Z. Anat. Entwickl.-Gesch. 141, 115—123 (1973)

Further Observations on Vertical Bundles of Dendrites in the Cerebral Cortex of the Rabbit*

Walter Massing and Kurt Fleischhauer
Anatomisches Institut der Universität Bonn

Received May 23, 1973

Summary. In tangential serial paraffin sections through the somatosensory cortex of the rabbit, apical dendrites were traced from the border of layer III/IV to their origin in layer V. From tracings obtained in area parietalis 1 a complete three dimensional reconstruction of several vertical bundles of dendrites was made in a square area measuring about 190 × 210 μm. It was found that (1) The majority of apical dendrites takes part in the formation of vertical bundles. (2) The composition of the bundles changes as they ascend from layer V through layer IV. (3) The apical dendrites of a bundle converge so as to form a "neck" below a point near the border of layer IV/III where the majority of the dendrites bifurcate into obliquely running branches. (4) In layer IV the bundles are interconnected by dendritic branches of large pyramidal cells the apical shafts of which bifurcate in layer V or deep in layer IV. (5) In addition, an extensive and complicated exchange of fibres running in different bundles through layer IV takes place in layer III.

Key words: Cerebral cortex—Apical dendrites—Dendritic bundles—Rabbit.

Introduction

Recently, it was shown that in the cerebral cortex of rabbit (Fleischhauer, Petsche and Wittkowski, 1972) and rat (Peters and Walsh, 1972) groups of apical dendrites of large layer V pyramids approach each other to run in close association through layer IV so as to form "clusters" or "vertical bundles of dendrites".

The composition and detailed structure of these vertical bundles of dendrites needs further clarification; and from an electrophysiological point of view it would be interesting to know whether, and if so to what an extent, adjacent bundles are interconnected with each other.

The present investigation deals with these questions and is a continuation of the earlier work in the rabbit. In order to obtain a better insight into the composition and detailed structure of vertical bundles, reconstructions were made from serial sections tangential to the surface of the cerebral cortex. The results will be described by illustrating and analysing a group of vertical bundles in area parietalis 1 of Rose (1931).

Material and Methods

An adult rabbit was anaesthetized with pentobarbitone sodium and fixed by perfusion from the aorta with a plasma expander (Periston N) followed by Bouin's solution. To avoid postmortal damage to nerve cells (Cammermeyer, 1962) a few hours were allowed to elapse before the brain was taken out and embedded in paraffin. As described in the previous paper (Fleischhauer, Petsche and Wittkowski, 1972) the brain was cut tangentially to the surface in a region indicated by 2 in Fig. 1 of that paper. This region includes large parts of area parietalis 1 and 2 and of regio praecentralis agranularis of Rose (1931).

* Supported by the Deutsche Forschungsgemeinschaft (Grant Fl 26/13).

Serial 8–10 μm sections were cut from the pia through the entire depth of the cortex. All sections were stained with Luxol fast blue followed by a perjodic-acid-Schiff reaction (PAS) and counterstaining with Ehrlich's hematoxylin because in the earlier paper it had been shown that with this staining each dendrite becomes visible as an empty profile against a stained background. In photographs of subsequent sections individual dendrites could be identified and followed downwards from the border of layer III/IV to the respective perikarya in layer V.

For three dimensional analysis several regions in area parietalis 1 and 2 as well as in area praecentralis agranularis were selected. Areas measuring about 200 μm in the antero-posterior and 300 μm in the medio-lateral direction were photographed in successive sections, the exact position of the area in subsequent sections being checked by the presence of blood vessels etc. Starting from a suitable section through layer IV the outlines of vertical dendrites with a diameter of more than about 4 μm were drawn on translucent paper and traced upwards and downwards in successive sections. The outlines of the perikarya giving rise to these dendrites were also drawn on the paper. Each dendrite and each perikaryon was numbered. Dendrites crossing the border of the selected area were also traced and given a number. By superimposing the drawings of the individual sections the exact spatial relationship of the dendrites could be analysed and followed throughout their course from the perikaryon to the upper layers.

After several regions had been investigated in this manner, a three dimensional reconstruction of one region in area parietalis 1 was prepared by using a drawing apparatus originally designed for geographical purposes ("Perspectomat", cf. Matter and Forster, 1970). Although in the series so treated one section was missing and one difficult to use because of stain deposits, all dendrites could be unequivocally identified. For technical reasons the vertical scale of the drawing (Fig. 2c) is 1.3 that of the scale in the horizontal plane.

When drawing the reconstruction it soon became clear that the picture of an area measuring 200×300 μm would be hopelessly complicated and confusing. Therefore a smaller area of about 190×210 μm but still containing several vertical bundles of dendrites was selected for being illustrated. But even for this small area a complete reconstruction of all dendrites and perikarya results in too tangled and crowded a picture. Therefore only those dendrites (and their respective perikarya) were drawn, which in a certain level of layer IV were found to take part in the formation of a bundle. The outlines of dendrites remaining isolated and without relation to a bundle as well as the dendrites coming in and leaving the area at various levels were marked and traced on the translucent paper but not drawn in the reconstruction.

Observations

Analysis of the origin, course and spatial relationship of the dendrites in various regions of area parietalis 1 and 2 and of area praecentralis agranularis has revealed the following consistent features. a) In a given area a considerable proportion of the apical dendrites present takes part in the formation of vertical bundles. b) The composition of a bundle differs in various levels of layer V, IV and III. c) Adjacent vertical bundles are interconnected by dendritic processes of cells the apical shaft of which bifurcates near its origin from the perikaryon or in layer IV. d) In layer III/II where the majority of apical dendrites have bifurcated, there is an intermingling of dendritic branches and bundles can no longer be detected. e) From the topographic position of a perikaryon in layer V no indication can be obtained as to whether or not its dendrite will take part in the formation of a particular bundle or run in isolation.

The various findings will be described with the help of a reconstruction of bundles made from a small region of area parietalis 1. Fig. 1 shows the uppermost section of the reconstructed block of tissue. In the area selected for reconstruction 123 profiles have been identified as dendrites and traced for at least some distance into the deeper layers. Of the 123 dendrites 90 are arranged in what appears as the cross section of 10 dendritic bundles. The other 33 dendrites appear to be

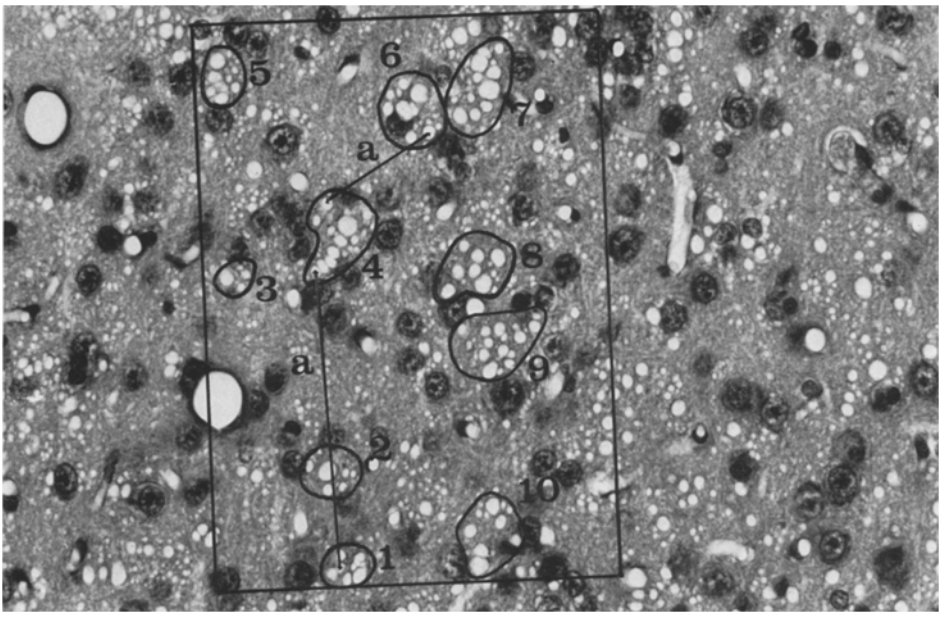

Fig. 1. Tangential section of area parietalis 1 of the rabbit's cerebral cortex. The section passes through the upper third of layer IV and shows cross sections of dendritic bundles. The square indicates the area chosen for the reconstruction Fig. 2 and corresponds to the uppermost level of Fig. 2c. The straight lines (*a*) indicate the distances bridged by branches arising from the deep bifurcations of apical dendrites illustrated in Fig. 4. Luxol fast blue-PAS-Htx. Magn. × 580

without direct relationship to a bundle. In Fig. 1 the bundles are encircled and numbered. In deeper levels (Fig. 2c) bundle 1 and 2 as well as bundle 3 and 4 and bundle 8 and 9 are shown to approach each other and to be part of larger bundles. To each of the main bundles a colour has been assigned and is used in Fig. 2a—d. For the sake of clarity, however, bundle 10 of Fig. 1 is left out in the reconstruction; but its dendrites are shown in the accompanying Fig. 2a and b and are marked with the symbol ⊕. In the drawings Fig. 2a and b all profiles identified as dendrites are shown at two different levels of the cortex in their correct position. Those belonging to the bundles are marked with the colour code, those remaining single throughout the depth of the cortex are given the symbol o and those moving out of the selected area are marked by the symbol +. In bundle 9 a myelinated fibre has been found to be associated with the dendrites throughout the cortex from layer III to layer V/VI; it is marked by the symbol ●.

The analysis of the sections and the reconstruction reveal that during their course from the perikaryon to the site of branching in layer III, the dendrites of one bundle are not exactly parallel but converging so as to form a "neck" in the upper third of layer IV. This region lies between the sections shown in Fig. 2a and b. At the neck the dendrites of each bundle are more tightly packed and there is considerably less neuropil between them than in the lower levels of layer IV.

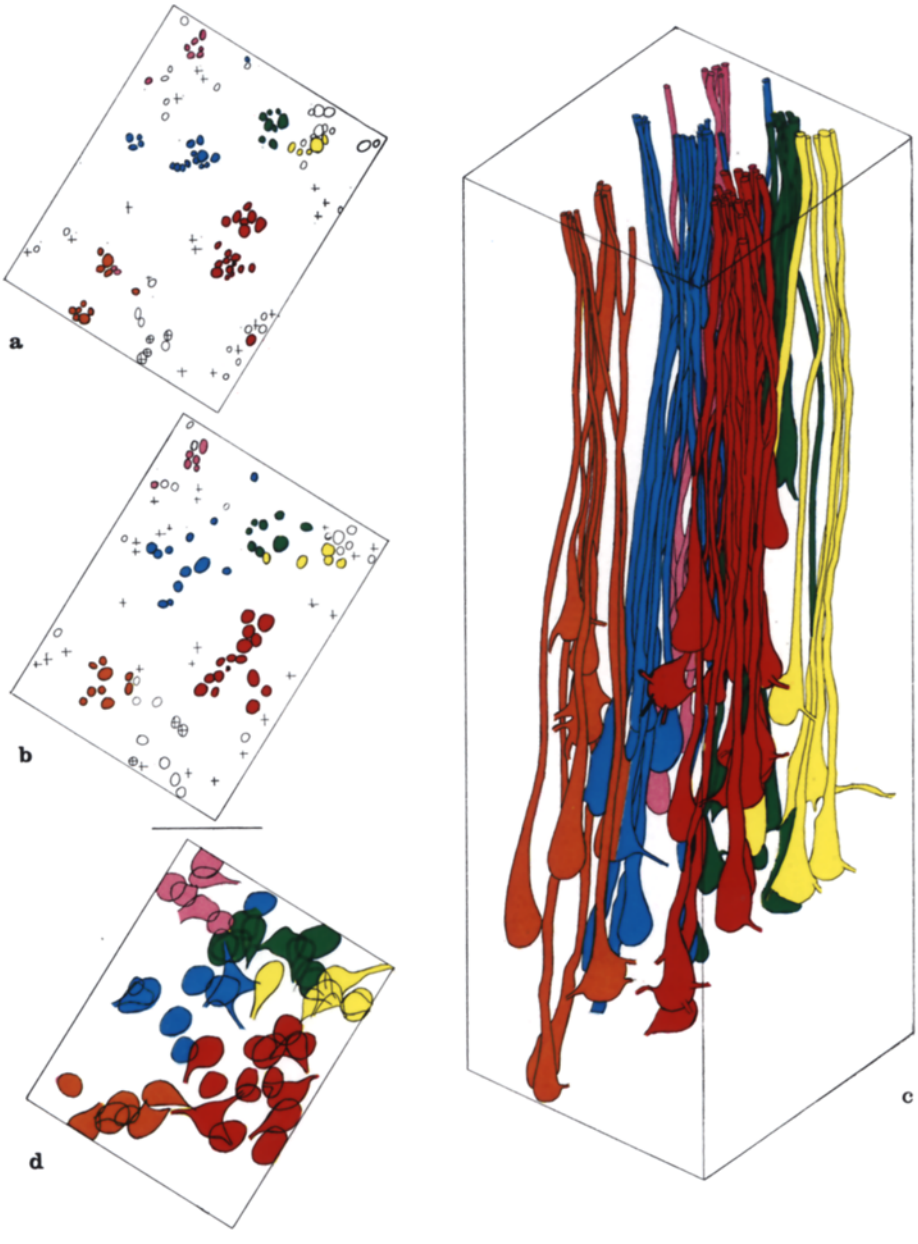

Fig. 2a—d. Reconstruction of the dendritic bundles 1–9 of Fig. 1. The three-dimensional picture (c) is accompanied by sections through its uppermost (a) and a somewhat deeper level (b) and by a drawing indicating the projection of the perikarya on one plane (d). Section (a) corresponds to the inset Fig. 1 but only the dendrites are indicated. In (a) and (b) dendrites leaving the reconstructed area are marked o; dendrites remaining single and without relation to a bundle + and the dendrites of bundle 10 ⊕; ● indicates a myelinated fibre accompanying bundle 9. In (a), (b), (c) and (d) the bundles of Fig. 1 chosen for reconstruction are colour coded as follows: orange = bundle 1 and 2; blue = bundle 3 and 4; pink = bundle 5; green = bundle 6; yellow = bundle 7; red = bundle 8 and 9. For further details see text

As seen from Fig. 2c the dendrites of each bundle originate from perikarya situated in various levels of layer V. At the same time these perikarya are scattered over a considerable area in the horizontal plane. There is no separation between the position of perikarya giving rise to dendrites running in adjacent vertical bundles. This is illustrated by Fig. 2d in which the outlines of the perikarya giving rise to the dendritic bundles are projected on a plane parallel to that of the sections shown in Fig. 2a and b. Of each perikaryon the outline is drawn at the level of its largest diameter. Comparison of Fig. 2d with Fig. 2a—c shows that the cells of origin of the apical dendrites are scattered over an area considerably larger than that occupied by the bundle in layer IV or at the neck. The dendritic branches arising from bifurcations above the neck could not be followed to their termination, and therefore the diameter and configuration of the space in layers II/I in which the terminal dendritic branches of one bundle are distributed could not be determined.

The scattering of the cells from which the dendrites forming a bundle originate is particularly well seen in the projection (Fig. 2d) of the blue bundle. The single perikaryon shown to be separated from the main group of blue cells by some neurones belonging to the green bundle gives rise to an apical shaft which branches in layer V. One of the branches joins the blue bundle and takes part in the formation of its neck (Fig. 2a); the other branch remains separated from the blue bundle and courses upward alongside a dendrite which does not join a particular bundle.

Within each vertical bundle individual dendrites change their position with respect to each other as they ascend through layer IV to layer III. To illustrate this change of position four sections of the red bundle of Fig. 2c are drawn separately in Fig. 3. The bundle is formed by the apical dendrites of 16 cells. Nine of these are situated in the lower half of layer V. Five perikarya are found in the region between plane A and B, and two more between B and C. The changes of position taking place within a bundle can be examplified by describing the relative positions of dendrites 7, 8 and 9 as they ascend to level D. In level A the two apical shafts of 7 and 8 are equally spaced near the perikaryon of cell 9. In plane B a new dendrite (13) has joined the bundle and is situated between 7 and 8 which are still seen at a considerable distance of each other. In plane C the relative position of 7 and 8 is reversed. Dendrite 8 is farthest away from 9, dendrite 7 and 8 are touching each other and between 7 and 9 a new dendrite is found which has arisen out of a bifurcation of dendrite 1. In plane D the relative position of the 3 dendrites is again different. Dendrite 9 has given rise to three branches (9a, b and c) which are running in different directions although still being within the bundle. 9a and b are far away from 9c, 7 and 8. These have come together with a branch of 1 and with the dendrites 6, 16 and 13 to form a particularly tightly packed part of the dendritic bundle. It is to be noted, however, that a systematic change in the position of dendrites such as, for instance, a twisting in a certain direction has never been found in any of the bundles investigated.

When following other dendrites of the bundle shown in Fig. 3 or when analysing other bundles (e. g. blue bundle Fig. 2c) it is obvious that above their neck the bundles usually consist of at least one apical dendrite which has not yet bifurcated and of one or more branches of dendrites which have bifurcated or branched into more than two smaller dendrites. Some of the dendrites originating from

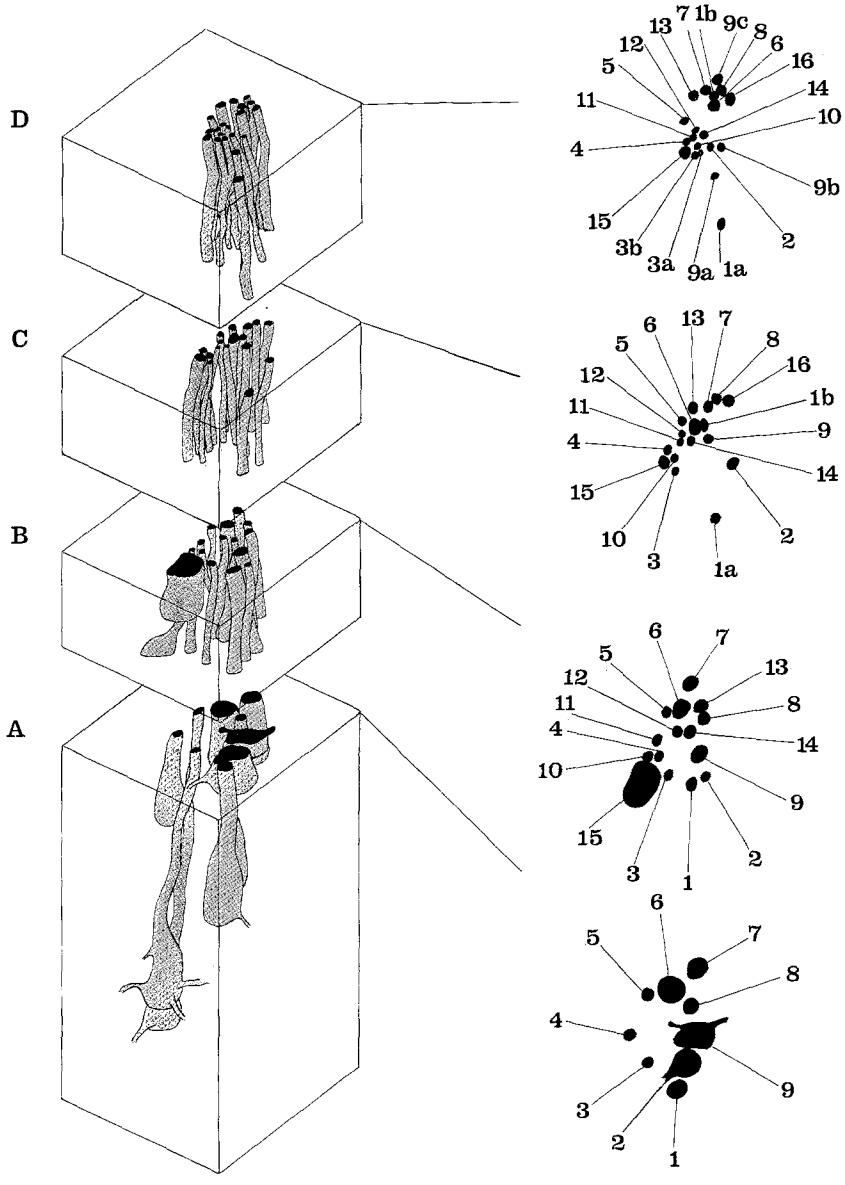

Fig. 3. Detail from reconstruction Fig. 2 to show changes in the relative position of dendrites within a bundle. Four slabs of the red bundle of Fig. 2 are drawn on the left. The accompanying figures on the right correspond to the upper surfaces of these slabs and give the exact outline and position of each dendrite. The 16 dendrites forming the bundle are numbered, and branchings are indicated by an additional a, b, c. For details see text

branchings near layer III move out of their bundle and join dendrites belonging to the branches of an adjacent bundle. For instance, in the series from which Fig. 3 was prepared, the dendritic branches 1a and 9a in plane D could be follow-

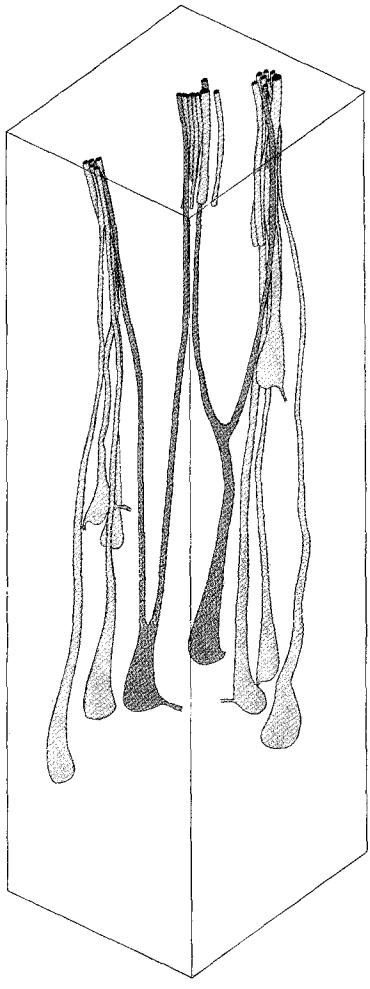

Fig. 4. Detail from reconstruction Fig. 2 to show interconnections between the orange, blue and green bundles by two neurones with dendrites bifurcating in layer V. For details see text

ed for some distance into layer III and at this level shown to be associated with dendrites of a different vertical bundle which was outside the reconstructed area. In this way many clusters of dendrites seen near layer III are composed of branches originally belonging to different bundles. When all dendrites have bifurcated in layer III their branches intermingle to such an extent that bundles can no longer be detected.

Connections between two different bundles of vertical dendrites are not only formed above the region of branching in layer III. There are some nerve cells the apical shafts of which bifurcate near the perikaryon or at least still in layer V into branches joining two different bundles. In the area reconstructed two such cells are found but their position and the course of their dendrites are not clearly seen in the reconstruction Fig. 2c because above the bifurcation the branchings

are shown in the colour of the bundle which they have joined. Therefore in Fig. 4 a picture is given in which bundle 1, 4 and 6 of Fig. 1 are partially reconstructed so as to clearly show the position of the perikarya and the course of the dendrites connecting the bundles. The considerable distance bridged by the dendrites of the two connecting cells is indicated in Fig. 1 by the two straight lines.

Discussion

Three dimensional reconstruction of vertical bundles of dendrites in the rabbit's cerebral cortex has revealed that the dendrites of one bundle are not running exactly parallel in a straight way towards the point of branching near layer III. In fact they were found to converge and to form a "neck" in the upper third of layer IV. The finding that in this region the bundle is particularly tightly packed so that there is less neuropil between the individual dendrites than in the deeper levels of layer IV may be of functional significance. It would be interesting to learn whether at the neck of the bundles membrane specializations are to be found with electron microscopy. This question is particularly intriguing since dendrodendritic contacts in the form of gap junctions have recently been shown to occur in the mammalian cerebral cortex (cf. Sloper, 1972).

The finding of a convergence of dendrites within a bundle towards a neck is not quite in agreement with the observations of Peters and Walsh (1972) in the rat. They describe (e. g. Fig. 12) the individual dendrites of a bundle as having almost the same or even greater distances between each other in the upper than in the lower levels of layer IV. Another difference between the results described in the present paper and the findings of Peters and Walsh (1972) is the confirmation of our earlier observation that within a given vertical bundle individual dendrites are constantly changing position with respect to each other as they ascend from layer V to layer III. Such an arrangement would enhance the possibility of the various dendrites within a bundle to influence each other at least by means of electrotonic summation of the electrical events taking place in the individual dendrites coming into close contact.

In an earlier paper (Fleischhauer, Petsche and Wittkowski, 1972) the hypothesis was put forward that the vertical bundles of dendrites are the morphological substrate of the grid of homologous vertical functional columns or generators extending up to about layer III which have been postulated in electrophysiological studies on the rabbit's cortex by Petsche, Rappelsberger and Frey (1971, 1972). From the results of their studies the authors also deducted, that between the densely packed and vertically orientated functional units or generators there seems to be a certain degree of connectedness. The present investigation has shown that adjacent vertical bundles of dendrites are in fact interconnected by special cells, i. e. by pyramids the dendrites of which bifurcate in layer V or deep in layer IV into branches joining two different bundles. As shown in fig. 4 one bundle may contain dendrites of two such connecting cells so that several bundles may be linked in this way. Therefore the present findings would seem to add further support to the hypothesis that the vertical bundles of dendrites are the morphological substrate of the grid of homologous vertical generators proposed by Petsche, Rappelsberger and Frey (1971, 1972). However, the finding of a connec-

tion between different vertical bundles by dendritic branches of apical shafts which bifurcate in layer V or deep in layer IV does not exclude the possibility that the connectedness observed between the vertical functional units in electrophysiological experiments is established by other morphological structures such as basal dendrites or recurrent axon collaterals.

Projection of the perikarya giving rise to the dendrites of adjacent bundles into one plane reveals a certain degree of grouping. However, the groups of neurones giving rise to dendrites joining different bundles are not clearly separated from each other. These findings correspond to what has been found in a similar projection made in the rat by Peters and Walsh (1972). Therefore, in rabbit and rat the bundling of dendrites is morphologically more conspicuous than the columnar arrangement of nerve cells. This observation agrees with the statement of von Bonin and Mehler (1970) that "A columnar arrangement of cortical cells is more evident in man and other higher primates than it is in most non-primates".

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Prof. Dr. K. Fleischhauer
Anatomisches Institut der Universität
D-5300 Bonn
Nußallee 10
Federal Republic of Germany