A COMPARATIVE STUDY OF THE NEURONAL PACKING DENSITY IN THE CEREBRAL CORTEX

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The differences that can be seen microscopically between stained preparations of various parts of the mammalian cerebral cortex have been studied for over a hundred years. Many attempts have been made to assess these differences and to separate those considered to be biologically insignificant from those thought to be essential to cortical organization. Far reaching generalizations have been built on the qualitative study of Nissl stained sections in which only the perikarya of the neurons are stained. These generalizations were severely criticized in an important paper by Bok (1929) and generally condemned by Lashley & Clark (1946).

The present work is a quantitative investigation of samples made under standard conditions from different well-defined anatomical regions of the cerebral cortex in several mammals. Replicated counts of the perikarya in the same section and in different sections of the same and other brains have been made.

It has been possible to measure the variations in neuronal density between counts on the same brain and between counts on different brains, and to look for an underlying pattern. An attempt has also been made to find a quantitative measure of the degree of similarity between the various cortical regions that have been studied.

MATERIALS AND METHODS

Cat and mouse brains were fixed by perfusing the anaesthetized animals with 10 % neutral formol saline (40 % neutral formaldehyde solution 10 ml., normal saline 90 ml.) after a preliminary perfusion with normal saline. The neutral formaldehyde solution was prepared by shaking the commercial solution with magnesium carbonate and allowing the mixture to stand for a few days. The brains were removed and kept in large volumes of the fixative which was changed whenever a yellowish tinge was shown by the bromo-thymol blue used as an indicator. One human brain was from a 50-year-old man who died from coronary thrombosis; this was perfused through both internal carotids 2 hr. after death. The details of a second human brain were taken from a careful study made by van Alphen (1945); this brain was also from an adult male of unstated age but was fixed in a formol-alcohol mixture.

Blocks about 3 mm. thick were cut from suitable parts of the cortex, dehydrated, cleared in toluene, infiltrated overnight at 37° C. in a mixture of paraffin wax (m.p. 52° C.) with sufficient benzole to remain just molten in a 37° C. oven. The blocks were then transferred to clean wax and finally embedded in 52° C. wax. Sections were cut with thicknesses varying between 10 and 20 μ and stained with 0·5 % aqueous cresyl violet.

Portions of these sections where the pial surface appeared to be plane were selected for examination. The thickness of each section was measured by means of

a calibrated fine adjustment and an oil-immersion objective, focusing first on the under surface and then on the upper surface of the section. These measurements were made at six randomly chosen spots on each section and the mean value of the six differences was assumed to be the thickness of the section under study. Little variation was found between the six values for any section when they were measured in this way but they sometimes differed by as much as 2 μ from the microtome setting. The depth of the cortex (pial surface to grey/white boundary) of the selected portion of the section was measured with an eyepiece micrometer that had been calibrated against a stage micrometer.

The number of perikarya in a unit volume of cortex may be computed from counts of nucleoli or from counts of perikarya and pieces of perikarya with the application of the Abercrombie (1946) correction. Either method leads to substantially the same result. The counts were made using an eyepiece graticule that enables the image of the section to be divided into a number of strips of known width, length and thickness, each strip being parallel to the pial surface. It is convenient to make the base of the outermost neuron-free layer the outer boundary of the outermost strip. The packing densities (number of perikarya/unit volume of cortex) can then be computed. In the present study the unit volume of cortex was taken as 0·001 mm.^{3} (10^6 μ^3).

It is, however, very difficult to make comparisons between the packing densities found for different samples of cortex since the thickness of the cortex, even under standard conditions with a plane pial surface, varies from region to region in the same brain and consequently the total number of strips surveyed may vary from sample to sample. In order to overcome this complication each sample of cortex, excluding the outermost, neuron-free, zone, has been divided into ten strips and the neuronal density computed from the raw data for each of these strips. This figure will be a measure of the neuronal packing density at the 'relative depth' of the midline of this strip. An example will make this process clear. Imagine that the packing density at different depths of a sample has been found by enumerating the nucleoli in twelve strips of cortex each 200 μ thick. The total thickness of cortex, excluding the outermost layer, is 2400 μ and we wish to find the mean density in each of ten strips 240 μ wide. Then the density at relative depth 0·1 will be the mean density of a strip centred at 120 μ, that at relative depth 0·2 will be the mean density of a strip centred at 240 μ and so forth. These new densities are easily obtained from those found for the observed 200 μ strips by a simple weighting of observations taken from adjoining strips. No corrections for shrinking, which is presumably of the order 20–25 %, have been made.

Details of the samples used in this study are given in Table 1. These figures do not include the repeated counts that were made on each sample. The absolute figures obtained for such repeated counts did not differ from each other by more than an occasional difference of the order of 2 units in a count of 50. The regions chosen from the cortex are recognizable on anatomical grounds and the reasons for selection are discussed on pp. 154–5.

In this way a number of replicated observations on the neuronal packing density in corresponding parts of the cortex of different adult animals were available for study. A method was then required for making a quantitative assessment of the

total difference with respect to neuronal packing at varying depths between these different regions in the various animals. From a consideration of the means and correlation coefficients subsisting between these variables, statistics known as the Generalized Distances (Mahalanobis, 1936) were calculated. Details of the computations may be found in Rao (1952). The characteristics of this statistical function and its biological significance will be considered later.

Table 1. *Details of sampling*

Animal and cortical region	Numbers of brains studied	Numbers of samples counted	
Man (pre-central gyrus)	2	13	Data for 1 brain from van Aphen
Man (striate area)	2	16	
Man (parastriate area)	2	6	
Cat (anterior sigmoid gyrus)	2	7	
Cat (post-lateral gyrus)	2	10	
Mouse (visual area)	2	5	Data from Haddara

RESULTS

Fig. 1 is a plot of points each representing the neuronal density found at various cortical depths in different sections of the visual cortex from two human brains. The densities are shown with black and open circles to distinguish the observations made from the two brains. This diagram is typical of the wide variation that is found whenever the counts are replicated. It will be noticed that the densities at any given depth show a considerable overlap between the two brains and from any single set of counts it would be impossible to distinguish between the two brains. Various methods of analysing these data are available, and results of using some of these methods will be described.

The total number of neurons in a cylinder of cortex

One may suspect that part of the variation in density may be due to the somewhat arbitrary divisions of the cortex which have to be made in order to study the possible changes in density with depth. If this were a major factor then the total number of neurons contained in a column of cortex under a pial surface of fixed area would be similar in various parts of the same cortical area. Table 2 gives the results of an investigation of this kind for the numbers of neurons in a cortical cylinder bounded by a pial surface with an area of 400 μ^2. It will be noticed that the range of the observations, rather than the standard deviation or confidence limits, has been given in the next column. This is because the distribution of the total number of neurons in such a column for any one cortical region is often so far from normality that confidence limits calculated in the usual way would be most misleading. Table 2 shows that not only do the mean number of neurons in these cortical columns for different cortical areas differ, but that, for the samples of human cortex considered, their ranges do not overlap. It may be noted that columns in the areas of cat cortex studied that are presumably comparable in function with areas of human cortex have neuronal populations of approximately the same sizes and within similar ranges. The neurons in the columns of the mouse visual cortex are few when compared

with any of the other animals. The last column of Table 2 gives the mean values of the total cortical thickness (including the outermost layer) and the standard deviation of these means. These figures call for little comment except to note that the mean value for the cortex of the human precentral gyrus from these samples is low compared with the usually accepted figure of about 4 mm.

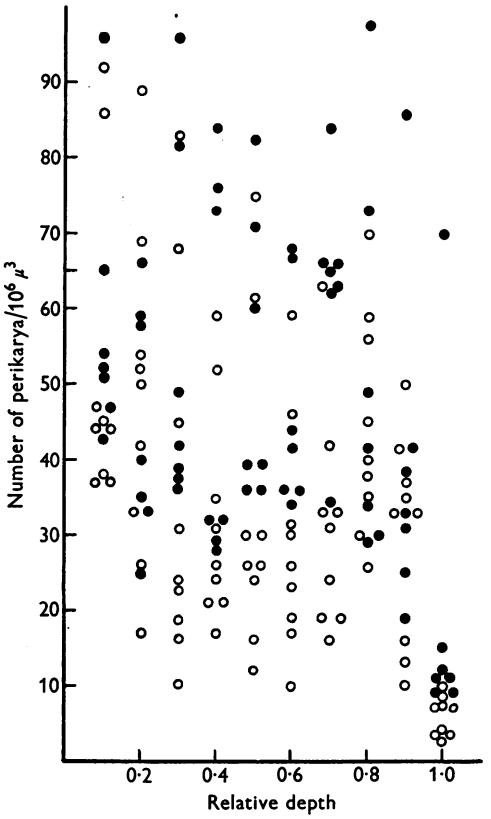

Fig. 1. The variation in neuronal density at different depths of the visual cortex in sections from two human brains. The measurements from the two brains are distinguished by black and open circles.

Table 2. *Numbers of neurons contained in a cortical cylinder with cross-sectional area 400 μ^2 in different regions*

Animal	Cortical region	Abbreviation	Mean number of neurons in column	Range	Mean total cortical thickness and its standard deviation
Man	Precentral gyrus	HM	23	21–25	2160 (130)
	Striate area	HV	60	47–69	1930 (150)
	Parastriate	HP	32	28–45	1780 (50)
Cat	Sigmoid gyrus	CSM	29	25–36	1790 (40)
	Post-lateral gyrus	CV	55	33–68	1520 (60)
Mouse	Visual	MV	14	13–16	680 (10)

The average neuronal densities at different depths of cortex

The publication of the whole of the original data is impracticable and they have been summarized in Table 3 which shows the mean packing density of perikarya per 10^6 μ^3 at different cortical depths, together with the standard deviation of these means. Generally speaking the coefficient of variation is about 10 %, but is very much smaller in parts of the human precentral cortex and in the mouse cortex. These values can be compared more easily if the mean values and their confidence limits are plotted as in Figs. 2–7. In these diagrams the mean values are shown as circles and 95 % confidence limits are marked off with bars. The extent of the limits implies that in repeated sampling the mean will lie within these limits in nineteen cases out of twenty if the distribution within the population from which the samples

Table 3. *General summary of neuronal densities at increasing depths of the cortex*

Type of cortex	No. of counts	Mean packing density of perikarya/10^6 μ^3 of cortex in terms of relative depth with corresponding standard deviation of mean									
		0·1	S.D.	0·2	S.D.	0·3	S.D.	0·4	S.D.	0·5	S.D.
Human visual (HV)	16	54·9	4·9	46·8	4·7	43·9	6·4	40·6	6·1	41·5	5·4
Human precentral (HM)	13	48·0	5·4	27·7	1·0	26·9	1·3	24·9	3·3	27·4	3·6
Human parastriate (HP)	6	76·7	8·4	66·3	5·0	55·2	5·4	43·5	3·1	56·3	8·2
Cat visual (CV)	7	124·0	11·5	95·9	6·6	72·5	4·9	78·2	10·8	76·0	4·8
Cat cruciate (CSM)	10	92·0	7·7	54·8	4·6	44·1	4·5	38·7	3·0	32·7	3·7
Mouse visual (MV)	5	86·0	3·3	81·8	0·7	77·6	3·3	78·6	4·1	52·6	3·5
		0·6	S.D.	0·7	S.D.	0·8	S.D.	0·9	S.D.	1·0	S.D.
Human visual (HV)	16	36·8	4·6	45·0	5·0	47·0	4·9	33·9	4·5	11·7	4·0
Human precentral (HM)	13	31·6	5·2	24·6	2·8	22·1	2·1	22·9	2·2	16·9	1·8
Human parastriate (HP)	6	60·0	10·1	50·2	8·1	40·3	4·5	27·7	3·3	8·7	1·9
Cat visual (CV)	7	84·2	6·5	88·7	11·8	71·2	8·9	56·9	12·5	25·9	2·7
Cat cruciate (CSM)	10	29·6	4·9	34·8	6·0	30·8	3·0	24·1	5·0	17·4	2·2
Mouse visual (MV)	5	48·4	2·1	65·2	2·4	60·6	3·9	61·2	1·3	39·2	5·8

have been drawn is normal. The wide boundaries of these limits are a measure of the hazard and difficulty involved in making general statements about the similarities and differences between different regions of cortex on the ground of the qualitative study of a few sections. In any case these limits must be treated with considerable reserve.

With this caution in mind, the mean values of the neuronal densities for the different animals may be plotted. These are shown in Figs. 8 and 9. Fig. 8 shows the mean values found for the three regions of the human cortex, Fig. 9 those for the visual cortices of the animals studied. Examination of these diagrams shows that although there may be considerable differences between the neuronal densities at any one depth of the cortex, there is a striking similarity in the forms of the graphs. Fig. 8 shows that at first the neuronal density is high and then decreases and, then, with a further increase in depth, increases to a new maximum and finally becomes very much smaller as the boundary between the grey matter and the white is reached. The similarity of the changes in neuronal density is especially clear when the parastriate (HP) and the precentral (HM) graphs are compared; if the HM graph

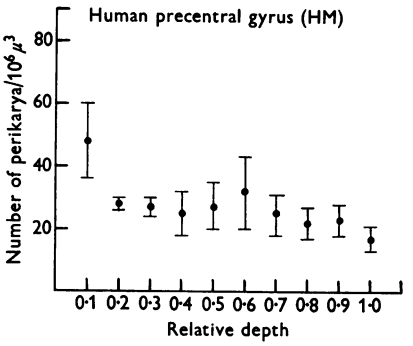

Fig. 2

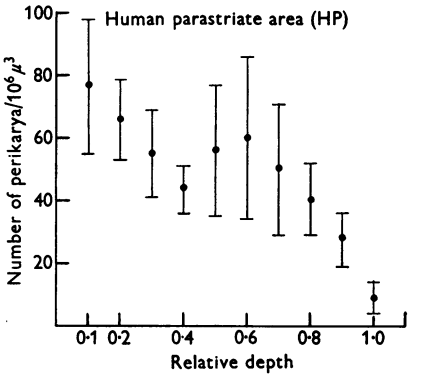

Fig. 3

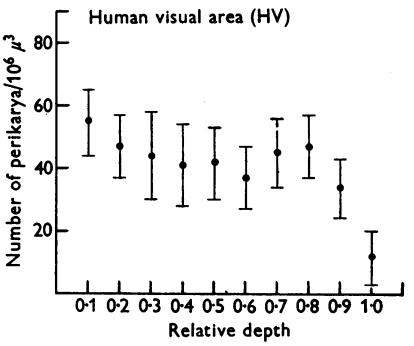

Fig. 4

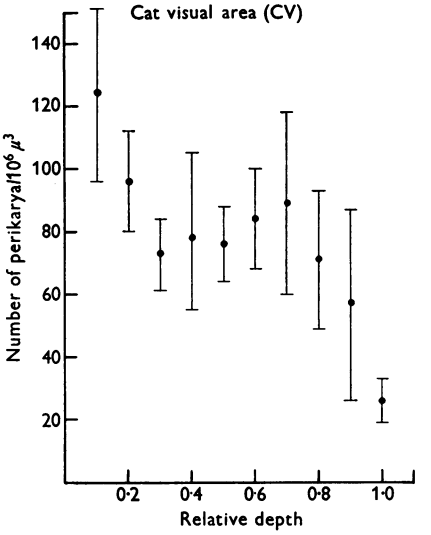

Fig. 5

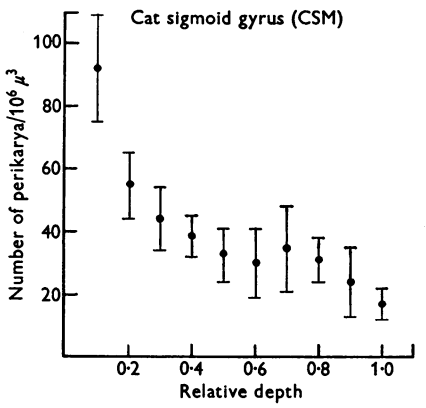

Fig. 6

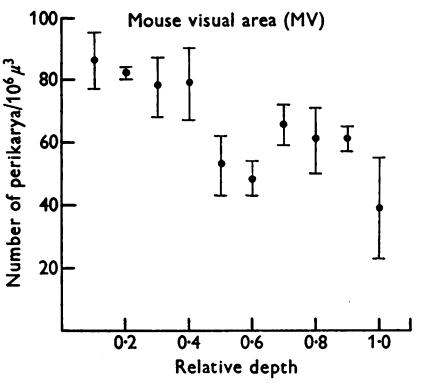

Fig. 7

Figs. 2–7. Mean values of the neuronal densities at different cortical depths with 95 % confidence limits for the various cortical regions studied.

were moved upward it would almost coincide with the HP graph. The small maximum shown at the relative depth 0·5 in the visual (HV) graph cannot be distinguished statistically from its neighbours but represents the aggregate of neurons which are assigned to various layers by the students of cyto-architectonics (to layer V by Campbell, Betz, Cajal and Meynert, to layer IV*c* by Brodmann and Economo and Koskinas).

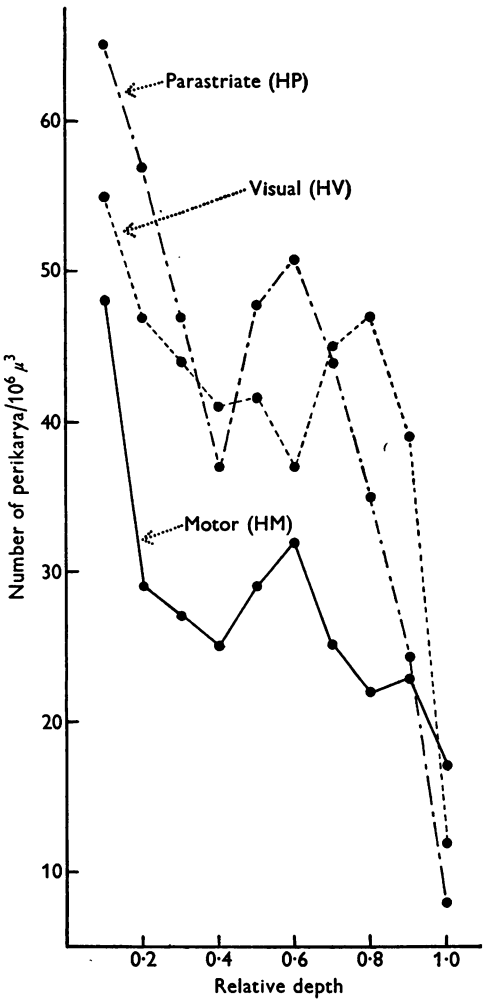

Fig. 8. Mean values of the neuronal densities at different cortical depths for three regions of human cortex.

Fig. 9 also shows the similarities between the patterns of neuronal packing in different visual cortices; again there are consistent differences between the mean densities at any one level but the patterns of density change with depth in the different cortices are similar—the rapid decrease followed by a small maximum at depth 0·4–0·5 with a second maximum deeper in the cortex.

The human precentral cortex (HM) and the cortex of the cat sigmoid gyrus show a single maximum at a depth roughly equal to that of the second maxima in the corresponding visual cortices.

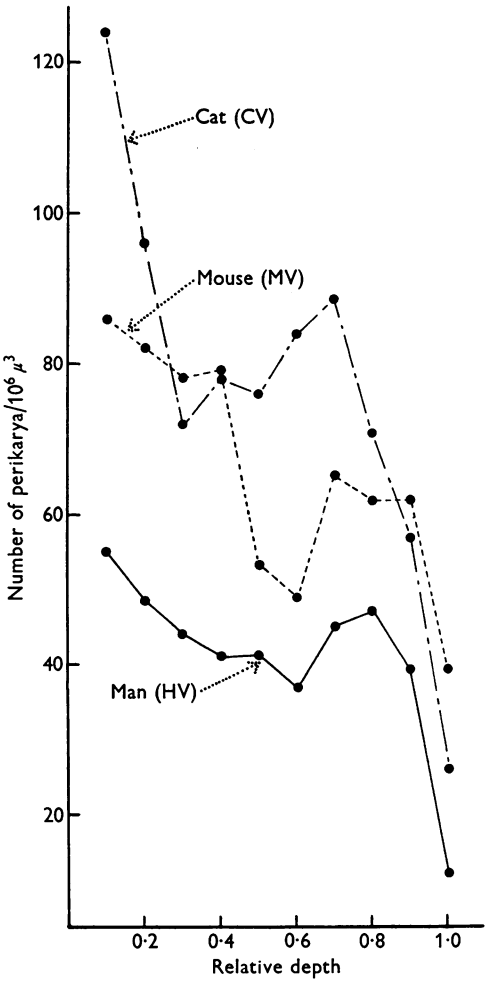

Fig. 9. Mean values of neuronal densities at different cortical depths of the visual cortices of mouse, cat and man.

The correlation between the neuronal densities at different depths of the cortex

The correlation coefficients between the neuronal densities at the various depths were calculated for each set of observations and from these different sets of coefficients the pooled correlation coefficients were computed by the standard process (e.g. Rao, 1952). Instead of quoting the coefficients it seems preferable to illustrate the results by a diagram such as Fig. 10. The relative depths are shown along the top and left-hand side of the figure and the strength of the correlation between the neuronal densities at any two depths is indicated by depth of shading in accordance

with the scheme: correlation coefficient greater than 0·5, heavy dots, between 0·25 and 0·5, small dots, 0·25 and less, left blank. It is immediately noticeable that the density at any depth is highly correlated with that at neighbouring depths: this is to be expected because there is no reason to think that the division of the cortex into ten layers of equal thickness has any biological significance and density measurements in such neighbouring layers might well be expected to be similar. One fact of interest emerges from this diagram; the densities in the lowermost quarter of the cortex are not significantly correlated with those in the outer part.

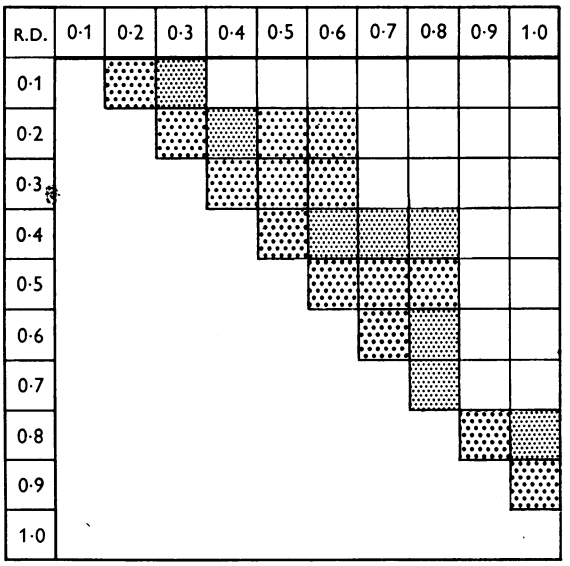

Fig. 10. Diagram to illustrate the correlation between neuronal densities at different depths. The degree of stippling of any square denotes the extent of the correlation between the densities in the two marginal 'relative depths' that form the co-ordinates of the square.

The measurement of the degree of similarity between cortices of different origin

A common problem in biological studies arises when one wishes to compare several, possibly different, groups of animals as a result of measurements of certain characters in a number of animals from each of the various groups. The choice of the characters to be measured can only be decided by the insight of the biologist; the assessment of the degree of similarity between the groups as a consequence of these measurements is a purely statistical problem.

It would be convenient if, on the basis of the measurements, a measure could be allotted to each pair of the supposed groups, which would have the properties of a 'distance'; the greater the 'distance' the more dissimilar the groups. Such a distance would have to possess certain properties which have been stated by Rao (1952) in the following way:

(*a*) The distance between two groups is not less than zero.

(*b*) The sum of the distance of a group from two other groups is not less than the distance between the other groups (triangle law of distance).

(*c*) The distance must not decrease when additional characters are considered.

(*d*) The increase in distance by the addition of some characters to a suitably chosen set must be relatively small so that the group constellations arrived at on the basis of the chosen set are not distorted when additional characters are considered.

The first two of these properties are mathematical and the last two are empirical in the sense that without them, the 'distance' might have little biological relevance. A suitable function of the observational measurements satisfying the four postulates was discovered by the Indian statistician Mahalonobis (1936), and is known as the Mahalonobis Generalized Distance. This function and the very lengthy computational procedure is fully described in the book by Rao (1952).

Table 4. A. *Distances when neuronal packing densities only are considered* (10 *variables*)

Distance from												
HV to		HM to		HP to		CV to		CSM to		MV to		
HP	16·6	HP	24·2	HV	16·6	CSM	28·8	HV	17·9	HV	33·3	
CSM	17·9	HV	27·6	HM	24·2	HV	32·5	HP	26·2	CSM	34·5	
HM	27·6	CSM	38·0	CSM	26·2	HP	33·4	CV	28·8	CV	35·3	
CV	32·5	CV	51·1	CV	33·2	MV	35·3	MV	34·5	HP	40·9	
MV	33·3	MV	52·7	MV	40·9	HM	51·1	HM	38·0	HM	52·7	

B. *Distances when absolute cortical thickness is also considered* (11 *variables*)

Distance from												
HV to		HM to		HP to		CV to		CSM to		MV to		
CSM	18·3	HP	24·9	HV	24·7	CSM	34·5	HV	18·3	HP	47·2	
HP	24·7	HV	30·2	HM	24·9	HV	35·8	HP	29·8	CSM	51·1	
HM	30·2	CSM	38·2	CSM	29·8	HP	47·0	CV	34·5	HV	54·0	
CV	35·8	CV	57·6	CV	47·0	HM	57·6	HM	38·2	HM	60·4	
MV	54·0	MV	60·4	MV	47·2	MV	66·7	MV	51·1	CV	66·7	

In the present investigation eleven characteristics were measured on each cortical sample—ten measurements of neuronal densities at various depths and the total cortical thickness. The Generalized Distance (D) was computed using the eleven variables and also using ten variables and omitting the cortical thickness in order to see if such an omission made very much difference to the comparisons. The results are shown in Table 4A and B. It is very difficult to visualize the interpretation of these distances and they are shown diagrammatically in Fig. 11, in which each cortical region has been denoted by a small labelled circle. Any two of the regions (e.g. HV and HM) are first plotted with the distance between them drawn to scale. The line joining these two regions is used as the base-line of a set of triangles whose third verticles represent the other cortical regions studied and whose sides are proportional to the distances that are given in Table 3. For example, the lengths of the sides of the triangle HV–HP–HM are proportional to the dissimilarity between these regions and it can be seen that HP is more like HV than like HM.

Nevertheless, this type of diagram is not entirely satisfactory because it represents a three-dimensional pattern as a drawing on a plane and some of the distances will

be distorted. Since any three points in a three-dimensional space are co-planar, the vertices HV–HM–CV are co-planar and so, for example, are the vertices HV–HM–MV but this does not imply that MV and CV are co-planar and almost coincident; Table 4 shows that the distance between them is 35·3 units in a plane roughly perpendicular to the paper. Consequently Figs. 11 and 12 must only be considered as visual aids to the understanding of Table 4.

The left-hand side of Fig. 11 maps some of the distances calculated from ten variables and the right-hand side shows the corresponding distances calculated from eleven variables. The relative positions of the points are little changed in the latter

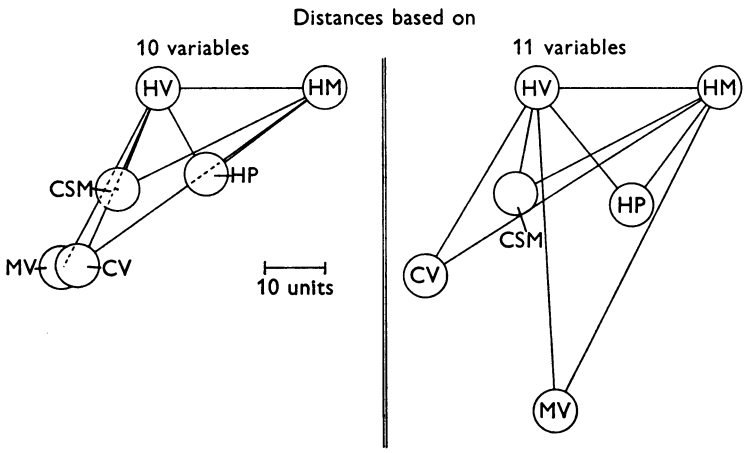

Fig. 11. Diagram to illustrate the relationship between some of the distances given in Table 4.

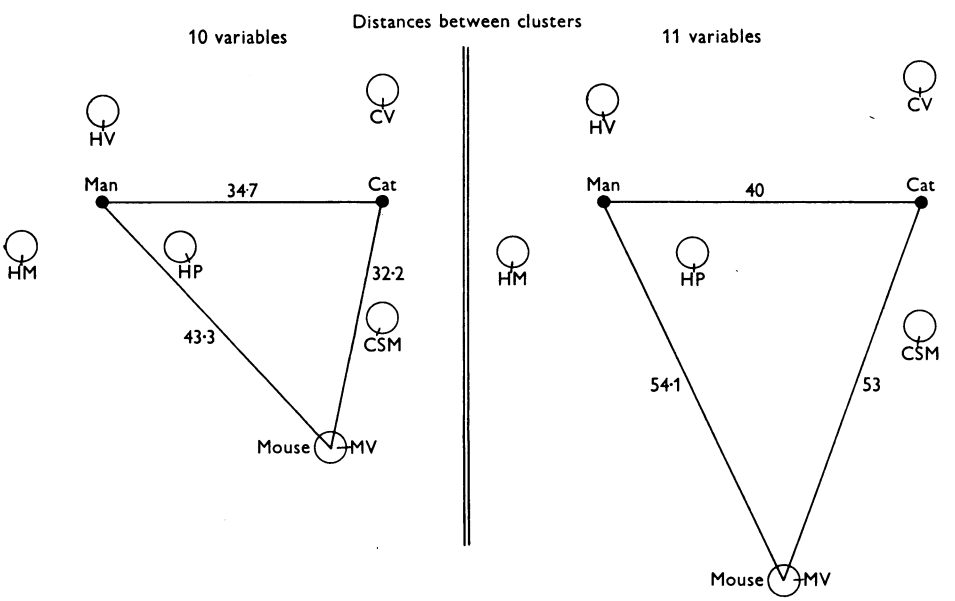

Fig. 12. Diagram to illustrate the concept of cortical 'clusters'.

case but the use of the additional parameter leads to a greater separation between the regions and especially to an increase in the distances of the mouse cortex from the other regions. In a general way it appears that as far as these variables are concerned, samples from different regions of the cortex of the same species are usually more similar to one another than are samples from cortices with similar functions from different species, e.g. HV is more like HM and HP than it is like CV or MV. This suggests that the different cortical regions from a single species form a kind of 'cluster'. This can be illustrated (Fig. 12) by calculating the mean distances between and within the 'clusters'. The general result that the distance between the cortex of man and cat is smaller than that between the cortex of man and mouse is clear and perhaps not at all surprising; but, in addition, an objective measure of this difference has been found. It is perhaps less intuitively obvious that the difference between cat and mouse visual cortex is as great as that between human and mouse visual cortex.

DISCUSSION

The neuron is the basic unit of the nervous system, and any attempt to understand the organization of this system must be interested in the packing density of these units and the patterns of interrelationship that subsist between them. The present study is only concerned with the first of these problems.

The qualitative study of the variations in neuronal density in the cerebral cortex dates back to the time of Meynert and the methods involving attempts to subdivide the cortex into areas of specific pattern with respect to perikaryal densities and sizes have been known as cytoarchitectonics for a number of years. The unsatisfactory results of these attempts were clearly shown by Lashley & Clark (1946) and, indeed, in a subsequent paper, Klotz & Clark (1950) emphasized that the variations between adult brains of the same species were so large that until the criteria for making the subdivisions were placed on an objective basis, such attempts were bound to fail. The fact remained that even an inexperienced observer could see that in many mammals the cortex associated with vision differed from that associated with motor activities. A compromise solution, still qualitative, was made by Bailey & von Bonin (1951) who made a considerable reduction in the number of distinguishable areas but also pointed out that different observers, using criteria thought to be common, did not entirely agree on the subdivisions—a conclusion similar to that of Lashley & Clark.

The only way out of this difficulty is by the use of quantitative methods and objective criteria of comparison. The present study attempts such a method but is still limited by the number of samples that it has been possible to examine. In spite of this limitation it has seemed worthwhile to indicate the kind of conclusion that further studies may justify more adequately.

An adequate study of the kind described in this paper would entail the examination of the total cortex of a number of animals of each species. At the present time such a study is impracticable and one has to select regions for study. Such a choice is not easy and the regions examined in the paper have been chosen because they are localizable by gross anatomical criteria and have some kind of well-defined activity in the living animal. The position of the cortex that receives afferent fibres from the

lateral geniculate body and is primarily associated with vision in man and cat is well-known and undisputed. One is a little less certain about the position of the visual cortex in mouse but the survey given by Haddara (1955) makes it reasonably certain that the correct region has been studied. The human parastriate region is easy to identify. If sufficiently large blocks of the occipital pole of man are sectioned in order to study the visual cortex around the calcarine fissure, the parastriate cortex is found on the sections by the sharp change in neuronal pattern that takes place at its boundary with the striate area.

Experiments with primates towards the end of the last century appeared to show that there was a sharply defined region of the cortex along the precentral gyrus that was concerned with the production of nerve impulses running to 'voluntary' muscles; this region became known as the 'motor area' and its circumscribed nature seemed to be confirmed by histological studies. This simple concept was upset by the work of Leyton & Sherrington (1917) on chimpanzees and, more recently, by the studies of Penfield & Jasper (1954) on the unanaesthetized human cortex. It became clear that there was considerable overlap between the predominantly motor function of the pre-central gyrus and the predominantly somato-sensory function of the post-central gyrus.

The situation in the cat is more confused. Some physiologists consider the cortex of the anterior sigmoid gyrus to be purely 'motor' while others consider that the cortex surrounding the cruciate sulcus, anteriorly and posteriorly, is 'sensori-motor'. A study of the literature shows that it is likely that in the lower mammals there is a true sensori-motor cortex and as the phylogenetic scale is ascended parts of the cortex adapted for somato-sensory integration become increasingly separated from parts associated with the activity of 'voluntary' muscles (van Crevel, 1958). This separation is never complete, even in man.

Considerations of the kind described in the last three paragraphs have led to the study of the cortical regions described in Table 1.

The extent of the variations in neuronal packing density in any one region has been illustrated in Fig. 1, which shows that statements based on the observations of a few sections are of little value for making general statements about these densities. Moreover, the variation with depth is not consistent from section to section of the same region of a single brain; one sample may show a higher density at some specified depth than a second sample, but a lower density at other depths. This lack of consistency is even more evident when samples from different animals are compared.

When the number of samples is increased, mean values of the neuronal densities at the different depths can be calculated together with the confidence limits for these means under the assumption of the normality of the distributions concerned.

Graphs showing the change in the mean values of the neuronal densities with depth show that the patterns of change in density are unexpectedly simple. In most of the regions of the cortex that have been studied the outermost neuron free 'layer' is followed by a region having a high neuronal density that decreases more or less sharply with the depth, reaches a minimum, increases again to a maximum and then decreases until the white matter is reached. The pattern is slightly

modified in the visual cortex where a second small maximum appears at a relative depth of about 0·4–0·5.

The reasons for this variation in density are unknown but a low neuronal density implies greater space between the perikarya. These spaces may allow additional room for blood vessels, neuroglia, dendrites and axons. Although no quantitative data are available, the density of dendritic fibres appears to be more or less uniform throughout any given region of cortex in a species with the possible exception of the outermost 'layer' containing the terminal ramifications of the apical dendrites. It is tempting to suggest that regions of lower neuronal density are associated with the presence of an increased number of axons. Such an hypothesis would suggest that the efficient operation of the cortex in higher mammals depends upon the complexity of the axonal pattern of connexions. If neuronal density is significantly related to complexity of axonal connexions, the outer minimum density would be associated with the terminal ramifications of incoming extrinsic afferent fibres and the decrease in density deeper in the cortex with the increasing number of axons leaving the cortex. The additional small maximum in the visual cortex corresponds to the aggregate of stellate neurons known to be associated with Gennari's line (Sholl, 1956; Mitra, 1955).

On the other hand, there is evidence that the neuronal density may be generally low in large animals as, for example, in the whale. Consequently, it cannot be assumed that the low neuronal density is an attribute only correlated with the efficiency of the primate cortex.

The examination of such diagrams as Figs. 8 and 9 may well lead to the conclusion that although the actual neuronal densities are different in various cortical regions, there is a basic pattern which is fundamentally similar in different regions of the cortex of the higher mammals. For example, if the HM curve of Fig. 8 were moved upwards it would approximate to the HP curve and the HV curve of Fig. 9 when moved upwards is not dissimilar to the CV curve. The problem of the interpretation of the differences in mean density between different species remains obscure even when cortical regions that are presumed to have similar functions (Fig. 9) are considered. The low neuronal density of the human visual cortex compared with that of the cat may be accounted for by the hypothesis of increased axonal density in man. The situation in the mouse then appears anomalous for there is no evidence that vision in the mouse is 'more efficient' than in the cat. It is, however, plausible that the general cortical organization in mammals low in the phylogenetic scale differs fundamentally from that in the cat and in primates. The macroscopic appearance of living cortex from mouse, rat and rabbit is much more translucent and it has a less firm consistency than the cortex in cat and the primates. These differences may indicate fundamental differences of organization.

It would be convenient if it were possible to discover reasonably simple mathematical formulae for the description and comparison of graphs such as those in Figs. 8 and 9. Various attempts were made to find such formulae and orthogonal polynomials were fitted to the data from several of the samples. The coefficients of the resulting formulae were very unwieldy and this fact, together with the impossibility of giving any biological interpretation to the polynomials, led to the abandonment of this method.

It is still desirable that some way should be found so that these various samples, each with eleven measured variables, could be compared. This type of problem was considered many years ago by Karl Pearson (Tildesley, 1921), but the Coefficient of Racial Likeness described in this paper has several disadvantages and depends upon the different variables being independent of one another. This condition is seldom fulfilled in biological investigations and is definitely unsatisfied in the present case. A different approach can be made by the use of Fisher's method of discriminant functions, but the method of Mahalanobis (1936) which has been briefly described above, was adopted.

This approach provides an objective method for estimating the relative differences between the various cortices and provides a set of numbers having the properties of Euclidean distances; the greater the difference between cortex A and cortex B, the greater the 'distance' between them. If cortex A is x units from cortex B and y units from cortex C, then the distance of cortex C from cortex B will not be greater than $(x+y)$. It must be borne in mind that the value of statements of this kind depends upon the biological insight of the investigator in the initial choice of adequate and appropriate variables, and it would be unjustifiable to regard the 'distances' given in this study as more than indications of the complete situation. No variable measuring connectivity has been considered and it is almost certain that further work will discover biochemical variations of great significance.

The present investigation nevertheless makes it possible to make certain comparisons objectively. The regions of the human cortex that have been studied are more similar to one another than they are to corresponding regions in the cat, and both human and cat cortices are more different from the mouse cortex than they are from each other. Other comparisons can be easily made from the figures given and these differences can be stated in objective and measurable terms.

The study of these cortical samples and the examinations of the data confirm the point of view (Sholl, 1956) that the cortex cannot be regarded as an organization of neurons with an invariant topographic arrangement of the perikarya. The wide variations in their density of packing suggest that the necessary invariance of pattern must be topological, maintained under changes of cortical thickness and curvature, and to be found in the connectivity pattern subsisting between the neurons and the afferent fibres to the cortex. This pattern can only be specified in statistical terms and by probability laws.

SUMMARY

1. The numbers of neuronal perikarya per unit volume ($10^6\ \mu^3$) of cerebral cortex were found at increasing depths in Nissl-stained sections from selected regions of the brains of man, cat and mouse.

2. Preparations were made from formalin fixed, paraffin embedded material from adult brains of each animal. Comparable counts made by van Alphen (1945) for the human brain and by Haddara (1955) for the mouse brain, were also used.

3. The total numbers of neurons in a cylinder of cortex with cross-sectional area 400 μ^2 were computed. These ranged from about 30–60 in man and cat, the higher number being found in the visual cortices in each case. The number of perikarya in a similar cylinder of mouse cortex was about 14.

4. The mean values of the neuronal densities at relative depths increasing from the pial surface to the boundary of the cortex with the white matter were calculated with the corresponding standard deviations.

5. When the mean densities are plotted against relative depth, a common pattern of density change is found in the non-visual cortices showing a single minimum density at about one-third of the cortical thickness followed by a single maximum at a greater depth.

6. In the visual cortices an additional small maximum is found in the region of the stellate cell concentration at a relative depth of 0·4.

7. The neuronal densities at different cortical depths are only correlated with those at neighbouring depths.

8. The Generalized Distance of Mahalanobis was used in order to obtain a numerical measure of the degrees of similarity between the different cortices. This method showed that for the parameters studied, intraspecific cortices were more similar to one another than to cortices presumed to have similar functional activities but derived from different species.

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REFERENCES

ABERCROMBIE, M. (1946). Estimation of nuclear population from microtome sections. *Anat. Rec.* **94**, 239–247.

ALPHEN, G. W. H. M. van (1945). *Kernmetigen in de Neocortex van de Mens.* Leiden: Eduard Ijdo.

BAILEY, P. & BONIN, G. von (1951). *The Isocortex of Man.* Urbana: University of Illinois.

BOK, S. T. (1929). Der Einfluss der in den Furchen und Windungen auftretenden Krummungen der Grosshirnrinde auf die Rindenarchitektur. *Z. ges. Neurol. Psychiat.* **121**, 682–750.

CREVEL, H. van (1958). *The Rate of Secondary Degeneration in the Central Nervous System.* Leiden: Eduard Ijdo.

HADDARA, M. A. H. (1955). Studies on the postnatal development of the visual cortex of the mouse. Thesis presented for the degree of Ph.D. of the University of London.

KLOTZ, D. A. & CLARK, G. (1950). An attempt at graphic cytoarchitectonic description. *J. comp. Neurol.* **92**, 215–225.

LASHLEY, K. S. & CLARK, G. (1946). The cytoarchitecture of the cerebral cortex of Ateles: a critical examination of architectonic studies. *J. comp. Neurol.* **85**, 223–305.

LEYTON, A. S. F. & SHERRINGTON, C. S. (1917). Observations on the excitable cortex of the chimpanzee, orang-utan and gorilla. *Quart. J. exp. Physiol.* **11**, 135–222.

MAHALANOBIS, P. C. (1936). On the generalized distance in statistics. *Proc. nat. Inst. Sci. India*, **2**, 49–55.

MITRA, N. L. (1955). A quantitative analysis of cell types in mammalian neo-cortex. *J. Anat., Lond.*, **89**, 467–483.

PENFIELD, W. & JASPER, H. (1954). *Epilepsy and the Functional Anatomy of the Human Brain.* London: J. and A. Churchill.

RAO, C. R. (1952). *Advanced Statistical Methods in Biometric Research.* New York: Wiley; London: Chapman & Hall.

SHOLL, D. A. (1956). *The Organization of the Cerebral Cortex.* London and New York: Methuen.

TILDESLEY, M. L. (1921). A first study of the Burmese skull. *Biometrika*, **13**, 176–262.