A QUANTITATIVE STUDY OF THE POSTNATAL CHANGES IN THE PACKING DENSITY OF THE NEURONS IN THE VISUAL CORTEX OF THE MOUSE

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INTRODUCTION

Previous studies on the ontogenetic development of the cerebral cortex have shown that the increase in the volume of the cortex as age advances is accompanied by the spacing out of cortical neurons (Vignal, 1888; Isenschmid, 1911; Sugita, 1918*a*, *b*; Conel, 1939, 1941, 1947, 1951; Peters & Flexner, 1950). The purpose of this paper is to study this phenomenon quantitatively in the visual cortex of the mouse.

MATERIAL

Breeding

The animals used in this study were descendants of C57 black mice obtained from the Department of Genetics, University College, London, through the kindness of Dr H. Grüneberg. For breeding purposes several adult males and females from different litters were left together in one cage; the pregnant females were subsequently isolated.

Determination of age

The isolated females were inspected for litters in the morning and the late afternoon. The animals were considered newly born when they were first found. This implies that the real age of the animal may be up to 16 hr. more than the age attributed to it in this work.

Determination of the visual area

Several investigators have produced cytoarchitectonic maps for the mouse cortex (Isenschmid, 1911; de Vries, 1912; Fortuyn, 1914; Rose, 1929). All except Isenschmid identified a visual area on the posterior part of the lateral aspect of each hemisphere. These investigators agree with Brodmann's localization of the visual cortex in his cytoarchitectonic map for the rodents (1909). Studies on the brain of rat by Lashley (1934) and Waller (1934) support the conclusions based on cytoarchitectonic studies.

METHODS

Histological technique

The animals were anaesthetized with ether and perfused with saline followed by Bouin's fluid. Fixation was completed in Bouin. The brains were embedded in paraffin and cut at 10 and 15μ; the sections were then stained with gallocyanin.

Twenty-five counts on the visual cortex were made from eleven animals representing five different ages. Estimates were made of the density of neurons and of the dimensions of the cell bodies. These were used to calculate (*a*) the mean volume of the perikarya at different ages, and (*b*) the actual number of neurons in a known volume of tissue. These numbers were determined from the crude counts by the method developed by Abercrombie (1946).

Counting

The numbers of perikarya and perikaryal fragments of the neurons in rectangular strips of cortex between the pia and the white matter were counted under a binocular microscope, using a $\times 40$ objective and $\times 10$ eyepieces. The counts of the 3-day-old mouse were made with an oil-immersion objective. Care was taken to ensure that the long axis of each strip was parallel to the pial surface. The counts were made by means of a grid in one of the eyepieces of the microscope.

Since the molecular layer contains very few nerve cells, this study has concentrated on the submolecular region of the cortex. It is necessary therefore to define clearly the boundary lines limiting the submolecular cortex on the counts.

Boundaries of the submolecular cortex

When the outermost line of the ocular grid was orientated along the pial surface, the lowest row of squares of the grid covering the cortex was often only partially occupied by cells; consequently, some approximation was necessary in order to define the lower boundary of the cortex. In the 3-day-old animal the approximation was made to one row, i.e. if the cells occupied half or more than half of the row, this was considered as a complete row, and if, on the other hand, the cells occupied less than half of the row the number of cells was added to the total found in the previous row. In animals 7 days old or older the approximation was to half a row, since the thickness of the row in these cases was nearly twice that found in the 3-day-old stage.

In cases where the molecular layer occupied half or more than half the thickness of a row that was partly in the submolecular cortex, this row was considered to lie wholly in the molecular layer. But if the molecular layer occupied less than half the thickness of that row, it was considered to fall wholly in the submolecular cortex.

Measuring the dimensions of the cell bodies

Two measurements were taken for each perikaryon: the maximum length (l) along the long axis, and the maximum breadth (d) perpendicular to the long axis. For each age studied at least eighty cells were measured. The thickness of the cells, i.e. the dimension perpendicular to the plane of the section, was considered to be equal to the breadth (d) of the cell.

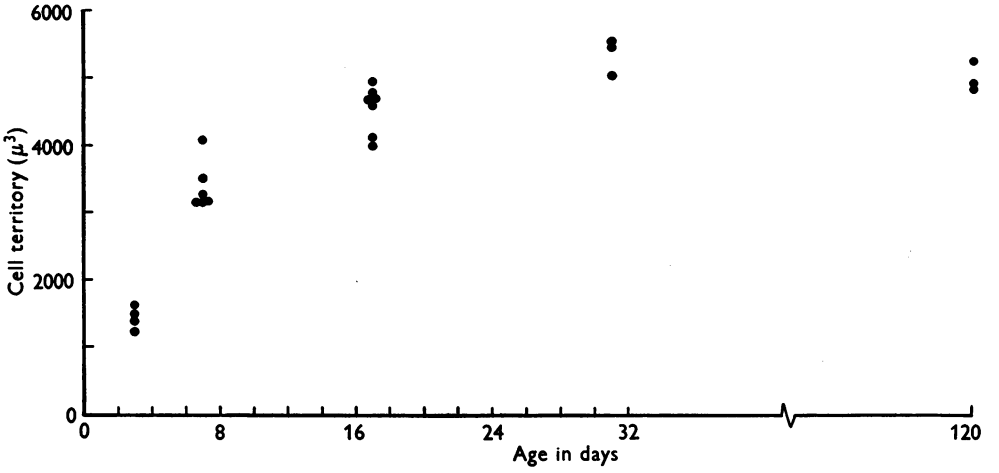
The mean measurements, $\bar{l}$ and $\bar{d}$, were calculated. These must be less than the true mean cellular dimensions, because one cannot differentiate whole perikarya from fragments. However, this error is not great, and was estimated by Abercrombie (1946) as being of the order of 6%.

These means were used to estimate:

(*a*) The mean volume of perikarya at various ages, by treating the perikaryon as a spheroid, using the formula $\frac{4}{3}\pi ab^2$, where $a=\frac{1}{2}\bar{l}$ and $b=\frac{1}{2}\bar{d}$.

(*b*) The actual number of cells in a particular cortical strip, correcting by Abercrombie's method.

The series of photographs in Plate 1 show five developmental stages of the visual cortex of the mouse in postnatal life, stained with gallocyanin. Examination of this series shows that with increasing age the cortex becomes broader and the perikarya of the neurons increase in size, becoming less densely packed. Three cortical zones (apart from the molecular layer) can be distinguished in all stages: a superficial zone of densely packed cells, a sparsely populated middle zone, a deep zone of more densely packed cells. The contrast in density between the superficial and the deep zones decreases gradually with age.



Text-fig. 1. The changes in the volume of cell territory with increasing age.

Changes in the packing density of cortical neurons during postnatal development

A. *Increase of cell territory with age*

The cell territory (S) for a particular cortical 'strip' is defined as the ratio of the total cortical volume in μ^3 to the number of whole neurons it contains. The greater the spacing between the cortical neurons the greater would be the cell territory in that strip.

B. *The growth of the cell territory*

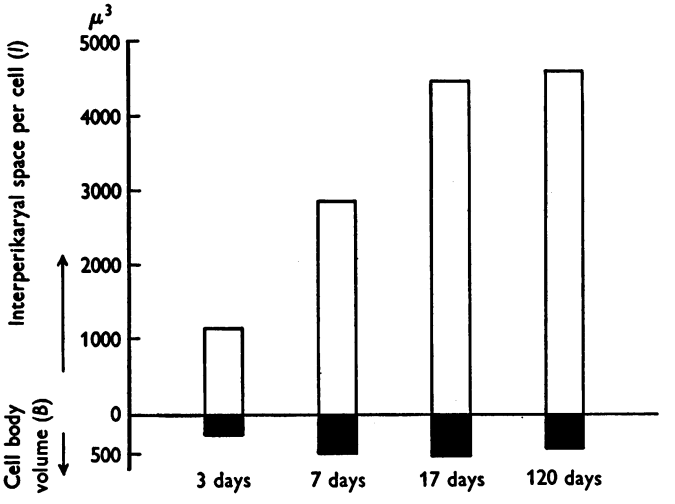
The cell territories (S) for twenty-five counts for four developmental stages in postnatal life were plotted against age (Text-fig. 1). The curve rises steeply between the age of 2 and 7 days, then becomes less steep between 7 and 17 days. After this stage it continues almost parallel with the time axis. This means that sizes of the spaces between the nerve cells of the cortex increase rapidly from 3 to 7 days, more slowly between 7 and 17 days, and almost cease to change after that time.

Table 1. *Components of the cell territory of the neurons in the cerebral cortex of the mouse*

Age (days)	Mean volume of the perikarya (μ^3)	Mean cell territory (μ^3)	Interperikaryal space per cell (μ^3)
3	281	1497	1216
7	535	3375	2840
17	576	5058	4482
120	467	5068	4601

C. *Components of the cell territory*

The cell territory as defined above contains the perikaryon together with a volume of interperikaryal space, the cortical tissue between the perikarya of the nerve cells as seen in preparations stained by gallocyanin. Table 1 gives the mean volumes of the perikarya and the mean cell territories found at different ages. In Text-fig. 2 the



Text-fig. 2. The changes in the components of the cell territory with increasing age.

length of each column represents the mean cell territory at a particular stage of development (based on all the counts for that age). The length of the black part of the column is proportional to the mean volume of the perikarya, while the length of the unshaded part of the column is proportional to the mean volume of the interperikaryal space per cell, at that particular stage of development. It is clear that the increase in cell territory that occurs in the growing cortex is mainly the result of the increase in interperikaryal space.

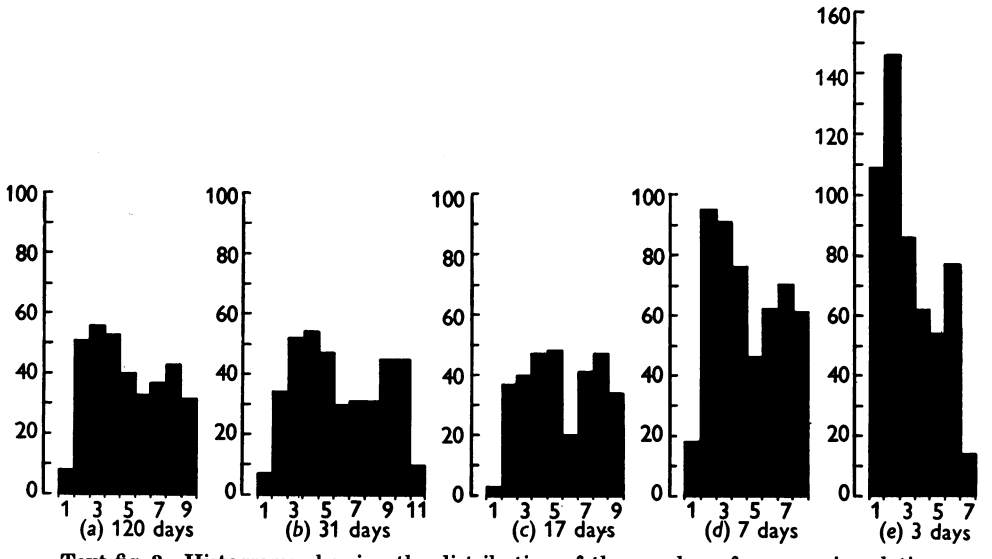
In the adult the interperikaryal space per cell forms about 90% of the cell territory; in other words, the cortical tissue between the perikarya forms 90% of the volume of cortex considered.

D. *Variation in the packing density with depth*

The photographs in Pl. 1 show that the packing density of the cells is not the same at all depths of the cortex at any of the ages studied. The series of histograms

in Text-fig. 3 illustrates the change in the number of neurons with depth in the visual cortex of mice in five developmental stages. Histograms *a*, *b*, *c* and *d* each represent a strip of cortex 150μ wide, and histogram *e*, which belongs to a 3-day-old stage, represents a cortical strip 140μ wide.

The number of neurons counted is shown vertically, and each horizontal unit is equivalent to an interval of 75μ in histograms *a*, *b*, *c* and *d*, and to an interval of 70μ in histogram *e*. The pial surface was taken as the origin.



Text-fig. 3. Histograms showing the distribution of the number of neurons in relation to their depth within the cortex.

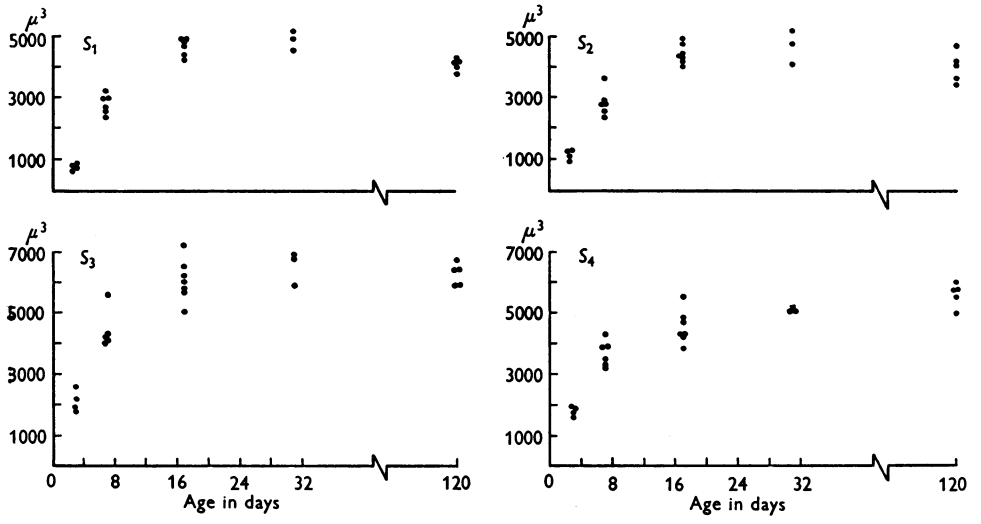
All five histograms show two peaks, separated by a trough. The peaks are high in the 3-day stage, but gradually become lower as age advances, and after the 17-day stage there is little change in their height. The difference between the first and the second peaks is greatest in the 3-day mice, but this difference in height decreases with age.

Briefly, the spaces between the neurons of the cortex increase with the age, the amount of the increase being related to the depth in the cortex at which the neurons are situated. This variation may be examined more precisely by comparing the cell territories at corresponding depths throughout the whole series.

The submolecular region of each cortical strip was divided into four zones for this purpose in such a manner that each zone contained 25% of the total number of cells present in the cortical strip. The cell territories in each of the four zones of each strip were calculated. These were termed S_1, S_2, S_3 and S_4, from the surface inwards.

Text-fig. 4 shows the result of plotting S_1, S_2, S_3 and S_4 against age. The patterns of the plotted points closely resemble those describing the average increase in cell territory throughout the total depth (Text-fig. 1). It may be concluded that in all four zones of the cortex there is a marked increase in the cell territory in the first 2 weeks of postnatal life. This increase is greatest in the first week and almost ceases after the seventeenth day.

The increase in the cell territory from 3 to 120 days is almost the same in the four zones and ranges from 4000 to 5000μ^3, but the extent of the cell territory at the earliest age studied varied between the different zones, being 800μ^3 in the outermost zone and 2000μ^3 in the third zone. The rates of increase in cell territory are consequently higher in the outer zones. The deeper zones show a greater volume of cell territory earlier in the life of the animal; this may indicate an earlier maturation in the deeper parts of the cortex.



Text-fig. 4. The changes in the magnitude of the cell territory at different depths of the cortex with increasing age.

DISCUSSION

The changes that take place in the organization of the cerebral cortex during development have been studied by many workers. The histological changes have been studied by Bolton (1903), Cajal (1911) and Lorente de Nó (1933), to name only a few investigations. Other workers have studied changes in the electrical activity of the growing cortex, for example, Lindsley (1936), Bishop (1950) and Hunt & Goldring (1951). The recent studies from Prof. Flexner's laboratory (Peters & Flexner, 1950; Flexner & Flexner, 1948; Flexner, Tyler & Gallant, 1950) have investigated the correlated histological, electrical and biochemical changes in the growing cortex of the guinea-pig.

The present work is a quantitative study of the changes in the packing density of the perikarya of the neurons in the developing visual cortex of mice. With each neuron is associated a 'cell territory', that is, an average measure of the volume of cortex apportioned to the perikarya of each neuron. It comprises the volume of the perikaryon itself, together with 'interperikaryal space'.

The packing density of the neurons decreases rapidly in the first week after birth, and then slows down until no change is found after the seventeenth day. The corresponding increase in cell territory is mainly due to an increase in the interperikaryal space. This increase in interperikaryal space presumably results from the growth of

the axons and dendrites of the cortical neurons and the ramifications of axons from the white matter (Stefanowska, 1898; Cajal, 1911; Lorente de Nó, 1933).

Bolton (1903) and Watson (1907), who studied the development of the cortex in man and small mammals qualitatively, divided the cortex into supragranular, granular and infragranular layers. They concluded that the infragranular layer became mature before the supragranular layer. A similar result was found in foetal pigs by Flexner, Flexner & Straus (1940), who noted that increased spacing of the neurons first appears in the deeper part of the cortex. The present work not only quantifies these results but shows that the magnitude of this increase at different depths of the cortex is independent of depth, although the increase in cell territory takes place earlier in the deeper parts of the cortex.

SUMMARY

1. The postnatal changes in the neuronal organization of the visual cortex of the mouse were studied in preparations stained with gallocyanin.

2. The packing density of the neurons decreases rapidly between the third and seventh days after birth and then more slowly, no change taking place after the seventeenth day.

3. The cell territory of a neuron was defined as the ratio between the volume of cortex considered and the number of neurons contained in that volume. The cell territory defined in this way comprises the volume occupied by the perikaryon and the interperikaryal space.

4. The greater part of the decrease in packing density of the neurons is due to the increase of the interperikaryal space. The increase in the size of the perikarya is only responsible for a small part of the change in cell territory.

5. The total increase in cell territory is the same at all depths of the cortex, but this increase occurs earlier in the deeper parts of the cortex.

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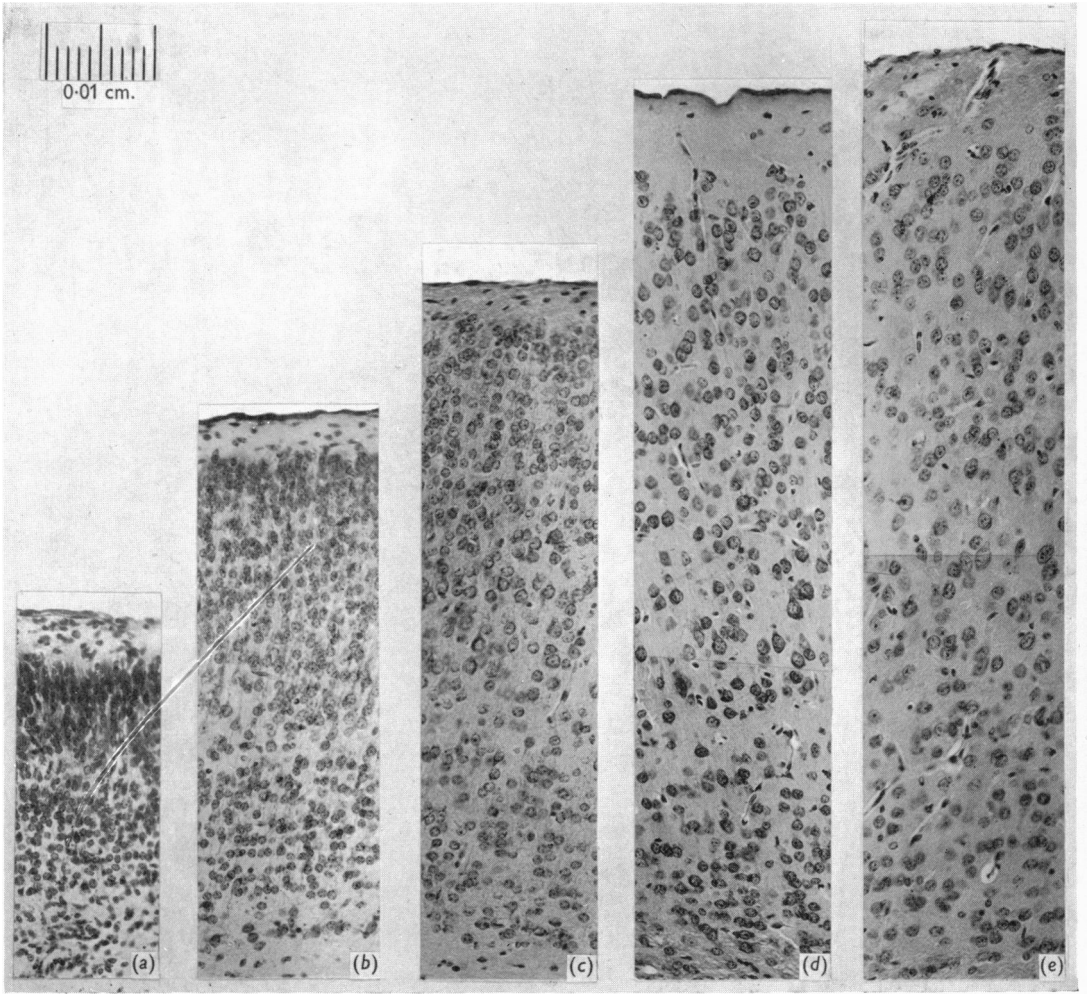

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EXPLANATION OF PLATE

Sections of the visual cortex of the mouse at different ages. Gallocyanin stain. (*a*) Newly born; (*b*) 3 days old; (*c*) 7 days old; (*d*) 17 days old; (*e*) 31 days old.