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Vertical Bundles of Dendrites in the Neocortex

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Summary. In frontal and tangential paraffin sections through the sensory-motor cortex of the rabbit, vertical bundles of dendrites have been found. Each bundle consists of several apical dendrites of layer V pyramids and extends through layer IV into layer III/II where the dendrites begin to ramify. Electron microscopy reveals that within the bundle some of the dendrites approach each other so closely as to be separated by the extracellular space only. A vertical bundling of dendrites has also been found in various regions of the sensory-motor cortex of the cat. — The hypothesis is put forward that the vertical bundles of dendrites are the morphological substrate of the vertical functional units deduced from electrophysiological observations.

Key words: Neocortex — Dendrites — Columns — Rabbit — Cat.

Introduction

Since the end of the last century it has been known that myelinated axons entering or leaving the cerebral cortex are not distributed at random but organized in small bundles or fascicles. As described in great detail by Kaes (1907), the number and composition of these bundles differs in the various lobi and gyri of the human neocortex. In more recent times it has been shown in addition that in certain layers of the neocortex nerve cells can also be grouped in the vertical direction so as to form columns or barrels. This has been strikingly demonstrated for layer IV in the somatosensory cortex of the mouse by Woolsey and van der Loos (1970).

In contrast to these observations, little attention has so far been paid to the spatial relations of the apical dendrites in the cerebral cortex. Most authors seem to assume that they are distributed more or less at random; but no special investigations are found in the literature. In the present paper, however, it will be shown that the apical dendrites are not distributed at random but running in distinct bundles. These may be of functional importance for the explanation of certain electrophysiological findings which indicate the presence of vertical structures in various regions of the cerebral cortex (cf. Mountcastle, 1957; Powell and Mountcastle, 1959; Hubel and Wiesel, 1963, 1965; Petsche, Rappelsberger and Frey, 1972; and others).

Material and Methods

Adult rabbits and cats were anaesthetized with pentobarbitone sodium and fixed by perfusion from the aorta with a plasma expander (Periston® or Macrodex®) followed by Bouin's solution. To avoid postmortal damage to nerve cells (cf. Cammermeyer, 1962) some hours were allowed to elapse before the skull was opened and the brain dissected. — In the *lissencephalic rabbit*, a particularly flat region of the neocortex which is indicated in Fig. 1

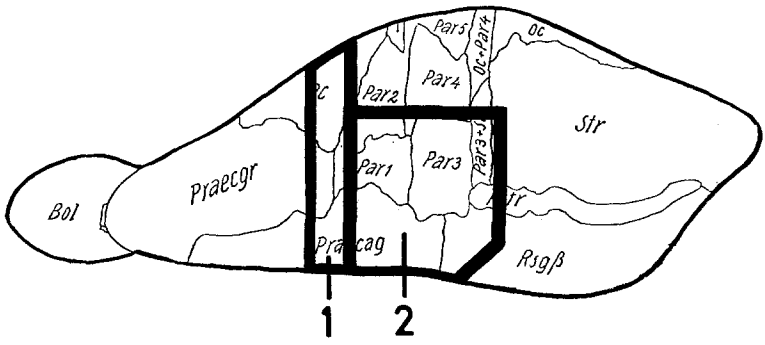

Fig. 1. The cortical regions investigated in frontal (*1*) and in tangential sections (*2*) are indicated on a diagram (Rose 1931, Fig. 3) of the cytoarchitectonic areas of the rabbit's brain as seen from above. Abbreviations: *Bol* Bulbus olfactorius; *Oc* Area occipitalis; *Par 1, 2, 3, 4* Area parietalis *1, 2, 3, 4*; *Pc* Regio postcentralis; *Praecag* Regio praecentralis agranularis; *Pstr* Area peristriata; *Rsgβ* Area retrosplenialis dorsalis; *Str* Area striata

was selected for being serially cut in a plane tangential to the surface after embedding in paraffin. Immediately rostral to this region the adjoining part of the hemisphere was taken out to be sectioned frontally. — In the *gyrencephalic cat* the brains were processed in toto and serially cut in either the frontal, horizontal or sagittal plane. — For studying the spatial relations of dendrites, 8–12 μm paraffin sections treated with one of the following methods were found to be particularly useful: Luxol-fast-blue according to Klüver-Barrera (1953) followed by Goldner's trichrome (cf. Fleischhauer, 1960); Luxol-fast-blue followed by a periodic acid-Schiff (PAS) reaction and counterstaining with Ehrlich's haematoxylin; silver-impregnation according to Bodian in the modification of Luna (1964) and sometimes followed by a Nissl-staining with gallocyanin-chromalaun according to Einarson (Romeis, 1968).

For electron microscopy an adult rabbit was perfused with a buffered solution of 2%, followed by 4% glutaraldehyde instead of Bouin's solution. Four hours later the brain was dissected and carefully orientated blocks were taken from the region indicated by 2 in Fig. 1. The tissue was postfixed in OsO_4, dehydrated in acetone and during dehydration treated with uranyl acetate and phosphotungstic acid.

After embedding in Vestopal® a series of large semithin sections was cut tangential to the cortical surface from layer I through layer II/III to the deeper part of layer IV. These sections were stained with methylene blue and azur II according to Richardson, Jarret and Finke (1960). In the last sections of this series a particular bundle of dendrites was marked. From this region ultrathin sections were cut, contrasted with lead citrate and investigated under the Siemens 101 electron microscope.

Observations

In paraffin sections stained with either the Klüver-Goldner or the Klüver-PAS-haematoxylin method, the main dendritic stems and their larger branches remain practically unstained and stand out against the neuropil, the perikarya and the cell nuclei. Cross sections of large dendrites have some features in common with cross sections of capillaries but can be discriminated because they lack basement membrane and endothelial sheath. Since in sections treated with Klüver-PAS even the finest basement membranes are clearly delineated so that cross sections of capillaries and large dendrites are not to be confused, this method was preferred for analysing the spatial arrangement of the dendrites in tangential sections. For this purpose Bodian sections are less suitable because poorly impreg-

nated cell nuclei may be mistaken for cross sections of dendrites. In frontal sections, however, the analysis of the dendritic pattern is greatly facilitated by comparing Klüver-PAS sections with adjacent sections impregnated according to Bodian.

Rabbit. The almost flat surface of the cortical region selected for this investigation (Fig. 1) permits a comparison of frontal and truly tangential sections through the same part of the cortex and thus facilitates the analysis.

When investigating at low magnification a frontal section stained with Klüver-PAS, a vertical striation is seen in layer IV and extending into layer III/II. At high magnification it becomes clear that this striation is brought about by groups of large dendrites. As shown in Fig. 2, the large apical dendrites of several nerve cells approach each other to run side by side until they bifurcate in layer III or II. Such groups of dendrites running in close apposition are called dendritic bundles.

The fact that most apical dendrites are organized in bundles is best seen in horizontal sections through layer IV. As illustrated in Fig. 3, the cross sections through large apical dendrites are not evenly distributed but arranged in groups with distances of about 40–50 μm in between. Each group corresponds to the cross section of a dendritic bundle. Most bundles have a diameter of between 20 and 35 μm and contain several large dendritic stems. There are some bundles, however, which are mainly formed by dendrites with a considerably smaller diameter. The picture changes at the border between layer IV and III because here many of the large apical dendrites begin to bifurcate.

Comparison of frontal sections with tangential sections as well as an investigation of serial sections in the tangential plane reveals that within a given vertical bundle the individual dendrites are constantly changing position with respect to each other as they ascend from layer V to layer III/II. As seen at high magnification, in every plane some of the dendrites are in close contact whereas others are separated by a thin layer of neuropil. Occasionally, a small myelinated fibre is seen within the confines of a bundle.

Details of the relations between the dendrites within a bundle can only be revealed by means of electron microscopy. In Fig. 4 a cross section of a dendritic bundle with a diameter of about 20 μm is illustrated at a low magnification of the electron microscope. The bundle is formed by 8 dendrites with diameters of between 3 and 6 μm and by some smaller ones. The dendrites seen in the lower right half of the bundle are separated from those seen in the upper left half and from each other by numerous very small profiles and some small myelinated fibres. The dendrites seen in the upper left part of the picture are more closely related: Two are separated by an extremely thin sheet of neuroglia and two are in direct contact so that for a great part of their circumference the two plasma membranes are separated by the extracellular space only. Between the dendrites in close apposition special membrane structures such as synapses or tight junctions have so far not been observed, but further and more detailed electron microscopic investigations are required to settle this question. It is most likely that cross sections through dendrites separated by the extracellular space only may appear as single, particularly large dendrites in light microscopical pictures such as Fig. 3.

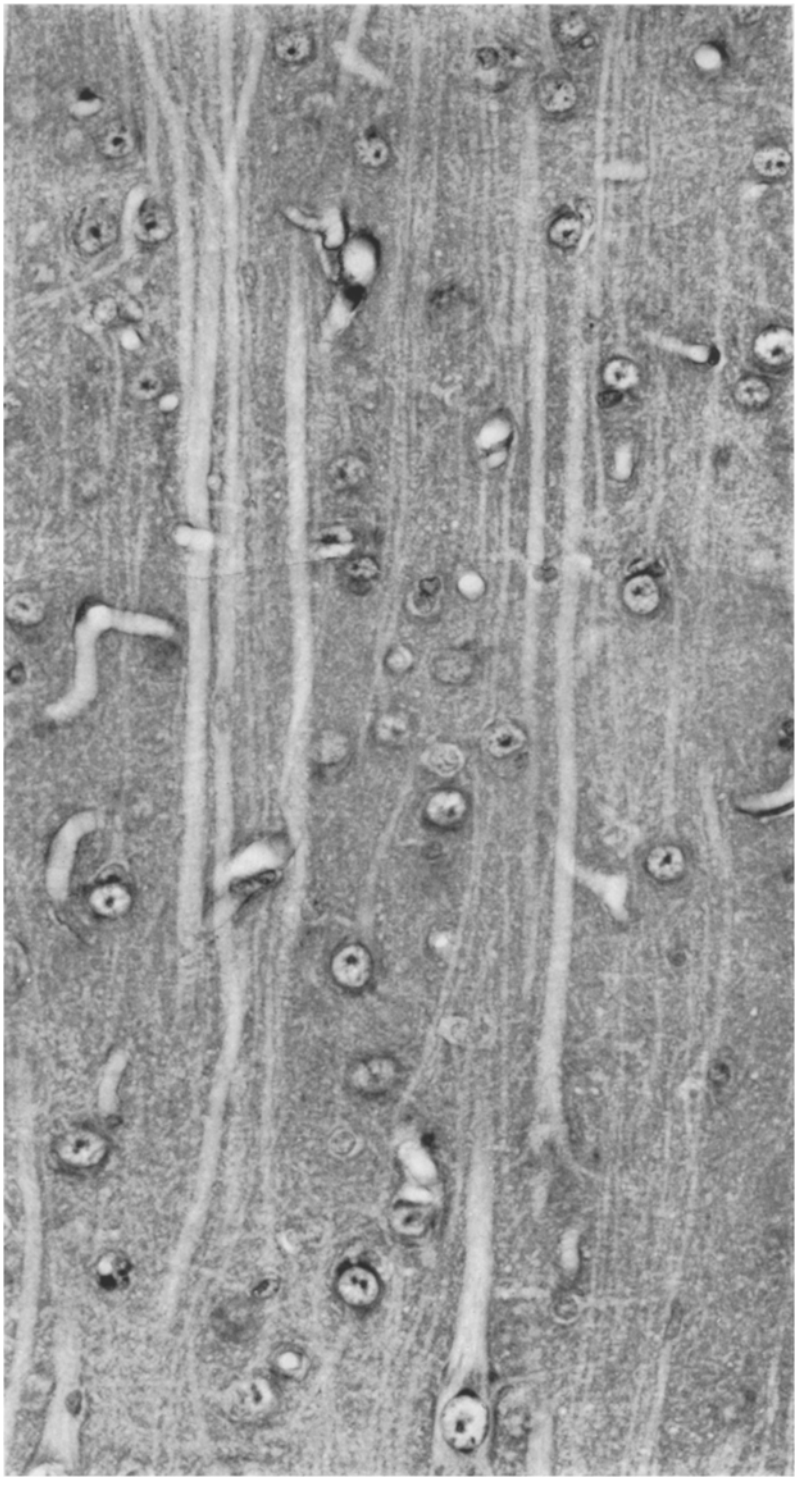

Fig. 2

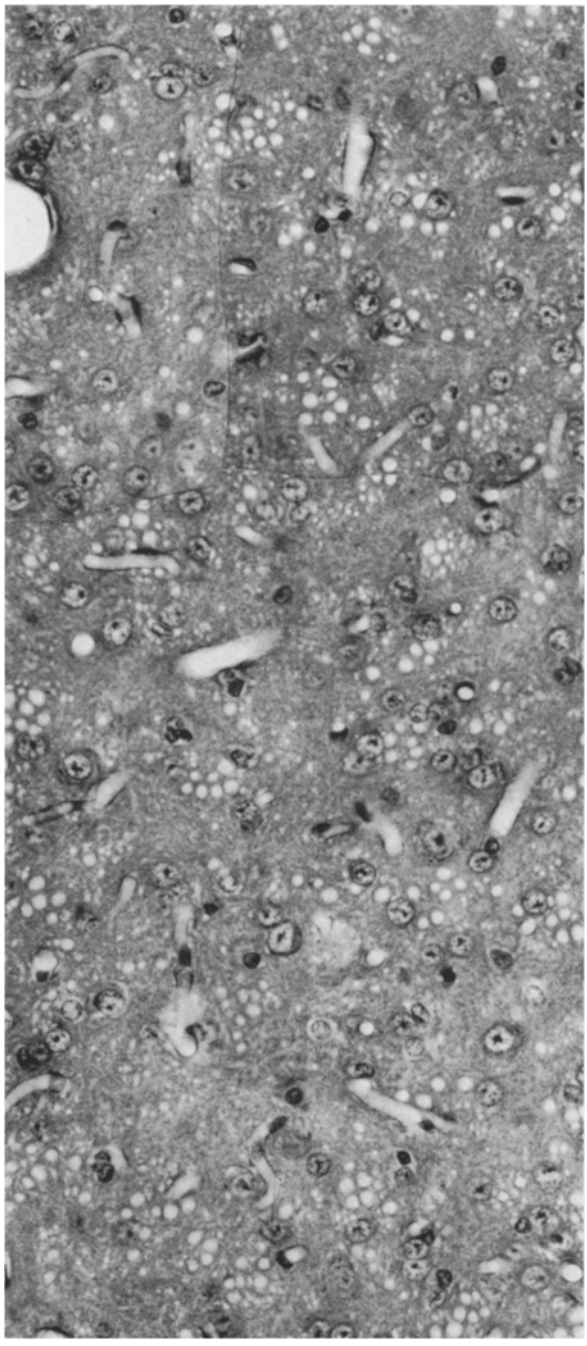

Fig. 3

Fig. 2. Frontal section through parietal cortex of the rabbit's brain. Bundles of apical dendrites being formed in the upper part of layer V and extending through layer IV into layer III/II. Klüver-PAS-haematoxylin. × 480

Fig. 3. Tangential section through parietal cortex of the rabbit's brain. In layer IV, many of the cross sections through large apical dendrites are organized in distinct groups, each indicating a vertical bundle of dendrites. Klüver-PAS-haematoxylin. × 350

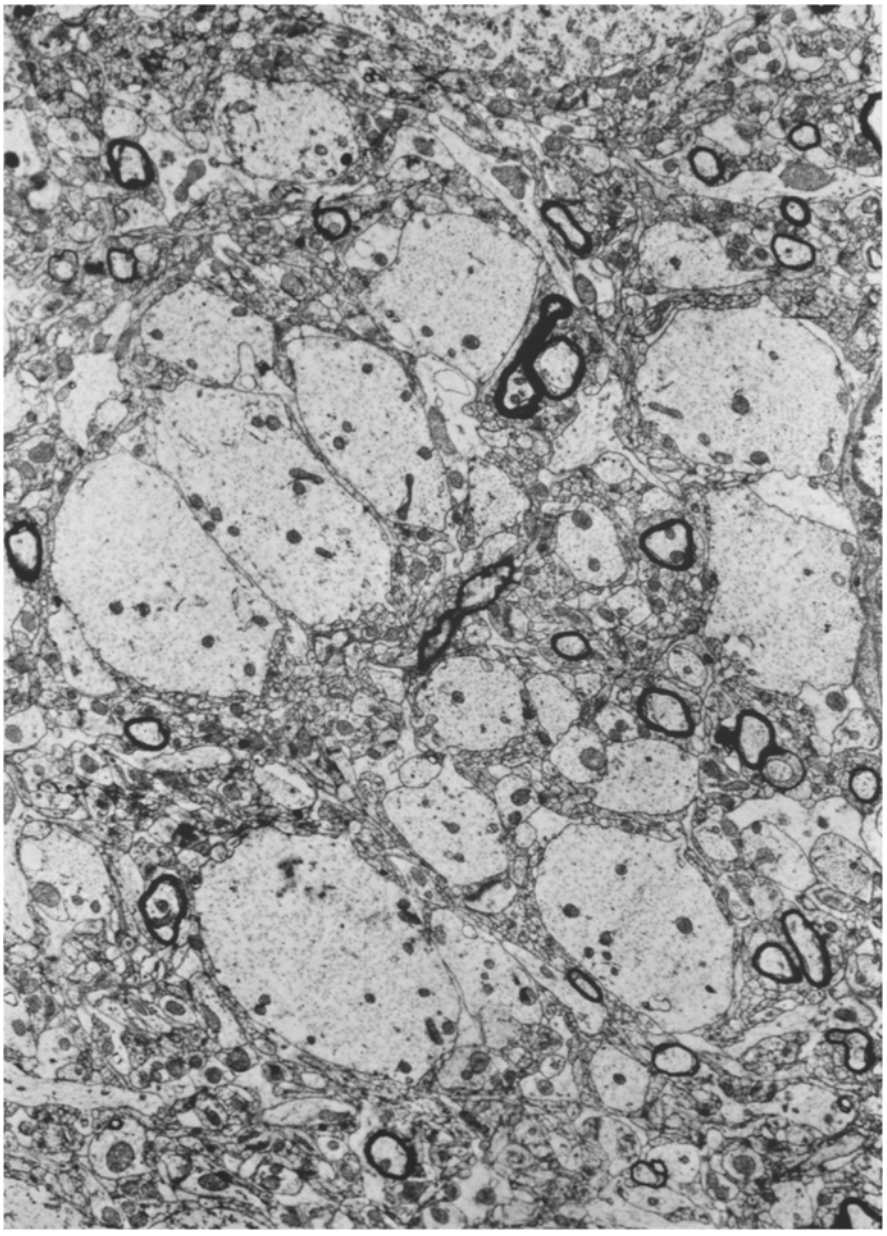

Fig. 4. Cross section of a vertical bundle of dendrites in layer IV of the parietal cortex of the rabbit's brain. For details see text. Glutaraldehyde-OsO_4. $\times 7000$

In subsequent sections through the same bundle, the individual dendrites seen in Fig. 4 have been traced to deeper levels. It was found that those dendritic stems which have direct contact in the plane of Fig. 4 have now become separated

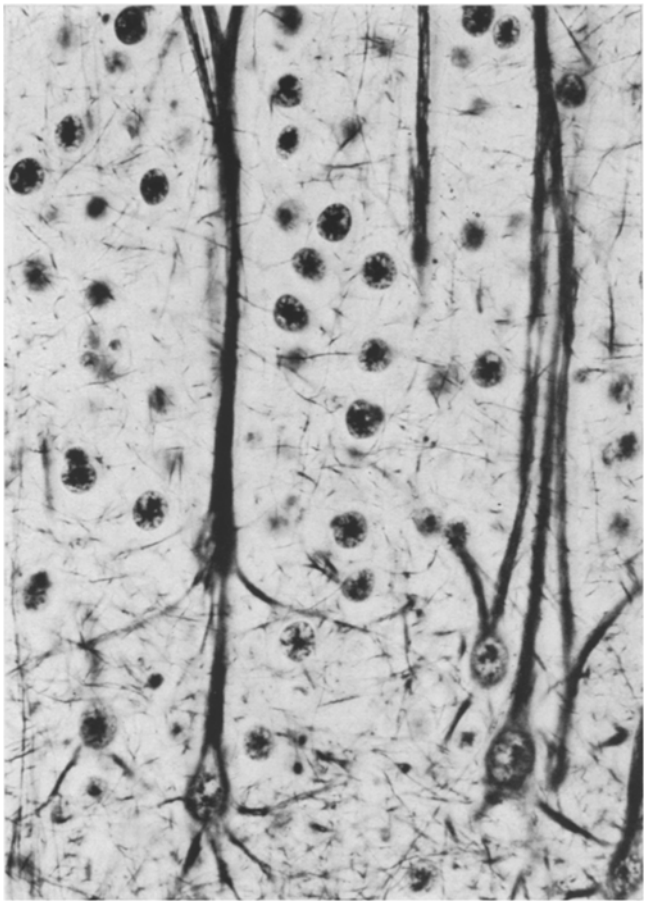

Fig. 5. Frontal section through the gyrus lateralis of the cat. Bundles of apical dendrites being formed in the upper part of layer V and extending through layer IV. Bodian × 350

by small profiles of neuropil whereas other dendrites of the same bundle have come into close contact. Therefore the vertical extent of the stretches of direct contact between two adjacent apical dendrites seems to be relatively short and not due to dendritic branching.

Cat. The internal structure of the cerebral cortex is more complex than in the rabbit, and the geometry is further complicated by the presence of gyri and sulci. However, in Klüver-PAS stained sections tangential to the surface of a gyrus, a grouping of cross sections of dendrites is observed in various regions of the neocortex: in horizontal sections, for instance, in the gyrus lateralis; and in tangential sections, in the gyrus fornicatus.

In Bodian sections, a distinct bundling of dendrites has been observed in many regions. In Fig. 5 two vertical bundles in the gyrus lateralis are illustrated. As in the rabbit, the bundles are formed by the large apical dendrites of several pyra-

midal cells. The dendrites approach each other shortly after having originated from the perikaryon and as in the rabbit the bundles extend through layer IV into layer III before they ramify.

In the cat, the picture is further complicated by the fact that immediately after leaving the perikaryon, many apical dendrites bifurcate. Usually, the two large dendritic stems of such cells join different dendritic bundles but sometimes one of the two is seen to run alone. In addition, the dendritic bundling in cats shows pronounced regional differences which require further investigation.

Discussion

The laminar arrangement of the nerve cells in the mammalian cerebral cortex has been studied in great detail and numerous cytoarchitectonic and myeloarchitectonic areas have been described. In addition, it has been known for several decades that the myelinated axons entering or leaving the cerebral cortex are organized in distinct bundles or fascicles (cf. Kaes, 1907). These fascicles could be an indication of some vertical or columnar organization within the cortex. So far, however, little is known with regard to the question of whether or not elements situated on top of each other in the various laminae of a given cortical region are morphologically organized so as to form vertical units. But in one particular region, i.e. in layer IV of the somatosensory region (S1) of the mouse, an arrangement of nerve cells in distinct vertical "barrels" has recently been described and strikingly illustrated by Woolsey and van der Loos (1970).

Since electrophysiological evidence strongly suggests the existence of vertical structures in other parts of the cerebral cortex as well as in other species, the present investigation was started in order to find out whether columns of nerve cells similar to those described for S1 in the mouse also exist in the rabbit's brain and if so, to correlate the morphological observations with electrophysiological findings obtained in the same species. However, a detailed study of serial sections in the frontal and horizontal plane did not reveal a picture similar to that described by Woolsey and van der Loos. Instead, a different kind of vertical organization was found in the form of dendritic bundles. This finding was unexpected, although during the course of this investigation an organization of dendrites in bundles was described for motoneurons in the spinal cord by Scheibel and Scheibel (1970 cat, monkey) and by Marsh, Matlovsky and Stromberg (1971 pig). Whereas the observations of dendritic bundles in the spinal cord are on the light microscopic level only, the electron microscopic investigation of dendritic bundles in the cerebral cortex of the rabbit has provided additional evidence for the close relationship that exists between the dendrites forming a bundle. And the finding that within the vertical bundles of the parietal cortex the dendrites may approach each other so closely as to be separated by the extracellular space only, is of obvious electrophysiological importance because it may provide the structural basis for an electrotonic summation of the events taking place in the dendrites running parallel in close association.

Although it is certain that in rabbits and cats most of the large dendrites within a bundle are apical dendrites arising from large pyramidal cells (Fig. 2),

the origin of the smaller components of the bundles could not be ascertained with the methods used in the present investigation. It remains to be shown whether the bundles also contain dendrites of stellate cells and descending apical dendrites of inverted pyramids situated in the upper layers of the cerebral cortex.

The morphological observations help to understand a number of electrophysiological findings which have accumulated during the past 15 years. When studying the field S1 in cats with microelectrodes, Mountcastle (1957) found that cells having the same type of reaction to peripheral stimuli are arranged in vertical columns. This kind of arrangement has since been confirmed by investigations of several other areas and in various species (Powell and Mountcastle, 1959; Hubel and Wiesel, 1963, 1965; Welt, Aschoff, Kameda and Brooks, 1967; and others). Whereas these observations are based on microelectrode studies, a vertical arrangement of functional units of a different order of magnitude than single nerve cells has also been postulated as the result of observations on the electrocorticogram (ECG) recorded with surface- and semi-microelectrodes.

It has been known for a long time (Li, Cullen and Jasper, 1956) that most ECG activity when observed simultaneously from epi- and subcortical electrode sites, is reversed in phase, with a zone of zero potential in between. This observation led to the hypothesis of the ECG generators being represented by vertically arranged dipoles. There has been much argument about whether or not the apical dendrites of pyramidal cells may be the morphological equivalent of these dipoles. The mechanism by which a depolarization of the soma region is correlated with a hyperpolarization of the apical dendrite arborizations is still unknown, and probably one or two interneurons play a part in these events (Raabe and Lux, 1972; Elul, 1972).

Investigation of penicillin spikes (Petsche, Rappelsberger and Frey, 1971; Prince and Wilder, 1967; Tharp, 1971) led to the conclusion that when acting as a distinguishable functional unit of discharges, i.e. as a generator, most likely a few tens of apical dendrites are synchronized. Similar conclusions were obtained from studies of the phenomenon of synchronization in epileptic seizures. Starting point was the observation that the potential gradients in the horizontal plane of the cortex are fairly steep. During seizures, gradients of up to 2 mV/mm have been measured (in penicillin foci even more than 3 mV/mm). As pointed out by Petsche, Rappelsberger and Frey (1972), this observation is not compatible with the assumption of the cortical tissue being a good passive conductor for its electric activity. Moreover, large brain waves such as seen in seizures, spread along the cortex with speeds in the range of between 10^{-2} to 10^{-1} m/sec.

To explain this phenomenon and its behaviour after cortical incisions (Petsche, Rappelsberger and Trappl, 1970), the existence of a grid of homologous, densely packed and vertically arranged functional units (generators) with certain degrees of mutual connectedness must be assumed. From the electrophysiological findings in the rabbit, a grid distance of about 50 μm has been postulated. It seems likely, that the morphological equivalent of this grid is provided by the vertical bundles of dendrites described in this paper. Taking into account the shrinkage of the tissue due to dehydration and sectioning, the diameter of and the distance between the bundles as observed in the histological sections are of the same order of magnitude as the values deduced from the electrophysiological studies.

Simultaneous recordings from several epi- and intracortical electrodes have thrown some light on the lenght of the functional columns (Petsche, Rappelsberger and Frey, 1972). From the shape of epicortically recorded ECG potentials which lack the higher-frequency components and look more uniform than the same potentials when recorded from deeper intracortical levels, the existence of a low-pass-filter effect of the superficial layers was concluded. This means that the postulated functional units, i.e. the generators, do not reach the cortical surface but end a few hundred micra below. This also holds true for the vertical dendritic bundles because they were shown to end where the dendrites begin to bifurcate in layer III/II.

The good agreement between the deductions made as a result of electrophysiological studies and the anatomical observations described in this paper strongly supports the hypothesis that the vertical bundles of dendrites in the cerebral cortex are the morphological substrate of the vertical functional columns or generators postulated in the electrophysiological investigations.

References

Cammermeyer, J.: An evaluation of the significance of the "dark" neuron. Ergebn. Anat. Entwickl.-Gesch. **36**, 1—61 (1962).

Elul, R.: Randomness and synchrony in the generation of the electroencephalogram. In: Synchronisation of EEG-activity in epilepsies, ed. by H. Petsche and M. A. B. Brazier. Wien-New York: Springer 1972.

Fleischhauer, K.: Fluorescenzmikroskopische Untersuchungen an der Faserglia. Z. Zellforsch. **51**, 467–469 (1960).

Hubel, D. H., Wiesel, T. N.: Shape and arrangement of columns in cat's striate cortex. J. Physiol. (Lond.) **165**, 559–568 (1963).

Hubel, D. H., Wiesel, T. N.: Receptive fields and functional architecture in two non-striate visual areas (18 and 19) of the cat. J. Neurophysiol. **28**, 229–289 (1965).

Kaes, Th.: Die Großhirnrinde des Menschen in ihren Maßen und in ihrem Fasergehalt. Ein gehirnanatomischer Atlas, 2 Bde. Jena: Gustav Fischer 1907.

Klüver, H., Barrera, E.: A method for the combined staining of cells and fibres in the nervous system. J. Neuropath. exp. Neurol. **12**, 400–403 (1953).

Li, C. L., Cullen, C., Jasper, H. H.: Laminar microelectrode analysis of cortical unspecific responses and spontaneous rhythms. J. Neurophysiol. **19**, 131—134 (1956).

Luna, L. G.: Further studies of Bodian's technique. Amer. J. med. Technol. **30**, 355–362 (1964).

Marsh, R. C., Matlovsky, L., Stromberg, M.: Dendritic bundles exist. Brain Res. **33**, 273–277 (1971).

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

Petsche, H., Rappelsberger, P., Frey, Zs.: Intrakortikale Mechanismen bei der Entstehung der Penicillinspitzen. EEG-EMG **2**, 176–180 (1971).

Petsche, H., Rappelsberger, P., Frey, Zs.: Intracortical aspects of the synchronisation of self-sustained bioelectrical activities. In: Synchronisation of EEG-activity in epilepsies, ed. by H. Petsche and M. A. B. Brazier. Wien-New York: Springer 1972.

Petsche, H., Rappelsberger, P., Trappl, R.: Properties of cortical seizure potential fields. Electroenceph. clin. Neurophysiol. **29**, 567–578 (1970).

Powell, T. P. S., Mountcastle, V. B.: Some aspects of the functional organization of the cortex of the postcentral gyrus of the monkey: a correlation of findings obtained in a single unit analysis with cytoarchitecture. Bull. Johns Hopk. Hosp. **105**, 133–162 (1959).

Prince, D. A., Wilder, B. J. F.: Control mechanisms in cortical epileptogenic foci. "Surround" inhibition. Arch. Neurol. (Chic.) **16**, 194–202 (1967).

Raabe, W., Lux, D.: Studies on extracellular potentials generated by synaptic activity on single cat motor cortex neurons. In: Synchronisation of EEG-activity in epilepsies, ed. by H. Petsche and M. A. B. Brazier. Wien-New York: Springer 1972.

Richardson, K. C., Jarett, L., Finke, E. H.: Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Stain Technol. **35**, 313–323 (1960).

Romeis, B.: Mikroskopische Technik. München-Wien: R. Oldenbourg 1968.

Rose, M.: Cytoarchitektonischer Atlas der Großhirnrinde des Kaninchens. J. Psychol. Neurol. (Lpz.) **43**, 353–440 (1931).

Scheibel, M. E., Scheibel, A. B.: Organization of spinal motoneuron dendrites in bundles. Exp. Neurol. **28**, 106–112 (1970).

Tharp, B. R.: The penicillin focus: a study of field characteristics using cross-correlation analysis. Electroenceph. clin. Neurophysiol. **31**, 45–55 (1971).

Welt, C., Aschoff, J. C., Kameda, K., Brooks, V. B.: Intracortical organization of cat's motorsensory neurons. In: Neurophysiological bases of normal and abnormal motor activities, ed. by M. D. Yahr and D. P. Purpura, p. 255–294. Hewlett, New York: Racen 1967.

Woolsey, T. A., Loos, H. van der: The structural organization of layer IV in the somatosensory region (S1) of mouse cerebral cortex. Brain Res. **17**, 205–242 (1970).

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