POSTNATAL DEVELOPMENT OF THE CEREBRAL CORTEX IN THE RAT

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Present knowledge of the development and structural organization of the cerebral cortex is based largely on qualitative observations, pioneered by the classical studies of Ramón y Cajal (1911) and later extended by Lorente de Nó (1922, 1949), von Bonin (1948*a*) and many others. Current treatment of the mode of functioning of the central nervous system, however, whether based on cybernetics, analogies with models or on mathematical concepts embracing information theory or conditional probability, lays emphasis on the pattern of connectivity between axons, perikarya and dendrites, and demands a closer knowledge of the quantitative aspects of the inter-relationship between neurones than is provided by purely descriptive neurohistology.

An early attempt to apply techniques of measurement to finer cortical structure was that of Bok (1936*a*, *b*), who investigated the relationship between the size and density of perikarya and depth beneath the pial surface, and established the idea of the 'cell territory' expressed in terms of the perikaryon and the extent of its associated dendritic field. These concepts have more recently been applied to the overall pattern of cortical organization (Campbell, 1954; Ryzen & Campbell, 1955) and further developed by Sholl (1953) whose data, combined with those relating to the distribution of axons (Sholl, 1955), have provided an anatomical basis for the estimation of neuronal connectivity (Sholl, 1956). Similar methods have been used in preliminary studies designed to correlate the histological abnormalities arising as a result of experimental cretinism with changes in adaptive behaviour (Eayrs, 1955).

Quantitative techniques have, as might be expected, been applied more freely to the study of changes taking place during development. Some workers have taken as their criteria of measurement the time of appearance of some relatively well-defined landmark of cortical maturation, such as the beginning of myelination (Flechsig, 1920), the first appearance of Nissl granules (Sugita, 1918*a*; Conel, 1947), a change in the refractive index of dendrites (Peters & Flexner, 1950) and modification of the electrical activity of the brain (Crain, 1952; Flexner, 1955). Others have plotted the course of cerebral development in terms of phenomena which are observable only as continuous processes, such as the increase in the surface area (Smith, 1934; Turner, 1950), volume (Kappers, 1926) and thickness of the cortex and its several layers (Sugita, 1917, 1918*b*; Conel, 1951), in the size of perikarya (Sugita, 1918*a*), in vascularity (Craigie, 1925), and alterations in the cerebral ground substance (Goodhead, 1957).

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An important phase of cortical maturation which has so far received but little quantitative treatment, however, is the growth and ramification of cell processes to form the neuropil and so vastly to increase the probability of synaptic interrelationship between neurones. The possible functional significance of this aspect of development was first suggested by von Economo (1926) who postulated, on the basis of comparative anatomical studies, that the relationship between the mass of the grey matter to that of the perikarya of its constituent neurones ('cell/grey coefficient') might be used as an index of the organizational status achieved by the brain. Interspecific differences in this coefficient appear to be inversely correlated with the size of the brain (Tower & Elliot, 1952; Shariff, 1953), while within the same species the coefficient decreases with advancing age (e.g. Sugita, 1918*b*; Brody, 1955). Although the prolongation of this phenomenon into old age is probably due to a loss of neurones and their replacement by non-conducting tissue, there can be little doubt that a decrease in cell density during a time when the brain is increasing in size must be associated with an elaboration of neuropil and, from a functional point of view, a corresponding shift of emphasis from the axo-somatic to the axo-dendritic mode of cortical integration whose possible significance has been pointed out and discussed by von Bonin (1948*b*).

These considerations must assume particular importance in any attempt to interpret the changes in cortical structure which result from hypothyroidism arising during early infancy. When this state is induced experimentally in the infant rat the expected decrease in the cell/grey coefficient fails to materialize (Eayrs & Taylor, 1951). Not only is the neuropil as a whole hypoplastic, but the growth of axons appears to be differentially retarded in certain regions of the cortex while, at the same time, the decay in the dendritic field of pyramidal neurones departs from the exponential pattern characteristic of the normal individual (Eayrs, 1955). So little quantitative data are available concerning the pattern of growth of cell processes in the normal individual that it has not been possible to assess whether these anatomical changes represent a distortion or merely a retardation of growth. The studies reported in the present paper have accordingly been undertaken to elucidate the mode of development of the cortical neuropil in the rat from birth to maturity.

MATERIALS AND METHODS

Animals

Forty-five young male rats and five male adults, all of the Birmingham strain, were used for this study. Variation in growth was minimized by breeding all the young from virgin females and raising them in litters reduced, on the day of birth, to a standard size of six.

Preparation of tissues

(*a*) *Histological treatment*

On the day of birth, and at ages of 6, 12, 18, 24 and 30 days post-partum, groups of rats were weighed and killed in chloroform vapour. Their brains were removed, weighed, and subjected to one of the following procedures:

(i) Fixation in a 25 % solution of chloral hydrate in 50 % ethanol and subsequent treatment by the method of Nonidez (1939) for the silver impregnation of

axons. Coronal paraffin sections were cut at 10μ thick, counterstained in Borel's methylene blue and mounted in DPX.

(ii) Processed by the Golgi-Cox method (modification of Sholl (1953)), embedded in celloidin and cut at 200μ in a coronal plane through one hemisphere and in a plane tangential to the frontal cortex through the other.

(iii) Fixation in a solution consisting of 90 parts of 70 % alcohol to 10 parts 40 % formaldehyde, coronal paraffin sections being cut at 10μ thick and stained with gallocyanin.

The brains of the adult rats used were similarly treated.

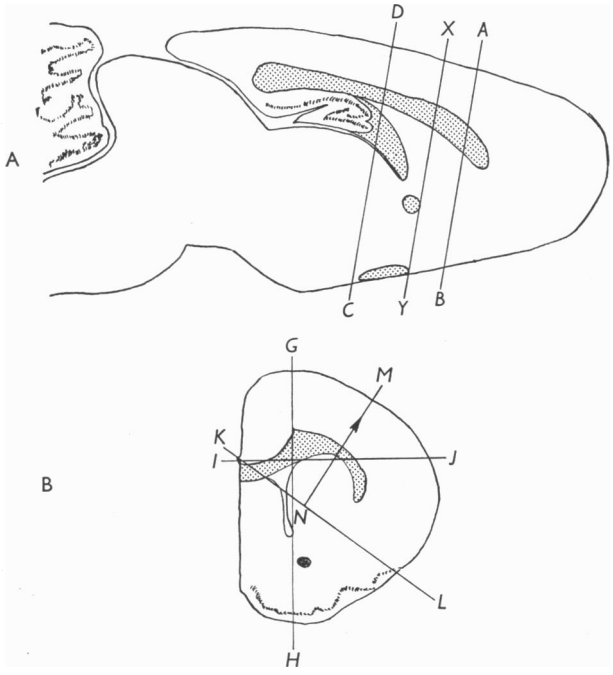

Fig. 1. Semi-schematic diagrams (approx. × 5) showing mode of preparation of tissues for histological examination. The line *XY* (top figure) marks the plane from which all coronal sections were cut. Sections tangential to the cortical surface were taken from the block of tissue represented by *ABCD*, whose anterior surface is illustrated in the lower figure. Sections were cut from the plane *KL* along the axis *NM* (for further explanation see text).

(*b*) *Planes of section*

Since the density of cortical neuropil varies from region to region care was taken to ensure that comparable areas were studied at all ages. Accordingly, before embedding, each brain was divided sagittally and one hemisphere divided in a plane, orthogonal to its medial surface, passing through the anterior margins of the anterior commissure and optic chiasma (*XY* in Fig. 1A). Coronal sections were cut from the block of tissue posterior to this plane, and a study made of those in which the anterior limits of the fornix first appeared. This plane of section passes through the sensori-motor cortex, and measurements were made in the region corresponding to that described as area 2 by Krieg (1946*a*, *b*), which has been previously studied quantitatively by Eayrs (1955) in normal and hypothyroid rats.

The second hemisphere of brains processed by the Golgi–Cox technique was used to obtain sections tangential to the cortical surface. The anterior surface of a block of tissue about 4 mm. thick cut in the coronal plane described above (see Fig. 1 A) was divided visually in the manner illustrated by Fig. 1 B in which the lines *GH* and *IJ* represent the planes previously used by Eayrs & Taylor (1951) and *M* is the mid-point of the cortical segment lying between them. The line *NM* is orthogonal to the cortical surface at *M* and passes through area 2. The cortex was removed by a cut in the plane *KL* which was subsequently used to orientate the specimen during embedding in celloidin and from which serial sections at 200 μ were cut in the direction of *NM* throughout the cortical thickness. Sections passing through layer 5*b* were identified by measuring the depth of this layer beneath the pial surface on coronal sections of the opposite hemisphere and counting off the number of tangential sections corresponding to this depth.

Quantitative estimations

(*a*) *Axon network*

The density of the axon network was estimated in a manner similar to that previously described by Eayrs (1955), using an optical system giving a magnification of $\times 900$. With the optical field centred in each of the cortical laminae 1, 3, 4, 5*a*, 5*b*, and 6*a* a grid ($100 \times 0{\cdot}5$ mm. squares) set in the focal plane of the eyepiece was used to count the number of axons intersecting a line of given length and to measure the proportion of this length occupied by other readily identifiable elements of the cortical tissue, i.e. perikarya, blood vessels, apical dendrites, and neuroglial nuclei. This proportion was subsequently used as a correction factor (see Table 4) to convert the number of axons counted within the cortex as a whole to that within the neuropil itself. The data were subsequently standardized, by taking into account the depth of focus of the optical system, to express the number of axons cutting an area of cortex of 1 mm.^{2}.

Estimates were carried out in different parts of each lamina; recounts later made on a number of randomly selected sections fell within 5 % of the original values.

(*b*) *Cell/grey coefficient*

The cell/grey coefficient was measured by recording the nature of the tissue underlying the intersections of the lines forming the grid. The number of coincidences between an intersection and a cell body relative to the total number of intersections was taken as an estimate of the proportion of cortical tissue occupied by perikarya.

Tests of reproducibility of the estimates were made by means of sample counts and gave results similar to those for axons.

(*c*) *Dendritic fields*

The dendritic fields formed by the basal dendrites of pyramidal cells in layer 5*b* were measured on coronal sections by the method described by Eayrs (1955) to estimate (i) the number of dendrites arising from the perikarya, (ii) the dendritic density, and (iii) the mean occurrence of points of branching and ending at successive intervals of 18 μ from the centre of the perikaryon. Similar observations were made on neurones in sections cut tangential to the cortical surface and identified by

measurement and inspection as passing through the middle of layer 5*b*. Ten randomly selected cells, five coronally and five tangentially orientated, were measured in this manner for each rat, the means of the data so derived being taken to characterize the dendritic fields of pyramidal cells in the tissues of the frontal cortex.

RESULTS

(*a*) *Qualitative changes in cortical structure*

(i) *The cerebral cortex at birth*

At birth local cortical differentiation has barely commenced, and cells are small and closely packed. Of the several laminae, only layer 1 can be distinguished with ease; layers 3 and 4 cannot be separated and consist of cells, strongly chromophilic, which are arranged in orderly columns. The position of a developing layer 5 can be identified as a thin band of somewhat larger neurones, while layer 6 is represented by a zone of cells in which Nissl substance is scanty and whose arrangement lacks any of the orderly pattern of those in layers 3 and 4. Very few axons indeed can be seen in the outermost layers of the cortex, and although considerably more are present in the deeper layers most of these fibres run a tangential course and no radially orientated bundles of axons (specific afferents of Lorente de Nó, 1922) are visible. The corpus callosum is poorly developed with few axons impregnated, its definitive position being largely occupied by migrating neuroblasts. No conclusion could be reached concerning the growth of dendrites, for Golgi–Cox preparations consistently failed to reveal any cells at all.

(ii) *The cerebral cortex at* 6 *days of age*

By the sixth day a marked increase in cortical differentiation has occurred and most of the laminae characteristic of the adult can be recognized without difficulty, although the perikarya are still closely packed together. The boundary between layers 3 and 4 is marked by the presence of larger cells which are apparently destined to develop into the 'border pyramids' found in this situation in the adult. Layer 4 itself is beginning to present its characteristic granular appearance and some of the cells in layer 5 are assuming a pyramidal shape, although none is yet argyrophilic as is the case in the adult. These cells presumably belong to sublayer 5*b* and no sublayer 5*a* (lamina interstriata), which is so characteristic of this region in the mature rat (Vaz Ferriera, 1951), has yet developed.

The number of axons has increased markedly since birth in all layers of the cortex, but bundles of radially orientated fibres traversing layers 5 and 6 have still not appeared. Most of the neurones demonstrated by the Golgi–Cox technique are pyramids located in layer 5. Each has a short apical dendrite which does not extend as far as layer 1, and does not branch. Few basal dendrites are present, many cells possessing none at all (Fig. 2).

(iii) *The cerebral cortex at* 12 *days of age*

The appearance of the cortex in the 12-day-old rat is, in general, characterized by: (1) an increase in the size of the perikarya; (2) a reduction in the packing density; and (3) a more precise demarcation between the several laminae, the lamina

interstriata now being well differentiated. The cells of the infragranular layers have developed conspicuous amounts of Nissl substance, and there is a marked tendency towards pyramidization in layer 5*b*, where a few cells now possess the argyrophilic properties of the adult. A marked increase in axon density has occurred, particularly in the granular and infragranular layers where many radially orientated fibres are now present, though not conspicuously assembled into fasciculi.

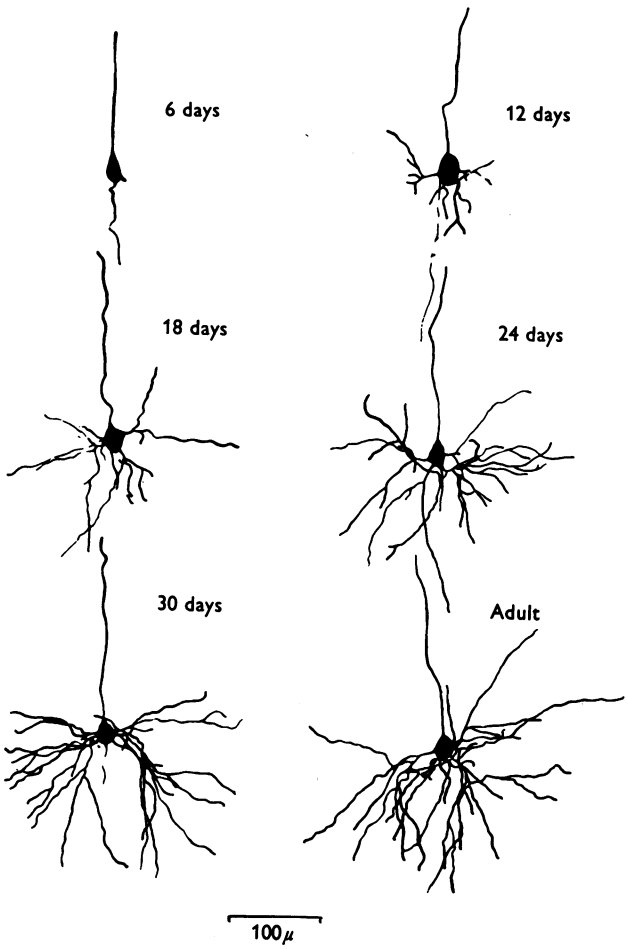

Fig. 2. Characteristic changes in appearance of pyramidal cells of the lamina ganglionaris from 6 days old to maturity.

One striking advance in development is seen in Golgi–Cox preparations, where many more neurones are impregnated. Considerable numbers of the apical dendrites now extend into layer 1, and numerous basal dendrites are present. Although these branch they do not extend far from the perikaryon, and there is, therefore, little if any overlap in the dendritic fields even of closely adjacent neurones (Fig. 2).

(iv) *The cerebral cortex from* 18 *days of age to maturity*

By the age of 18 days the cerebral cortex has assumed all the features characteristic of the adult, and changes between that age and maturity are quantitative rather than qualitative. All the laminae are now well marked, the cells of layer 5*b* are becoming conspicuously pyramidal in shape, and there is a marked increase in the extent of the dendritic fields, which now interlace with those of neighbouring neurones. Many such cells are now argyrophilic. The apical dendrites have increased in thickness and the majority extend into layer 1 and frequently branch. On a few of the apical dendrites, pedunculated bulbs can now be seen. The density of the axon network has markedly increased even to macroscopic inspection, particularly in the granular and infragranular layers, and fasciculi of radially coursing axons are now prominent in layer 5. These features become progressively more fully developed in the cortex of the 24- and 30-day-old rat, the most prominent changes being an increase in the complexity of dendritic ramification in layer 1 which is accompanied by a decrease in the number of axons in this region, an increase in the number of pedunculated bulbs and, apart from layer 1, a steadily increasing density of the axonal component of the neuropil.

(*b*) *Quantitative changes in cortical structure*

(i) *Cell/grey coefficient* (Fig. 3)

The cell/grey coefficient decreases most rapidly during the first days of life, whereafter the increment follows a slow and linear course until maturity. Not all laminae develop in the same way, however. Layer 1, which possesses very few identifiable neurones, shows very little change throughout the period of growth. The density of perikarya is greatest in the granular layer and remains so throughout the course of maturation. On the other hand, this density falls rapidly in both layers 3 and 5*a*, a feature which may to some extent be attributed to the rapid growth of apical dendrites of neurones in the subjacent layers which occurs between the 6th and 12th days of age. The somewhat later expansion of basal dendrites may account, to some extent, for the relatively delayed reduction in the cell/grey coefficient in layers 5*b* and 6*a*.

(ii) *Density of axons*

The density of axons does not increase at a uniform rate during cortical maturation. Only a small increase occurs during the first 7 days, but between the 6th and 18th day the increase is so rapid that at the end of this period the mean density is more than half the adult value (Fig. 4). Thereafter, the rate of increment is reduced, but by the 30th day falls short of the density in the fully mature cortex by a factor of only 15 %. The period of greatest decrease in the cell/grey coefficient (birth–6 days) does not, therefore, coincide with that during which axons multiply most rapidly, although the points at which the curves for the packing density of perikarya and axon density begin to reach a plateau show a rough inverse correspondence (Figs. 3, 4).

The axon network does not develop at a uniform rate throughout the cortex, for although the fibre densities in the several laminae maintain approximately the

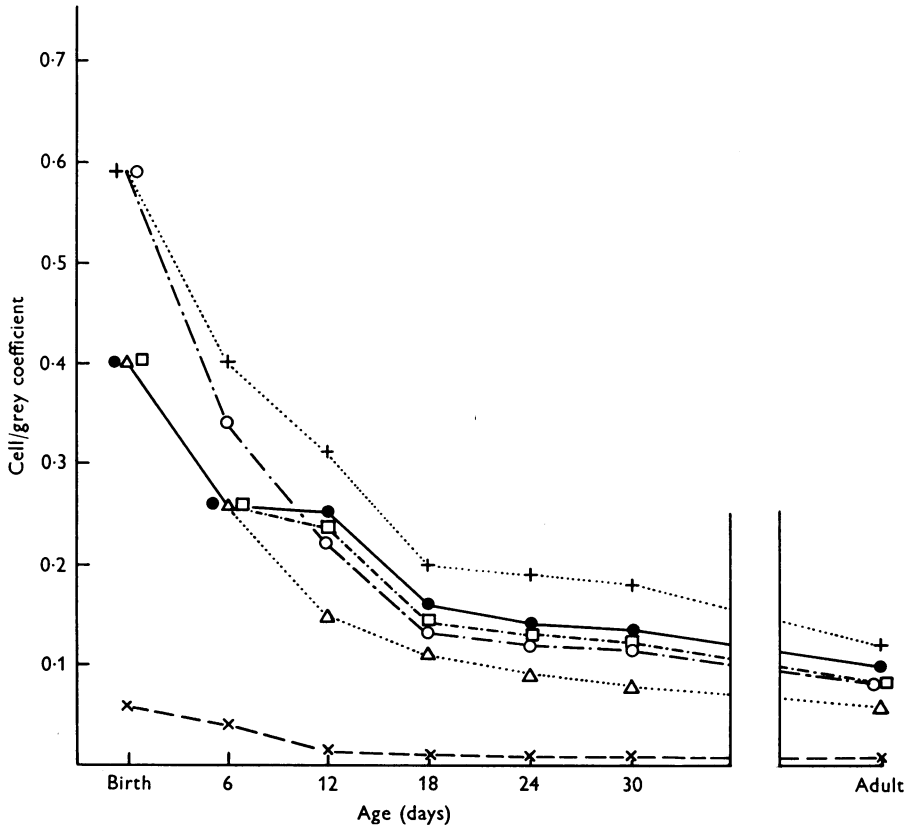

Fig. 3. Changes in the cell/grey coefficient in the several cortical laminae from birth to maturity. Key: layer 1, ×----×; layer 3, ○—·—·—○; layer 4, +·····+; layer 5*a*, △·····△; layer 5*b*, ●————●; layer 6, □-·-·-·-□.

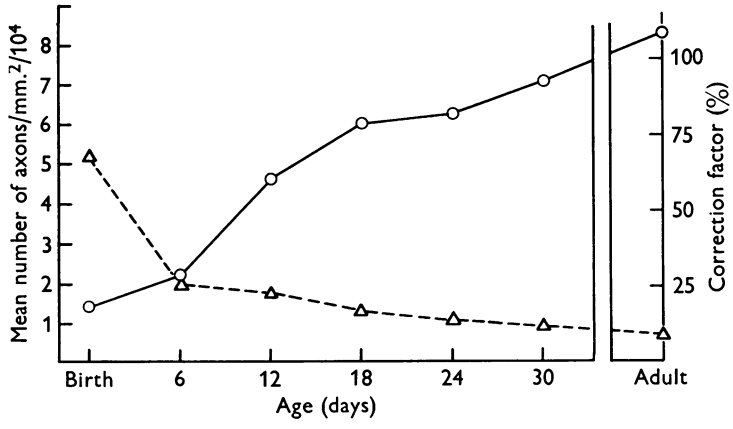

Fig. 4. Increase in number of cortical axons (○————○) and decrease in correction factor (i.e. the proportion of cortical tissue occupied by formed elements other than axons) (△-------△) with advancing age.

same relationship with respect to each other from birth to maturity (Fig. 5) there are interlaminar differences in the periods during which growth is most rapid. In layers 3, 4, 5*b* and 6*a* the rate of increment is greatest between 6 and 18 days of age (Fig. 6), whereas in layer 1 the number of axons increases rapidly to reach a peak at 12 days old, after which there is a steady decrease in density. By contrast, layer 5*a* lags behind the remainder, a considerable proportion of its axons (32 %) appearing after the age of 30 days.

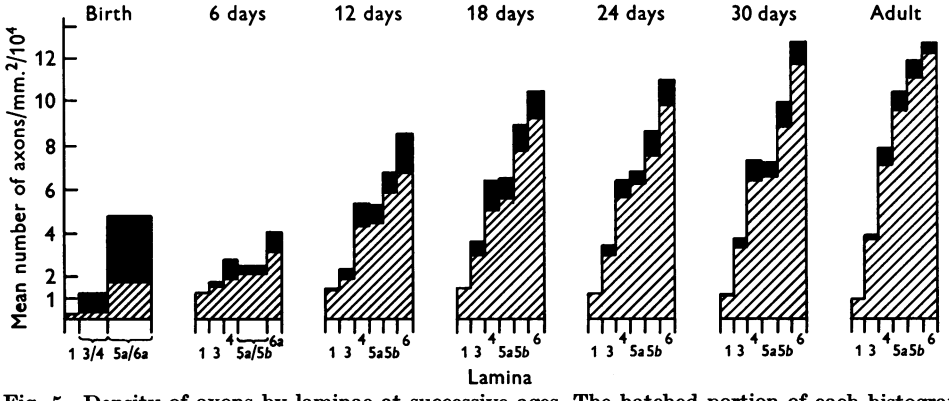

Fig. 5. Density of axons by laminae at successive ages. The hatched portion of each histogram gives the density in the cortex taken as a whole; the solid portion is the increment resulting from correction for the presence of formed elements other than axons, the total giving the axonal density within the neuropil.

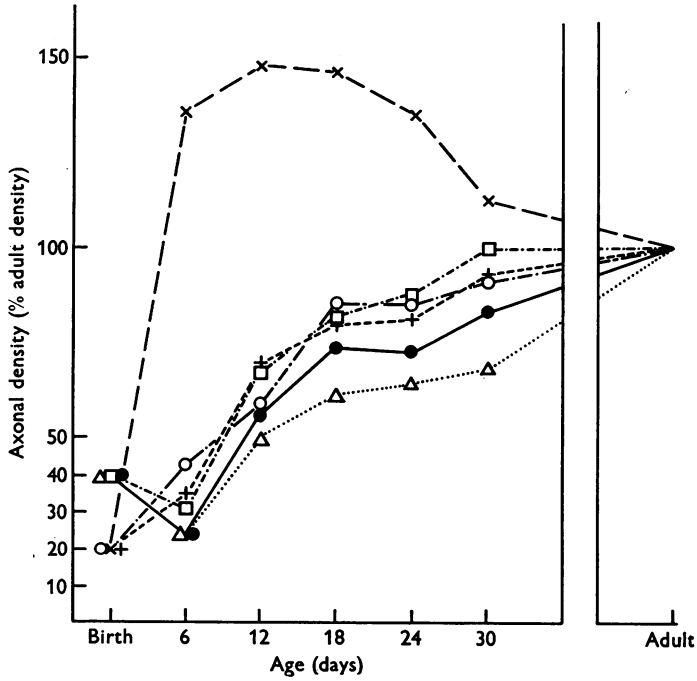

Fig. 6 Changes in axonal density with increasing age. Densities at each age are expressed as a percentage of their ultimate value in the adult. Key: layer 1, ×----×; layer 3, ○-·-·-○; layer 4, +----+; layer 5*a*, △ ····· △; layer 5*b*, ●————●; layer 6, □-·-·-·-□.

(iii) *Dendritic fields*

Measurement was restricted to the dendritic fields of the pyramidal cells of the lamina ganglionaris. As described earlier, none of these neurones was impregnated at birth, and at 6 days old the few basal dendrites present extended but a negligible distance from the perikaryon. For this reason useful quantitative data are available only from the age of 12 days onwards.

Table 1. *Changes in pattern of dendritic fields formed by basal dendrites of pyramidal cells in the lamina ganglionaris from birth to maturity*

Property	Age					
	6 days	12 days	18 days	24 days	30 days	Adult
(1) Mean number of dendrites arising from perikaryon	0·6	5·4	5·2	5·3	5·3	5·2
(2) Mean number of branching sites	0·0	4·7	8·3	18·0	19·4	23·1
3) Mean number of endings	0·6	10·1	13·5	23·3	24·7	28·3
4) Branching index, i.e. ratio of (1) to (3)	1·0	1·9	2·6	4·4	4·7	5·5

Table 1 shows that the expansion of the dendritic field is characterized by two processes: an increase in the number of dendrites arising from the perikaryon and the peripheral branching of existing dendrites. The first of these is complete by the 12th day of age, at which time the number of primary basal dendrites has reached that characteristic of the adult. On the other hand, there is a steady increase in the amount of branching; the period of most rapid increment, as shown by changes in the branching index, occurs between the ages of 18 and 24 days, further increase in the dendritic field until maturity being relatively small.

The mode of distribution of dendrites at successive stages of development is illustrated by Fig. 7A, B, which shows the distribution of the points at which dendrites branch and end in relation to the perikaryon. At 12 days old the main weight of branching occurs between 18 and 36μ from the centre of the perikaryon, and although the amount of branching taking place both at this distance and more peripherally increases steadily with advancing age the position of the peak remains unchanged. On the other hand, changes in the length of dendrites are shown by a shift of the peak of endings from the second zone (18–36μ) in the case of the 12-day-old rat to about the 6th zone (90–108μ) in the 30-day-old rat. Little change seems to occur in this respect between 30 days and maturity, but growth during this period is manifest by an increase in the number of dendrites which extend beyond the zone in which ending is maximal. This increase in the incidence of very long dendrites is further illustrated by the numbers which extend beyond the 180μ limit of measurement. Such dendrites, which were first observed in the 24-day-old rat, have risen in number to a mean of 2·5 per cell in the adult.

The density of the dendritic field at increasing distance from the cell body is demonstrated by the families of curves shown in Figs. 7C and 8. In the former, the maximum density in the 12-day-old rat is seen to occur as close as 18μ to the perikaryon and thence to decay rapidly. At later ages, however, not only is there an

overall increase in density at successive ages, but an initial predominance of branching over ending causes a rise in density to a peak which tends to move outwards as the animal grows older. In Fig. 8 the same data are expressed, as first described by Sholl (1953), in terms of the densities of dendrites emerging from the surfaces of a series of concentrically arranged spheres whose radii differ by

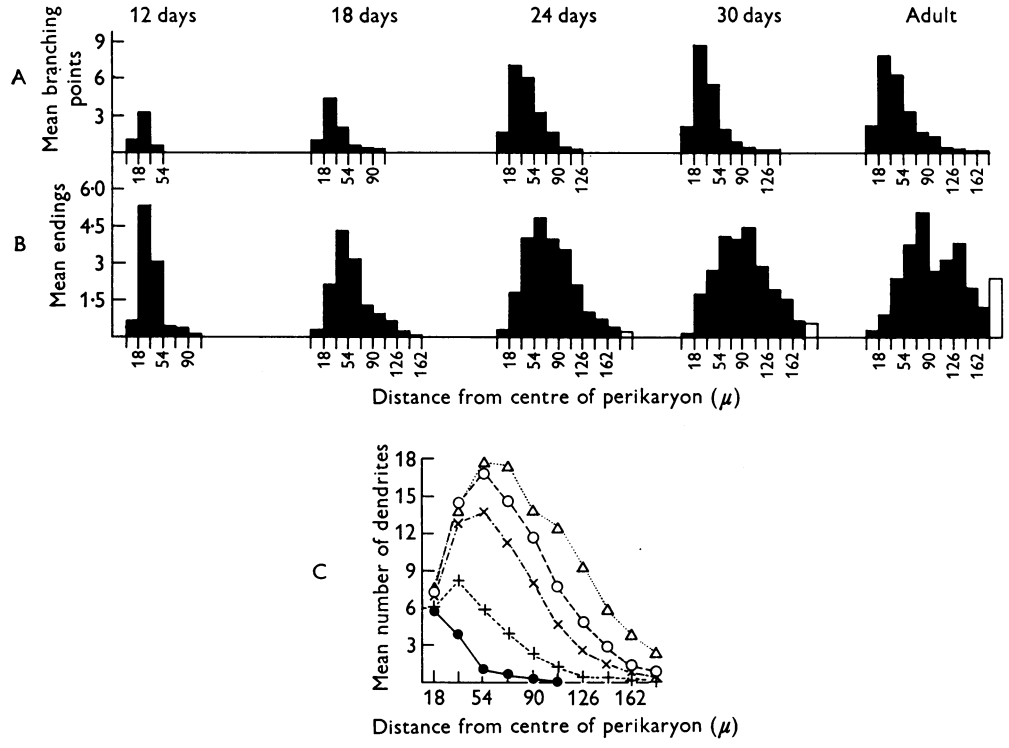

Fig. 7. Distribution of branching (A) and ending (B) points of basal dendrites in relation to the centre of the perikaryon. The white areas of the histogram for the distribution of endings show the increasing number of dendrites extending beyond the 180 μ limit of estimation in the older animals. C gives the resultant dendritic densities at successive distances from the perikaryon. Key: 12 days old, ●————●; 18 days, +---+; 24 days, ×—·—·—×; 30 days, ○— — —○; adult, △·····△.

a distance of 18 μ. The obviously good fit of the calculated regression lines to the data shows that an exponential relationship of dendritic density and distance from the perikaryon is preserved throughout the course of development, the expansion of the dendritic field being expressed by a reduction in the size of the regression coefficient with advancing age.

DISCUSSION

Although there have been several previous accounts of the changes which occur during the maturation of the cerebral cortex (e.g. Paton, 1900; Ramón y Cajal, 1911; Sugita, 1917, 1918*a–c*; Tilney, 1933; Peters & Flexner, 1950) the present qualitative and quantitative findings, by emphasizing certain aspects of develop-

ment not touched upon by previous investigators, provide a more complete picture of the sequence of events than has so far been presented.

It is clear that, in the rat, only an elementary stage of differentiation has been reached at birth: the laminar arrangement of cells seen in the adult has not yet been attained, the perikarya are closely packed and few processes are present. Three phases: (i) the growth of axons, (ii) the growth of dendrites, and (iii) an increase in the spacing of perikarya, characterize the subsequent development of the neuropil but, while these overlap and are clearly inter-related, they do not coincide.

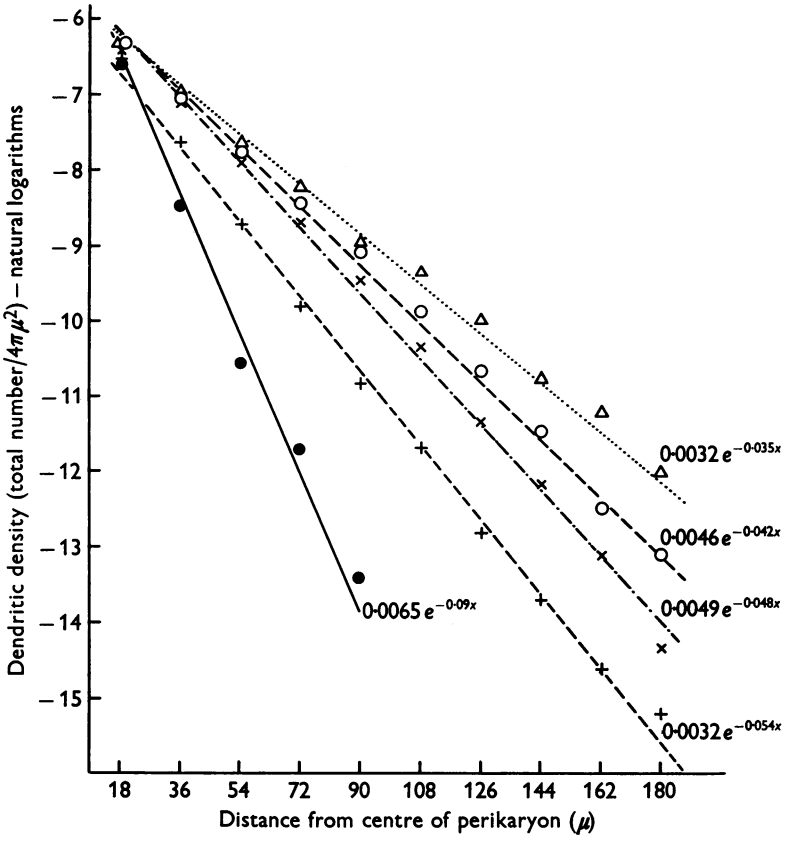

Fig. 8. Decay in density of dendritic fields with distance from the perikaryon at successive ages, together with parameters estimated for each regression. Key as for Fig. 7C.

The period of greatest reduction in cell density occurs during the first 6 postnatal days, in spite of the fact that during this period the total number of cells present is increasing as a result of mitotic activity (Allen, 1912) and the growth of cell processes is minimal. Thereafter, a markedly smaller rate of decrease in packing density is associated with the growth of cell processes, the period of most rapid increment in the density of axons (6–18 days) preceding that for the dendrites (18–24 days). Somewhat similar findings have been recorded by Peters & Flexner (1950), who showed that a major increase in the spacing of perikarya occurred between the 30th and 40th day of gestation in the guinea-pig, although even at the end of this

period very few cell processes could be identified by phase-contrast microscopy. It would thus appear that, judged by these criteria, the development of the cerebral cortex of the rat at birth has reached a stage similar to that of the guinea-pig at a gestation age of 30 days, and thereafter follows an essentially similar course.

These observations imply that the spacing of perikarya is not, as at first seemed likely, governed primarily by the growth of neuropil, but by the development of some histologically amorphous medium within which neural processes subsequently grow, branch and mature, and which they eventually come in part, at least, to replace. Several additional pieces of evidence support this view. First, in the deeper regions of the cortex there is actually a fall in the density of axons during the first 6 days of life, suggesting that the growth of the interstitial medium has exceeded that of the axons. Secondly, the size of the factor used to adjust the density of axons within the cortex as a whole to represent that within the amorphous background decreases very slowly at a time when cell processes are proliferating rapidly (Fig. 4) whereas, were the growth of processes the major cause of a reduced packing density of perikarya, it might be expected that the dimension of this factor would decrease in proportion to the increase in the density of cell processes. Finally, were the interstitial medium to remain constant in volume, the increased spacing of perikarya being solely due to proliferation of cell processes, then the density (as opposed to the total number) of axons in the neuropil would tend to remain constant, or even fall as axons thicken and myelinate. This, in the light of the results presented, is clearly not the case.

The nature of the interstitial medium within which the cell processes proliferate remains open to conjecture. The possibility that this is provided by neuroglial material cannot be excluded, but while no specific studies have been carried out to determine the amount of such material present, estimates of the volumes of such neuroglial elements as could be identified in silver-impregnated specimens gave no reason to suppose that a hyperplasia of this component could account for a decreasing cell/grey coefficient. There is, on the other hand, some evidence to suggest that the medium is extracellular, for in the guinea-pig there occurs, between the 30th and 40th days of gestation, a marked increase in chloride space (Flexner & Flexner, 1949) which coincides with an increase in the spacing of perikarya. Moreover, the extracellular space steadily decreases in volume during the time when cell processes are proliferating. These events are associated with a progressive increase in the intensity of staining by the periodic acid–Schiff reaction (Goodhead, 1957) which may therefore be interpreted as indicating an increasing concentration of the ground substance as processes grow within it.

These observations call into question the functional significance of the cell/grey coefficient. Nissl (1898), on the basis of studies made on the mole, dog, and man, first observed that cells were more closely packed in lower species than in higher, thus drawing attention to the possible importance of the neuropil separating the perikarya. This view was subsequently further developed by von Economo (1926) who, pointing to the greater possibility of neuronal inter-connexions in brains where cells were widely spaced, proposed that the cell/grey coefficient might be used as an index of the degree of organization, and hence presumably of functional capacity, of the cerebral cortex. This concept, whose significance has been discussed

by von Bonin (1948*b*), has been widely accepted. The present results show that the coefficient can be applied as an ontogenetic index only with caution, for although it shows a steady decrease from birth to maturity, this decrease now appears to be due to factors other than the growth of neuropil, while its major portion precedes the period of maximal dendritic growth. Furthermore, the value of the coefficient as a phylogenetic index is equally open to doubt and is in any case vitiated by inadequate and inconsistent data. The present measurements for the rat, although designed to provide a basis for comparison between ages rather than absolute criteria, conform almost exactly with the earlier estimates of Eayrs & Taylor (1951), for the 24-day-old rat and are of the same order as, though slightly smaller than, those of Peters & Flexner (1950) who used the statistical sampling method of Chalkley (1943) in their study of the foetal guinea-pig. The mean cell/grey coefficient found for the adult rat (9 %) is, as might be expected, larger than that proposed by von Economo for man (3·7 %), which, in the light of Agduhr's (1941) criticism, must be regarded as being on the high side. Von Bonin (1952) and Shariff (1953), on the other hand, using similar methods, derived cell/grey coefficients for eulaminate cortex of 21 % for man and 36 % for *Tarsius*, while Sholl (1953) has computed a value of 25 % for the striate cortex of the cat, all these estimates far exceeding those obtained for both rat and guinea-pig and the earlier and more widely accepted data for man. Such inconsistencies presumably have their origin in such variables as the mode of preparation of the tissues, thickness of section, the region of cortex examined and, particularly where the method of Chalkley (1943) is used to measure the coefficient, in the depth of focus of the optical system and the subjective judgement of various observers as to what is, or is not, in focus. Until such factors are adequately standardized there can be little value in attempting to use the cell/grey coefficient as a comparative index of cerebral organization (for further critical discussion see Haug, 1956).

A more valuable criterion of functional capacity, though technically more laborious to acquire, would appear to be the probability of interaction between axons and dendrites within the neuropil. Sholl (1956) has recently drawn attention to the importance of the quantitative aspects of cortical organization in relation to function and has used concepts such as those of Cragg & Temperley (1954), Beurle (1954) and Sholl & Uttley (1953), which are based on the apparently statistical arrangement of connexions within the neuropil, to stress the view that the excitation of neurones may be governed more by the probability of their interaction under varying conditions than through the medium of precisely organized circuits. Such probabilities will clearly depend, among other things, on the densities of both the axons and dendrites, and since throughout the course of development the density of the basilar dendritic field associated with each pyramidal neurone decays exponentially with distance from the centre of the perikaryon it is possible, by applying the formulae of Uttley (1955), to derive the changes in probability of connexion between neurones which occur during cortical maturation in the rat. Approximate values obtained in this way are plotted in Fig. 9, and it is perhaps of interest to note that the placing reflex, which depends on the integrity of the region of cortex studied (Brooks, 1933) can first be elicited at about 17 days of age when the probability of interaction may be estimated, by interpolation, at 100

functional 'contacts' per neurone. The maturation of behaviour is retarded in the hypothyroid individual (Eayrs & Lishman, 1955), and it may be significant that at the time when the placing reaction first appears (24 days) the factor of connectivity, derived from the data of Eayrs (1955), proves to be of the same order (95 connexions) as in the normal. Such observations indicate a useful field of study, but clearly insufficient work has so far been undertaken to assess the value of the correlations between anatomically determined connectivity and behavioural capacity.

Fig. 9. Changes in estimated connectivity resulting from the maturation of neuropil.

The data provided by present experiment do, however, serve to throw further light on the effects of thyroid deficiency on cortical maturation. Earlier, it has been shown that in the 24-day-old hypothyroid rat the growth of the axon network in layer 4 is more severely retarded than in other layers and that the decay of the dendritic field departs from the normal exponential pattern. The form of the curves showing the mode of increase in axon density in the several laminae (Fig. 6) and the fact that the dendritic field decays exponentially throughout development provide no evidence to suggest that the cortex of the hypothyroid rat resembles that of a younger animal. It must therefore be inferred that the changes arising as a result of thyroid deficiency represent a distortion rather than a retardation of growth.

SUMMARY

1. A qualitative and quantitative study has been made, using Nissl, silver and Golgi–Cox preparations, of the changes which take place in the sensorimotor cortex of the rat during the course of development.

2. At birth the neurones are very closely packed throughout the cortical thickness, and individual laminae cannot readily be distinguished. An increase in cell size and spacing is accompanied by laminar differentiation, layers 3 and 4 becoming distinguishable at 6 days old and layer 5*a* at 12 days.

3. Few axons are present at birth, and during the early stages of development these run a predominantly tangential course. Bundles of radially orientated axons, presumably specific thalamic afferents, do not appear until between 12 and 18 days of age. Very few dendrites appear until after the 6th day of life, but after the 12th day growth and multiplication by branching are rapid. By the 18th day cortical structure has attained its adult characteristics and thereafter changes are quantitative rather than qualitative.

4. The periods of most rapid change in the cell/grey coefficient, and in the density of axons and dendrites do not coincide. The cell/grey coefficient decreases most rapidly between birth and 6 days old; the density of axons increases maximally between the ages of 6 and 18 days and that of dendrites between 18 and 24 days.

5. Changes in axonal density in the several cortical laminae do not coincide. Increments in layers 3, 4, 5*b* and 6*a* follow much the same time course, but the density of axons in layer 1 reaches a maximum at about 12 days old and thereafter decreases, while that in layer 5*a* is delayed by comparison with the remainder.

6. The mean number of dendrites arising from the perikaryon has reached the adult figure as early as the 12th day of age. Subsequent development of the dendritic field is marked by a peripheral extension of dendrites and by an increased amount of branching. Branching remains maximal at a distance of 18–36 μ from the centre of the perikaryon throughout the course of development. On the other hand, the number of dendritic endings reaches a peak which lies between 18 and 36 μ in the 12-day-old rat and is steadily shifted away from the perikaryon to a distance of about 108 μ in the adult. The density of the dendritic field preserves an exponential pattern of decay throughout the course of its development.

7. These findings are discussed from a functional point of view and with reference to the somewhat different pattern of development which occurs in hypothyroidism.

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