites within the clusters (*arrows*). Scale bar = 50 μm

as they enter layer 4, and whether they join the clusters as they ascend through upper layers has not been ascertained.

As first shown by Escobar et al. (1986) in the neocortex of the mouse, the apical dendrites of the pyramidal cells in layer 6a do not join the clusters formed by the apical dendrites of the pyramidal cells in layers 5–2. Instead they form their own independent groupings, which we have referred to as "bundles." Since the pyramidal cells of layer 6a are small, their apical dendrites are thinner than those of the pyramidal cells in upper layers and they form numerous bundles that ascend as far as layer 4, where they terminate and form their apical tufts (see Figs. 3 and 4).

A comment needs to be made about the difference in measurements of the center-to-center spacing of these dendritic bundles in rat visual cortex as determined by various investigators. As seen in Table 1, most of the measurements of center-to-center spacing range between 40 and 60 μm. At the high end is the value of 76 μm obtained by Winkelmann et al. (1975), and at the lower end the value of 27 μm obtained by Vercelli et al. (2004). The reason for these large differences is yet not fully apparent, but Vercelli et al. (2004) suggest that it might be due to the fact that most investigators have only determined the frequency of occurrence of counted dendritic clusters at the level of layer 4, and have concentrated only on clusters that contain large diameter dendrites. Curtetti et al. (2002) point out that there are

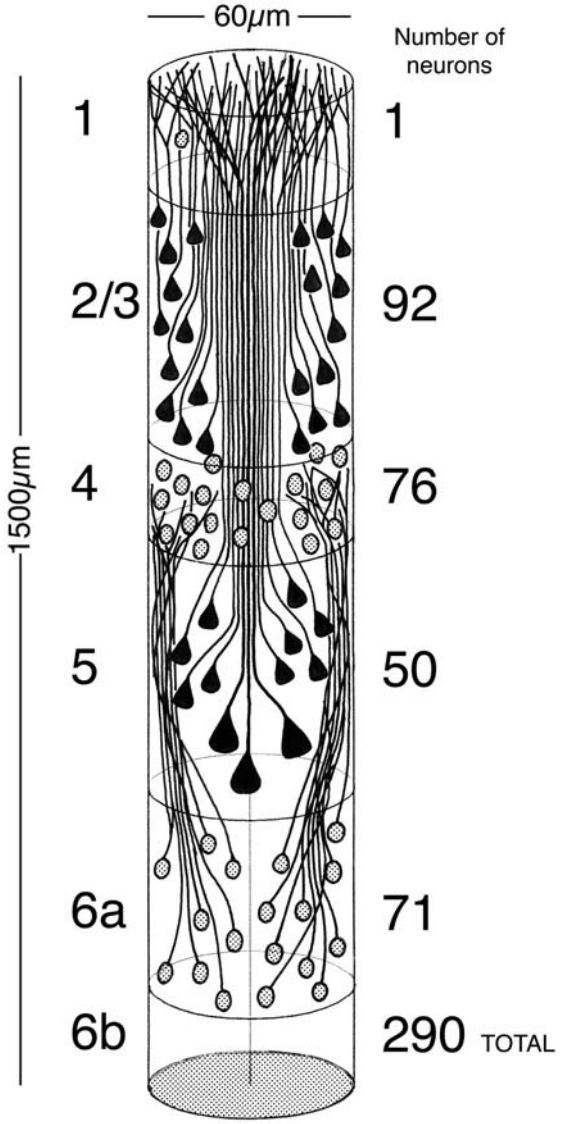

Fig. 3 Diagrammatic representation of the pyramidal cell module in rat primary visual cortex, area 17. The cortical layers are indicated on the *left* and the number of neurons in each layer contributing to the module is given on the *right*. After Peters (1993)

several types of apical dendritic clusters, some consisting of apical dendrites that arise exclusively from layer 5, others that arise from pyramidal cells in layers 5, 3, and 2, and yet others that are formed from neurons in the supragranular layers. Vercelli et al. (2004) state that they included all of these types of dendritic clusters in their analyses, so that their value for the number of clusters per unit area of tangential sections is greater than that obtained by previous authors. But as will be noticed in Table 1, the value obtained by Vercelli et al. (2004) is very similar

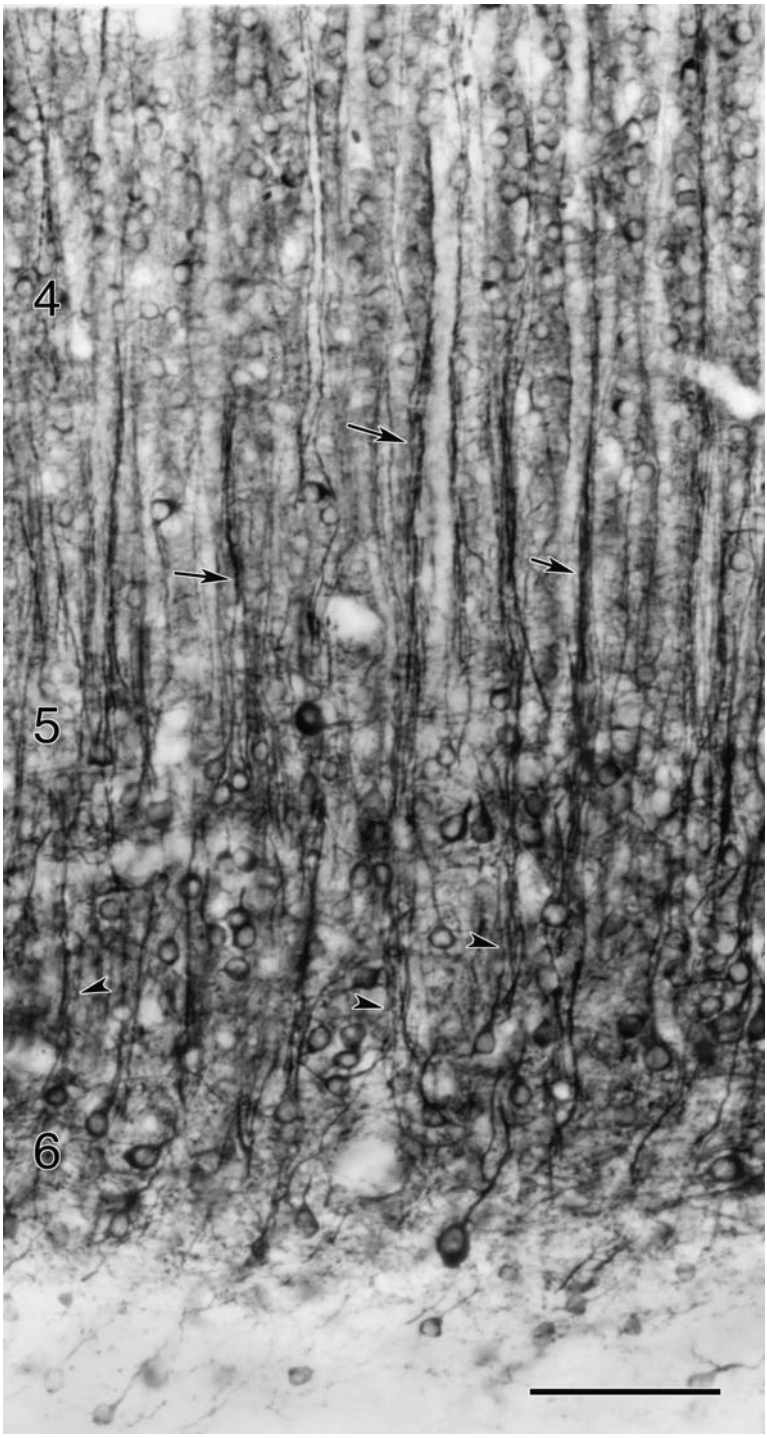

Fig. 4 (continued)

to that obtained by White and Peters (1993) in mouse barrel field and by Lev and White (1997) in mouse motor cortex. However, it should be noted that White and Peters (1993) found some variation in the spacing of apical dendritic clusters, in that the clusters in the walls of barrels have an average spacing of 22 μm, while in the barrel hollows their average spacing is 25 μm. The reason is that, as shown by Detzer (1976), apical dendrites at the periphery of the hollows in mouse barrel field often bend, or become deflected, as they approach layer 4, so that they enter the walls of the barrels, thereby increasing the concentration of clusters in the barrel walls.

Apical Dendritic Clusters in the Neocortices of Other Animals

In other cortices there is a similar aggregation of apical dendrites of layer 5 pyramidal cells into clusters and the aggregation of apical dendrites of layer 6a pyramidal cells into bundles. This is true for the organization of pyramidal cell apical dendrites in sensory–motor cortex of the rabbit (Fleischhauer et al., 1972), areas 41 and 17 of the cat (Feldman and Peters, 1974; Peters and Yilmaz, 1993), area 17 of the monkey (see Figs. 4 and 5; Peters and Sethares, 1991; 1996), and medial prefrontal cortex of the human brain (Gabbott, 2003). The one exception appears to be area 18 of the monkey, in which apical dendrites of layer 5 pyramidal cells aggregate with those from layer 6a pyramidal cells to form swathes of apical dendrites that ascend into layer 4, where they are joined by the apical dendrites of layer 4 pyramidal cells (Fig. 5). In area 18 the majority of the apical dendrites from layers 6a, 5, and 4, form their apical tufts in layer 4 and lower layer 3, with only a few of them ascending as far as layer 1. Consequently, it is the apical tufts of layer 2 and 3 pyramidal cells that dominate layer 1. Thus, in area 18 of monkeys, there are essentially two separate tiers of apical dendrites: a lower tier containing apical dendrites from pyramidal cells in layers 6a–4 and an upper tier containing apical dendrites of layer 2/3 pyramidal cells (Fig. 5).

Another interesting variation in the clustering of apical dendrites occurs in the cat sensorimotor cortex. There it has been shown by Fleischhauer (1974) that in the posterior sigmoid gyrus the apical dendrites of the layer 5 pyramidal cells frequently bifurcate soon after they emerge from the large cell bodies. The secondary dendritic branches then run obliquely for a short distance and at the level of layer 3 they join secondary branches from other apical dendrites to form dendritic clusters

Fig. 4 (continued) Monkey primary visual cortex. Vertical sections labeled with an antibody to MAP 2. The illustration shows the apical dendritic clusters (*arrows*) arising from layer 5 pyramids and the bundles of apical dendrites (*arrowheads*) arising from layer 6a pyramidal cells. The locations of layers 4, 5, and 6 are indicated. From Peters and Sethares (1991). Scale bar = 100 μm

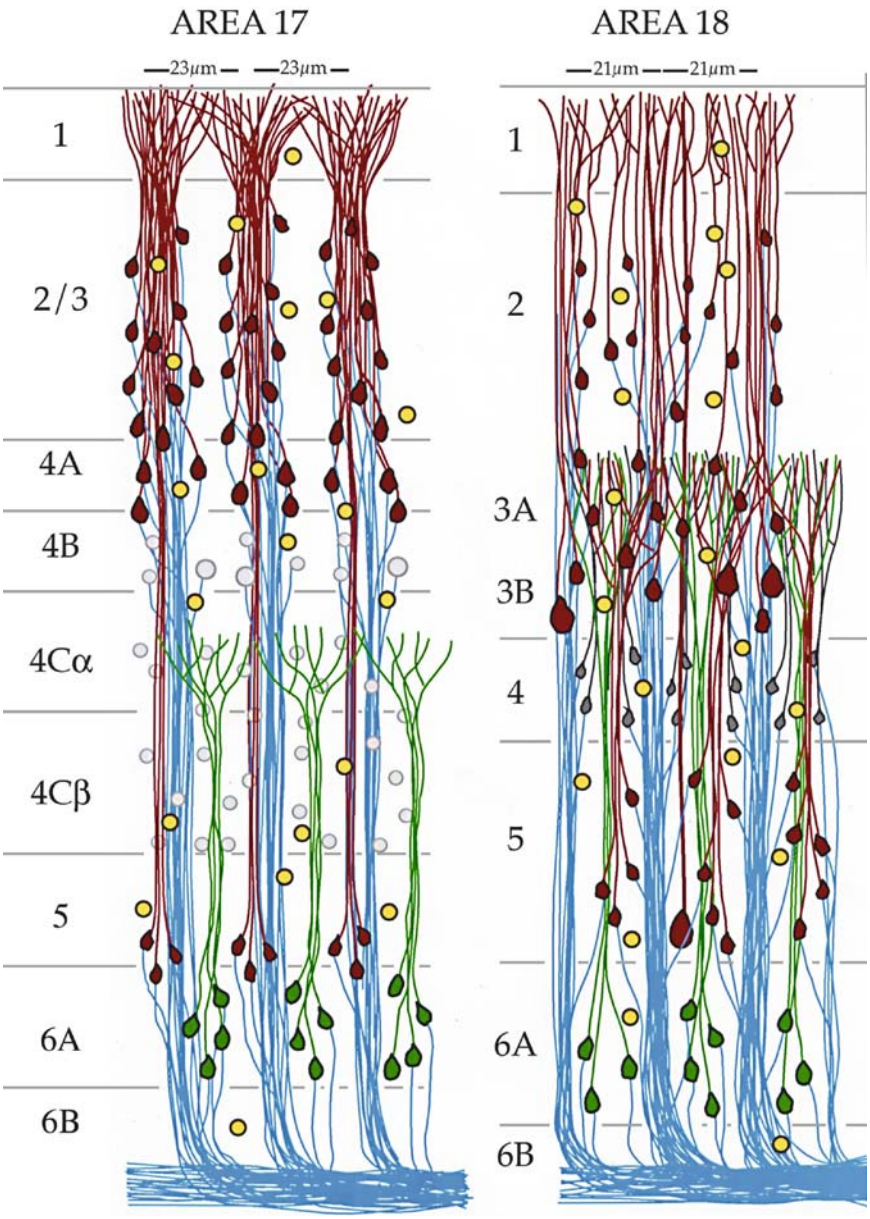

Fig. 5 Diagram to show the differences in the arrangement of pyramidal neurons in areas 17 and 18 of monkey visual cortex. Not all of the neurons in a module are shown in the diagram. The pyramidal cells in layers 5, 4A, 3, and 2 are shown in *red*, and the pyramidal cells in layer 6A are in *green*. Neurons in layer 4 are *grey*, while inhibitory neurons are *orange*. The bundles of myelinated nerve fibers that extend from the modules to enter the white matter are shown in *blue*.

that extend up to layer l. A similar bifurcation of layer 5 apical dendrites, with secondary branches entering neighboring clusters, has also been noted by Massig and Fleischhauer (1973) in the somatosensory cortex of the rabbit.

The Spatial Arrangement and Connections of Apical Dendritic Clusters

Peters and Kara (1987) proposed that dendritic clusters are basically arranged in a hexagonal pattern. The pattern is not perfect, but in tangential sections through rat primary visual cortex taken at the level of layer 4, most dendritic clusters appear to be at the center of six other clusters. This same distribution pattern is also found in monkey primary visual cortex (Peters and Sethares, 1996); in cat primary visual cortex (Peters and Yilmaz, 1993); prelimbic cortex of the rat (Gabbott and Bacon, 1996); and in Brodmann areas 25, 32, and 24 of the human medial prefrontal cortex (Gabbott, 2003). However, after carrying out a mathematical analysis of the distribution of dendritic clusters in primary somatosensory cortex of the rat, Skoglund et al. (2004) concluded that there is not a hexagonal pattern of cluster distribution in this cortex, since the distances between individual clusters range between 24 and 121 μm.

Obviously, the question of the geometric distribution of dendritic clusters needs to be examined further, and any modular theory of cortical function based on the concept that the apical dendritic clusters are the axes of functional cortical modules, or minicolumns, has to take into account that there is biological variation in the composition of the modules, as shown by studies like those of Lev and White (1997) and Vercelli et al. (2004).

Lev and White (1997) labeled callosally projecting neurons in MsI cortex of the mouse by retrograde transport of horseradish peroxidase deposited onto severed callosal fibers in the contralateral hemisphere. They found that in some dendritic clusters in the contralateral hemisphere all of the apical dendrites were labeled, whereas in adjacent clusters none of the dendrites were labeled. This suggests that some pyramidal cell modules are composed exclusively of callosally projecting neurons, while other clusters have different functions. Vercelli et al. (2004) examined the output neurons in rat visual cortex using lipophilic tracers to label different pyramidal cell populations and determined that neurons contributing to dendritic clusters can have different specific targets. Pyramidal cells projecting to ipsi- and contralateral cortex cluster together and the same clusters contain neurons that project to

Fig. 5 (Continued) Note that in area 17 the apical dendrites of layer 5 pyramids pass into layer 2/3 where they are joined by the apical dendrites pyramidal cells in that layer, but the bundles of apical dendrites of the layer 6A pyramidal only extend as far as layer 4C. In area 18, the apical dendrites of the layer 5 and layer 6A pyramidal cells both extend only up to layer 3A. Nearly all of the apical dendrites in the clusters in the upper layers of the cortex arise for the layer 2 and 3 pyramidal cells. From Peters et al. (1997)

the striatum. But the clusters that contain callosally projecting cells do not contain neurons that project to the superior colliculus, the dorsal division of the lateral geniculate body, or project through the cerebral peduncle. Consequently not all pyramidal cell modules are identical and there is not a random mixing of projection neurons, but some degree of specificity.

Myelinated Axon Bundles

Although they are not very evident in rodent cortex, vertical bundles of myelinated axons are prominent in cortices such as those of the cat and primates. These vertical bundles of myelinated axons generally become evident at the level of layer 3 and extend through the depth of the cortex, ultimately blending with the myelinated nerve fibers in the underlying white matter. In the primary visual cortex (Peters and Sethares, 1996) and in area 18 (Peters et al., 1997) of the rhesus monkey cortex, it has been shown that these vertical bundles of myelinated axons arise from the groups of pyramidal cells whose apical dendrites aggregate together to form the clusters. It is assumed that each vertical bundle of myelinated axons represents the output or efferent fibers from the neurons associated with individual clusters of apical dendrites. Consequently, it is not surprising to find that the center-to-center spacing of the vertical bundles of myelinated axons is the same as that of the apical dendritic clusters within the same cortical area (Figs. 5 and 6).

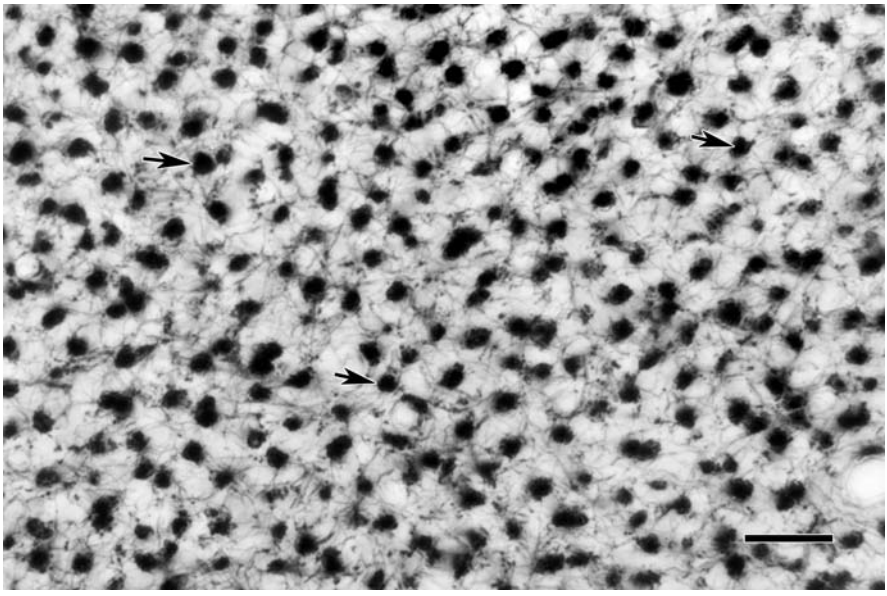

Fig. 6 Monkey primary visual cortex transverse section taken at the level of layer 5 and stained to show the regularly spaced bundles of myelinated axons (*arrows*). Scale bar = 25 μm

Lohmann and Köppen (1995) have also shown that the dendritic clusters and the vertical axonal bundles in rat visual cortex originate from the same neurons, and that the axonal bundles and the dendritic clusters have similar center-to-center spacing. Recently Casanova et al. (2008) have concluded that in the human cortex the pyramidal cell arrays and the vertical bundles of myelinated axons have similar spacing.

Unmyelinated Axon Bundles

In addition to vertical bundles of myelinated axons, the cerebral cortex of monkeys (e.g., DeFelipe et al., 1990) and of humans (e.g., del Rio and DeFelipe, 1995) also contains vertically oriented bundles of unmyelinated axons that are referred to as horsetails. These horsetails are the axonal plexuses of the inhibitory double bouquet cells and can be demonstrated in monkey neocortex by immunolabeling with antibodies to calbindin and tachykinin. As shown by DeFelipe et al. (1990), in the monkey these axonal bundles are widespread and form a regular columnar system descending from layer 2 to layers 3–5. The bundles are most evident in tangential sections taken at the level of layer 3, where they can be seen to have a center-to-center spacing of 15–30 μm. In a later study of the calbindin labeled double bouquet cells in monkey striate cortex, Peters and Sethares (1997) showed that there is one double bouquet cell, and therefore one vertically oriented double bouquet cell axonal plexus, or horsetail, per pyramidal cell module (Fig. 7). Within layer 2/3 the double bouquet axons run alongside the apical dendritic clusters, while in layer 4C they are closely associated with the vertical myelinated axonal bundles. DeFelipe et al. (1989; 1990) proposed that the axon terminals of the double bouquet cell synapse with the shafts and spines of basal dendrites and oblique shafts of apical dendrites of pyramidal cells, but the exact role of these vertical bundles of inhibitory axons is not known. It is likely that they constitute a vertical inhibitory system that acts upon pyramidal cells within the minicolumns.

Yanez et al. (2005) have carried out a survey of the distribution of double bouquet cells in the cortices of various mammalian species. There are no double bouquet cells in the neocortices of rodents and rabbits, and compared to primates there are relatively few double bouquet cells in the cortices of carnivores such as cats, dogs, lions, and cheetahs. Consequently there is great variation in the occurrence of double bouquet cells with horsetail axons, and Yanez et al. (2005) conclude that although double bouquet cells are an important neuronal element in the organization of minicolumns in primate neocortex, this is less true in other mammalian species.

A Conclusion

It is evident that the modules of pyramidal cells whose apical dendrites form clusters and the vertical bundles of myelinated axons are facets of the same basic, modular organization of neurons into vertical units that we can refer to as minicolumns. And

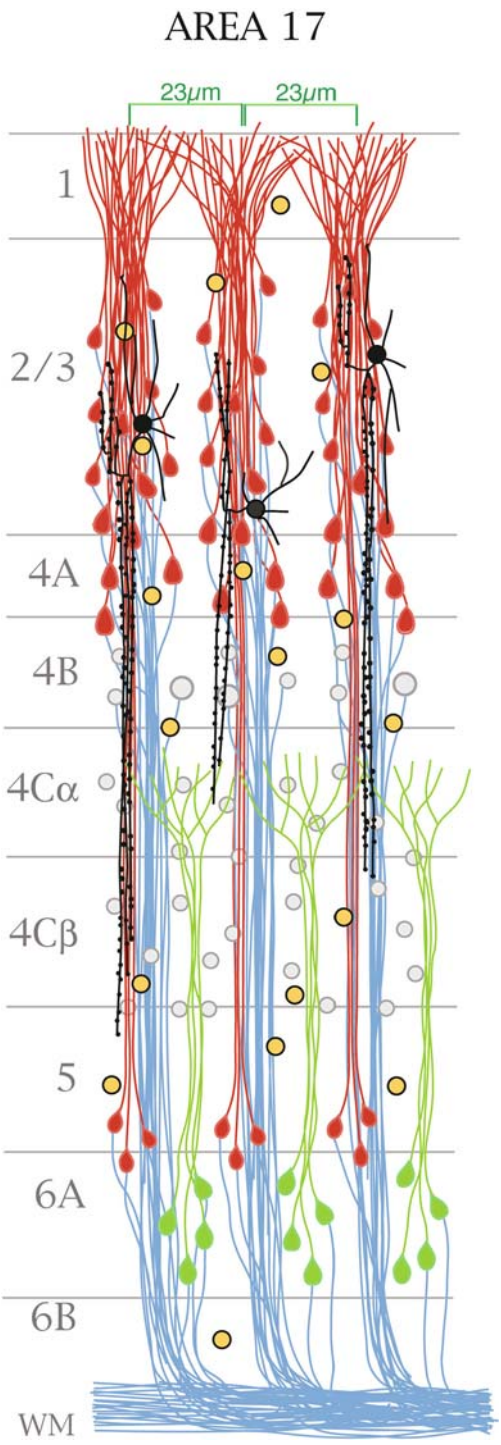

Fig. 7 Diagram of the microcolumn in monkey visual cortex to show that there is one double bouquet cell horsetail (*black*) per pyramidal cell module. The other colors correspond to those in Fig. 5, which explains the composition of the pyramidal cell modules in monkey area 17. From Peters and Sethares (1997)

furthermore, in primates the horsetail bundles of axons from double bouquet cells are strongly associated with the pyramidal cell modules.

As suggested by Peters and Sethares (1996) it is not likely that individual minicolumns have definite boundaries. For example, although the apical dendrites of pyramidal cells may be clustered, the basal dendrites of these same neurons extend for some distance laterally and intertwine with the basal dendrites of neurons in other clusters. In addition the axons of the thalamic input to the cortex have terminal plexuses that spread over several hundred microns, and the axonal plexuses from different thalamic neurons overlap each other. Nevertheless if the minicolumns are looked upon as being arranged in a two-dimensional sheet, even though the thalamic inputs to this sheet overlap, it is likely that an individual minicolumn receives an input that is slightly different from that received by its neighbors. Thus, in visual cortex, for example, the neurons in an individual minicolumn could respond to a slightly different part of the receptive field than the neurons in its neighboring minicolumns, and perhaps respond better to a different orientation of an image, and a different color or eye preference than its neighbors. Given these possibilities it may be expected that the output of the neurons in each minicolumn carried through its vertical bundle of myelinated nerve fibers, is unique to that minicolumn.

Minicolumns Based on Vertical Arrays of Neuronal Cell Bodies Seen in Nissl Stained Sections

In some areas of the cerebral cortex, and especially in the primate temporal lobe, vertical arrays, or strings, of neuronal cell bodies are evident in Nissl stained vertically oriented sections. The Nissl stained vertical arrays are one or two cell bodies wide and they are especially obvious in layers 2–4 (Fig. 8). Intervening between these vertical arrays of neuronal cell bodies are pale zones that presumably contain dendrites and axons, which have little Nissl substance. However, it should be borne in mind that the Nissl image is two-dimensional and how the cell bodies are arranged in three dimensions is not obvious from such sections. von Bonin and Mehler (1971) examined sections of human cortex cut obliquely, but basically in the tangential plane, and although their description is sketchy, it implies that the neurons are arranged in rather indistinct vertically oriented groups, strings or rows, that are as much as 80 μm apart. How this image fits with what is known about the arrangement of apical dendrites into clusters and myelinated axons into bundles is not fully evident, but it is likely that the pale spaces between the strings of neuronal cell bodies correspond to the clusters and bundles of apical dendrites. An issue that has not been resolved is how the cell bodies of cortical neurons are arranged. From studies of clusters it is generally assumed that the neuronal cell bodies surround the dendritic clusters, whereas Nissl preparations would suggest that the neurons are in discrete vertical strings. Which arrangement is correct, needs to be resolved by making three-dimensional reconstructions of the disposition of neuronal cell bodies through the depth of the cortex, and this has not yet been done.

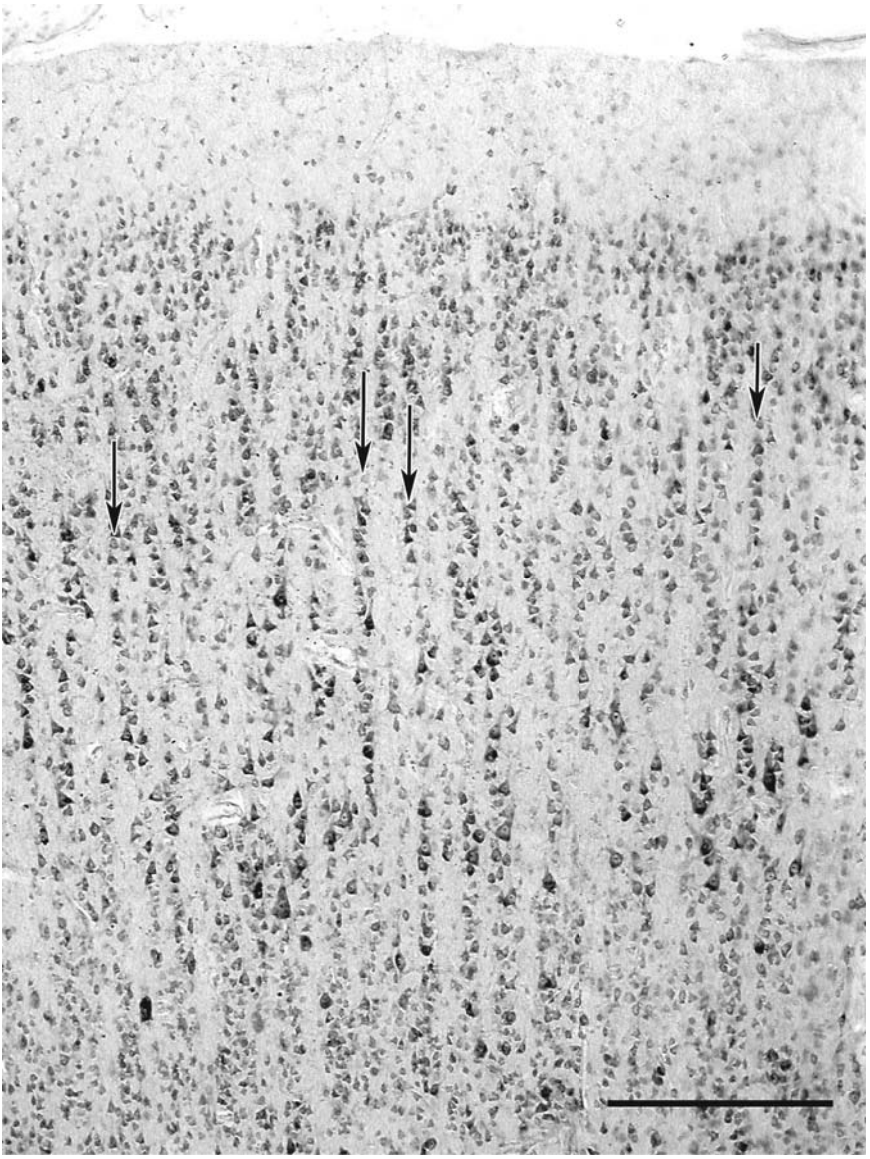

Fig. 8 Nissl stained section of primary auditory cortex, area 41, from a human cerebral hemisphere. In this cortical area there are obvious vertical strings of neurons (*arrows*) separated by pale spaces that are largely occupied by the apical dendrites of pyramidal cells. Scale bar = 100 μm

The only study in which both the disposition of dendritic clusters and of vertical arrays of neurons in the same cortical areas have been compared in the same cortical area is that of Gabbott (2003). He examined human prefrontal cortex using both MAP2 labeling to show dendritic clusters in sections cut parallel to the cortical

surface and in Nissl stained sections cut in the vertical plane to show the vertical strings of neuronal cell bodies. Gabbott (2003) found the center-to-center spacing of the dendritic clusters to be 52–59 μm, and the center-to-center spacing of the vertically oriented strings of neurons to be 49–60 μm, suggesting that the two entities are different views of the same neuronal organization in the cortex. However, Gabbott (2003) makes no comment about how the neuronal cell bodies are disposed.

One reason why more studies like that of Gabbott (2003) have not been carried out is that most studies of the composition and dimensions of dendritic clusters and pyramidal cell modules have been carried out in mice, rats, cats, and rabbits, with a few studies in monkeys and only one in humans (see Table 1). In contrast all of the studies of the dimensions of minicolumns based on spacing of vertical strings of neurons in Nissl stained sections have been carried out in monkeys, ape, and human cortex (see Table 2).

Typically, even in temporal cortex the vertical arrays of neurons are not always visible throughout the entire depth of the cortex, but they are generally obvious in layers 2 and 3 (see Fig. 8). Consequently it is in these layers that most analyses and measures of these vertical arrays of cells have been carried out using digitized images. Since this is a young field of research, the methods of analysis and the nomenclature used by various sets of investigators vary, making it sometimes difficult to compare the numerical data generated in various studies and to even discern what is meant by the term "minicolumn." Some investigators equate the vertical string of cell bodies with minicolumns, and others regard a minicolumn as being an entity that extends from the midline of one vertical string of cell bodies through the pale staining interval to the midline of the adjacent string. In this chapter we are taking the stance that the latter definition is correct and in terms of optical density measures taken from digitized images, this means that the size of a microcolumn would be the distance from one density peak, i.e., the axis of a vertical string of cell bodies, to the next density peak. Some examples of the dimensions of minicolumns measured in this way are given in Table 2.

Table 2 Center-to-center spacing of vertical strings of neurons in normal neocortex

Species	Area examined	Center-to-center spacing (μm)	Authors
Monkey	Area 22	36	Buxhoeveden et al. (2001)
Monkey	Areas 46,TL, and TC	22–27	Cruz et al. (2005)
Chimpanzee	Area 22	35–36	Buxhoeveden et al. (2001)
Human	Area 22	50	Buxhoeveden et al. (2001)
Human	Areas 22, 23, and 41	37–47	Seldon (1981)
Human	Medial prefrontal	49–60	Gabbott (2003)
Human	Cingulate cortex	80	Schlaug et al.(1995)
Human	Frontal cortex	51–58	Buxhoeveden et al. (2006)
Human	Visual cortex	34	Buxhoeveden et al. (2006)
Human	Areas S1, 4, 9, and 17	34 average	Casanova et al. (2006)

One of the first investigators to examine the vertical strings of neurons in Nissl stained sections was Seldon (1981), who examined three cytoarchitectonic areas in human auditory cortex, areas TA (area 22), area TB (area 42), and area TC (area 41), using celloidin-embedded material. In these cortical areas the arrangement of the somata of the neurons into vertical strings is very obvious. Seldon (1981) found that in his material the minicolumn width, namely the average width of a string of cells plus the average width of the intervening pale zone, was 36.7–39.7 μm on the right side of the brain and 44.8–46.7 μm on the left side. Consequently, Seldon (1981) suggested that although they are basically organized in similar fashions, auditory cortical areas on the right side of the brain are more diffusely organized than those on the left side.

Among those who have used digitized images to determine the dimensions of minicolumns are Buxhoeveden et al. (2000). Using their method, this group has determined that the minicolumns in area 22 in the superior temporal gyrus of nonhuman and human primates have different dimensions. They conclude that in monkeys, chimpanzees, and orangutans, the minicolumns are 33–36 μm wide, while in area 22 of the human brain the minicolumns are 54 μm wide (Buxhoeveden et al., 2001; 2002a). Other examples of the dimensions of minicolumns revealed by Nissl staining are given in Table 2.

The real point of making measurements of minicolumns from digitized images of Nissl stained material is to determine if there are alterations in the dimensions of the minicolumns between species, in disease states, and in the brains of humans with behavioral disorders.

Alterations in Nissl Stained Minicolumns

There have been a number of studies on the features and dimensions of minicolumns seen in digitized images of vertical Nissl stained sections taken from the brains of patients with a variety of disorders. They will not all be considered here. Consideration will only be given to the differences that have been reported in Alzheimer's disease, in normal aging and in autism, which is the focus of this book.

Alzheimer's Disease

Buldyrev et al. (2000) used a quantitative method derived from condensed matter physics to examine the disposition of neurons in Nissl stained sections from the inferior bank of superior temporal sulcus in normal human brains, in brains from patients with Alzheimer's disease, and in patients with dementia resulting from Lewy body disease. In control brains they find evidence for the presence of minicolumnar ensembles with a periodicity of about 80 μm, but in brains from Alzheimer's patients in whom there is a loss of neurons, they report an almost complete loss of the minicolumnar organization. Further, the relative degree of loss of the minicolumnar organization appears to be directly proportional to the number of neurofibrillary tangles present, but not to the amount of β-amyloid. In brains

from patients with dementia with Lewy bodies, there is a similar disruption of the minicolumnar organization, even though there is little neuronal loss. The authors conclude that this approach is a useful tool for analyzing the anatomical basis for brain disorders.

Normal Aging

Some of the changes in minicolumnar organization seen in Alzheimer's disease will probably due to normal aging, and in area 46 of the rhesus monkey, in which senile plaques are uncommon, Cruz et al. (2004) using a density map method found that there is no age-related reduction in total neuronal density or in microcolumn width, length or periodicity. However, they did found subtle changes that indicate some disorganization of the minicolumns with age.

In a study of human material, Chance et al. (2006) examined minicolumnar spacing and the organization of cells in the cortices of 17 neurologically normal adult humans aged between 40 and 90 years. They examined Nissl stained sections from the planum temporale (area 22), primary auditory cortex (A1), and middle temporal gyrus (area 21) and concluded that compared to individuals under 65, brains from individuals over 65 years of age show a reduction in the width of minicolumns in the medial temporal gyrus and in the planum temporale, but not in primary auditory cortex. This analysis suggests that although there is no extensive loss of neurons in normal aging, the packing density of the neurons in the vertical strings is increased, implying that there is some loss of neuropil with age. This is consistent with reported loss of dendritic branches, dendritic spines, and axon terminal with increasing age (e.g., Peters et al., 2001). Chance et al. (2006) stained some additional cortical sections for Alzheimer-type pathology and determined that although none of their cases met the criteria for diagnosis of Alzheimer's disease, increased plaque load in these normal brains correlates with a decrease in the width of minicolumns.

Autism

Casanova et al. (2002) have compared the brains from autistic individuals with normal brains and found that in Nissl stained vertical sections taken from Brodmann's areas 9, 21, and 22, the minicolumns in the normal and autistic brains have a somewhat different structure. In the autistic brains, the minicolumns are smaller in width, and the neurons within the vertical strings are less tightly packed, leading the authors to conclude that per unit volume of cerebral cortex, the minicolumns are more numerous in the cortices of autistic individuals. Since studies by earlier authors have shown that there is no abnormality in cell density in the cortices of autistic individuals compared to controls (Coleman et al., 1985; Bailey et al., 1993), Casanova et al. (2002) suggest that autism is the consequence of a defect in migration of cells into the cortex during development.

In a later study Buxhoeveden et al. (2006) examined the frontal cortex from the brains of two autistic individuals, one a 3-year-old child and the other a 41-year-old man, and compared the minicolumnar spacing in the dorsal, mesial, and orbital frontal cortices with those of normal brains. They report that in the adult autistic brain the minicolumnar spacing in dorsal frontal cortex is 15% less than in control brains, 23% less in the orbital cortex, and 10% less in the mesial frontal cortex, although the latter difference was not significant. In contrast to the frontal cortex the minicolumn spacing in area 17 was the same in the autistic and control brains. Buxhoeveden et al. (2006) then compared the minicolumnar spacing in the same frontal cortical areas of the 3-year autistic brain with those of controls and found no difference in the spacing in the dorsal and orbital areas, but a statistically smaller spacing in the mesial frontal cortex. For area 17, again there was no difference. The authors suggest that the existence of smaller minicolumns in the frontal cortices of autistic brains means that there is an increase in the number of minicolumns in autistic brains, producing larger than normal frontal lobes in autistic children (see Courchesne, 2004; Carper and Courchesne, 2005). While there might be an increase in the overall numbers of minicolumns in the autistic brain, because the minicolumns are smaller, this would not necessarily lead to an increase in the sizes of the frontal lobes in autistic children.

Essentially these conclusions about autistic brains have been substantiated in a more thorough study of primary sensory (area S1), primary motor (area 4), primary visual (area 17), and frontal association (area 9) cortex using six autistic brains and six age-matched controls (Casanova et al., 2006). Again Casanova and his colleagues find the widths of the strings of neurons to be narrower by 1.5 μm, or 5.5% in the autistic brains, so that the minicolumnar spacing, measured from one density peak to another, is narrower in the autistic brains. The consequence is that the number of minicolumns per linear distance of the cortical sections is greater in autistic brains, even though the number of cells per minicolumn is the same in the autistic and control brains. But because there are more minicolumns in autistic brains, the overall density of neurons is 23% greater than in control brains, although the sizes of the neuronal cell bodies and of their nucleoli are smaller. Since the brains of autistic adults are basically the same size as normal ones, the implication is that the autistic cortex contains more minicolumns that normal brains (see Courschesne and Pierce, 2005). Since there is less neuropil in the autistic brains, this may mean that the dendritic trees, and even the number of synapses per neuron, may be fewer in cortices of the autistic brains.

In a recent review article on the anatomy of autism Amaral et al. (2008) point out that in these studies by Casanova and his colleagues, only 14 cases of autism, 9 of which had seizures and at least 10 with mental retardation, have been examined for minicolumn pathology. Consequently, more studies using a greater number of autistic brains with fewer other complications need to be carried out before any definite conclusions can be reached about changes that can only be attributed to autism. It would also be appropriate to examine brains in which the apical dendritic clusters and myelinated axon bundles have been stained to confirm the sizes of the minicolumns as detected in digitized images from autistic brains.

At present the causes underlying this potential increase in the numbers of minicolumns in autistic brains in unknown, although it must be related to the manner in which neurons are generated to form the minicolumns during development. It is generally assumed that a minicolumn, as the term is used here, is comprised of the neurons that migrate along the same radial glial fiber during development, and in both humans and monkeys the cell divisions that generate the pyramidal cells in the minicolumns occur before embryonic day 40 (Rakic, 1974; 1985). There is evidence that the brains of autistic infants 2–4 months of age are, on average, 10% larger than those of normal infants, and that there is a spurt of growth, and presumably neuron formation, in autistic infants during the first years of life, a spurt that is not present in normal infants. However, after this spurt of growth there is a plateau, so that in adolescents and adults the autistic brain is no larger than the normal brain (see Courchesne et al., 2004; Courchesne and Pierce, 2005). It is suggested that it is during this time of excessive growth in infancy that the minicolumns are laid down and when they would be generated in excess in autistic brains. Casanova et al. (2006) suggest that the reduction in both the sizes of neuronal cell bodies and of the nucleoli in these neurons in the smaller minicolumns could reflect a bias toward shorter connecting fibers in the autistic cortex, since the distances between adjacent neurons are shorter. They further suggest that this bias would favor local computation by neurons, at the expense of the formation of connections between cortical areas and connectivity across the corpus callosum.

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