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THE BASIC UNIFORMITY IN STRUCTURE OF THE NEOCORTEX

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INTRODUCTION

SEVERAL quantitative studies have shown that the cell density may vary in different laminæ and in different areas of the neocortex in the same brain and between different species (*see* Tower, 1954; Brody, 1955; Cragg, 1967). The cells are usually most closely packed in layer IV, the density is high in the visual cortex and low in the motor and in general the neurons are more widely separated in larger brains. In an electron microscopic study of the motor cortex of area 4 and of area 3b of the somatic sensory area of the monkey (Sloper, 1973; Sloper, Hiorns and Powell, 1979) the number of neuronal cell bodies was counted in a narrow width through the full depth of the cortex from the pia to the white matter. Surprisingly it was found that despite the marked difference in the thickness of the cortex of these two areas, and their different cytoarchitecture and function, the absolute number of neurons through the cortex was the same and the proportions of the two main cell types, the pyramidal and stellate, were similar. A comparison has now been made of the number of cells through the entire thickness of the cortex in most of the major structural and functional areas in the monkey and in several other species, ranging from mouse to man. With the exception of area 17 of the visual cortex of primates the figures are similar for the different areas, and despite the marked differences in the size of the brains the absolute number of cells through the thickness of the cortex has been found to be constant in the brains of different animals. The results may be of relevance to our understanding of the evolution of this part of the brain, and perhaps to the question of the anatomical basis of the functional columnar organization which is a feature of many areas of the cortex (Mountcastle, 1957, 1978; Hubel and Wiesel, 1962, 1977). A preliminary communication of the results has already appeared (Rockel, Hiorns and Powell, 1974).

MATERIAL AND METHODS

All of the animals (Table 1) were normal adults and the brains were fixed by perfusion through the heart with 0.9 per cent saline followed by 10 per cent formalin. The two human brains were from 26-year-old males who had been killed suddenly in accidents not involving the brain; these were fixed by immersion in 10 per cent formalin after dividing the right and left cerebral hemispheres within a few hours of death. From the human and macaque monkey brains blocks were cut approximately 10 × 5 mm and sufficiently deep to include the entire cortex to be studied together with some of the underlying white matter; the blocks were cut as perpendicular to the surface as possible. The motor and somatic sensory areas were included in one block across the pre- and post-central gyri and which reached to the depth of the central sulcus. The visual cortex of the macaque monkey was studied in blocks which included areas 17 and 18 on the exposed medial and lateral surfaces of the hemispheres, together with part of area 17 lying in the wall of the buried calcarine sulcus; in addition samples were taken throughout area 17 in a 1:10 series of sections of brains in which the entire occipital lobe had been cut in the coronal plane. The visual cortex of the human brains was taken from the posterior and middle thirds of the calcarine sulcus of both hemispheres. The frontal cortex was probably area 9 according to the map of Brodmann, the parietal that of area 7 and the temporal from area 22 in the superior temporal gyrus. Fig. 1 shows the sites in one macaque brain which was typical of all three.

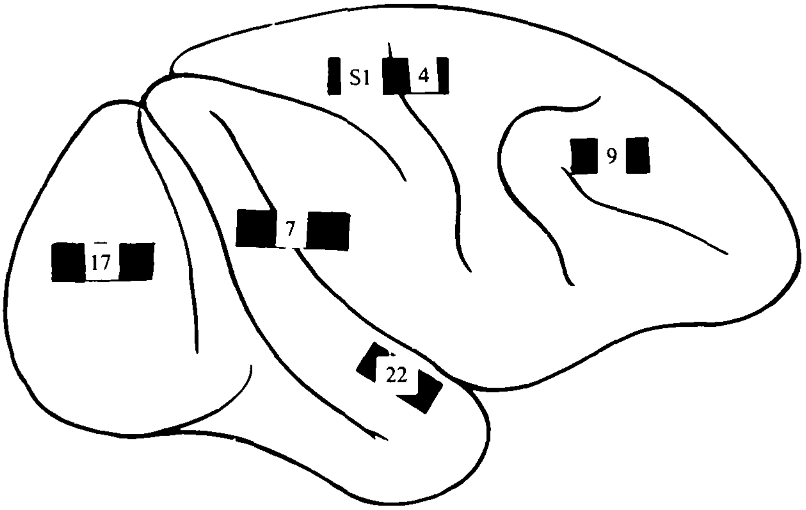


FIG. 1. The sites from which blocks of the neocortex were taken in the macaque monkey.

The cytoarchitectonic features seen in the sections were in accord with the classic descriptions of the respective areas. In the case of the mouse, rat, cat and Tupaia, the entire forebrain was processed as one block. After fixation in formalin the blocks were immersed in 70 per cent alcohol and 2 per cent acetic acid for a few days before being dehydrated and embedded in paraffin wax. Serial sections were cut at 25 μm. The monkey and human blocks were cut perpendicular to the surface of the cortex, with the larger rectangular surface as the cutting edge and the entire forebrain of the smaller animals cut in either the coronal or sagittal planes. A 1:10 series of sections was mounted and stained with thionine. All stages of the histological processing were kept as constant as possible apart from slight differences according to the size of the blocks.

Cell counts of these areas were made using a 10 × eye piece and 100 × oil immersion objective with a ruled graticule in the eyepiece. All neuronal cell somata with distinct nuclei and nucleoli through the thickness of the sections were counted in a width of 30 μm (3 graticule squares) between the pial surface and the white matter. The counts were begun either at the surface or at the level of the white matter and were made in traverses through the cortex as perpendicular to the surface as possible; to do this with a curved surface, an attachment on the moving stage was used by which sections could be rotated independent of the X and Y axes of the moving stage. Thus the absolute number of cells has been counted in a volume of tissue 30 μm (width) by 25 μm (section thickness) through the depth of the cortex.

The cortex of the three macaque monkeys was first sampled and the number of cells in 20 traverses through sections of each area were counted independently by two observers, 40 samples of each area. As there were no significant differences between the mean counts and standard deviations of the two observers the brains of other species were sampled independently, each observer making 10 traverses through each area of one brain of each species.

When it was found that the absolute number of cells in area 17 of the macaque monkey was significantly higher than that in other areas, counts were made in most parts of area 17 of several macaque monkey brains, and blocks of this area were taken from other primates (Table 1) and processed in the same manner. The cortex of the peristriate region, areas 18 and 19, was also sampled in most species.

Although a correction for shrinkage was not required for the interpretation of the results in this study, it was thought of interest to estimate this in the human brain in case the data are used for other studies. One young adult brain, not used for the counts, was obtained unfixed and needles were inserted in the cortex at precisely known distances apart. The brain was bisected and the hemispheres immersed in 10 per cent formalin for several days, and blocks of a similar size and site as those for the counts were taken. The blocks were measured, processed *and cut* (*cf.* Olszewski, 1952, page 12; Phillips, Powell and Shepherd, 1963) in the same way as those for the counts. From measurements of the sections it was found that a linear shrinkage of 18 per cent had occurred due to the fixation and histological processing.

TABLE 1. BRAINS USED FOR SAMPLES OF SIX DIFFERENT CYTOARCHITECTURAL AND FUNCTIONAL AREAS

Species	*No. of brains*	*Areas studied*
Mouse	2	Frontal, motor, somatic sensory, parietal, area 17, temporal
Rat	2	,,
Cat	2	,,
Monkey	3	,,
Man	2	,,

Brains used for quantitative studies of area 17 only: Tupaia 1; Galago 1; Marmoset 1; Squirrel monkey 2; Baboon 1; Chimpanzee 1.

RESULTS

The counts in the different areas of the cortex of the macaque monkey confirmed the initial observations (Sloper, 1973; Sloper *et al.*, 1979) that the absolute number of cells in the same narrow width through the entire thickness of area 4 and of area 3b of the somatic sensory area were the same, and they also showed that the same number of cells was present in the association cortex of the frontal, parietal and temporal areas (Table 2). Within the first somatic sensory area in the post-central gyrus the absolute number of cells through the thickness of the cortex was the same in three of its cytoarchitectural subdivisions, areas 3b, 1 and 2. In the striate cortex of area 17 containing the representation of the central 10 degrees of the visual field, however, the number was approximately 2.5 times greater, and it was quite clear at the time of counting that this was due to a much greater density of cells through most of the depth of the cortex but especially in layers II, III and IV. In order to investigate the differences between the observers in counts of the five areas (excluding the visual area) taken from three monkeys a three factor analysis of variance was performed. Observers and areas were taken as 'fixed effects' factors and monkeys taken as a 'random effects' factor. The results of this analysis indicate no difference between the observers ($F = 0.47$; d.f. = 1,2) or between the areas ($F = 0.36$; d.f. = 2,4). A difference between monkeys is suggested at the 1 per cent significance level ($F = 5.97$; d.f. = 2,570). A further analysis of the visual area for monkeys (excluded from the above) indicates no difference between the monkeys counted by two observers ($F = 0.33$; d.f. = 1,76) but a slight difference at the 5 per cent significance level between the observers ($F = 224.97$; d.f. = 1,1).

TABLE 2. *MACACA MULATTA*. MEAN AND SD OF 40 COUNTS IN EACH CORTICAL AREA

Motor	110.2 ± 9.4
Somato-sensory	109.4 ± 9.4
Frontal	112.0 ± 11.1
Temporal	109.8 ± 10.3
Parietal	114.6 ± 9.9
Striate	267.9 ± 13.7

Further counts of different parts of area 17, including that in the buried walls of the calcarine sulcus, gave the same figures as in the cortex on the lateral surface of the hemisphere. Although it was thought to be important to count the number of cells in the region of representation of the temporal monocular crescent, we were unable to identify it on any qualitative morphological features in the 'stem of the calcarine sulcus' (Wiesel, Habel and Lam, 1974). In the adjoining peristriate cortex, however, the figures were the same as in other areas ($\simeq 110$) and qualitative examination readily shows this difference in the density of the cells in areas 17 and 18 (fig. 2).

For the corresponding areas of the cortex in the brains of other species the counts were all remarkably similar, both within and between species (Table 3). In all species, except other primates, the figures for area 17 were the same as in other areas of the cortex. Within area 17 of the normal and Siamese cat there was no difference between the cortex containing the representation of the binocular and monocular parts of the visual field, nor between these and areas 18 and 19 and of the

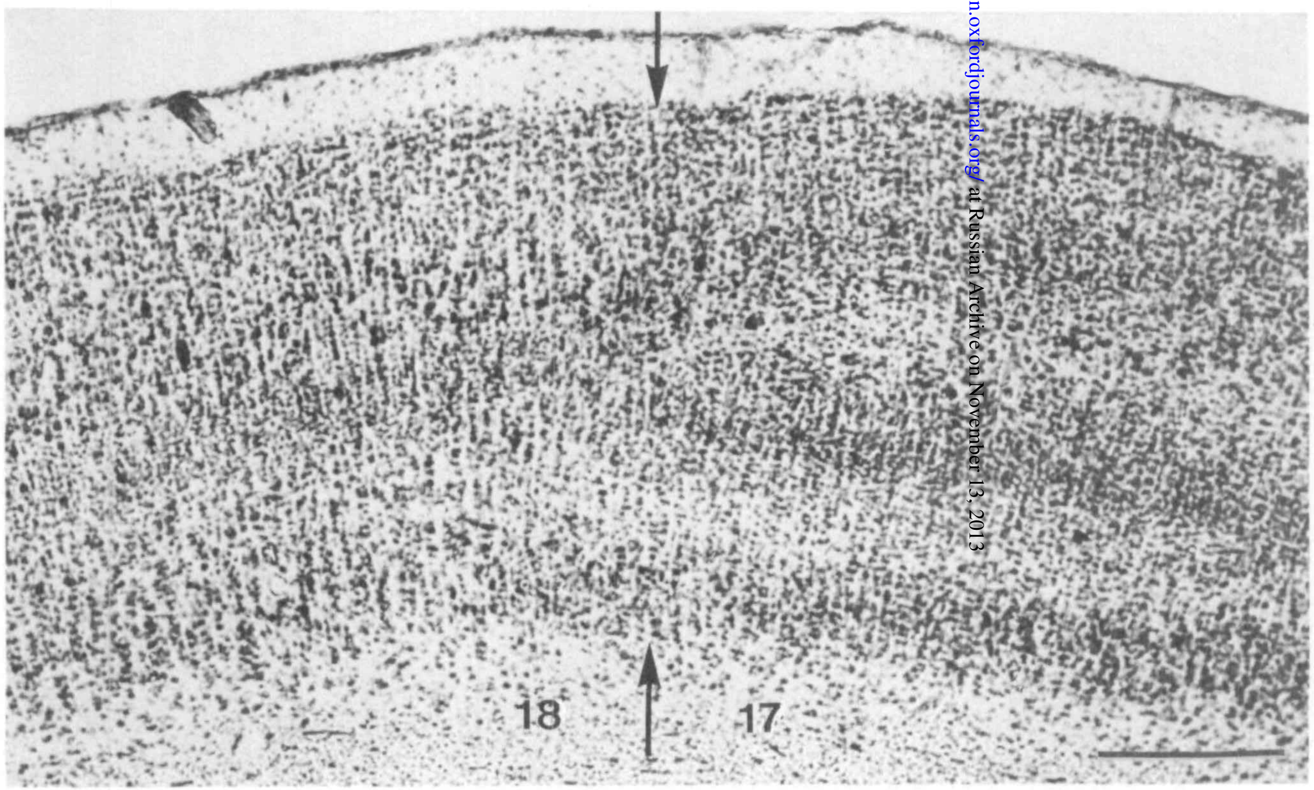


FIG. 2. The boundary between areas 17 and 18 in the macaque monkey; the greater density of neurons in area 17 as compared with area 18 is readily seen. Bar = 0.5 mm.

TABLE 3. COMPARISON OF CELL COUNTS

	Motor	*Somato-sensory*	*Frontal*	*Temporal*	*Parietal*	*Visual*	*Mean of means*
Mouse	109.2 ± 6.7	111.9 ± 6.9	110.8 ± 7.1	110.5 ± 6.5	104.7 ± 7.2	112.2 ± 6.0	109.9 ± 6.8
Rat	108.2 ± 5.8	107.0 ± 6.7	104.3 ± 7.2	107.7 ± 9.2	105.2 ± 6.8	107.8 ± 7.9	106.7 ± 7.4
Cat	103.9 ± 7.6	106.6 ± 7.2	108.0 ± 6.2	113.8 ± 7.3	110.6 ± 7.4	109.8 ± 9.9	108.8 ± 7.7
Monkey	110.2 ± 9.4	109.4 ± 9.4	112.0 ± 11.1	109.8 ± 10.3	114.6 ± 9.9	267.9 ± 13.7	—
Man	102.3 ± 9.5	103.7 ± 5.8	103.3 ± 8.6	107.7 ± 7.5	104.1 ± 12.5	258.9 ± 15.8	—

The mouse, rat, cat and man values are means of two samples of ten counts, one from each of two animals, in each area. The monkey values are based upon three animals, with two samples of twenty counts being made for each animal. The standard deviations shown are obtained from within animal pooled variances.

first auditory area of the cat. An analysis of variance was conducted with the 5 species (mouse, rat, cat, human and monkey) and the 5 areas (motor, somatosensory, frontal, temporal and parietal) being treated as fixed effects and two animals of each of the species being considered as random effects. The results show no significant differences between the species (F = 0.614; d.f. = 4,5) or between the areas (F = 1.75; d.f. = 4,470). A test for the human alone between the areas showed no difference (F = 0.64; d.f. = 4,4). Separate tests between all areas, *including the visual* as well as the five above, within the species indicates no difference in the rat (F = 1.06; d.f. = 5,5) and in the mouse (F = 3.63; d.f. = 5,5) but there is a suggestion of difference in the cat (F = 5.51; d.f. = 5,5; $P < 0.05$). The cells were more densely packed in the cortex of the smaller brains of the mouse and rat, and were more widely dispersed in the larger brains and especially the human. In general the cells were somewhat smaller in the smaller brains than in the larger brains and in some areas, such as area 17, than in the cortex of area 4. In certain areas, for example, the motor cortex of the monkey and the auditory cortex of the cat, it was quite evident during the traverse through the cortex that one was following and counting a definite row or 'column' of cells.

The higher figure for the number of cells in area 17 of the macaque monkey led to a study of this area in certain other primates which were available (Table 4). In all of these examples (except Tupaia) a similar count to that of the macaque was obtained and statistical analysis of them showed no significant difference. In testing for difference between these primates, as only one animal of each species was used, the between animal variability was estimated from the three macaque monkeys. The between primates difference was not significant when compared to this assumed between animal variability (F = 1.47; d.f. = 5,8). The peristriate cortex of these animals had, like the macaque, the lower figure of approximately 110, the same as in other areas. One finding of interest was a distinct difference in the binocular and monocular parts of area 17 of Tupaia, where it was possible to sample them because they have been identified with anatomical and physiological techniques (Kaas, Hall, Killackey and Diamond, 1972). In the monocular region on the medial and callosal surfaces of the hemisphere it was the same as in other functional areas of all species, but in the binocular part on the dorsal surface of the hemisphere the number was higher and about midway between that in area 17 of the other primates and that in the other functional areas. This finding led to the comparison of the monocular and binocular parts of area 17 of the cat and attempts to identify the monocular area in the macaque monkey. The counts of area 17 of the human brain were checked repeatedly and further blocks taken.

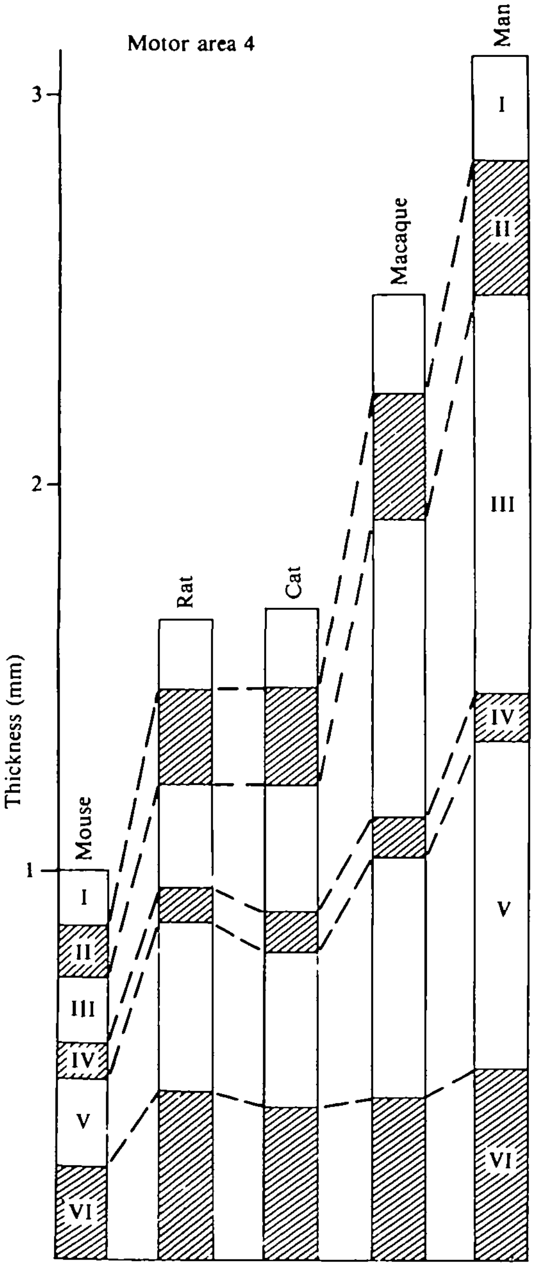
TABLE 4. MEAN AND SD OF COUNTS IN AREA 17 OF VISUAL CORTEX

Mouse	112.2 ± 6.0
Rat	107.8 ± 7.9
Cat	109.8 ± 9.9
Tupaia, Monocular	109.6 ± 9.19
Binocular	192.8 ± 8.40
Galago	277.40 ± 8.40
Marmoset	277.00 ± 9.65
Squirrel monkey	273.60 ± 13.16
Macaque	267.9 ± 13.7
Baboon	268.4 ± 7.2
Chimpanzee	279.00 ± 5.66
Man	258.9 ± 15.8

The thickness of the cortex and of the individual laminæ of certain functional areas in several brains (in the same sections as were used for the cell counts) were drawn with a drawing apparatus and a comparison of these (figs. 3-6) indicates the relatively small degree of difference in the cortex as a whole between mouse and man. In the motor and parietal cortex the increase occurs mainly in the supra- and infra-granular layers and in area 17 in layers III and IV. Within the non-human primates the thickness of area 17 is essentially the same in brains which varied in size from Tupaia to chimpanzee, but layer IV progressively thickens in the larger brains.

DISCUSSION

The major finding of this quantitative study of the neocortex has been the consistency of the absolute number of nerve cells in a strip of constant width through the entire thickness of the cortex from the pial surface to the white matter in several quite distinct structural and functional areas, both within the brain of one species and also in the brains of animals as widely different in size as mouse and man. The only exception to this similarity is the binocular part of area 17 of the visual cortex in a number of primate brains, in which there are approximately 2.5 times as many neurons. It would seem that in evolution the *number* of cells in the thickness of the neocortex, and thus underlying unit area of cortical surface, of the mammalian brain has remained constant, except in area 17 of certain primates where it has increased considerably. A width of 30 μm within which to count the cells was chosen partly because of the evidence suggesting that this is approximately the width of the simplest functional column in the neocortex (Welt, Aschoff, Kameda and Brooks, 1967; Hubel and Wiesel, 1974), and the number of cells found in it would therefore represent one quantitative feature of the anatomical basis of the



FIGS. 3–5. The change in thickness of the cortex in the motor, parietal and visual areas between mouse and man.

functional column. As well as being the functional unit of the cortex the findings suggest that the column may also be a morphological and developmental unit. The thickness of the neocortex does vary between different areas within the same brain and between corresponding areas of different brains, and in the latter case it is due to variations in thickness of certain laminæ, the particular laminæ involved depending upon the function and connections of the cortical area.

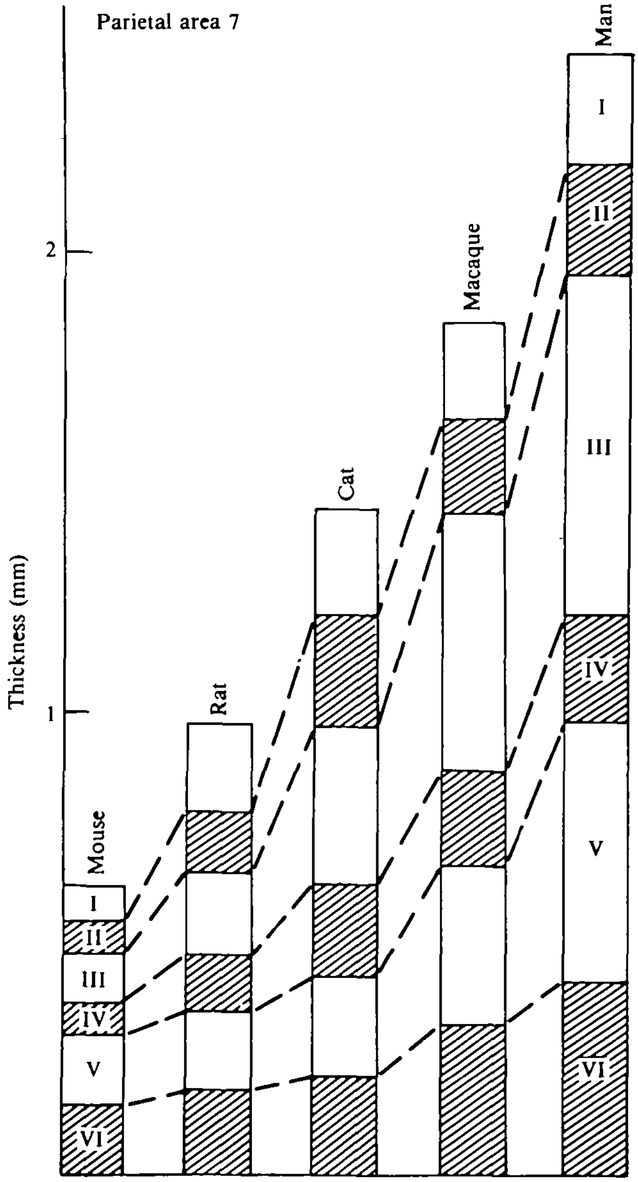


Fig. 4

The number of cells in the cortex of different areas and brains may indeed be identical, and certainly the size of the standard error for the mean number over the whole sample (except for area 17 in primates) would not contradict this hypothesis. The slight variations which have been found could have been due to technical factors such as the occasional inclusion of a glial cell, omission of some nerve cells in lightly stained sections, some blocks not being cut strictly perpendicular to the surface, or the sampling traverse not passing perpendicularly; the boundary between grey and white matter is not sharp in some areas so all the cells may not have been included. With one exception, area 17 of the human brain, counts of

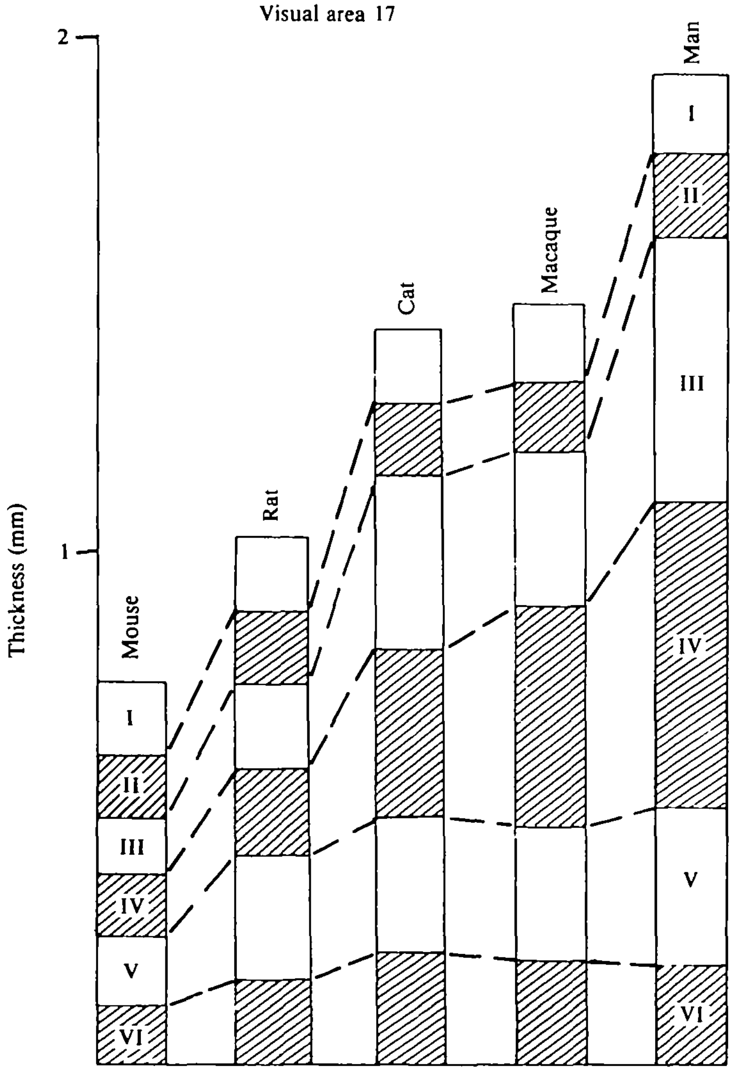


FIG. 5

all areas repeated after an interval of some years showed no significant difference. In contrast to the other primate brains it had been found earlier that there was a significantly lower number of cells in area 17 of the human, but as this finding was difficult to interpret counts were repeated and further blocks and sections cut. When it was realized that the low figures might have been due to many of the small cells not being adequately stained, probably due to imperfect fixation, the coverslips on the slides were removed and the sections were restained deeply; the number of cells was then found to be almost as high as in other primates, but in these restained sections the figure in the peristriate cortex was still approximately 110. In the visual cortex of the two human brains Cragg (1967) studied, the density of cells in area 17 in one brain was approximately half that in the other, and although he could offer no explanation it might have been for the same reason. One finding which was initially a marked discrepancy actually provided support for the validity of the counts. In one macaque monkey brain (not included in Table 1) the counts of area 17 were about 170 instead of around 270, and this was disturbing until it was

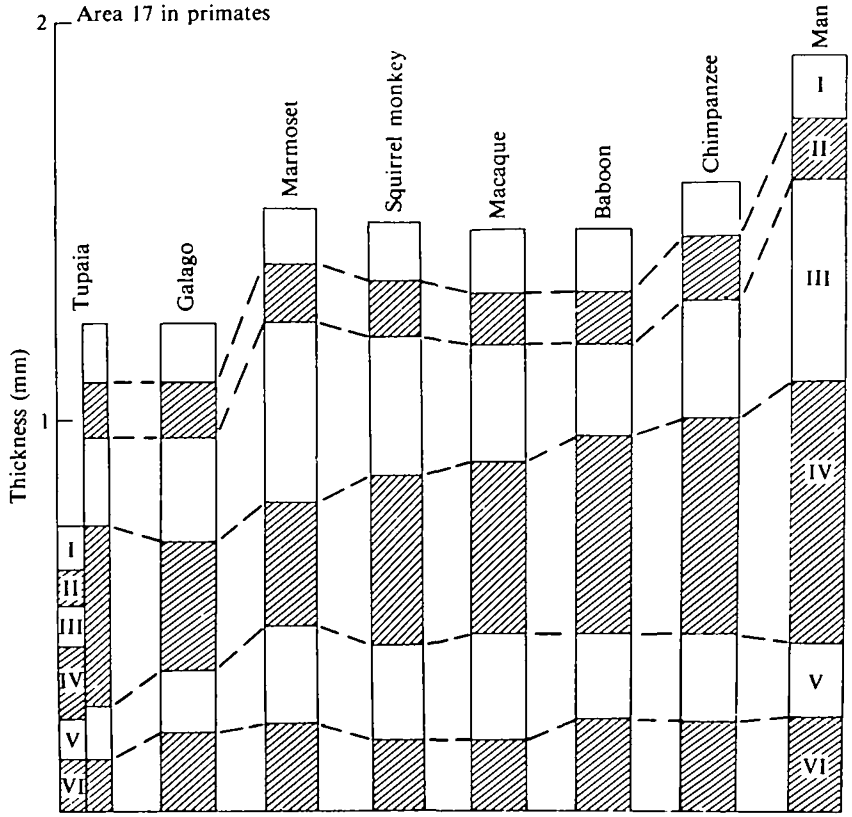


FIG. 6. The thickness of the cortex of area 17 in several primates; in Tupaia, in which it was possible to measure the monocular and binocular regions, the monocular (*left*) is approximately half the thickness of the binocular (*right*).

found that the sections had been cut at a thickness of 15 μm rather than 25 μm; in the thinner sections this was the figure one should have obtained. The actual figures obtained in counts of cells through the thickness of the cortex may vary with different methods of fixation and histological processing, and if the sections are not cut strictly perpendicular to the surface increasing angles of obliquity would result in the cortex being thicker and the counts greater.

In a study of ageing in the cerebral cortex Brody (1955) counted the cells through the thickness of the cortex in a much wider strip (1125 μm) and in thinner (10 μm) sections than in the present study in several areas in many brains. Analysis of his data shows that he found the number of cells to be the same in the adult pre- and post-central and inferior temporal gyri, but almost twice the number in the superior temporal and striate cortex. From the figures provided by Brody one can calculate that in the volume sampled in the present study he would have counted 80 to 90 cells in the pre- and post-central and inferior temporal gyri. If allowance is made for several differences in methodology the figures are in good agreement with ours and indicate that sampling a wide strip of cortex would not alter our conclusion about the constancy of cell numbers in different areas.

An exception to the similarity in absolute number in the different areas and species was area 17 of the primates, and a much higher density of neurons in primate visual cortex than in several non-primate brains was also found and discussed by Cragg (1967). In all brains the figures for the adjoining peristriate cortex of areas 18 and 19 were the same as in other functional areas, $\simeq$ 110. In the visual cortex of Tupaia the binocular part had a number that was midway between the high figure characteristic of area 17 of the other primates and that of the other species, but the monocular area was the same as in other functional areas. It was not possible to identify the region of representation of the monocular segment in the other primate brains, so it is not known whether the increase in number of cells occurs only in the binocular part. In view of the difference found in Tupaia it is obviously important to count the cells in primates in which the monocular segment is clearly identifiable after injection of labelled amino acids into the eye (Wiesel *et al.*, 1974). Until this has been done one can only speculate about the functional significance of the increase in number of cells in area 17 of primates, but it may be significant that in the binocular part of Tupaia, in which the number of cells is midway between that of area 17 of other primates and of the other functional areas of all animals, the projection of the different laminæ of the lateral geniculate nucleus (related to different eyes) is upon different laminæ rather than to adjoining columns as in the macaque (Harting, Diamond and Hall, 1973; Casagrande and Harting, 1975; Hubel, 1975). The larger number would not appear to be related solely to a pronounced ocular dominance columnar arrangement, however, because the same number of cells has been found in area 17 of squirrel and macaque monkeys in this and a previous study (Cowey, 1964) but ocular dominance columns have not been shown with histological techniques in the squirrel monkey (Hubel and Wiesel, 1977; Hendrickson, Wilson and Ogren, 1978). Whatever the explanation proves to be, it may be noted that information about an individual stimulus coming through two eyes and two pathways is first brought together in area 17, and the relative size of the uncrossed pathway increases in phylogeny.

The large number of cells in area 17 of the primate, in which vision is probably the most important and dominant sensory pathway, raises the possibility that there may be more cells in other 'specialized areas' such as the supratemporal plane and Broca's area in the human brain, the auditory cortex of echo-locating bats and the barrel fields related to the vibrissæ in certain rodents. That this may indeed be so is suggested by the figure for the cortex of the human superior temporal gyrus in Brody's (1955) study being higher than in other areas (except area 17).

In the electron microscopic study of the motor and somatic sensory areas of the monkey the proportions of pyramidal and non-pyramidal cells, as identified on the ultrastructural features of their cell somata, were found to be the same, as well as the absolute number of neurons (Sloper, 1973; Sloper *et al.*, 1979). Similar proportions have been found in area 17 of the monkey (Tömböl, 1974), in the visual and motor areas of the rat and cat (Winfield, Gatter and Powell, 1980) and in human temporal cortex (unpublished observations). Although the proportions of the two major types of cells in the cortex remain constant, their laminar distribution may vary, as has been found between the motor and somatic sensory areas of the monkey (Sloper *et al.*, 1979).

The pattern of distribution of the intrinsic cortical connections is also essentially similar in areas of the monkey as different in cytoarchitecture and function as area 17 (Fisken, Garey and Powell, 1975), the motor cortex (Gatter and Powell, 1978), the somatic sensory area and area 5 (Shanks, Pearson and Powell, 1978; Vogt and Pandya, 1978). Several studies with retrograde axonal transport methods have shown that with few exceptions the laminar origin of the efferent fibres from the cortex is similar in most areas, the cortico-cortical arising mainly from layer III but with some from layer V, the cortico-thalamic from layer VI and those to other subcortical sites from layer V (*see* Gilbert and Kelly, 1975; Lund, Lund, Hendrickson, Bunt and Fuchs, 1975; Jones and Wise, 1977). These findings of a basic uniformity in structure of the cortex are apparently in contrast to the differences in cytoarchitecture between areas and they raise the question of the cause of the differences in morphology that are readily seen between the motor, sensory and association areas. The apparent contradiction may be resolved if one considers that the absolute numbers and proportions of the main cell types are constant and that the pattern of intrinsic fibre connections is essentially uniform throughout the neocortex, but the morphological differences which form the basis for cytoarchitectonic differentiation are correlated with variations in the relative sizes of the major afferent and efferent fibre pathways of individual areas, and particularly the relative proportions of cortico-cortical connections compared to those with subcortical structures. Certain architectonic features are, however, due to differences in intrinsic structure of the cortex; in area 17 of primates there are more cells and the accentuation of the outer band of Baillarger into the prominent stria of Gennari probably results from the greater number of non-pyramidal interneurons.

Many studies with a variety of techniques in recent years have shown that variations in connections are present, that the changes in density of the afferent or efferent pathways occur precisely at architectonic boundaries and that correlations can be made between the relative strength of the connections and cytoarchitecture. The extensive literature will not be reviewed but only the more relevant findings and mainly from the monkey will be considered (*cf.* Powell, 1973; Jones and Burton, 1976). The granular or koniocortex of the sensory areas receives a large number of fibres from the thalamus and the number and size of these diminish abruptly at their limits. Marked variations in the size of the thalamic projection have been found between several areas of the parieto-temporal lobe and again the changes coincide with boundaries determined on morphological criteria (Jones and Burton, 1976). The number of afferent and efferent cortico-cortical fibres also varies considerably, the koniocortex of area 17 and of area 3b having few but the adjoining peristriate areas and areas 1 and 2 of the somatic sensory area, together with the association areas, having far more. There is increasing evidence to indicate that thalamo- and cortico-cortical fibres terminate in narrow bands of cortex (*cf.* Hubel and Wiesel, 1977; Imig and Brugge, 1978; Shanks *et al.*, 1978), and the latter are often situated at boundaries of architectonic areas which may have clearly distinguishable morphological features. In the subdivisions of the visual (Zeki, 1977) and somatic sensory areas (Shanks *et al.*, 1978) the parts of the cortex of one hemisphere which have commissural connections are connected with each other by association fibres and regions devoid of commissural connections only with other non-commissural parts; in the auditory cortex a similar principle seems to be present as both commissural and association connections are present throughout most of the individual subdivisions (Diamond, Jones and Powell, 1968*a*, *b*; Pandya, Hallett and Mukherjee, 1969). The change in strength of the various afferent and efferent pathways which coincides with architectonic boundaries is associated with differences in the laminar termination of afferent fibres, with the demarcation and degree of development of individual laminæ and with the arrangement of the cells in rows or columns. When the thalamic pathway is strong the fibres end mainly in layer IV, but as the number of thalamic fibres progressively diminishes their termination becomes more restricted to layer III (Jones and Burton, 1976). Although cortico-cortical fibres terminate in all layers, when these increase to form the major afferent pathway to a particular area most of them terminate in layer IV (Martinez-Millán and Holländer, 1975; Pearson and Powell, 1978). Thus layer IV appears to be the major site of termination of the strongest afferent pathway to any individual area. The cortico-cortical fibres arise mainly from layer III and in areas which send many cortico-cortical connections this layer is more prominent with larger and more widely spaced pyramidal cells. The degree of columnar arrangement of the cells in any cortical area is a reflection of the number of cortico-cortical connections as these fibres run perpendicular to the surface, with the efferent fibres from layer III passing deeply through the other layers and the afferent fibres ascending through most layers. In areas of the cortex in which early descriptions emphasized the columnar arrangement of cells, recent work has shown that there are strong cortico-cortical connections. This is true of the koniocortex of the auditory area which has numerous commissural and association connections in contrast to the koniocortex of area 3 of the somatic sensory area and area 17 of the visual cortex, both of which have few cortico-cortical connections and little columnar arrangement; the increasing columnar arrangement as one moves from the koniocortex of areas 17 or 3b into adjoining areas can be explained on the same basis.

The significance of recent observations on the origin and termination of connections with other cortical and sub-cortical sites in narrow bands or columns, and the fact that subcortical sites such as the amygdala, basal nucleus and locus coeruleus are sending fibres to the cortex, is not clear. Further studies with more refined methods will show an even greater complexity but on the present evidence some general conclusions may be drawn. The heavier the afferent pathway is from the thalamus *and* the sparser the cortico-cortical connections, the cytoarchitecture is that of the granular or koniocortex as typified by area 17 and area 3b and the granular barrel fields of the somatic sensory area of certain rodents (Woolsey and Van der Loos, 1970; Welker, 1976). Here, layers II, III and IV are difficult to distinguish and there is little arrangement of the cells in columns. In the koniocortex of the auditory area, where there are more cortico-cortical fibres, layer III is prominent and there is a columnar arrangement of the cells. In other subdivisions of the sensory areas where the thalamic afferents are sparser *and* the cortico-cortical fibres increase, layer III has larger pyramidal cells and is more clearly demarcated together with the cells becoming columnar in arrangement. Although area 4 also has a strong afferent pathway from the thalamus the dominant cytoarchitectural feature is the greater size of the pyramidal cells in layers III and V, correlated with the widespread efferent projections to cortex and subcortical sites by larger or longer axons or more axon branching. The clear lamination of the homotypical association cortex probably reflects a more even balance between the various afferent and efferent projections. There are complex interrelationships between the proportions of the several diverse afferent and efferent pathways so the cytoarchitectural changes may occur in gradients. The greater differences in relative proportions between the pathways to certain areas in larger brains may underlie the clearer architectonic differentiation; in the thalamic connections of the subdivisions of the somatic sensory and visual areas definite differences are found between the cat and monkey (Wilson and Cragg, 1967; Jones and Powell, 1970; Hendrickson *et al.*, 1978). The differences in architecture found in the neocortex are mainly due to these variations in the density of connections, changes in cytoarchitecture being accompanied by changes in connections.

A cytoarchitectonic area can be defined not only on the basis of its intrinsic cellular structure but also as one having the same pattern of afferent and efferent connections and the same function throughout, the only difference within it being related to topographical representation. The functions of individual areas are dependent upon their connections, and differences in the origin and termination of these, and of the ratios between them, determine the differences in function that have been shown between architectonic areas; the number and proportions of the neurons remain constant. Although the proportions of the pyramidal and non-pyramidal neurons are the same in areas as diverse as the motor and somatic sensory, the details of the form of the cells need not be identical, because the dendritic ramifications, density of axonal arborization and the layers of termination of the afferent pathway may vary. It is impressive, however, how similar are the descriptions of the types of Golgi-impregnated neurons in different areas and species (*cf.* Lorente de Nó, 1949; Jones, 1975; Feldman and Peters, 1978).

The variations in density between the various pathways which underlie cytoarchitecture may be governed by certain principles determining the interrelations between the proportions, the origin and the termination of the different connections. There is, for example, the reciprocity in origin and termination of most commissural connections, the relationship of the commissural and non-commissurally connected regions in the same hemisphere to each other, a possible complementarity between strength of the thalamo-cortical and cortico-cortical connections of any individual area, and a reciprocal relationship in the strength of thalamo-cortical and cortico-thalamic pathways. If all pyramidal cells send their axons into the white matter, and non-pyramidal do not, the total number of efferents is the same from each unit cylinder through the depth of the cortex (except area 17 in primates) because the total number of cells and the proportion of pyramidal cells remain constant, and there must therefore be an interrelationship between the proportions of the efferent fibres to different sites. Whether there is also a similar relationship in the afferent connections must remain speculative, but the available evidence is suggestive.

The thickness of the cortex does not vary much between brains of different sizes and the figures show that even in the motor cortex there is barely a three-fold increase between mouse and man. In the motor and parietal areas the major increment is in layers III and V, which are the main sites of origin of the efferent fibres, and the apical dendrites of the pyramidal cells will become longer. In the visual cortex layers III and IV show the greatest increase, but within the primates it is surprising how little change there is in total thickness between Tupaia and Man. The obvious change that does occur in the cortex with increase in size of the brain is increase in its *area. From the findings of the constancy in number and proportions of cell types in the cortex between different areas and species it seems probable that early in mammalian evolution these two quantitative features of the cortex are determined genetically and this specification remains constant. It may be that the number of cells through the depth of the cortex is not specified directly, but rather the specification is for a constant packing density for cells in the horizontal plane, that is when projected upon the cortical surface in the mature brain* (figs. 7 and 8).

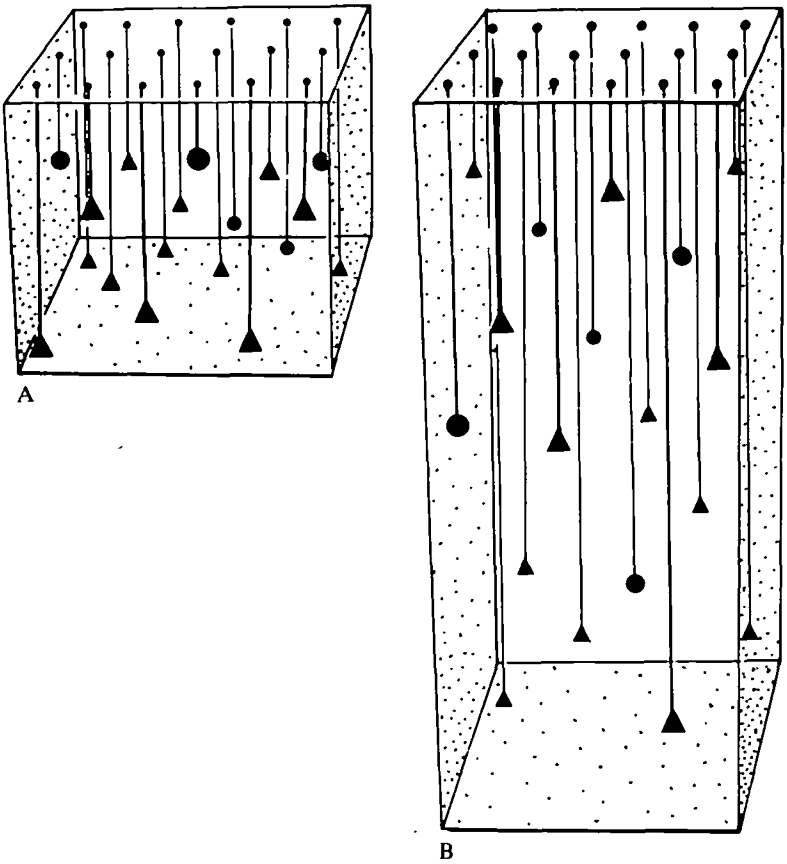


FIG. 7. Schematic figure of mature cortex to illustrate the same number of neurons through the depth of the cortex, and constant packing density when projected upon the surface, in the cortex (A) of small brains (mouse or rat) and in thicker cortex (B) of larger primate brains. Pyramidal (▲) and stellate (●) cells retain the same proportions in different species. The lines extending vertically from the cells to the surface are projection lines, and in the case of pyramidal cells they could also represent the apical dendrites.

This constancy in number of cells (and proportions) through the depth of the cortex may be an expression of a basic structural and functional unit or module. Although the volume of the cylinder of cortex that was sampled in this study is approximately the size of the smallest functional column (Welt *et al.*, 1967; Hubel and Wiesel, 1974) it should be emphasized that this is not necessarily the size of the simplest anatomical unit. In the cortex of larger brains the number of these units or modules increases, by repeated specification, and the additional ones are assembled side by side. The increase in the number of modules would be accompanied by a corresponding increase in the interconnections between themselves and other parts of the central nervous system. In the neocortex of primate brains the increase in number of modules occurs particularly in the association areas

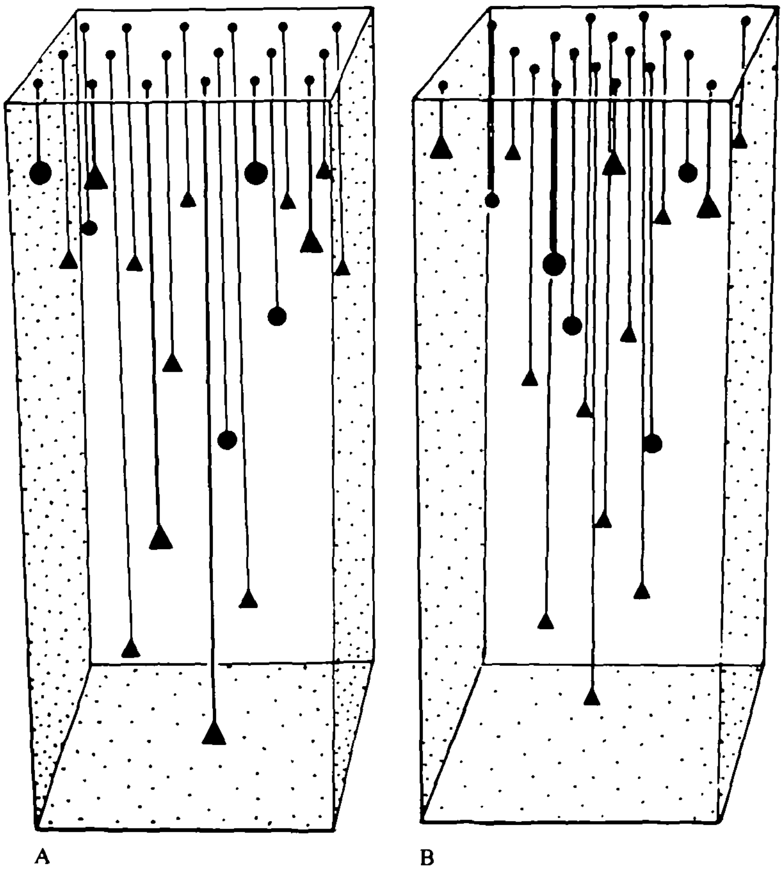


FIG. 8. Schematic figure to show that when the cells in the cortex are arranged in 'columns', as in the auditory cortex or in area 4 in primates, the packing density when projected upon the surface remains constant (as in fig. 7) and it could either be uniform (A) or there could be local variations within the same overall density (B). The much greater density of cells actually present in the cortex than in the figures would accentuate the appearance of a columnar arrangement.

of the frontal and parieto-temporal lobes and it is significant that in these areas the increase in thickness of the cortex is predominantly in layers III and V which are the source of intracortical connections. Increase in area, but not in thickness or number of cells through the depth of the cortex with increase in size of the brain, has been shown to be true for area 17; the thickness of the cortex and the number of cells through its depth are the same in squirrel and macaque monkeys, but the area is twice as great in the macaque (Cowey, 1964). The greater thickness of the cortex and vertical spacing of the cells in the larger brains is no doubt due largely to the more numerous and extensive dendritic and axonal ramifications because with an increase in area of cortex by one square centimetre there will be several million more cells and axons, many of which are terminating within the cortex either in the immediate vicinity or in other areas of the same or opposite hemispheres. The uniformity of structure and function throughout the binocular part of area 17 in the monkey has been emphasized by Hubel and Wiesel (1977), and they have concluded that the same basic units are present throughout but more of them 'are used per degree of visual field the greater the detail of analysis'. These authors have also pointed out the advantages in development of such uniformity of structure with replication of the same specification. From the present data and the evidence of the pattern of intrinsic connections, it is suggested that essentially the same structural units are present throughout the whole of the neocortex. The differences in function between cytoarchitectonic areas depend upon differences in the total pattern of connections of these modules, and increases in number of modules within a particular functional area, or between areas and species, will be an accurate reflection of the greater analysis of detail and of the functional importance of that region. Preliminary counts of areas of the cerebral cortex other than the neocortex (pyriform, hippocampal) suggest that although the absolute number of cells in individual areas is different from that of the neocortex and from each other there is the same constancy in the absolute number in the same structure between different species.

The width of 30 μm within which the cells have been counted is of the same order of size as the smallest functional column in the cortex where it has been possible to measure them (Welt *et al.*, 1967; Hubel and Wiesel, 1974). Whether this indicates more than a chance association and that the absolute number of cells found here form a structural and functional unit (together with their connections), the basis of the functional column, must remain conjectural until more is known of the intrinsic synaptic organization of the cortex. The presence of a columnar functional organization in all areas of the neocortex that have so far been investigated (Mountcastle, 1978), however, is in accord with the basic uniformity of structure of the neocortex that has been suggested here. The significance of the columnar organization is in the processing of incoming information, vertically within a column and horizontally between adjoining columns, with a wide and diverse distribution of the results through the numerous pyramidal cell axons; the form of the processing might be essentially similar in all areas but the differences in function between areas would be due to the differences in the *sources and termination* of their connections.

The constancy of the number of cells that is present through the depth of the cortex in different areas irrespective of the variation in thickness of the cortex should permit accurate assessments of the number of cells in the whole cerebral cortex after its surface area has been determined (Mountcastle, 1978). For such estimates a correction factor for shrinkage must be used, *both* for the total surface area and for the number of cells in fixed tissue; in the present study (Material and Methods) it has been found that for the sections of human brains the linear shrinkage was of the order of 18 per cent. If the conclusion about the correlation of cytoarchitectural differentiation with connections is correct, emphasis should be placed upon the more objective results of investigations in which the total pattern of connections of areas of the cortex are determined rather than upon the still somewhat subjective criteria of cytoarchitecture. This suggestion would apply particularly to the association cortex of the frontal and parieto-temporal lobes, where rigorous physiological studies are now making progress. It may also be worth investigating whether there are changes in the number or proportion of cells *through the depth* of the cortex in the brains of animals and patients with disorders of known genetic basis. In several mutant strains of mice genetically associated variability in numbers of neurons has been found in all regions of the hippocampus (Wimer, Wimer, Vaughn, Barber, Belvanz and Chernow, 1976), and in the dentate gyrus the total number of cells varies in different strains (Wimer, Wimer, Vaughn, Barber, Belvanz and Chernow, 1978), being severely reduced in the reeler mouse (Stanfield and Cowan, 1979). There is a common genetic specification for cell number throughout the entire granule cell lamina of the dentate gyrus (Wimer *et al.*, 1978) and the effects of mutation upon the structure of the hippocampus appear to be only in the radial dimension (Stanfield and Cowan, 1979). These observations are in accord with our hypothesis that the number of cells through the depth of the cortex (or the constancy in packing density of cells as projected upon the surface) is determined genetically, and in mutant strains of animals and in human patients with genetic disorders this parameter of cortical development may be selectively affected.

SUMMARY

The number of neuronal cell bodies has been counted in a narrow strip (30 μm) through the depth of the neocortex in several different functional areas (motor, somatic sensory, area 17, frontal, parietal and temporal and in many species (mouse, rat, cat, monkey and man). With the exception of area 17 of the visual cortex in a number of primates the same absolute number ($\simeq$ 110) of neurons has been found in all areas and in all species. In the binocular part of area 17 of the primates there are approximately 2.5 times more neurons. Thus in mammalian evolution the area of the neocortex increases in larger brains but the number of neurons through the depth remains constant, except in area 17 of primates. From these and other findings it is suggested that the intrinsic structure of the neocortex is basically more uniform than has been thought and that differences in cytoarchitecture and function reflect differences in connections.

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