PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Network Analysis of Dendritic Fields of Pyramidal Cells in Neocortex and Purkinje Cells in the Cerebellum of the Rat

T. Hollingworth and M. Berry

Phil. Trans. R. Soc. Lond. B 1975 **270**, 227-264
doi: 10.1098/rstb.1975.0008

NETWORK ANALYSIS OF DENDRITIC FIELDS OF PYRAMIDAL CELLS IN NEOCORTEX AND PURKINJE CELLS IN THE CEREBELLUM OF THE RAT

By T. HOLLINGWORTH† and M. BERRY‡

† *Department of Pathology and* ‡ *Department of Anatomy, Medical School, University of Birmingham, Birmingham B15 2TJ, England*

(*Communicated by Sir Francis Knowles, F.R.S.* – *Received* 5 *April* 1974 – *Revised* 5 *August* 1974)

CONTENTS

The connectivity within the dendritic array of Purkinje cells in the cerebellum and pyramidal cells of the neocortex of the rat, stained by the Golgi–Cox method, has been quantified by the method of network analysis. Connectivity was characterized either by applying the system of Strahler ordering, which assigns a relative order of magnitude to each branch of the arborescence or by the identification of unique topological branching patterns within the tree.

The former method has been used to define the entire dendritic array of the Purkinje cell and the apical system of neocortical pyramids It has been shown that the relation between the numbers of branches of successive Strahler order in Purkinje cells form an inverse geometric series in which the highest order is unity and the ratio between successive orders approximates to 3. On the other hand, the apical dendrites of neocortical pyramids exhibit two bifurcation ratios, i.e. a ratio of 3 between low orders and a ratio of 4 between higher orders.

A computer simulation technique was used to generate networks of a size comparable with the Purkinje cell networks and grown according to two hypotheses

namely, a 'terminal growth model' in which additional segments were added randomly to the terminal branches only and a 'segmental growth model' in which additional segments were added randomly to any branch within the array including terminal branches. Subsequent ordering of the simulated trees revealed that the relation between the numbers of successive orders for networks generated according to the 'segmental model' tended towards an inverse geometric series with a ratio of 4 and that generated according to the 'terminal model' tended towards a ratio of 3. This result showed that the dendritic tree of Purkinje cells grow in a manner indistinguishable from a system adding branches to random terminal segments and that neocortical apical dendrites add their collateral branches to random segments of the apical shaft but that the collateral branches themselves grow by random terminal branching. The possibility that such conclusions may be influenced by loss of branches incurred by either a failure of impregnation, by sectioning, or by environmental influences was investigated by means of a computer technique. It was shown that providing such losses occur randomly there is no significant disturbance between the relative number of successive orders.

The method of ordering used gives more precise information about connectivity if the branches are further divided into segments by noting the order of branches converging at each node. It has been shown that the majority of the dendrites of the Purkinje cells are organized into systems of fourth order or less which merge with relatively few fifth, sixth and seventh order branches. These differences in the two parts of the tree were further reflected by equivalent differences in the lengths of branches in the two parts of the tree.

The analysis of unique topological branching patterns was used to study the growth of both the basal dendrites of neocortical pyramids, the side branches of apical dendrites, and the dendrites of Purkinje cells. The frequency distribution of distinct topological branching patterns were enumerated for networks with from 4–7 terminal branches and compared with those expected from networks grown according to the 'terminal' and 'segmental' growth models. In every case the analysis showed good agreement with the terminal model and no agreement with the alternative hypothesis, strongly suggesting that these dendritic systems of both cells grow by branching randomly on terminal segments. With a complete series of topological types of branching patterns for networks with a given number of terminal branches, generated according to either hypothesis, 'absolute bifurcation ratios' may be computed between each order by applying the same method of ordering that was used for the dendrites in Purkinje cells. The absolute bifurcation ratios of networks with a given number of terminal branches are defined as the ratios between adjacent orders in a complete series of topological types. This latter parameter was also used to test the above hypotheses of growth in the small networks of the basal dendrites of neocortical pyramids and substantiated the findings that the topology of these dendrites is established by branching randomly on terminal segments only.

The above results are discussed in relation to the basic principle of network analysis, nature–nurture influences on the growth of dendritic fields and the implications of differing branching patterns on the neurophysiology of the dendritic system.

I. Introduction

Bok (1936) was the first to analyse dendrites quantitatively by measuring the lengths of segments between the branching points of the dendrites of neocortical pyramids in cats. In 1953, Sholl estimated the density of dendritic fields and later (Sholl 1955) introduced the statistical concept of neuronal connectivty between axons and dendrites. At the same time, Eayrs (1955) developed the target method which, like the technique of Sholl (1953), also gave an estimate of dendritic density. Despite the introduction of, for example, new stereological techniques (Mannen 1966), computer reconstruction methods (Levinthal & Ware 1972; Valverde & Ruiz-Marcos

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

1969) and different ordering systems (see, for example, Coleman & Riesen 1968), the methods of Sholl (1953) and Eayrs (1955) have had the widest application and remain the most used.

More recently, Berry *et al.* (1975) and Berry & Bradley (1975*a*) have claimed that the method of network analysis of the branching patterns of dendrites precisely defines their intrinsic connectivity and have suggested that the method might be used both for quantitative comparative studies and neuroanatomical–neurophysiological correlative investigation of the role of dendritic branching in the integration of neural activity. It has also been claimed that the method defines underlying modes of growth of dendritic systems by an analysis of the mature branched structure.

Network analysis tackles the problem of defining an arborescence from two standpoints, by the analysis of distinct topological branching patterns and by applying a centripetal method of ordering to the tree and subsequently analysing the frequencies of orders of differing magnitude. The obvious advantage of network analysis over the conventional target methods of Sholl (1953) and Eayrs (1955) is that the former analysis is independent of the geometry of a given network whereas the latter is applicable to radially orientated fields only. It is perhaps for this reason that asymmetrical dendritic fields such as the apical dendritic system of neocortical pyramids or the dendrites of Purkinje cells in the cerebellum have never been defined quantitatively. Moreover, the need for a new technique of analysis of dendritic fields has been emphasized by Berry, Hollingworth, Flinn & Anderson (1972*b*) in view of the errors inherent in the methods of Sholl and of Eayrs. Berry *et al.* (1972*b*) further pointed out that although the target method of Eayrs may be applicable to comparative studies, absolute measurement using this technique is impossible.

The clear advantage of examining the dendritic branching patterns of whole cells, rather than the dendritic density of a part, is that, along with the synaptic geometry and the surface area characteristics, the branching patterns of dendrites in the entire dendritic system of a cell form the structural basis for integration of post-synaptic dendritic potentials whose summed effects influence spike generation. Accordingly, the documentation of dendritic topology is an essential step towards understanding neuronal function. The observation that dendrites may change their branching patterns and geometry in response to environmental manipulations (Valverde 1968; Coleman & Riesen 1968; Greenough & Volkmar 1973) is interesting because in these circumstances network analysis of dendritic plasticity offers a means of understanding arborescences in terms of their information processing capacity.

The work of Bray (1973*a*), Bunge (1973), Yamada, Spooner & Wessels (1970, 1971) and Ludueña & Wessels (1973) on the growth of neuroblasts in tissue culture has provided valuable information about the properties of the growth cones of 'neurites' and, in conjunction with network analysis, a clearer picture may emerge of how dendritic patterns are generated although the related problem of how genetic and/or environmental factors influence these structures remains obscure. In particular, it now appears certain that branching occurs at growth cones located at the tips, or along the shafts of dendrites (Bray 1973*a*, *b*; Morest 1969*a*, *b*) and it follows that the branching patterns of dendrites are established by branching at the tips of dendrites or along the shafts of any segment in the growing tree. The event appears to occur quite spontaneously in tissue culture (Bray 1973*a*) but, *in vivo*, may be triggered by the random contact of an axon forming a synapse with a filopodium (Vaughn, Hendrikson & Grieshaber 1974) and may be constrained by the elements which surround the dendrities and the pattern of synaptic input converging on the growing network (Berry & Bradley 1975*b*; Bradley & Berry 1975).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

This paper describes the application of network analysis to the quantitative description of the morpholology and growth of dendritic fields of neocortical pyramidal cells and Purkinje cells in the central nervous system of the rat. Two hypotheses of growth have been tested namely branching on random terminals and branching on random segments of the tree. These stochastic models have been chosen arbitrarily but may be useful in high-lighting any constraint deviating growth from a purely random process. The graph theoretical considerations of network analysis have been given elsewhere (Berry *et al.* 1975; Berry & Bradley 1975*a*).

II. Materials and methods

1. *Animals*

Sixteen Wistar rats were killed at 30 days *post partum*, their brains removed and treated by the method of Golgi–Cox as modified by Sholl (1953). Celloidin sections 150 μm in thickness were cut in a standard coronal plane orthogonal to the surface of the cortex (Eayrs & Goodhead 1959). Ten pyramidal cells from each of layers IV and Vb of the sensorimotor cortex of each animal were projected at a magnification of ×600 and their basal dendrites traced. One

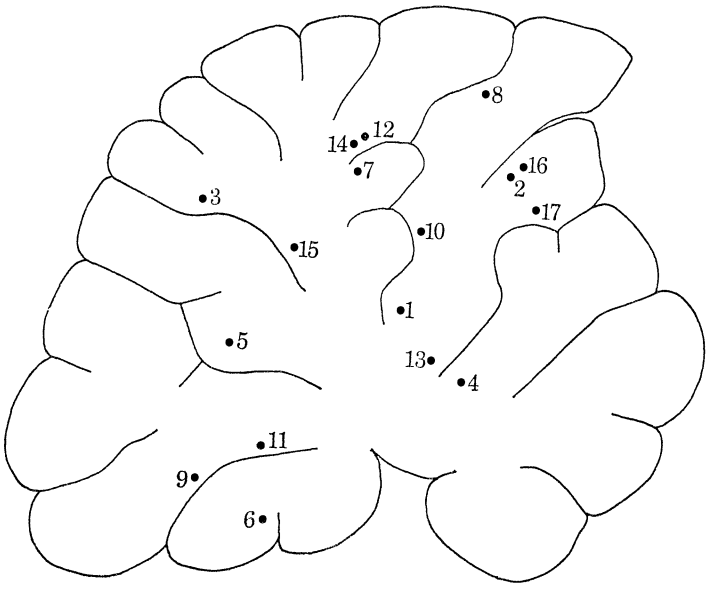


Figure 1. Sagittal section of the vermis of the rat cerebellum (right is anterior) showing the positions of Purkinje cells 1–17 within the folia. The frequency of Strahler orders for each cell is shown in table 2.

hundred complete apical dendrites were also traced from pyramidal cells in both layer IV and layer Vb. A second series of 17 brains from adult animals was prepared in an identical manner and a single 80 μm thick celloidin section was cut through the mid-saggital plane of the cerebellar vermis. One Purkinje cell, which appeared to have a complete and well impregnated dendritic tree, was selected from each section, projected at a magnification of ×1000 and the ramifications of the dendritic tree drawn to their terminations. The positions of the Purkinje cells chosen in sagittal sections through the vermis of the cerebellum are shown in figure 1.

No note of the sex of the animals was made in either series.

2. *Topological analysis*

(*a*) *Terminology*

The method of network analysis reduces the branching pattern of a given dendritic field into a plane graph composed of points, called *vertices*, interconnected by lines, designated *arcs*. Vertices at the outermost tips of the dendritic tree connect one arc and are called *pendant vertices*, the respective *pendant arcs* are the terminal segments. Vertices connecting arcs are the sites of branching and both arcs and pendant arcs form the *segments* of the dendritic array. The *connectivity* of a dendritic tree is defined as the mode of interconnection of arcs and pendant arcs in the network and is not used in the context of synaptic geometry.

Dendritic networks have a functional orientation since they are afferent systems channelling information towards a spike generating area at the *root point* of the network and hence are composed of *true* or *directed* arcs. Dichotomously branching trees have three arcs converging at each vertex constituting one *sink* and two *sources*; the former is defined as the next consecutive arc nearest the root point and the latter are the two arcs which drain into the sink.

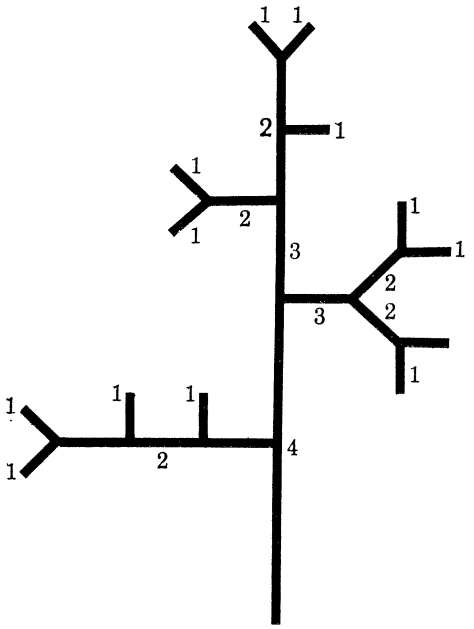


FIGURE 2. Diagrammatic explanation of the method of Strahler ordering. Terminal segments are assigned order 1. The confluence of order 1 branches forms order 2 branches, the confluence of order 2 forms order 3 branches, etc., collateral branches converging on branches of higher order do not change the order of the branch into which they drain but divide it into segments.

(*b*) *Strahler ordering system*

By using the method of Strahler ordering all pendant arcs are first order and the junction of two first order arcs produces a second order arc. Second order arcs receive only first order arcs at their vertices. The junction of two arcs of second order produces an arc of third order. Third order arcs may recieve first and second order arcs at their vertices. Thus a junction of an arc of order n with an arc of similar order produces a resultant arc of order $n+1$. The method is shown diagrammatically in figure 2. A *branch* is defined as a series of consecutive segments of identical Strahler order. A *daughter branch* or *collateral* is defined as any branch of '$n-1$' Strahler order or less which drains into a *parent branch* of Strahler order 'n'.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

(*c*) *Hypotheses of growth*

Figure 3 illustrates how branching patterns are formed by monochotomous branching on segments and dichotomous branching on pendant vertices. The frequency of topological types which are produced at each stage of branching is a function of the mode of growth of the network. For the two forms of branching mentioned above we have worked out the frequencies of topological types that occur over the initial stages of growth (1) when arcs are added randomly to existing arcs (random segmented growth hypothesis) and (2) when arcs are added randomly to pendant arcs or vertices (random terminal growth hypothesis). It can be seen from figure 3 that the frequency analysis of topological types with four or more terminals will distinguish between the two hypotheses of growth.

Monochotomous branching increases the number of pendant arcs in a network by one with the formation of each new vertex and can only occur on segments. *Dichotomous branching* can occur on segments and pendant vertices. In the former case the number of pendant arcs is increased by two with the formation of each new vertex; in the latter, one pendant arc is added with the formation of each new vertex. Berry *et al.* (1975) have pointed out that monochotomous branching on pendant arcs and dichotomous branching on pendant vertices produce identical networks.

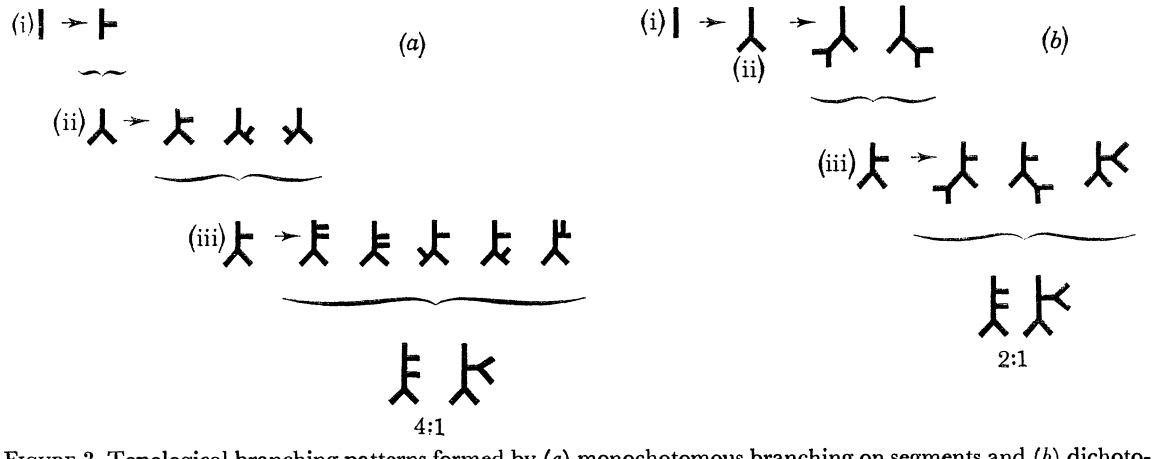


FIGURE 3. Topological branching patterns formed by (*a*) monochotomous branching on segments and (*b*) dichotomous branching on pendant vertices. The Roman numerals refer to the consecutive stages of branching and the patterns to the right of each arrow depict where branching can occur on the pattern to the left of the arrow. The branching patterns seen under each bracket represent the topological types drawn in a standard format, used throughout this paper, into which the patterns above the bracket may be resolved. The ratio against the products of the third stage of branching refers to the frequency distribution of each topological branching pattern.

(*d*) *Pyramidal cells*

A number of basal dendrites arises separately from the perikarya of pyramidal cells of layer IV and Vb. A small proportion of these dendrites remains unbranched whilst much the greater number branch to a varying extent. All basal and apical dendrites were classified into topological types (see figure 4). The topology of the side branches and the apical tuft of branches subtending 4 and 5 pendant vertices was recorded (figure 5). Topological types subtending greater numbers of pendant vertices were too few in number to be included in the analysis. All basal dendrites and the entire apical dendritic system was ordered by the Strahler method.

(*e*) *Purkinje cells*

The original tracings of the cells were overlain with 'Chartpack' tape by using a different colour for each Strahler order. The tape was then removed from the drawing and used to reconstruct the dendritic tree in plane graph form. All arcs subtending 4, 5, 6 and 7 pendant vertices were identified and the topological type distal to each was classified. It will be seen in figure 5 that the less complex types may be included more than once in the analysis when they comprise the sub-units of larger groupings.

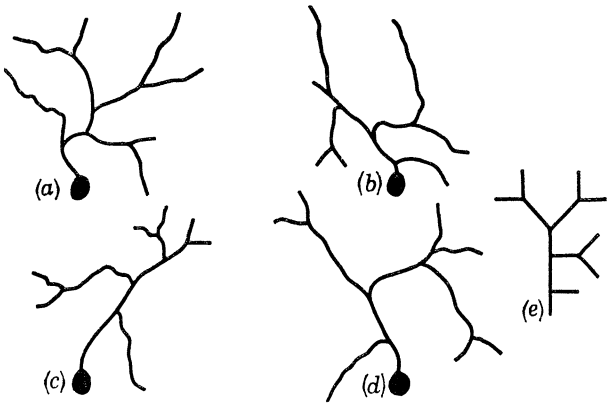


FIGURE 4. Typical basal dendrites are illustrated in (*a*), (*b*), (*c*) and (*d*) and are representative of a single topological branching pattern (*e*). The rationale for resolving mirror images and rotational differences is given by Berry *et al.* (1975).

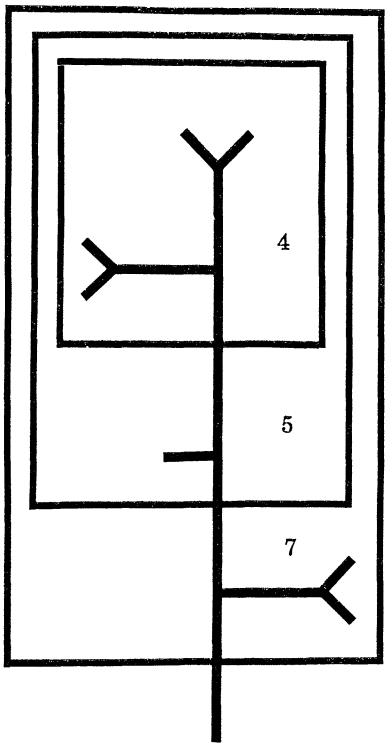


FIGURE 5. Illustration of the method of subidividing the dendrites of Purkinje cells and the apical dendrites of neocortical pyramids according to their numbers of pendant arcs. Topological types contained within each box have the number of pendant arcs shown. The validity of this form of analysis is discussed by Berry & Bradley (1975*a*).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Each dendritic segment was numbered. Pairs of numbers were then fed into a KDF 9 computer each comprising the number of a designated segment and the number of the segment into which this designated segment drained. When all segments were entered the computer was programmed to print out (1) the sources and sink of each segment (see §II 2 (*a*) for definition of source and sink); (2) the total number of branches of each Strahler order and (3) the number and Strahler order of daughter branches stemming from parent branches of given Strahler order. These data were checked by running them through the computer until errors such as duplications or missing segments, loops etc. were eliminated.

3. *The lengths of the dendrites of Purkinje cells*

The length of each dendritic segment was measured directly from the taped reconstructions. The mean lengths of segments and branches were computed together with the mean *path lengths* of each Strahler order. This latter parameter is defined as the mean length of the path from the axon hillock to the most distal part of each branch of a given order.

4. *Dendritic density of Purkinje cells*

The density was given by the mean dendritic length occupying 1 μm^2 of dendritic area. Total dendritic length was computed by summing all the segment lengths and the outline of the silhouette of the field was drawn by joining all the pendant vertices stemming from major branches, the paper cut-outs were weighed and dendritic field area calculated in μm^2 (figure 6).

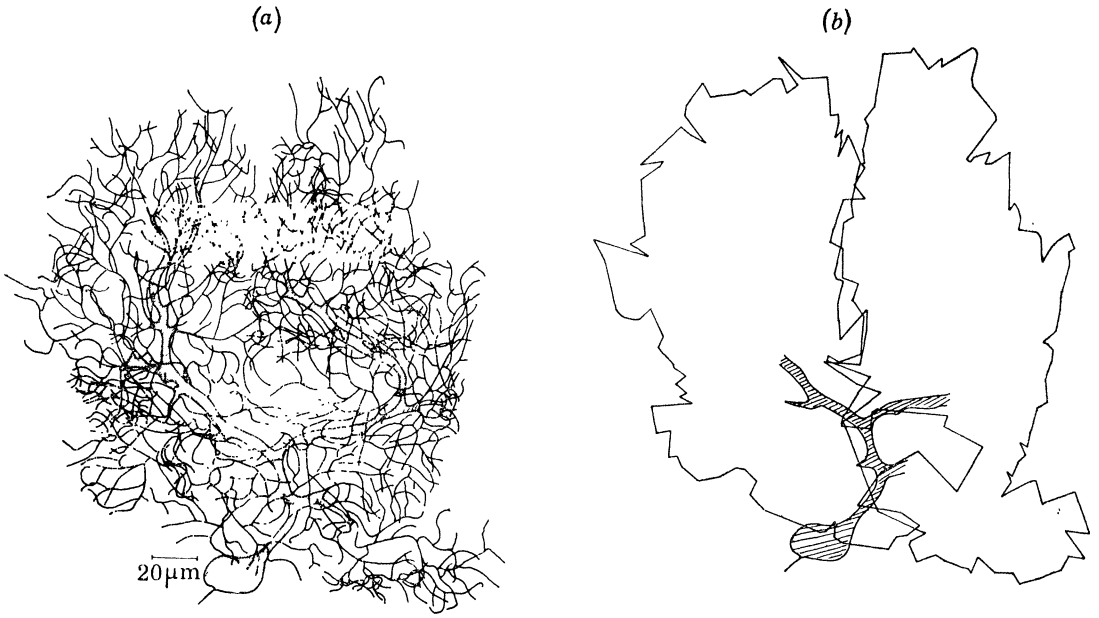


FIGURE 6. (*a*) Dendritic field of Purkinje cell no. 5 (see table 2 for quantitative data). The large hatched dendrites are smooth and the lined dendrites the spiny branches. (*b*) Area of the dendritic tree drawn by joining all the terminals in figure 6*a*.

5. *Treatment of results*

The results obtained by the topological analysis and by Strahler ordering of the cell types were compared with expected results for the segmental and terminal models.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

(*a*) *Topological analysis*

The frequency distributions of observed topological types, with from 4–7 pendant vertices were compared using a χ^2 analysis with the expected distributions for the two models each of which produces markedly different frequency distributions. Because of the small size of some of the expected values, the results of the χ^2 analysis were compared with a frequency distribution of 'pseudo χ^2' obtained by using a KDF 9 computer. The computer was programmed to

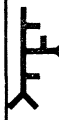
item		observations	pendant arcs	possible topological types											
															
pyramids in layer IV	basal	103	4						63	40					
	apical	93							50	43					
pyramids in layer Vb	basal	104							73	31					
	apical	208							124	84					
Purkinje cells		666							469	197					
															
pyramids in layer IV	basal	88	5						49	29	10				
	apical	65							33	18	14				
pyramids in layer Vb	basal	106							57	29	20				
	apical	130							80	31	19				
Purkinje cells		435							203	166	66				
															
pyramids in layer IV	basal	55	6				15	10	12	10	6	2			
	apical	55					11	11	9	12	9	3			
pyramids in layer Vb	basal	94					22	16	15	20	17	4			
	apical	73					19	16	13	10	10	5			
Purkinje cells		338					98	67	58	40	51	24			
															
pyramids in layer IV	basal	47	7	11	7	5	4	6	4	1	3	4	1	1	
pyramids in layer Vb	basal	54		8	5	12	5	5	4	4	3	2	4	2	
Purkinje cells		238		40	42	23	32	22	25	18	11	8	10	7	

FIGURE 7. Frequency of topological branching patterns from basal and apical dendrites of neocortical pyramids in layer IV and V b and Purkinje cells. Note that the topological patterns of basal dendrites represent whole dendrites whereas topological types of apical dendrites and Purkinje cells represent the patterns in the peripheral tree subtending a given number of pendant arcs. Compare these observed frequencies with the expected frequencies for branching on random arcs and random pendant vertices shown in figures 8 and 9.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

sample randomly two populations of topological types distributed according to the frequency distributions obtained for the terminal and segmental models. The size of each sample was equal to the total number of observations made histologically in the appropriate group and each was repeated 200 times. The distributions obtained by random sampling were then tested by χ^2 analysis against the expected distributions for the two models to produce the pseudo χ^2 value.

(*b*) *Analysis of frequency of Strahler orders*

The results obtained by ordering the dendritic array of Purkinje cells were compared with those obtained from networks with comparable numbers of pendant vertices produced by a KDF 9 computer programmed to simulate the terminal and segmental models. A hundred models of each type were produced.

(i) *Segmental model*. Starting from a simple three arc tree, with two pendant arcs and a third arc, representing the first part of the dendritic tree between the perikaryon and the first bifurcation, a network with the required number of pendant vertices was generated, A tree with 'n' pendant arcs has $2n-1$ segments (i.e. arcs including pendant arcs). A number K was chosen between 1 and $2n-1$ by using a random number generator. A list of segment numbers was kept and the tree branched monochotomously on the Kth segment in this list thus generating a new pendant arc. The list of pendant arcs was updated (i.e. n increased by 1), and the process repeated until the required number of pendant vertices was obtained.

(ii) *Terminal model*. Networks were constructed in a similar manner to that described for the segmental model except that, for a total of n pendant arcs, K was randomly chosen between 1 and n and the network branched on the vertex of the Kth pendant arc. As with the segmental model the number of pendant arcs in the network increased by 1 with the addition of each branch.

(*c*) *Simulation of loss of portions of the dendritic tree of the Purkinje cells*

A subroutine was added to each of the above programs to simulate the loss of portions of the dendritic tree due to incomplete impregnation and to sectioning. Complete models with the number of pendant vertices varying from 500–600 were produced and ordered. Any arc in the network, with the exception of pendant arcs, was then randomly chosen and that portion of the network distal to the arc was considered lost. The remainder of the model was reordered and treated as before. This random loss was repeated six times on each of 50 examples of each growth pattern.

III. Results

1. *Growth of dendritic trees*

(*a*) *Topological analysis of the dendritic trees of Purkinje cells and neocortical pyramids*

All nodes of neocortical pyramidal cells were dichotomous but 5% of the nodes of Purkinje cells were trichotomous. All trichotomous nodes were resolved as being dichotomous with a separation distance between arcs on the parent branch of 1 μm. The error introduced by this procedure is minimal and a full discussion of the significance of trichotomous branching in the growth of Purkinje cells is given by Berry & Bradley (1975*b*). Analysis of the frequencies of topological branching patterns recorded in dendrites with from 1–7 pendant arcs for each cell type (figure 7) showed that the apical dendrites of neocortical pyramids may exhibit both 'segmental' and 'terminal' growth but that their basal dendrites and the dendrites of Purkinje cells grow entirely by 'terminal' branching.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

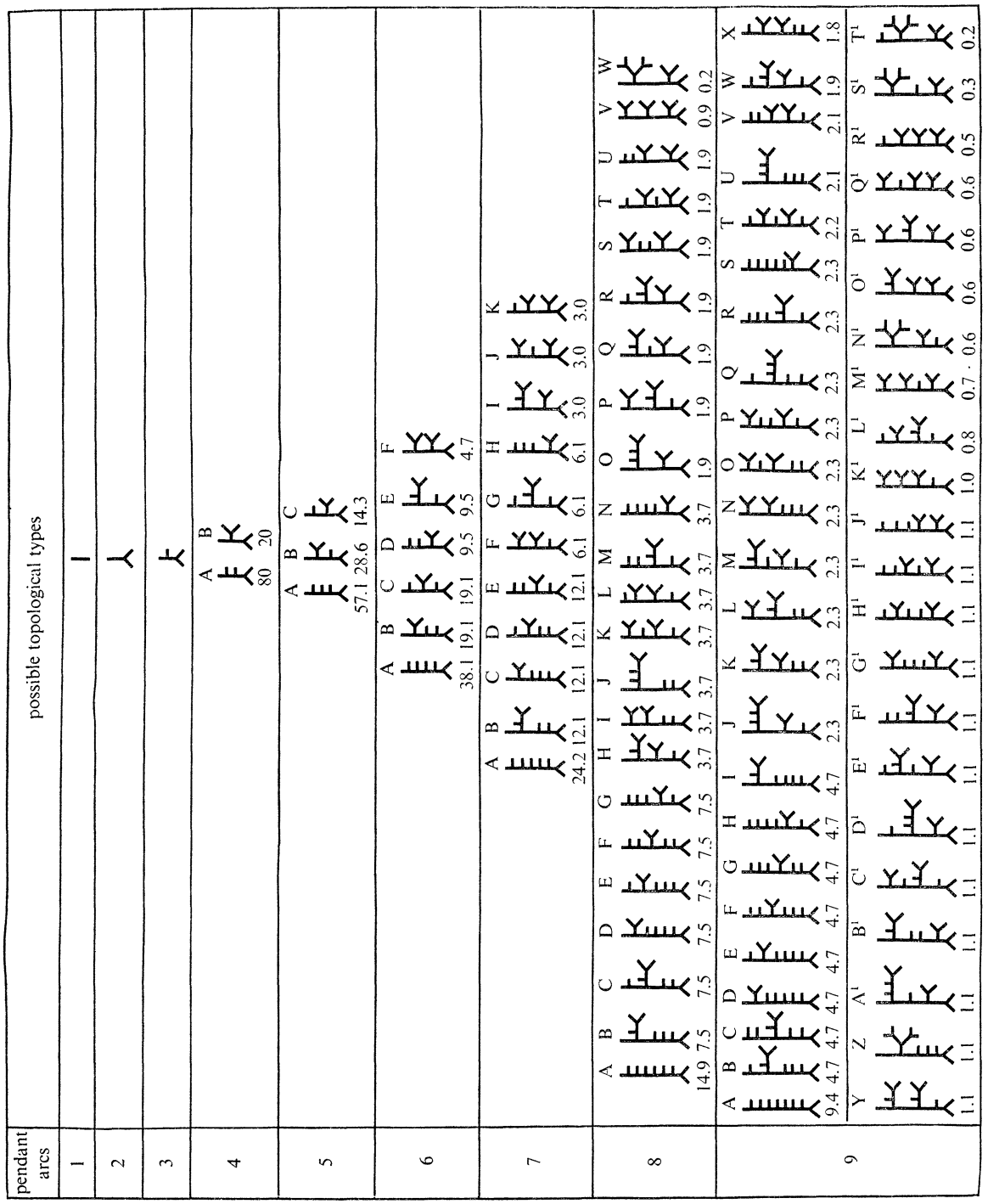


FIGURE 8. Possible topological types generated by monochotomous branching on segments (segmental growth model) in networks with from 1 to 9 pendant arcs are listed together with the frequency of occurrence (%) of types when branching occurs on random segments. The letters associated with each topological type may be used for identification in table 1. (See Berry *et al.* (1975) for source of data.)

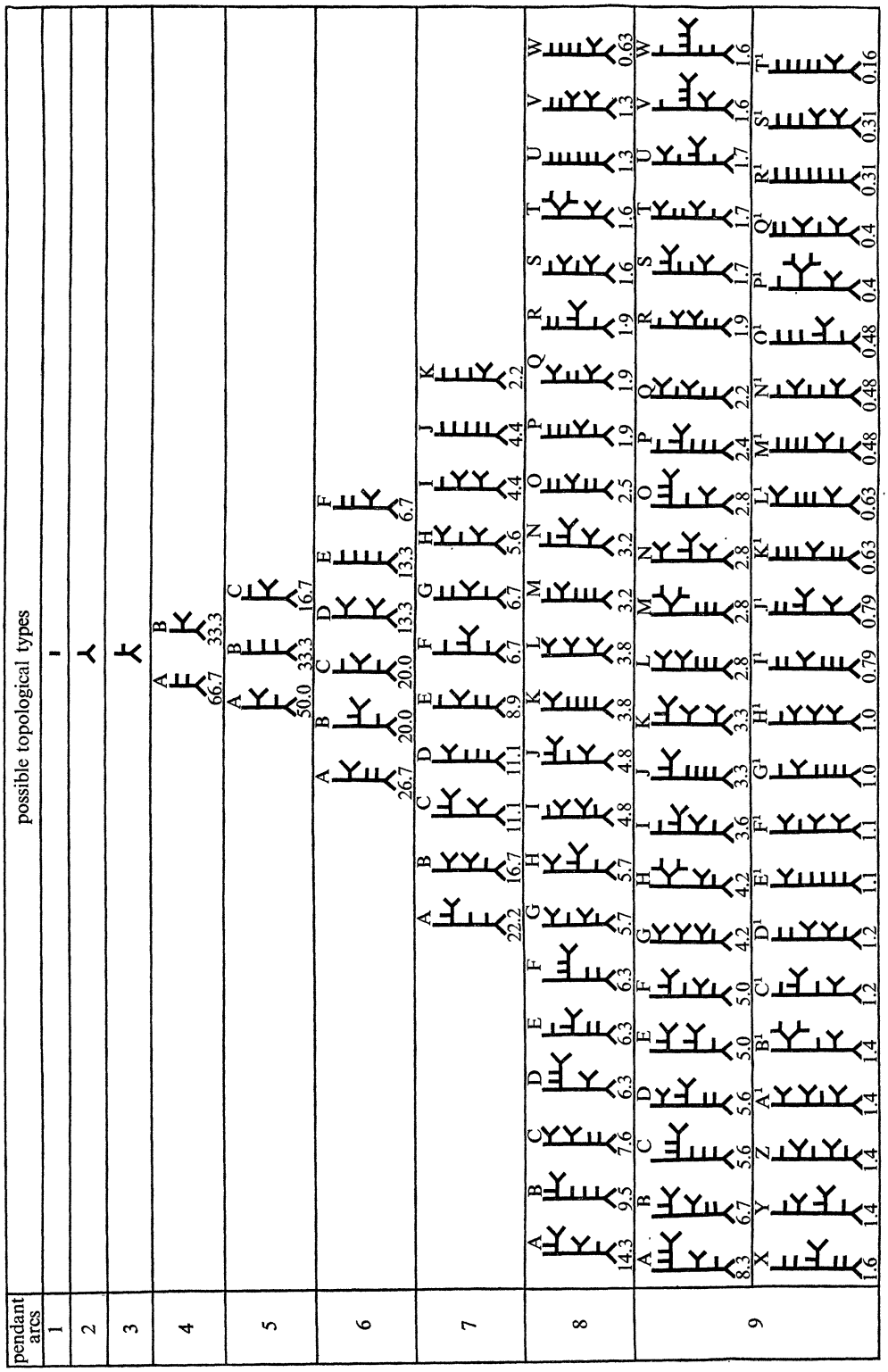


FIGURE 9. Possible topological types generated by dichotomous branching on pendant vertices (terminal growth model) in networks with from 1 to 9 pendant arcs are listed together with the frequency of occurrence (%) of types when branching occurs on random pendant vertices. The letters associated with each topological type may be used for identification in table 1. (See Berry *et al.* (1975) for source of data.)

(i) *Growth of the Purkinje cell dendritic tree*. The frequencies of topological types in the fourth to seventh pendant arc series, inclusively, are set out in figure 7 and the χ^2 analysis of these observed frequencies against the expected frequencies for the segmental and terminal growth models (figures 8, 9) are set out in table 1. Examination of these data shows that the branching pattern of the Purkinje cell dendritic tree grows in a manner which is indistinguishable from 'branching' on random pendant arcs.

(ii) *Growth of the basal dendrites of neocortical pyramids*. The observed number of topological types for each group of pendant arcs for the basal dendrites of neocortical pyramidal cells are set out in table 1, column (iii). In cases where the number of observations was sufficiently large for χ^2 analysis to be interpreted meaningfully, the observed distribution was tested against the expected distributions for the terminal and segmental growth hypotheses set out in figures 8 and 9. It will be seen that the observed distributions differ significantly in all cases from the segmental growth distribution (table 1, columns (v), (vi)). By contrast, comparison with the expected distribution obtained by terminal growth reveals no significant differences. When only small numbers of observations were available the χ^2 values were compared with pseudo χ^2 values obtained by random sampling of topological types distributed according to the two growth hypotheses. For example, only a small number of topological types with seven pendant arcs were identified in neocortical neurons of layer Vb and thus χ^2 values could only be compared with pseudo χ^2 values. The χ^2 values of 11.0 (column (v)), and 76.6 (column (vi)), are close to the mean pseudo χ^2 values obtained by computer sampling of a terminal growth model distribution, i.e. 9.5 ± 4.0 and 81.3 ± 28.6 (column (vii)) but differ markedly from pseudo χ^2 values obtained following sampling of a segmental distribution of topological types, i.e. 50.7 ± 14.9 and 10.0 ± 4.6 (column (viii)).

Inspection of table 1 shows that there is consistent agreement with the hypothesis that dendrites grow by 'terminal branching' and no agreement with the alternative hypothesis.

(iii) *Growth of the apical dendrites of neocortical pyramids*. The topological analysis of the apical dendrites of neocortical pyramids was confined to the side branches and apical tufts. It is clear from examination of figures 7–9 that these side branches grow by branching on pendant arcs (terminal growth model). Evidence will be given later however that these branches arise from the apical shaft by monochotomous branching on random segments.

(*b*) *Strahler ordering of Purkinje cell dendritic trees*

The results of the application of the Strahler method of ordering are shown in table 2 and figure 10. Table 3 sets out the means of successive Strahler orders of the branches of the dendrites of Purkinje cells and of those of the two computer growth models grouped according to the maximum Strahler order attained.

The maximal Strahler order of the 17 Purkinje cells studied was either 6 or 7, the higher order cells being characterized by a much greater mean number of order 1 branches

The segmental computer model, unlike the Purkinje cells and the terminal computer growth model produced many systems with a maximal order of 5 in addition to networks with a maximal Strahler order of 6. Only one example of a seventh order system was produced by segmental growth. The marked differences in the connectivity of the networks produced by the segmental method of growth is further reflected in the greater mean number of Strahler order 1 segments required to generate any given maximal Strahler order and in the more rapid decrement of the numbers of successive Strahler orders.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE 1. TABLE OF THE FREQUENCIES OF TOPOLOGICAL TYPES OBTAINED FROM THE HISTOLOGICAL ANALYSIS OF PURKINJE CELLS AND NEOCORTICAL PYRAMIDS TOGETHER WITH THE RESULTS OF THE χ^2 AND PSEUDO χ^2 ANALYSIS ASSUMING THE NULL HYPOTHESIS: * THE EXPECTED FREQUENCIES FOR THE TERMINAL AND SEGMENTAL GROWTH HYPOTHESES FOR EACH TOPOLOGICAL TYPE A–K ARE SHOWN IN FIGURES 8 AND 9

(i) origin of cell	(ii) number of pendant arcs	(iii) observed number of each topological type*											(iv) degrees of freedom	(v) terminal growth model		(vi) segmental growth model		(vii) Mean pseudo χ^2 values derived from sampling of terminal distribution tested against		(viii) mean pseudo χ^2 values derived from sampling of segmental distribution tested against	
		A	B	C	D	E	F	G	H	I	J	K		χ^2	P	χ^2	P	terminal distribution	segmental distribution	terminal distribution	segmental distribution
pyramidal cells from sensorimotor neocortex layer IV	4	73	31	—	—	—	—	—	—	—	—	—	1	0.6	0.5–0.4	6.3	0.025–0.01	—	—	—	—
	5	49	29	10	—	—	—	—	—	—	—	—	2	2.1	0.4–0.3	32.3	<0.001	—	—	—	—
	6	15	10	12	10	6	2	—	—	—	—	—	5	2.2	—	40.1	—	5.3 (±3.4)	38.9 (±14.5)	32.1 (±11.2)	5.0 (±3.1)
	7	11	7	5	4	6	4	1	3	4	1	1	10	5.3	—	42.0	—	10.3 (±4.5)	70.5 (±28.4)	48.4 (±16.9)	10.3 (±4.0)
pyramidal cells from sensorimotor neocortex layer Vb	4	63	40	—	—	—	—	—	—	—	—	—	1	1.4	0.3–0.2	22.8	<0.001	—	—	—	—
	5	57	29	20	—	—	—	—	—	—	—	—	2	1.7	0.5–0.4	42.1	<0.001	—	—	—	—
	6	22	16	15	20	17	4	—	—	—	—	—	5	8.4	—	70.6	—	4.7 (±3.4)	61.5 (±18.1)	49.4 (±14.3)	4.9 (±3.0)
	7	8	5	12	5	5	4	4	3	2	4	2	10	11.0	—	76.6	—	9.5 (±4.0)	81.3 (±28.6)	50.7 (±14.9)	10.0 (±4.6)
Purkinje cells from vermis of cerebellum (see figure 1)	4	469	197	—	—	—	—	—	—	—	—	—	1	4.2	0.05–0.025	38.2	<0.001	—	—	—	—
	5	203	166	66	—	—	—	—	—	—	—	—	2	4.5	0.2–0.1	77.6	<0.001	—	—	—	—
	6	98	67	58	40	51	24	—	—	—	—	—	5	3.5	0.7–0.6	140.4	<0.001	—	—	—	—
	7	40	42	23	32	22	25	18	11	8	10	7	10	12.0	—	150.4	—	9.9 (±4.2)	302.7 (±64.4)	191.6 (±31.9)	9.8 (±4.5)

TABLE 2. RESULTS OF THE ANALYSIS OF PURKINJE CELLS SHOWING THE FREQUENCIES OF STRAHLER ORDERS OF THE 17 PURKINJE CELLS: THE POSITION OF PURKINJE CELLS IN THE FOLIA OF THE VERMIS IS SHOWN IN FIGURE 1

Purkinje cell number	number of segments of Strahler order						
	1	2	3	4	5	6	7
1	544	168	50	14	3	1	—
2	435	143	38	16	4	1	—
3	264	86	27	7	2	1	—
4	331	103	34	12	3	1	—
5	525	171	53	18	4	2	1
6	446	149	50	15	5	1	—
7	321	98	28	9	2	1	—
8	358	113	40	11	3	1	—
9	388	124	37	15	2	1	—
10	421	138	45	13	4	2	1
11	263	87	25	5	2	1	—
12	489	162	55	17	4	2	1
13	402	138	44	12	4	1	—
14	503	159	48	18	5	2	1
15	416	141	48	12	4	2	1
16	443	136	40	12	4	2	1
17	549	174	60	19	5	2	1

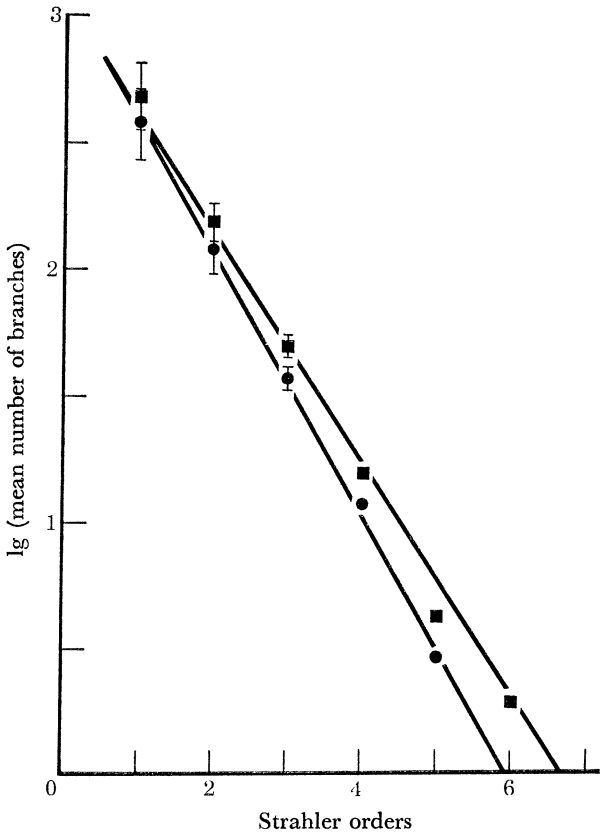


FIGURE 10. Graph of the mean frequency of Strahler orders in 6 (●) and 7 (■) order networks of Purkinje cell dendrites (6 order network: intercept, 3.10; slope, −0.52; r, 0.99; 7 order networks, intercept 3.08; slope 0.46; r, −0.99).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The relationship between the numbers of successive Strahler orders for the Purkinje cells are shown graphically in figure 10. On a $\log_{10}$ scale there is an inverse relation with a high degree of correlation, the slope of which represents the overall bifurcation ratio. Comparison of the slopes of sixth order and seventh order cells reveals a significant difference on 't' testing which

TABLE 3. MEAN NUMBER OF STRAHLER ORDERS IN THE NETWORKS OF PURKINJE CELLS AND IN NETWORKS CONSTRUCTED BY COMPUTER ACCORDING TO THE SEGMENTAL AND TERMINAL BRANCHING HYPOTHESES

	maximum order attained	no. of examples	Strahler orders 1	2	3	4	5	6	7
histology	6	10	375 ± 87	121 ± 28	37 ± 9	12 ± 4	3 ± 1	1	—
Purkinje cell	7	7	478 ± 52	154 ± 16	50 ± 7	16 ± 3	4.3 ± 0.5	2	1
terminal model	6	49	350 ± 72	117 ± 25	38 ± 9	12 ± 3	3.5 ± 0.9	1	—
	7	51	434 ± 70	145 ± 23	48 ± 8	16 ± 3	5.7 ± 1	2.2 ± 0.4	1
segmental model	5	40	356 ± 72	89 ± 18	23 ± 5	5.6 ± 1.6	1	—	—
	6	59	423 ± 89	106 ± 22	27 ± 6	7.1 ± 1.9	2.3 ± 0.5	1	
	7	1	513	132	34	11	4	2	1

TABLE 4. TABLE OF COMPARISON OF REGRESSIONS OF FREQUENCY OF STRAHLER ORDERS ON THE TYPES OF STRAHLER ORDERS FOR THE HISTOLOGICAL DATA FROM PURKINJE CELLS AND DATA OBTAINED BY COMPUTER SIMULATION (SEE TEXT FOR FULL EXPLANATION)

	networks	slope	intercept	correlation	pair	P values for comparisons of slopes by Student's 't' test
A	histology pooled networks	−0.4807	3.0529	0.9852	—	—
B	histology 7 order networks	−0.4595	3.0770	0.9945	B and C	< 0.001
C	histology 6 order networks	−0.5197	3.1005	0.9920		
D	terminal model pooled networks	−0.4619	3.0116	0.9874	A and D	0.01–0.005
E	terminal model 7 order networks	−0.4452	3.0199	0.9909	B and E	0.25–0.125
F	terminal model 6 order networks	−0.5070	3.0803	1.0000	C and F	0.10–0.05
					E and F	< 0.001
G	segmental model pooled networks	−0.5497	3.0729	0.9840	A and G	< 0.001
H	segmental model 6 order networks	−0.5331	3.0720	0.9911	C and H	0.10–0.05
I	segmental model 5 order networks	−0.6301	3.2019	0.9940	H and I	< 0.001
J	histology pooled networks Strahler orders 1–4	−0.5036	3.1201	0.9799	—	—
K	histology 7 order networks Strahler orders 1–4	−0.4970	3.1782	0.9946	K and L	0.35–0.30
L	histology 6 order networks Strahler orders 1–4	−0.5083	3.0796	0.9800		
M	terminal model pooled networks Strahler orders 1–4	−0.4851	3.0735	0.9832	J and M	0.4–0.3
N	terminal model 7 order networks Strahler orders 1–4	−0.4805	3.1139	0.9903	K and N	0.15–0.10
O	terminal model 6 order networks Strahler orders 1–4	−0.4897	3.0311	0.9852	L and O	0.15–0.10
					N and O	0.15–0.10
P	segmental model pooled networks Strahler orders 1–4	−0.5984	3.1856	0.9866	J and P	< 0.001
Q	segmental model 6 order networks Strahler orders 1–4	−0.5949	3.2082	0.9883	L and Q	< 0.001
R	segmental model 5 order networks Strahler orders 1–4	−0.6044	3.1506	0.9889	Q and R	0.15–0.10

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

questions the validity of the use of the overall bifurcation ratio as a means of comparison of networks of differing branching patterns. The use of bifurcation ratios to detect the mode of growth of networks has been discussed by Berry *et al.* (1975). The problem has been investigated by separately comparing data relating to (i) complete networks irrespective of the maximal order attained (pooled information); (ii) complete networks grouped according to the maximal Strahler order attained (comparison both within and between the two models and the Purkinje cells); (iii) the peripheral parts of the networks grouped according to the maximal Strahler order attained both within and between the two computer models and the Purkinje cells.

TABLE 5. THE EFFECT OF RANDOM LOSS OF SEGMENTS ON THE DISTRIBUTION OF FREQUENCY OF ORDERS IN A COMPUTER SIMULATION MODEL (SEE TEXT FOR FULL EXPLANATION)

	networks	loss	number of pendant arcs	slope	correlation	pair	*P* values for comparison of slopes by '*t*' test
A	terminal model 6 order networks, Strahler orders 1–4	no loss	524.7 ± 47.6	−0.4932	0.9963	—	—
B	terminal model 6 order networks, Strahler orders 1–4	maximal loss	470.0 ± 41.2	−0.4905	0.9887	A and B	0.45–0.40
C	terminal model 7 order networks, Strahler orders 1–4	no loss	572.8 ± 54.6	−0.4794	0.9960	—	—
D	terminal model 7 order networks, Strahler orders 1–4	maximal loss	523.5 ± 78.0	−0.4817	0.9916	C and D	0.40–0.35
E	segmental model 6 order networks, Strahler orders 1–4	no loss	549.5 ± 60.5	−0.6017	0.9954	—	—
F	segmental model 6 order networks, Strahler orders 1–4	maximal loss	485.9 ± 82.2	−0.5960	0.9928	E and F	0.25–0.20

(i) *Complete pooled networks.* The analysis of this information revealed significant differences between Purkinje cells and both growth models (comparisons A and D, A and G, table 4).

(ii) *Complete networks grouped according to maximal Strahler order. A. Intra-group comparison.* Comparison of the sixth with seventh order networks of both Purkinje cells and the terminal computer model and the fifth with sixth order networks of the segmental computer growth model were in each case significantly different by '*t*' testing (comparison B and C, E and F, H and I, table 4).

B. Inter-group comparison. Comparison of the seventh order Purkinje cells with the seventh order terminal models showed no significant difference (comparison B and E, table 4). Sixth order Purkinje cells did not significantly differ from sixth order networks arising from either terminal or segmental growth (comparisons C and F, C and H, table 4). Only one seventh order network was produced by the segmental model and hence no comparisons could be made.

(iii) *Pooled information from peripheral portions of the network.* Comparison of the information relating to Strahler orders 1–4 irrespective of the maximal order of the networks showed a highly significant difference in the case of the Purkinje cell data and the segmental model (comparison J and P, table 4). Comparison of the Purkinje cell data with the terminal mode indicated no significant difference (comparison J and M, table 4).

(iv) *Peripheral portion of networks grouped according to maximal order. A. Intra-group comparison.* There were no significant differences between sixth and seventh order Purkinje cells, sixth and seventh order terminal models and fifth and sixth order segmental models (comparisons K and L, N and O and Q and R, table 4).

B. Inter-group comparison. Comparisons of sixth order Purkinje cells and sixth order terminal model networks and seventh order Purkinje cells with seventh order terminal models revealed no significant differences (comparisons L and O and K and N respectively, table 4). By contrast the comparison of sixth order Purkinje cells with sixth order segmental models showed a highly significant difference (comparison L and Q, table 4).

(*c*) *Simulation of loss of portions of the dendritic tree*

The results of the computer simulation of loss of portions of the dendritic tree are summarized in table 5. The slopes of the regression lines for numbers of successive Strahler orders for complete models and models after maximal loss for both growth hypotheses showed no significant differences. Furthermore, there were no significant differences between models after maximal loss and those models used for comparisons with the histological material in table 4.

(*d*) *Absolute bifurcation ratios of the basal dendrites of neocortical pyramids*

Growth hypotheses for small networks such as the basal dendrites of neocortical pyramids can be tested by comparing the frequencies of topological types and bifurcation ratios in an observed pendant arc series with those in the corresponding complete pendant arc series (Berry *et al.* 1975).

Figures 8 and 9 list the number of distinct topological types that occur in pendant arc series 1–9 when growth occurs by monochotomous branching on random arcs and when growth is constrained to branching on random pendant arcs. By summing the products of the frequency and number of Strahler orders of each topological type in a pendant arc series an *absolute bifurcation ratio* can be calculated between each Strahler order of the series for a given hypothesis of growth. The absolute bifurcation ratios in the pendant arc series 1–9 of networks grown by monochotomous branching on random segments and branching on random pendant arcs is given in tables 6 and 7. Berry *et al.* (1975) have shown that networks generated by a given mode of growth establish a consistent bifurcation ratio between Strahler orders which first appears between the lowest orders. This ratio has been called the established bifurcation ratio and has been shown to be 3 for branching on random pendant arcs and 4 for monochotomous branching on random arcs. Examination of table 6 shows that the established bifurcation ratio has not been attained in networks grown by monochotomous branching on random arcs by the ninth pendant arc series but that branching on random pendant arcs produces an established bifurcation ratio of 3 between the first and second Strahler orders as early as the third pendant arc series.

Table 8 lists the percentage frequencies of each Strahler order and the bifurcation ratios between adjacent orders calculated from the observed frequencies of topological types in pendant arc series. Comparison of table 8 with tables 6 and 7 shows how closely the bifurcation ratios between orders approximate to the absolute bifurcation ratios in the complete pendant arc series formed by branching on random pendant arcs but bears no relation to the ratios calculated from the pendant arc series formed by monochotomous branching on random segments. Pooling of topological types with from 10–20 pendant arcs shows that the bifurcation

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE 6. ABSOLUTE BIFURCATION RATIOS OF NETWORKS EXHIBITING MONOCHOTOMOUS BRANCHING ON RANDOM SEGMENTS (SEE TEXT FOR FULL EXPLANATION AND BERRY *ET AL.* (1975) FOR SOURCE OF THE DATA)

pendant arcs no.	1 (%)	ratio 1:2	2 (%)	ratio 2:3	3 (%)	ratio 3:4	4 (%)	overall bifurcation ratio (slope of linear regression)	order rank
1	100 (100)	—	—	—	—	—	—	—	1
2	200 (66.6)	2	100 (33.3)	—	—	—	—	2	2
3	300 (75.0)	3	100 (25.0)	—	—	—	—	3	
4	400 (74.1)	3.3	120 (22.2)	6	20 (3.7)	—	—	4.5	3
5	500 (72.9)	3.5	142.9 (20.8)	3.3	42.9 (6.3)	—	—	3.4	
6	600 (72.4)	3.6	166.6 (20.1)	2.69	61.9 (7.5)	—	—	3.1	
7	699.3 (72.4)	3.7	190.7 (19.8)	2.52	75.7 (7.8)	—	—	3.0	
8	801.6 (72.7)	3.7	215.8 (19.6)	2.5	85.5 (7.8)	427.5	0.2 (0.02)	13.2	4
9	892.8 (73.0)	3.8	237.3 (19.4)	2.6	90.9 (7.4)	82.6	1.1 (0.09)	8.2	

TABLE 7. ABSOLUTE BIFURCATION RATIOS OF NETWORKS EXHIBITING DICHOTOMOUS BRANCHING ON RANDOM PENDANT VERTICES (SEE TEXT FOR FULL EXPLANATION AND BERRY *ET AL.* (1975) FOR SOURCE OF THE DATA)

pendant arcs no.	1 (%)	ratio 1:2	2 (%)	ratio 2:3	3 (%)	ratio 3:4	4 (%)	overall bifurcation ratio (slope of linear regression)	order rank
1	100 (100)	—	—	—	—	—	—	—	1
2	200 (66.7)	2	100 (33.3)	—	—	—	—	2	2
3	300 (75.0)	3	100 (25.0)	—	—	—	—	3	
4	400 (70.6)	3	133.4 (23.5)	4	33.3 (5.9)	—	—	3.5	3
5	500 (66.8)	3	166.7 (23.3)	2.5	66.7 (9.9)	—	—	2.7	
6	600 (67.7)	3	200 (22.6)	2.3	86.7 (9.8)	—	—	2.6	
7	700 (68.0)	3	233.4 (22.7)	2.4	95.6 (9.3)	—	—	2.7	
8	799.4 (68.5)	3	266.6 (22.8)	2.7	100.2 (8.5)	62.6	1.6 (0.1)	7.1	4
9	904.2 (68.4)	3	300.5 (22.7)	2.8	109 (8.2)	12.4	8.8 (0.6)	4.4	

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

ratios between first and second, and second and third Strahler orders approximates to the established bifurcation ratio of 3 in the fourth order rank.

(*e*) *Strahler ordering of the apical dendrites of neocortical pyramids*

The analysis of topological types of dendrites stemming from the apical dendrites was confined to trees with 4 and 5 pendant arcs since few examples were available with a greater number of pendant arcs. This analysis supports the hypothesis that the *branches* of apical dendrites are generated by branching on random terminals (figures 7–9). However, the Strahler analysis of apical dendrites demonstrates that the collaterals stemming from the shafts of the apical dendrites of neocortical pyramids may be formed by monochotomous branching on the segments of the shaft randomly since, although the ratio between first and second Strahler orders approximates to 3 (the established bifurcation ratio for terminal branching), the ratios between second and third orders approximate to 4, the established ratio for monochotomous branching on random segments (table 9).

TABLE 8. TABLE OF OBSERVED BIFURCATION RATIOS: A, NEOCORTICAL PYRAMIDS FROM LAYER IV; B, NEOCORTICAL PYRAMIDS FROM LAYER Vb (SEE TEXT FOR FULL EXPLANATION)

number of observations	number of pendant arcs	Strahler orders: 1 (%)	ratio 1:2	2 (%)	ratio 2:3	3 (%)	ratio 3:4	4 (%)	overall bifurcation ratio
		A. Neocortical pyramids from layer IV							
146	1	146	—	—	—	—	—	—	—
132	2	264	—	132	—	—	—	—	—
126	3	378	—	126	—	—	—	—	—
103	4	412 (69.24)	2.88	143 (24.03)	3.58	40 (6.72)	—	—	3.21
106	5	530 (67.09)	2.90	183 (23.17)	2.38	77 (9.75)	—	—	2.62
94	6	564 (67.79)	2.95	191 (22.96)	2.48	77 (9.26)	—	—	2.71
54	7	378 (68.23)	3.0	126 (22.74)	2.52	50 (9.025)	—	—	2.75
53	8	416 (70.39)	3.3	126 (21.32)	2.57	49 (8.29)	—	—	2.91
45	9	405 (67.84)	2.91	139 (23.28)	2.84	49 (8.2)	12.25	4 (0.67)	4.44
120	10–20	1295	3.1	418	2.97	141	4.55	31	—
		B. Neocortical pyramids from layer Vb							
145	1	145	—	—	—	—	—	—	—
133	2	266	—	123	—	—	—	—	—
115	3	345	—	115	—	—	—	—	—
104	4	416 (71.48)	3.08	135 (23.20)	4.36	31 (5.33)	—	—	3.67
88	5	440 (68.1)	2.99	147 (22.76)	2.49	59 (9.13)	—	—	2.73
55	6	330 (66.94)	2.90	114 (23.12)	2.35	49 (9.94)	—	—	2.59
47	7	329 (67.56)	2.94	112 (23.0)	2.44	46 (9.45)	—	—	2.67
23	8	184 (69.17)	3.12	59 (22.18)	2.57	23 (8.65)	—	—	2.83
16	9	144 (67.93)	3.0	48 (22.64)	2.67	18 (8.49)	9.0	2 (0.94)	4.01
22	10–15	245	2.82	87	3.0	29	4.14	7	—

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE 9. MEAN FREQUENCIES OF STRAHLER ORDERS OF 100 COMPLETE APICAL DENDRITES FROM PYRAMIDAL CELLS IN LAYERS Vb and IV OF THE NEOCORTEX

(Note how the bifurcation ratio between first and second Strahler orders approximates to 3, and second and third Strahler orders approximates to 4, supporting the hypothesis that the small second order side branches grow by branching on random pendant vertices but that they are themselves formed by branching on random segments of the apical dendrite.)

		mean number of Strahler orders						
cell type	number of cells	1	ratio 1:2	2	ratio 2:3	3	ratio 3:4	4
layer IV pyramids	16	17.44 ± 0.89	2.70	6.44 ± 0.29	3.22	2	2	1
	84	13.89 ± 0.38	3.04	4.57 ± 0.16	4.57	1	—	—
layer Vb pyramids	74	34.42 ± 0.93	2.88	11.97 ± 0.36	4.43	2.70 ± 0.10	2.70	1
	26	25.62 ± 1.20	3.10	8.27 ± 0.42	8.27	1	—	—

2. *Definition of connectivity*

(*a*) *Breakdown of Strahler orders of the dendritic tree of Purkinje cells*

The bifurcation ratio obtained by the frequency analysis of Strahler orders is a useful parameter by which to describe networks but this quantity gives only general information about the way in which dendrites are joined together. However, the Strahler method does permit intricate dissection of networks if the parent branches of dendrites of a given order are recorded. Table 10 shows the breakdown of Strahler orders into daughter and parent branches (see § 2(*a*) for definition of daughter and parent branches). Examination of table 8 shows, for example, the mean number of Strahler order 1 branches is 375.2 in order 6 networks. Of these 241.8 (64.4 %) join together to produce Strahler order 2 branches, 99.1 (26.4 %) end as collaterals on

TABLE 10. TABLE OF THE DISTRIBUTION OF COLLATERAL DAUGHTER BRANCHES ON PARENT BRANCHES OF PURKINJE CELLS: A, SEVENTH ORDER; B, SIXTH ORDER NETWORKS (SEE TEXT FOR FULL EXPLANATION)

		daughter Strahler branch						
		1	2	3	4	5	6	7
A. 7th order networks								
parent branches	1	308.9	154.4	—	—	—	—	—
	2	124.4	99.7	49.8	—	—	—	—
	3	36.4	40.3	31.3	15.6	—	—	—
	4	7.7	11.8	12.0	8.6	4.3	—	—
	5	0.6	1.7	4.7	5.3	4.0	2.0	—
	6	0	0.3	1.4	1.3	0.14	2.0	1
	7	0	0.6	0.4	0.4	0.14	0.0	1
total frequency		478.0	154.4	49.8	15.6	4.3	2.0	1
B. 6th order networks								
parent branches	1	241.8	120.9	—	—	—	—	—
	2	99.1	74.6	37.3	—	—	—	—
	3	27.1	32.0	23.2	11.6	—	—	—
	4	6.7	11.4	10.5	6.0	3.0	—	—
	5	0.5	2.7	2.9	5.1	2.0	1	—
	6	0	0.2	0.7	0.5	1.0	1	—
total frequency		375.2	120.9	37.3	11.6	3.0	1	—

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Strahler order 2 branches, 27.1 (7.2 %) as collaterals on Strahler order 3 branches, 6.7 (1.8 %) as collaterals on Strahler order 4 branches and 0.5 (1.1 %) on Strahler order 5 branches. The pattern for all orders in sixth and seventh order networks is illustrated in figure 11 and shows that the majority of the dendrites of Purkinje cells join with one another to produce fourth order networks which end as collaterals on relatively few fifth order branches. The mean number of segments per branch increased linearly over the first five Strahler orders but this relation was lost over the remaining orders of the network (figure 12).

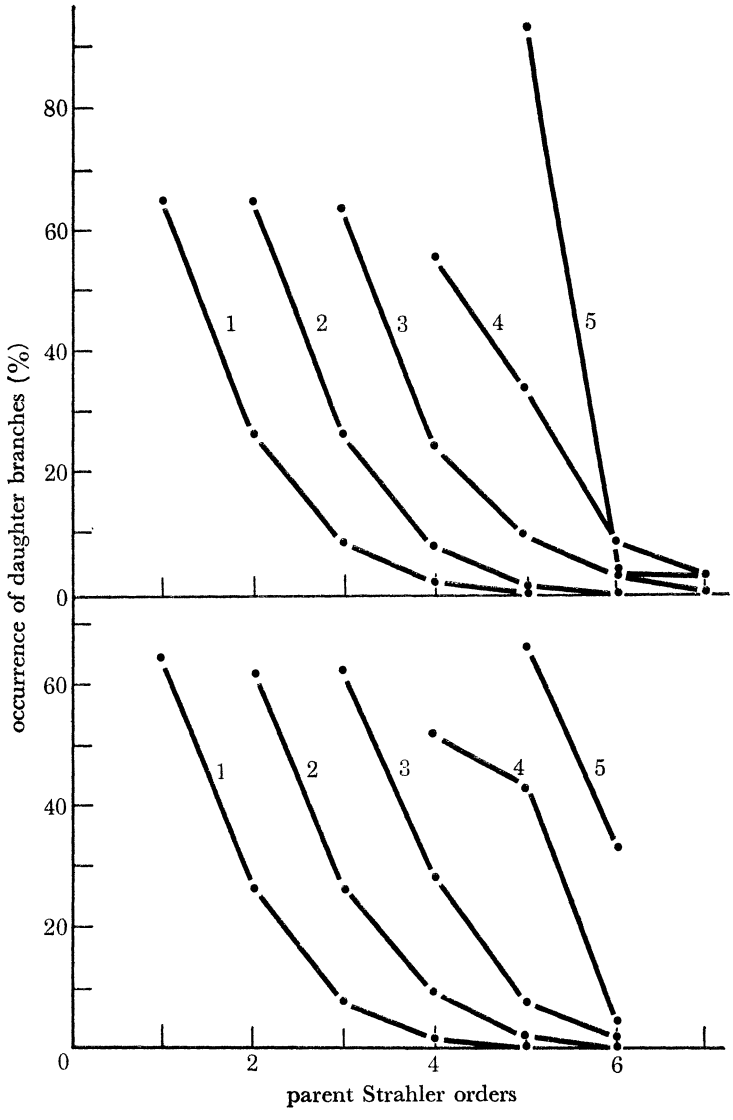


FIGURE 11. Graph of collateral distribution of the mean number of daughter Strahler orders on parent Strahler orders in (*a*) 7 and (*b*) 6 order networks of Purkinje cells. The numbers associated with each line refer to the Strahler order of the daughter branch (see text for full explanation).

(*b*) *Geometry of the dendritic tree of Purkinje cells*

The spatial arrangement of the branches of the dendritic tree of a typical Purkinje cell is illustrated in figure 13*a*. In all cells the higher Strahler order branches are arranged in a uniform density over the area of the field. The network is further made up of a 'skeleton' of

the mainly smooth branches of fifth, sixth and seventh order segments (figure 13*b*) into which fourth or smaller order networks drain, the latter being composed of the spiny branches of the tree. The number of daughter branches draining into a parent branch increases with increasing order but the probability of the parent branch and a given daughter branch being of the same order, and thus effecting a change in the order of the parent branch, decreases with increasing order of the parent branch. Accordingly, the sixth and seventh order networks of Purkinje cell dendrites tend to be arranged into subnetworks of fourth or less order which drain into relatively few fifth, sixth and seventh order branches (figure 13). The lengths of these higher order branches are longer than the lower order branches and thus the overall path lengths in the two parts of the tree are also different (figures 12, 15, 16 and 17).

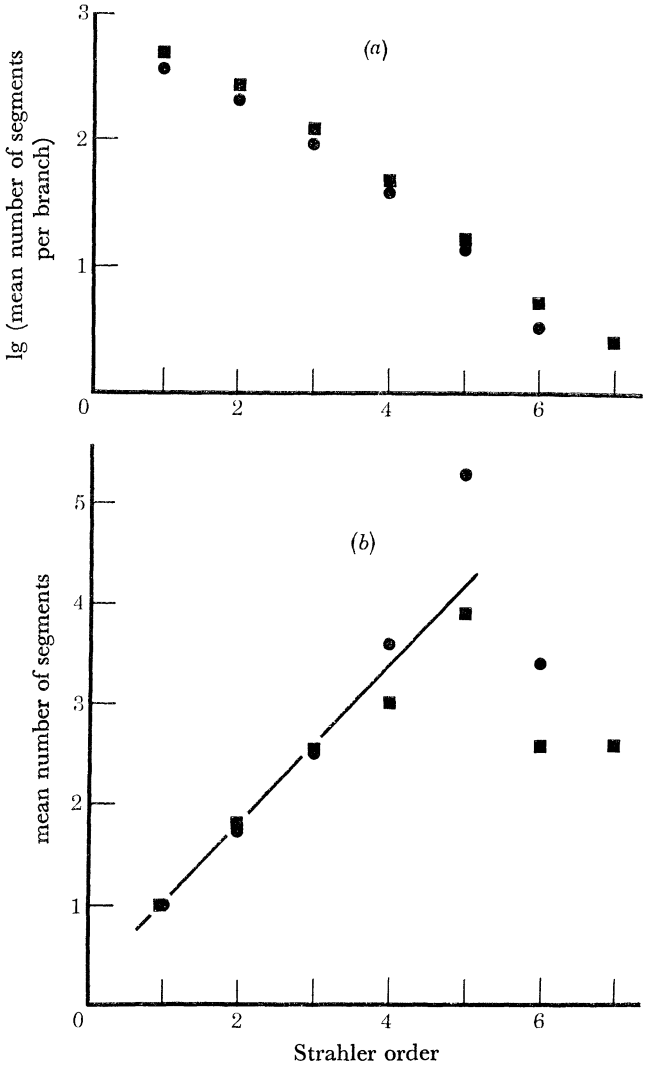


FIGURE 12. (*a*) Graph of the mean number of total segments of each Strahler order in dendritic fields of Purkinje cells – note the exponential relation. ●, 6 order networks; slope, 0.42; intercept, 3.12, $r = 0.96$; (■), 7 order networks; slope -0.41, intercept 3.22, $r = 0.99$. (*b*) Graph of the mean number of segments per branch for each Strahler order. ●, 6 order networks; intercept order 1–5, 0.28; slope orders 1–5, 1.04; *r* orders 1–5, 0.82; ■, 7 order networks; intercept orders 1–5, 0.35; slope orders 1–5, 0.70; *r* orders 1–5, 0.91.

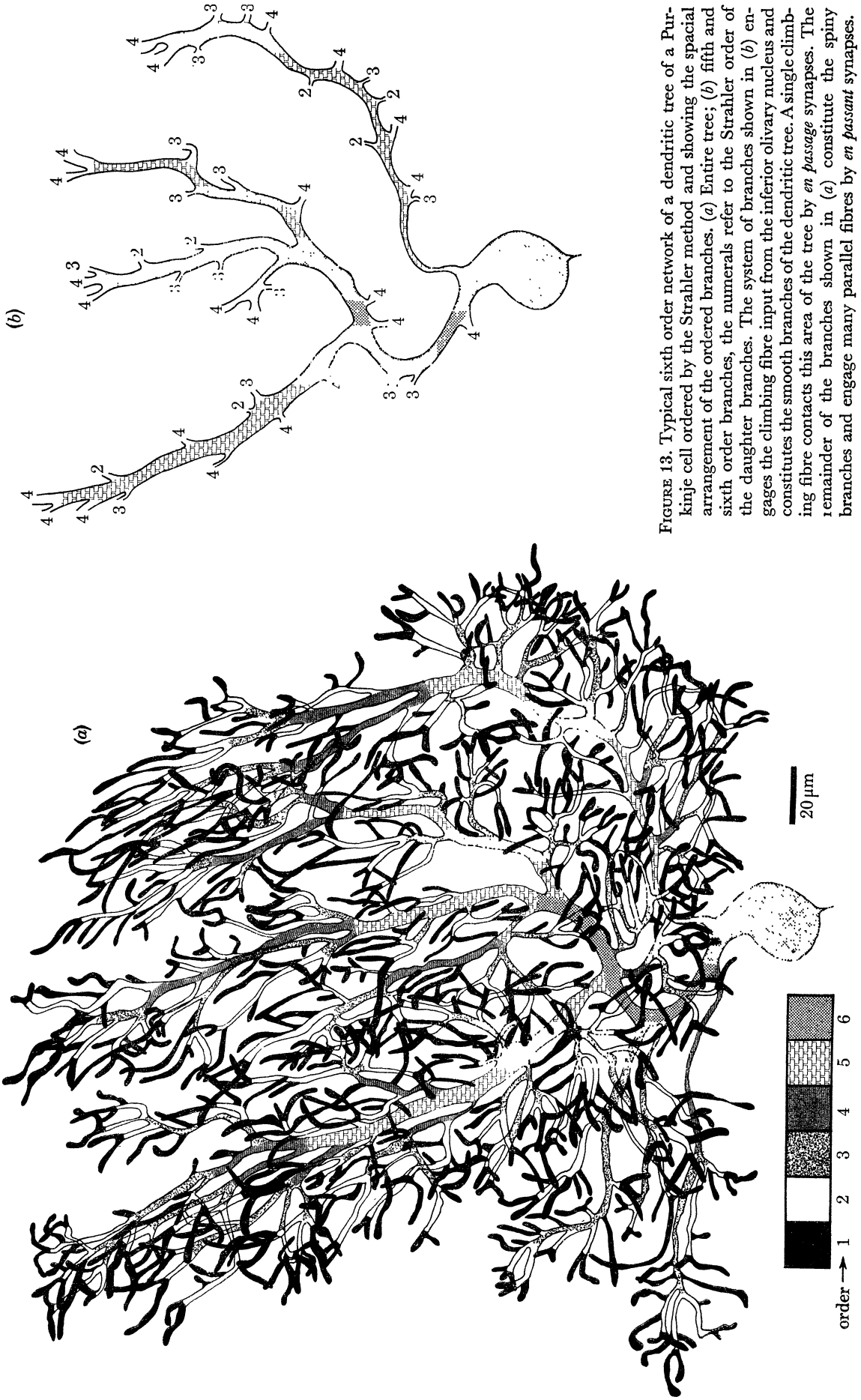


FIGURE 13. Typical sixth order network of a dendritic tree of a Purkinje cell ordered by the Strahler method and showing the spacial arrangement of the ordered branches. (*a*) Entire tree; (*b*) fifth and sixth order branches, the numerals refer to the Strahler order of the daughter branches. The system of branches shown in (*b*) engages the climbing fibre input from the inferior olivary nucleus and constitutes the smooth branches of the dendritic tree. A single climbing fibre contacts this area of the tree by *en passage* synapses. The remainder of the branches shown in (*a*) constitute the spiny branches and engage many parallel fibres by *en passant* synapses.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

(*c*) *Geometry of neocortical pyramids*

Basal dendrites and the side and apical tuft of branches of the apical dendrite have a similar pattern of branching; however the apical dendrites form their side branches by segmental growth.

Figure 14 illustrates the geometry of typical neocortical pyramids and shows how the shaft

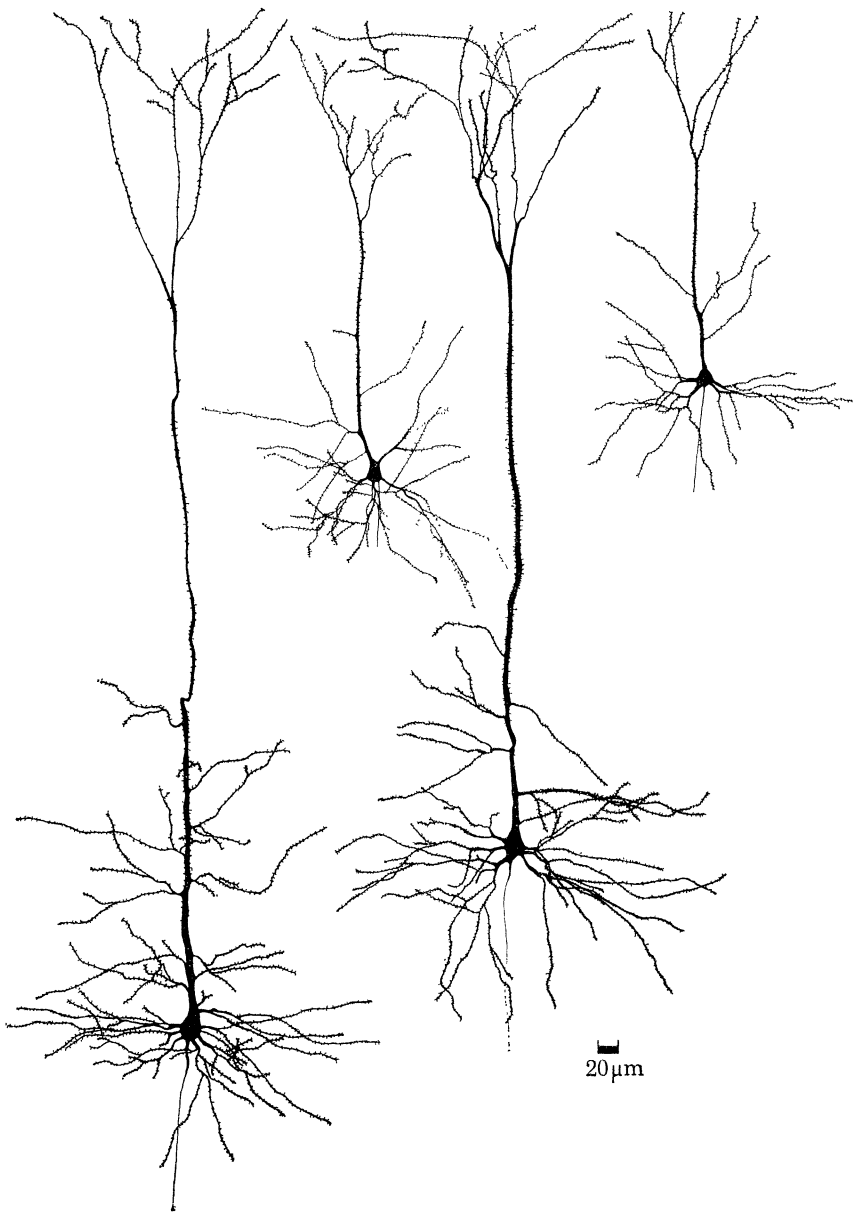


FIGURE 14. Geometry of neocortical pyramids from layer Vb (lower pair of cells) and layer IV (upper pair of cells). The fibre systems of the cortex run in a radial plane parallel with the long axis of the apical dendrites. The side branches and apical tuft of branches of the apical dendrite and the basal dendrites sample axons predominantly running in an orthogonal plane and are thus likely to be contacted by the *en passant* synapses of many axons. The apical dendrite, on the other hand, runs in a plane parallel to the direction of the axons and is likely to be engaged by *en passage* synapses of a relatively small number of axons. It is conceivable that the apical dendritic shaft samples information in a small number of axons and that the basal and apical side and tuft branches sample contextual information in the surrounding field of axons.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

of the apical dendrite is directed in a plane parallel to the orientation of axons in the cortex while basal dendrites, collateral side branches of the apical dendrites and the apical tuft of dendrites course predominantly in an orthogonal plane.

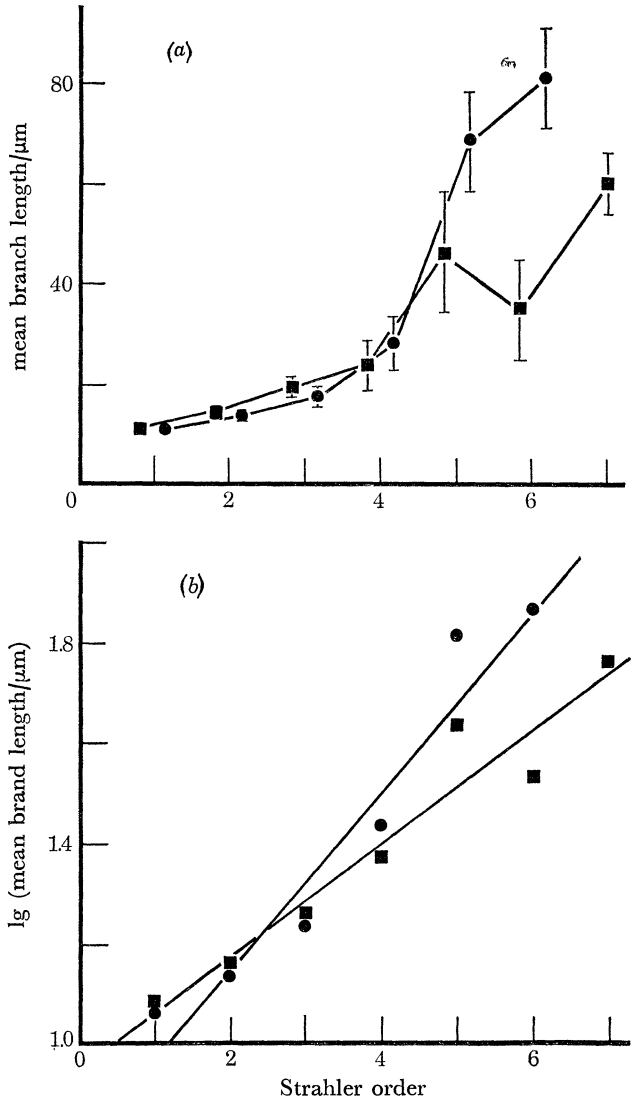


FIGURE 15. Graph of the mean branch lengths of Strahler orders, in 6 (●) and 7 (■) order dendritic trees of Purkinje cells. (*a*) Raw data; (*b*) logged data; 6 order networks, intercept 0.80; slope 0.18; $r = 0.92$; 7 order networks, intercept 0.95; slope 0.11; $r = 0.9$.

(*d*) *Analysis of the length of the dendrites of Purkinje cells*

The mean lengths of branches of successive Strahler orders increased exponentially. Increments were greater in order 6 networks than order 7 networks (figure 15*a*, *b*). The mean lengths of segments of successive branches remained constant for second, third and fourth Strahler orders but increased and showed more variability thereafter (figure 16). The mean length of first order branches, which by definition have no segments, were significantly larger than the segments of second, third and fourth Strahler order branches. The mean path lengths of branches

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

of successive Strahler order showed linear decrements of two distinct magnitudes over the first five orders with respect to all subsequent orders (figure 17).

(e) *Analysis of dendritic density of Purkinje cells*

The mean total dendritic length was 7627.4 ± 140 μm and the mean dendritic area 26316.32 ± 318 μm². Dendritic density was very consistent with a mean value of 0.3133 ± 0.0015 μm/μm² and together with the linear relation between dendritic length and dendritic area

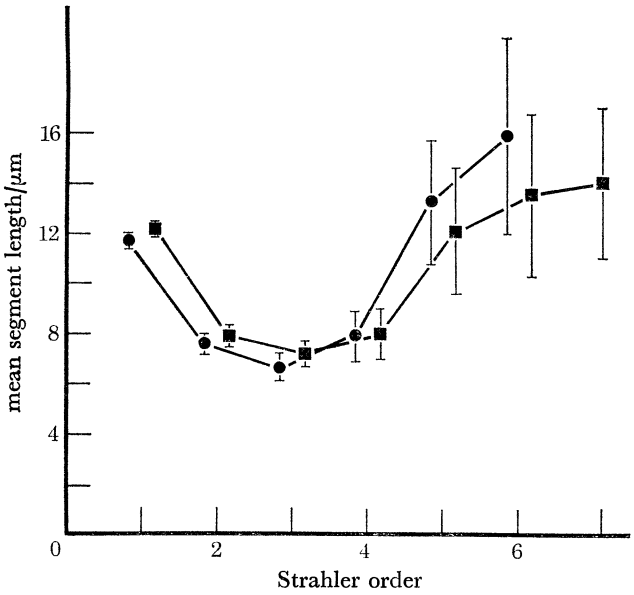


FIGURE 16. Graph of the mean segment lengths of Strahler orders of 6 (●) and 7 (■) order networks of the dendritic tree of Purkinje cells.

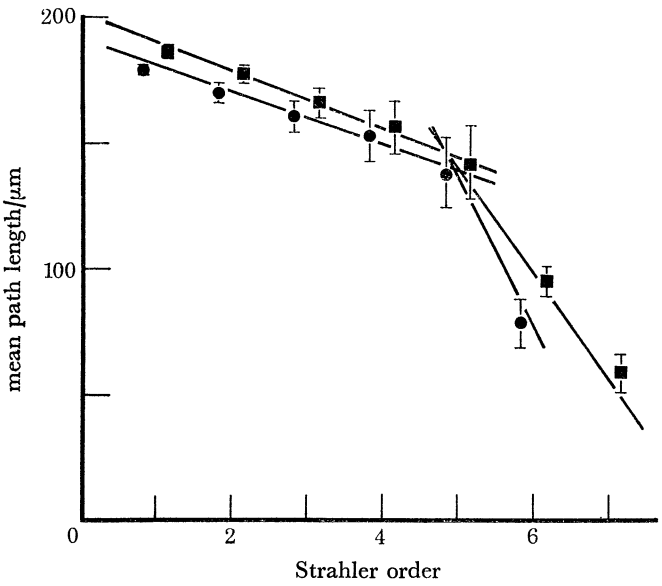


FIGURE 17. Graph of the mean path lengths of Strahler order in 6 (●) and 7 (■) order networks of the dendritic fields of Purkinje cells (6 order networks: Strahler orders 1–5, slope -10.43; $r = -0.81$; Strahler orders 5–6, slope -58.0; $r = -0.8$; 7 order networks: Strahler orders 1–5, slope -11.77; $r = -0.62$; Strahler orders 5–7, slope -40.57; $r = 0.87$.

(illustrated in figure 18) these results show that dendrites fill space at a constant density. Thus assuming that axon density also remains constant throughout the vermis, Purkinje cell dendrites may sample a fairly constant number of axons per unit area.

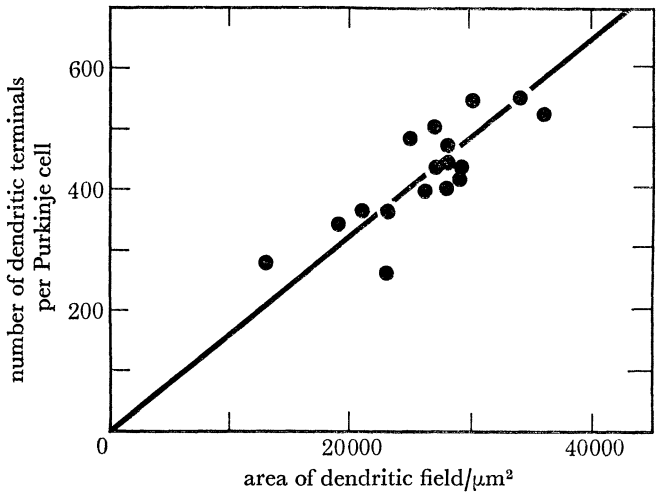


FIGURE 18. Graph of the number of dendritic terminals of each Purkinje cell against the area of the dendritic field plotted for the 17 cells analysed.

IV. DISCUSSION

Network analysis of dendritic fields provides valuable information about the growth of branching networks and the precise quantification of the connectivity of dendritic trees allows the method to be used both for comparative studies and for obtaining a better understanding of the role of branching in the integrative functions of dendrites.

1. *The growth of dendrites*

It is now established that the points of growth on dendrites are the growth cones (Bray 1970; Bunge & Bray 1970) which may be located at the tips (Bray 1973*a*; Tennyson 1970; Grainger & James 1970) or on the shafts of dendrites (Morest 1969*a*, *b*; Scheibel & Scheibel 1971) and it has become generally accepted that the vesicles within the growth cones and/or the filopodia eminating from them are the sources of new membrane for the growing tip (Morest 1969*b*; Bray 1970; Bunge & Bray 1970; Yamada *et al.* 1971; Bray 1973*b*). The structural lability of the growth cones can be demonstrated cinematographically as extension, retraction and ruffling movements of the filopodia (Bray 1970; Bunge & Bray 1970; Bray 1973*a*; Yamada *et al.* 1970, 1971; Ludueña & Wessels 1973), whereas the dendrites supporting the growth cones are structurally stable. It has been suggested that a dense array of longitudinally orientated microtubules may serve both as a skeletal framework for the latter and as an antegrade and retrograde transport system between the soma and the growing tip. The lability of the growth cone may be correlated with the absence of microtubules but the presence of a complex arrangement of contractile microfilaments (Yamada *et al.* 1970, 1971; Ludueña & Wessels 1973). The branches of growing dendrites are supported by a microtubular skeleton (Bunge 1973), are very stable and become left behind by the growing tip (Bray 1970, 1973*a*).

(*a*) *Growth at pendant arcs*

From what has been said already, it seems self evident that growth on pendant arcs occurs at the growth cones located at the tips of dendrites. Although new branches may also stem from the shafts of dendrites (segmental branches), their subsequent growth would also proceed terminally since growth cones are carried to the tips of such newly formed dendrites. Thus, as we have found, terminal growth predominates in dendritic systems. Fine structural studies of growth cones in the developing central nervous system (Vaughn *et al.* 1974; Kawana, Sandri & Akert 1971; Skoff & Hamburger 1974) have shown that synapses can engage filopodia and Vaughn *et al.* (1974) have suggested that when a filopodium is engaged all other filopodia are retracted and the original growth cone becomes an extension of the dendritic shaft. A new growth cone is formed distal to the point of synapse.

Thus simple extension, dichotomous branching or trichotomous branching will occur if one, two of three filopodia are contacted respectively. It is probable that synaptic formation on filopodia is a random event and this would account for our finding that dendritic arborescences arise by a random branching process. The factors controlling the frequency of synaptic contact may include such items as the potential for synaptic formation in the surrounding axon field and the number, length and speed of retraction of filopodia. Thus the direction of growth, order of branching and length of segments may be designated by the interaction of dendritic growth cones with neighbouring axons and it is possible that a low level of interaction between these elements in the neocortex may account for the lack of nodes whose order of magnitude is higher than dichotomy and the long segment lengths of dendrites, whereas, in the cerebellum, a relatively high degree of interaction may give rise to trichotomy and short segment lengths.

(*b*) *Growth at segments*

Branching may also occur on the segments of dendrites and this is borne out by an observation of Bray (1973*b*) and by the results of Morest (1969*a*, *b*) and Scheibel & Scheibel (1971). They identified the growth cones as varicose enlargements on growing dendrites of a variety of neurons in the mammalian brain. The interpretation of the significance of the varicose enlargements on growing dendrites may be verified if the branching patterns of the various cells analysed could be correlated with the location of growth cones over the dendritic surface.

The apical dendrites of neocortical pyramids exhibit two forms of growth, namely random terminal and random segmental branching and an explanation of this may be sought from a brief review of their natural history. During the migratory phase of neocortical development neuroblasts move from the subventricular zone into the cortical plate and the first dendritic process to appear is the apical dendrite. The apical tuft of branches is seen at this early period arborizing within the marginal zone (Berry 1974). As later migratory cells attain positions above the post-migratory neurons the apical tufts of these latter cells do not leave the marginal zone and thus the shafts of their apical dendrites increase in length as the cells occupy increasingly deeper positions in the cortex as migration proceeds. Thus if new branches are to appear on apical dendrites they must arise from the apical shaft. The new collateral branches do, however, grow by random terminal branching.

2. *Plasticity of dendritic fields and the validity of the method*

The pattern of branching *per se* does not determine the final form of the dendritic tree since networks with identical growth produce the same topological arrays but exhibit widely differing overall morphology, e.g. the dendritic trees of Purkinje cells and those of the basal dendrites of neocortical pyramids exhibit random terminal branching but clearly have quite different trees. Segment length and angles of bifurcation determine the final form of the tree. The degree to which these latter parameters and branching patterns are environmentally and/or genetically determined is unknown. Several workers have emphasized the importance of axonal relationships during the development and growth of dendrites (Marin-Paddilla 1970, 1971; Morest 1969*a*; O'Leary, Inukai & Smith 1968; Larramendi 1969; Kornguth & Scott 1972; Rakic 1972) and the role played by climbing and parallel fibres in the growth of the dendritic trees of Purkinje cells (O'Leary *et al.* 1968; Larramendi 1969; Kornguth & Scott 1972; Rakic 1972) will be discussed in other communications (Berry & Bradley 1975*b*; Bradley & Berry 1975).

Dendrites can change their morphology in response to environmental influences (Jones & Thomas 1962; Matthews & Powell 1962; Holloway 1966; Ruiz-Marcos & Valverde 1969; Coleman & Riesen 1968; Sumner & Watson 1971; Volkmar & Greenough 1972; Greenough & Volkmar 1973; Berry & Hollingworth 1973; Berry 1975) and it is probable that dendrites are most susceptible to morphological change during the development of axo-dendritic connexions. Thus Valverde (1968) showed that the dendritic fields of layer IV neurons in the optic cortex of mice enucleated at birth became abnormally orientated although dendritic branching was not reduced. This observation has been substantiated by the results of Altman & Anderson (1971), who attributed changes in orientation and morphology of the dendritic trees of Purkinje cells to a paucity of axons following the destruction of the dividing precursors of cerebellar microneurons during development. On the other hand, Van der Loos (1965) did not find that the dendritic fields of improperly orientated pyramidal cells in the neocortex were influenced by the planar organization of the cortical neuropil but bore a constant spacial relationship to the orientation of the perikaryon. But although there is this difficulty if resolving the findings of workers with respect to the effects of nurture and nature on the orientation of dendritic fields there is general agreement that dendritic branching is modified by environmental changes.

Coleman & Riesen (1968) showed that the dendrites of stellate cells in layer IV of the visual cortex of cats reared in darkness have a smaller length and branch less when compared with those of normally reared littermates. Volkmar & Greenough (1972) and Greenough & Volkmar (1973) studied the branching patterns of the dendrites of neurons in the occipital cortex reared in complex environments branched more than those in other groups. The degree to which these changes are reversible has not been elucidated but Sumner & Watson (1971) have shown that the dendritic fields of hypoglossal neurons of rats retracted after axotomy but re-expanded when the nerve reinnervated muscle. Sumner & Watson (1971) used Sholl's method of analysis and thus did not look at the branching patterns of the dendrites. It is relevant to note here that Globus & Scheibel (1967) found no quantitative difference between the dendritic fields of stellate and pyramidal cells in the striate cortex of normal and dark reared rats using the Sholl technique of analysis.

In the main, the above evidence does indicate that dendrites are normally subjected to remodelling according to the level of afferent activity converging on neurons either by

re-orientation, by retraction and/or by regrowth. This work has indicated that dendritic networks are established by branching on random segments or by branching constrained to random pendant vertices. The factors operating in dendritic membrane which underly these random growth processes may also function both during retraction and during regrowth. If such were the case, the fundamental patterns of branching would be retained during environmentally induced morphological change. Thus, in the case of terminal branching, for example, random pendant arcs may be retracted and if regrowth occurs on random pendant vertices, then the fundamental pattern of branching of the network will be maintained. This contingency has been investigated in part, by using a computer simulation model (see §5 of Discussion) and the results indicate that the random loss of segments does not substantially change the branching pattern of a network. The degree of remodelling that normally occurs during development and adult life is not known but such morphological changes might be confined to the peripheral part of the basal dendritic trees of neocortical pyramids (Coleman & Reisen 1968; Greenough & Volkmar 1973) and thus if a change in the branching patterns occurs as a result of environmental pressure it may be reflected in a change in the bifurcation ratio between the proximal and distal Strahler orders. However, this work shows that the branching patterns of neocortical cells and cerebellar dendrites exhibit particular patterns of topological types and bifurcation ratios that infer that dendrites maintain a constancy in their connectivity although they are vulnerable to environmental manipulation. Such a conclusion might be expected, if the unique branching pattern in a given system forms the structural basis of integration. Accordingly, changes in dendritic density would be directed at handling data of differing magnitudes without concomitant changes in information processing.

3. *Definition of the connectivity of dendritic networks*

Consistent results both between and within groups were only obtained using the Strahler ordering system, when the peripheral portions of the dendritic tree of Purkinje cells were compared. Such treatment may seem artificial but is justified if one views the network as the outcome of a stochastic growth process which results in a statistical relationship between orders. The application of the Strahler method of ordering to river networks, for example, commonly produces a deviation from a true inverse geometric series involving the higher orders. Smart (1967) and Shreideggar (1966) have shown that these deviations are statistically predictable. In fact a single network, no matter how large, can be placed within a pendant arc series and the pendant arc series has a place in a hierarchy of order ranks (Berry *et al.* 1975). We suggest that the variability of the overall absolute bifurcation ratio can be accounted for if these factors are known. It can therefore be argued that comparisons between networks are best made at the periphery where the large number of individual networks have established a consistent bifurcation ratio and to ignore the more proximal portion where bifurcation ratios are unstable because the number of daughter Strahler orders able to change the parent order become increasingly less common. Our results show that the Strahler system of ordering describes networks sufficiently accurately to analyse meaningfully the growth of different networks.

The early establishment of a stable bifurcation ratio between the low Strahler orders in small dendritic trees may mean that the integrative relation between lower orders has a high probability of being maintained despite the fact that it is the terminal branches which are engaged in the active branching process during growth. Thus the relation between orders tends to be unstable in the region of the soma during growth of the tree since it is in the proximal dendrites

that the hierarchy of Strahler orders changes with respect to both rank order and the bifurcation ratio between adjacent orders.

A possible advantage of the early establishment of a stable bifurcation ratio is that growing networks, or mature systems which depend on a number of small networks, like the basal dendritic trees of neocortical cells, may have a high probability of possessing the structural constancy which is presumably one prerequisite to meaningful integration. The exact role of branching in the integrative process is little understood and may be interpreted differently if dendritic membrane is construed to be active, on the one hand (Chang 1952; Tasaki, Polley & Orrego 1954; Spencer & Kandel 1961; Wall 1965; Arshavskiy *et al.* 1966; Spear 1972), or passive, on the other (Clare & Bishop 1955; Purpura 1959; Rall 1959, 1962).

In the latter case 'cable theory' is directly applicable to the problem of the conduction of the electrotonic potential and, in some cases, entire dendritic trees may be viewed as being equivalent to a single cylindrical conductor (Rall 1964). The advantage of this simplification is that any point on the dendritic tree can be represented as being at a given electrotonic distance from the soma and thus the amplitude attenuation at the soma of a given postsynaptic potential generated at any locus on the tree can be defined (Rall 1970). Since electrotonic distance tends to increase in a stepwise manner over orders of branching, amplitude attenuation similarly tends to increase by a factor of the order of branching of the system. The net result is that over a symmetrical linear system small multiple inputs injected at the periphery of the tree over different branches may be as effective at the soma as the equivalent of the sum of those inputs delivered as a single impulse at one locus at a similar electrotonic distance. Although Rall (1962) transformed a hypothetical dichotomously branching tree, with a stable bifurcation ratio of 2 established between all orders, into an equivalent cylinder, it is possible that asymmetrical trees with bifurcation ratios greater than 2 can be transformed into equivalent cylinders provided firstly, that the correct relation between diameters of branches meeting at a node is maintained, i.e. that the conductance into the parent branch is proportional to the 3/2 power of the diameter and that, secondly, all branches of a given order possess the same total electrotonic distance. Diameters have not been recorded in this work particularly since the presence of spines over the peripheral parts of the Purkinje cell tree makes measurement of this parameter difficult. Dendritic path lengths for a given order have however been computed, from which electrotonic distance can be calculated, either intuitively (Rall 1962), or by electrophysiological measurement (Rall 1970).

The possibility however that dendrites generate all-or-none responses (Arshavskiy *et al.* 1966) either throughout their length or only at nodes introduces other dimensions into the significance of branching in the integrative function of the dendritic tree. As Rall (1970) has commented, the firing of a given node would become dependent on a complex set of contingent probabilities resulting from the interaction of inhibition and excitation within the daughter branches which would provide a single neuron with a very large logical capacity. Under these circumstances it is difficult to begin to comprehend the role of a given branching pattern in such integrative processes without more information concerning the electrophysiology of dendritic membrane, although some of the initial ground work has been reported for both closed and open networks (Arshavskiy *et al.* 1966; Smolyaninov 1966). The quantitative definition of the topology of branching patterns attained by network analysis does permit the construction of accurate models from which the logic of dendritic networks may be worked out for a given set of assumptions about the physical properties of their membrane.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

4. *Length of Purkinje cell dendrites*

The dendritic length of Purkinje cells only has been measured. The difficulty of computing absolute length from the projected length of neocortical dendrites already foreshortened by sectioning precluded measurement of these dendrites (Berry *et al.* 1972, 1973). The depth of the plane in which the dendrites of Purkinje cells lie is some 20–50 μm thick. However, assuming that all dendrites course along randomly directed lines within the depth of their plane of orientation, the means of the lengths of orders and the relation between these measures should be good approximations to the true mean values.

The exponential increase in branch length with successively increasing Strahler order is expected for although segment length is fairly constant the number of segments per branch increases exponentially. The constancy of segment length is marked over the second, third and fourth orders but the length of segments increases in fifth, sixth and seventh Strahler order branches. The mean path lengths of each Strahler order also shows a different relation between the distal and proximal parts of the tree indicating that the mean distances impulses must travel between the vertices of first and fourth Strahler orders and the region of the axon hillock in the soma decreases by a constant small amount with increasing order but the mean path length from the vertices of fifth and subsequent Strahler orders decrease by a greater constant amount. This result perhaps emphasizes the differences between the peripheral and proximal part of the tree already alluded to. The peripheral part of the tree appears to be organized into fourth order networks which may function mainly to receive and integrate incoming information from parallel fibres whereas the proximal part of the tree is perhaps concerned with summing and conducting this information to the soma and receiving input from climbing and basket cell axons.

Whereas the spiny distal parts of the dendrites of Purkinje cells establish contacts with the axons of granule and stellate cells (Hámori & Szentágothai 1964; Fox & Barnard 1957; Fox, Siegesmund & Dutta 1964) climbing fibres engage mainly the primary and secondary 'smooth' branches of Purkinje cell dendrites (Scheibel & Scheibel 1954; O'Leary *et al.* 1968; Larramendi & Victor 1967; Hámori & Sentágothai 1966) along with the axons of basket cells (Chan-Palay & Palay 1970) and possibly association fibres (Eager 1965). The primary and secondary branches described by these workers make up the proximal system consisting of fifth, sixth and seventh order branches. Thus the two parts of the network receive quite separate inputs. Marr (1969) and Blomfield & Marr (1970) have suggested that climbing fibres carry items of information from the primary motor cortex and that mossy fibres relay contextual information from central and peripheral sources through parallel fibres directly or via interneurons to Purkinje cells. Although the discovery that climbing fibres also engage granule and Golgi cells in the granular layer (Chan-Palay & Palay 1970) has complicated the model, it is none the less apparent that the proximal system of dendrites may receive rather more specific information than the distal network and, accordingly, it is possible that the branching characteristics of the distal network have been biologically selected to sample and handle a multiplicity of inputs injected by '*en passant*' synapses and that of the proximal network has been adapted to receive a particular 'bit' of information over '*en passage*' synapses whilst the entire dendritic tree of the Purkinje cells has evolved to integrate both forms of input. It is possible that similar mechanisms are operational in the neocortex but in this part of the central nervous system the shaft of the apical dendrites of pyramidal cells may receive items of information by '*en passage*' synapses

and the basal dendrites and collaterals of the apical dendrite receive information possibly about context, by '*en passant*' synapses. The gross structural differences between afferent systems in the cerebellum and neocortex are thus adapted to engage a planar arrangement of axons. In the cerebral cortex all categories of axons are arranged in a uniplanar array so that the item sampling (shaft of the apical dendrite) and context sampling systems (basal dendrites and apical collaterals) are set in a plane orthogonal to each other and the item sampling membrane is drawn out to offer a large surface area for contact with a smaller number of axons. In the cerebellar cortex contextual and itemized inputs run in orthogonal planes and the long smooth proximal branches offer a large surface area for synaptic contact with axons carrying items of information whilst the planar array of their daughter branches offer a network suited to sample and integrate contextual information.

5. *Technical limitations of the method*

It is unlikely that a single entire dendritic tree has been analysed in this work, since parts of the dendritic tree may be cut away during sectioning or not become impregnated. Purkinje cells were contained in 80 μm sections cut in a plane parallel to the long axis of the tree and thus, in this case, loss was small but the basal dendrites of neocortical pyramids may be grossly foreshortened by sectioning since the diameter of the dendritic field is often much greater than the thickness of the sections used (Berry *et al.* 1972*b*; Berry, Hollingworth, Flinn & Anderson 1973). Loss may also occur from incomplete impregnation (Blackstad 1970; Stell 1965) while some dendrites may be too small to be resolved by means of the light microscope (Schadé, Van Bacher & Colon 1964). These and the related problems of a possible selective preference of impregnation of particular neuronal types are discussed by Kiernan & Berry (1975).

Since it is possible that the results of this work represent an artefact produced by losses from the tree due to sectioning and a partial failure to impregnate, or to a possible influence of the environment on the tree, we have compared our histological data with those obtained from both complete networks grown by computer simulation and these same networks subsequently subjected to the random loss of segments (having assumed that the above histological losses occur randomly). The results show that, over the magnitude of loss studied, the bifurcation ratios of network are not affected. We therefore conclude that, accepting the limitations set by the Golgi–Cox method and the resolving power of the light microscope, the technique of network analysis may be used to obtain meaningful information about the growth and organization of dendritic fields providing any structural deficiencies in the tree analysed are the result of random losses.

We are grateful to Dr R. M. Flinn and Mrs E. M. Anderson for their assistance with the mathematics and computer programming, to Professor J. T. Eayrs for his assistance in preparing the manuscript and to Mrs S. Buckley for her expert technical assistance.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

References

Altman, J. & Anderson, W. T. 1971 Irradiation of the cerebellum in infant rats with low level X-rays; histological and cytological effects during infancy and adulthood. *Expl Neurol.* **30**, 492–509.

Arshavskiy, Yu, I., Berkinblit, M. B., Kovalev, S. A., Smolyaninov, V. V. & Chaylakhyan, L. M. 1966 An analysis of the functional properties of dendrites in relation to their structure. In *Models of the structural–functional organisation of certain biological systems* (ed. I. M. Gelfand, V. S. Gurfinkel, S. V. Formin & M. L. Tsetlin), pp. 25–77. Moscow. Originally published in 1966 under the auspices of the Academy of Science of the U.S.S.R. (Institute of Biological Physics). English translation 1971. Cambridge, Massachusetts: M.I.T. Press.

Berry, M. 1974 Development of the neocortex of the rat. In *Studies on the development of behaviour and the nervous system*, vol. II (ed. G. Gottlieb), pp. 7–67. New York: Academic Press.

Berry, M. 1975 Plasticity in the visual system and visually guided behaviour In *Advances in psychobiology* (ed. G. Newton & A. H. Riesen). (In the Press.)

Berry, M., Anderson, E. M., Hollingworth, T. & Flinn, R. M. 1972*a* A computer technique for the estimation of the absolute three-dimensional array of basal dendritic fields using data from projected histological sections. *J. Microsc.* **95**, 257–267.

Berry, M. & Bradley, P. M. 1975*a* The application of network analysis to the study of branching patterns of large dendritic fields. *Brain Res.* (submitted for publication).

Berry, M. & Bradley, P. M. 1975*b* The growth of the dendritic tree of Purkinje cells in the cerebellum of the rat. *Brain Res.* (submitted for publication).

Berry, M. & Eayrs, J. T. 1966 The effects of X-irradiation on the development of the cerebral cortex. *J. Anat.* **100**, 707–722.

Berry, M. & Hollingworth, T. 1973 Development of isolated cortex. *Experientia* **29**, 204–207.

Berry, M., Hollingworth, T., Anderson, E. M. & Flinn, R. M. 1975 The application of network analysis to the study of the branching patterns of small dendritic fields. In *Advances in neurology* (ed. G. W. Kreutzberg). New York: Raven Press. (In the Press.)

Berry, M., Hollingsworth, T., Flinn, R. M. & Anderson, E. M. 1972*b* Dendritic field analysis – a reappraisal. *T-I-T- J. Life Sci.* **2**, 129–140.

Berry, M., Hollingworth, T., Flinn, R. M. & Anderson, E. M. 1973 Morphological correlates of functional activity in the nervous system. In *Macromolecules and behaviour* (ed. G. B. Ansell & P. B. Bradley), pp. 217–240. London: Macmillan.

Blackstad, T. W. 1970 Electron microscopy of Golgi preparations for the study of neuronal relations. In *Contemporary research methods in neuroanatomy* (ed. W. J. H. Nauta & S. O. E. Ebesson), pp. 186–217. Berlin, Heidelberg, New York: Springer Verlag.

Blomfield, S. & Marr, D. 1970 How the cerebellum may be used. *Nature, Lond.* **277**, 1224–1228.

Bok, S. T. 1936 The branching of the dendrites in the cerebral cortex. *Proc. Acad. Sci. Amst.* **36**, 1209–1218.

Bradley, P. M. & Berry, M. 1975 The effects of reduced climbing and parallel fibre input on Purkinje cell dendritic growth. *Brain Res.* (submitted for publication).

Bray, D. 1970 Surface movements during the growth of single explanted neurons. *Proc. natn. Acad. Sci. U.S.A.* **65**, 905–910.

Bray, D. 1973*a* Branching patterns of sympathetic neurons in culture. *J. Cell. Biol.* **56**, 702–712.

Bray, D. 1973*b* Model for membrane movements in the neural growth cone. *Nature, Lond.* **244**, 93–96.

Bunge, M. B. 1973 Fine structure of nerve fibres and growth cones of isolated sympathetic neurons in culture. *J. Cell Biol.* **56**, 713–735.

Bunge, M. B. & Bray, D. 1970 Fine structure of growth cones from cultured sympathetic neurons. *J. Cell. Biol.* **47**, 241*a*.

Chang, H. T. 1952 Cortical neurons with particular reference to the apical dendrites. *Cold Spring Harb. Symp. quant. Biol.* **17**, 189–202.

Chan-Palay, V. & Palay, S. L. 1970 Interrelations of basket cells axons and climbing fibres in the cerebellar cortex of the rat. *Z. Anat. Entwicklungs-gesch.* **132**, 191–227.

Clare, M. H. & Bishop, G. H. 1955 Properties of dendrites; apical dendrites of the cat cortex. *Electroenceph. clin. Neurophysiol.* **7**, 85–98.

Coleman, P. D. & Riesen, A. H. 1968 Environmental effects on cortical dendritic fields. I. Rearing in the dark. *J. Anat.* **102**, 363–374.

Eager, R. P. 1965 The mode of termination and temporal course of degeneration of cortical association pathways in the cerebellum of the cat. *J. Comp. Neurol.* **124**, 243–258.

Eayrs, J. T. 1955 The cerebral cortex of normal and hypothyroid rats. *Acta Anat.* **25**, 160–183.

Eayrs, J. T. & Goodhead, B. 1959 Postnatal development of the cerebral cortex in the rat. *J. Anat.* **93**, 385–402.

Fox, C. A. & Barnard, J. W. 1957 A quantitative study of the Purkinje cell dendritic branchlets and their relationship to afferent fibres. *J. Anat.* **91**, 299–313.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Fox, C. A., Siegesmund, K. A. & Dutta, C. R. 1964 The Purkinje cell dendritic branchlets and their relation with the parallel fibres: light and electron microscopic observations. In *Morphological and biochemical correlates of neural activity* (ed. M. M. Cohen and R. S. Snider), pp. 112–141. New York: Harper and Row.

Globus, A. & Scheibel, A. B. 1967 The effects of visual deprivation on cortical neurons: a Golgi study. *Expl Neurol.* **19**, 331–345.

Grainger, F. & James, D. W. 1970 Association of glial cells with the terminal parts of neurite bundles extending from chick spinal cord *in vitro*. *Z. Zellforsch.* **108**, 93–104.

Greenough, W. T. & Volkmar, F. R. 1973 Pattern of dendritic branching in occipital cortex of rats reared in complex environments. *Expl Neurol.* **40**, 491–504.

Hámori, J. & Szentágothai, J. 1964 The 'crossing-over' synapse: an electron microscope study of the molecular layer in the cerebellar cortex. *Acta biol. Hung.* **15**, 95–119.

Hámori, J. & Szentágothai, J. 1966 Identification under the electron-microscope of climbing fibres and their synaptic contacts. *Expl Brain Res.* **1**, 65–81.

Holloway, Jr. R. L. 1966 Dendritic branching; some preliminary results of training and complexity in rat visual cortex. *Brain Res.* **2**, 393–396.

Jones, W. H. & Thomas, D. B. 1962 Changes in the dendritic organisation of neurons in the cerebral cortex following differentiation. *J. Anat.* **96**, 375–381.

Kawana, E., Sandri, C. & Akert, K. 1971 Ultrastructure of growth cones in the cerebellar cortex of the neonatal rat and cat. *Z. Zellforsch.* **115**, 284–298.

Kiernan, J. A. & Berry, M. 1974 Neuroanatomical methods. In *Methods in brain research* (ed. P. B. Bradley). London: John Wiley.

Kornguth, S. E. & Scot, G. 1972 The role of climbing fibres in the formation of Purkinje cell dendrites. *J. comp. Neurol.* **146**, 61–82.

Larramendi, L. M. H. & Victor, T. 1967 Synapses on spines of the Purkinje cell of the mouse. An electron-microscopic study. *Brain Res.* **5**, 15–30.

Larramendi, L. M. H. 1969 Analysis of synaptogenesis in the cerebellum of the mouse. In *Neurobiology of cerebellar evolution and development* (ed. R. Llinas), pp. 803–844. Chicago: Proceedings of the First International Symposium of the Institute for Biomedical Research. American Medical Association/Education and Research Foundation.

Levinthal, C. & Ware, R. 1972 Three dimensional reconstruction from serial sections. *Nature, Lond.* **236**, 207–210.

Ludueña, M. A. & Wessells, N. R. 1973 Cell locomotion, nerve elongation and microfilaments. *Dev Biol.* **30**, 427–440.

Mannen, H. 1966 Contribution to the morphological study of dendritic arborization in the brain stem. In *Correlative neurosciences. Progress in brain research*, vol. 21 A (ed. T. Tokizane & J. P. Schadé). Elsevier: Amsterdam, London, New York.

Marin-Padilla, M. 1970 Prenatal and early postnatal ontogenesis of the human motor cortex. A Golgi study. II. The basket-pyramidal system. *Brain Res.* **23**, 185–191.

Marin-Padilla, M. 1971 Early prenatal ontogenesis of the cerebral cortex (neocortex) of the cat (*Felis domestica*). A Golgi study. I. The primordial neocortical organisation. *Z. Anat. Entwicklungsgesch.* **134**, 117–145.

Marr, D. 1969 A theory of cerebellar cortex. *J. Physiol., Lond.* **202**, 437–470.

Matthews, M. R. & Powell, T. P. S. 1962 Some observations of transneuronal cell degeneration in the olfactory bulb of the rabbit. *J. Anat.* **96**, 89–102.

Morest, D. K. 1969*a* Differentiation of cerebral dendrites: a study of the post-migratory neuroblasts in the medial nucleus of the trapezoid body. *Z. Anat. EntwGesch.* **128**, 271–289.

Morest, D. K. 1969*b* The growth of dendrites in the mammalian brain. *Z. Anat. EntwGesch.* **128**, 290–317.

O'Leary, J. L., Inukai, J. & Smith, J. M. 1968 Histogenesis of the cerebellar climbing fibre in the rat. *J. comp. Neurol.* **142**, 377–392.

Purpura, D. P. 1959 Nature of electrocortical potentials and synaptic organisations in cerebral and cerebellar cortex. *Int. Rev. Neurobiol.* **1**, 48–163.

Rakic, P. 1972 Extrinsic cytological determinants of basket and stellate cell dendritic patterns in the cerebellar molecular layer. *J. comp. Neurol.* **146**, 335–354.

Rall, W. 1959 Branching dendritic trees and motorneuron membrane resistivity. *Expl Neurol.* **1**, 491–527.

Rall, W. 1962 Theory of physiological properties of dendrites. *Ann. N.Y. Acad. Sci.* **96**, 1071–1092.

Rall, W. 1964 Theoretical significance of dendritic trees of neuronal input-output relations. In *Neural theory and modelling*. Proceedings of the 1962 Ojai Symposium (ed. R. F. Reiss), pp. 73–97. Stanford, California: Stanford University Press.

Rall, W. 1970 Cable properties of dendrites and effects of synatpic location. In *Excitatory synaptic mechanisms*. Proceedings of the Fifth International Meeting of Neurobiologists (ed. P. Andersen and J. K. S. Jansen), pp. 175–188. Oslo, Bergen, Tronsö Universitetsforlaget.

Ruiz-Marcos, A. & Valverde, F. 1969 The temporal evolution of the distribution of dendritic spines in the visual cortex of normal and dark raised mice. *Expl Brain Res.* **8**, 284–294.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Schadé, J. P., Van Bacher, H. & Colon, E. 1964 Quantitative analysis of neuronal parameters in the maturing cerebral cortex. In *Progress in brain research* (ed. D. P. Purpura & J. P. Schadé), pp. 150–175. Elsevier: Amsterdam.

Scheibel, M. E. & Scheibel, A. B. 1954 Observations on the intracortical relations of the climbing fibres of the cerebellum. *J. comp. Neurol.* **101**, 733–763.

Scheibel, M. E. & Scheibel, A. B. 1971 Selected structural–functional correlations in postnatal brain. In *Brain development and behaviour* (ed. M. B. Stern, D. J. McGinty & A. M. Adinolfi), pp. 1–21. New York: Academic Press.

Scheideggar, A. E. 1966 Stochastic branching processes and the law of stream orders. *Water Res. Res.* **2**, 199–203.

Sholl, D. A. 1953 Dendritic organisation in the neurons of the visual and motor cortices of the cat. *J. Anat.* **87**, 387–407.

Sholl, D. A. 1955 The organization of the visual cortex in the cat. *J. Anat.* **89**, 33–46.

Skoff, R. P. & Hamburger, V. 1974 Fine structure of dendritic and axonal growth cones in embryonic chick spinal cord. *J. comp. Neurol.* **153**, 107–148.

Smart, J. S. 1967 A comment on Horton's Law of Stream Numbers. *Water Res. Res.* **4**, 1001–1014.

Smit, G. J., Uylings, H. B. M. & Veldmaat-Wansink, L. 1972 The branching patterns in dendrites of cortical neurons. *Acta Morphol. Neerl-Scand.* **9**, 253–274.

Smolyaninov, V. V. 1966 The problem of the electrical properties of syncytia. In *Models of the structural-functional organisation of certain biological systems* (ed. I. M. Gelfand, V. S. Gurfinkel, S. V. Formin & M. L. Tsetlin), pp. 132–154. Moscow. Originally published in 1966 under the auspices of the Academy of Science of the U.S.S.R. (Institute of Biological Physics). English translation 1971. Cambridge, Massachusetts: M.I.T. Press.

Spear, P. J. 1972 Evidence for spike propagation in cortical dendrites. *Expl Neurol.* **35**, 111–121.

Spencer, W. A. & Kandel, E. R. 1961 Electrophysiology of hippocampal neurons. IV. Fast prepotentials. *J. Neurophysiol.* **24**, 272–285.

Stell, W. K. 1965 Correlation of retina cytoarchitecture and ultrastructure in Golgi preparations. *Anat. Rec.* **153**, 383–397.

Sumner, B. E. H. & Watson, W. E. 1971 Retraction and expansion of the dendritic tree of motor neurones of adult rats induced *in vivo*. *Nature, Lond.* **233**, 273–275.

Tasaki, I., Polley, E. H. & Orrego, F. 1954 Action potential from individual elements in cat geniculate and striate cortex. *J. Neurophysiol.* **17**, 454–474.

Tennyson, V. 1970 The fine structure of the axon and growth cone of the dorsal root neuroblast of the rabbit embryo. *J. Cell. Biol.* **44**, 62–79.

Valverde, F. 1968 Structural changes in the area striata of the mouse after enucleation. *Expl Brain Res.* **5**, 274–292.

Van der Loos, H. 1965 The 'improperly' orientated pyramidal cell in the cerebral cortex and its possible bearing on the problems of neuronal growth and cell orientation. *Bull. Johns Hopkins Hospt.* **117**, 228–250.

Volkmar, F. R. & Greenough, W. T. 1972 Rearing complexity affects branching of dendrites in the visual cortex of the rat. *Science, N.Y.* **176**, 1445–1447.

Vaughn, J. E., Henrikson, C. K. & Grieshaber, J. A. 1974 A quantitative study of synapses in motor neuron dendrite growth cones in developing mouse spinal cord. *J. Cell. Biol.* **60**, 664–672.

Wall, P. D. 1965 Impulses originating in the region of dendrites. *J. Physiol., Lond.* **180**, 116–133.

Yamada, K. M., Spooner, B. S. & Wessels, N. K. 1970 Axon growth: roles of microfilaments and microtubules. *Proc. natn. Acad. Sci. U.S.A.* **66**, 1206–1212.

Yamada, K. M., Spooner, B. S. & Wessels, N. K. 1971 Ultrastructure and function of growth cones and axons of cultured nerve cells. *J. Cell Biol.* **49**, 614–635.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES