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The density of synapses and neurones in the motor and visual areas of the cerebral cortex

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INTRODUCTION

Cortical synapses can be seen by electron microscopy, but their density in tissue sections does not appear to have been estimated. Since the diameter of a synapse is much greater than the section thickness, synaptic profiles must be measured as well as counted to obtain the density. Many authors have counted neurones in tissue sections by light microscopy after staining the Nissl bodies, but in general the tissue shrinkage caused by histological preparation has not been measured. The volume shrinkage with paraffin embedding may be 52% (Brizzee, Vogt & Kharetchko, 1964), 73% (Ramon-Moliner, 1961) or even 83% (Robins, Smith & Eydt, 1956) and is variable according to the method of preparation. Many of the published estimates of neuronal density are thus too high by an unknown factor between two and six, and the dependence of this factor on the exact method of preparation makes the comparison of the results of different authors of doubtful validity. Neurones have therefore been counted again in a representative selection of mammals using a method that eliminates shrinkage and yields an absolute density.

When the density of synapses and the density of neurones have been estimated on the same tissue, division of one result by the other gives an average value for the number of synapses on each neurone in the tissue. This number has been found to be considerably larger than previous estimates made without electron microscopy, and shows interesting variations between species and in different cortical areas. It is possible that the average number of synapses on each neurone may be a measure of the quality or sophistication of cerebral cortex. This idea derives from the findings of Nissl (1898) on the greater separation of human neurones, compared with those of mole or dog brain, and the remarks of Von Economo (1926) on the greater opportunity for neuronal interaction provided by the more extensive fibre plexus that fills the space between the widely separated neurones. The average number of synapses on each neurone may be a useful parameter to measure in investigating the structural basis of mental retardation, or the question of whether there are detectable structural changes in learning, for example, in the formation of a learning set for visual pattern problems. The motor and visual areas were selected for this study because they can be recognized in all species and probably represent opposite extremes of neo-cortical specialization.

METHODS

Electron microscopy

The two mouse and two monkey brains used for electron microscopy were perfused with formalin by the method of Pease (1962). After 2–24 h of immersion in formaldehyde, thin slices of the appropriate cortical areas were cut, and the cortex in each slice was trisected by two cuts parallel to the pia to yield strips representing the upper, middle and lower thirds of the cortex. These strips were chopped up in separate pools of 2% osmium tetroxide in phosphate buffer at pH 7·3 (Millonig, 1962). Small blocks were subsequently dehydrated in acetone and stained with uranyl acetate, and lead citrate as described by Westrum (1965). Silver-grey sections were cut in Araldite and examined on a Siemens Elmiskop 1*b* at a fixed magnification of 14000. Fields were selected for good definition and photographed without reference to their contents. Prints were made at a magnification of 3·5 to give an overall magnification of 49000, and all synapses counted and measured. For the latter, an average of the longer and shorter diameters of each synaptic profile was used. The criterion used to distinguish synapses was the presence in a membranous profile of numerous small membrane-bound vesicles, regardless of whether the thickened region of synaptic apposition was included in the profile or not. Histograms of the number of synaptic profiles of various diameters were drawn, and an average diameter calculated.

The expected distribution of synaptic profile diameters can be approached as follows: suppose that all the synapses were spherical and of uniform diameter D, and that the electron microscope section intersected a particular sphere at a distance r from its centre to make a circular profile of diameter x. Then all values of r are equally probable, so the number of profiles intersected by the sections in the range of diameters between x_1 and x_2 will be proportional to the corresponding (r_1-r_2). Now since $x^2+4r^2 = D^2$ by Pythagoras's theorem,

$$2(r_1-r_2) = (D^2-x_1^2)^{\frac{1}{2}}-(D^2-x_2^2)^{\frac{1}{2}}.$$

This distribution is plotted in Fig. 1*e* with $D = 0{\cdot}8$ μm, and comparison with the observed distributions shows that there must be some range of diameters in the synapses. A good fit (Fig. 1*f*) may be obtained on the assumption that 10% of the synapses have a diameter of 1·0 μm, 33% of 0·8 μm, 40% of 0·6 μm and 17% of 0·4 μm, though this is by no means a unique solution.

If the profiles were generated by intersection of the electron microscope section with spherical synapses all of one diameter D, as in Fig. 1*e*, then the average diameter $\bar{x}$ of a synaptic profile would be related to the maximum synaptic diameter D by the equation $4\bar{x} = \pi D$ (Abercrombie, 1946). This simplified situation probably represents the best way of making an approximate estimate of the average synaptic diameter, in spite of the fact that the synapses vary in diameter and are usually non-spherical. The measured synaptic profiles were therefore averaged, and this result multiplied by $4/\pi$ to yield an estimate of the average synaptic diameter.

The average diameter of cortical synapses was of the order of 0·6 μm, or 6000 Å, and silver-gray sections are about 600 Å thick (Peachey, 1958). The synapses counted in the area covered by the prints must have been distributed in the tissue in a slice of

thickness equal to the average diameter of the synapses plus the section thickness (Abercrombie, 1946). Since the measured diameter of the synapse is about 10 times greater than the presumed section thickness, the uncertainty in the latter has little effect on the overall result. The density of the synapses was thus calculated as the number counted in the micrographs distributed in a volume equal to the area covered by the micrographs multiplied by the sum of the section thickness and the average synaptic diameter. This calculation assumes that shrinkage does not occur in preparation. This point was checked by cutting two thin slices from a formalin-fixed rectangular block of cortex, mounting one slice on a gelatinized slide, and taking the other through osmium tetroxide, acetone, uranyl acetate and Araldite. This slice embedded in a thin layer of araldite was then compared with the slide-mounted slice, and no measurable shrinkage was found. In order to relate the magnification of the electron microscope to that of the light microscope used for counting neurones, a platinum wire of about 5 μm diameter was photographed at a magnification of 14000 on the electron microscope, and the same grid-mounted wire was measured under oil immersion on the light microscope, and compared with the stage micrometer. The validity of applying the Abercrombie calculation to counts of synapses by electron microscopy has been established empirically by Clementi, Whittaker & Sheridan (1966).

Light microscopy

The brains studied are listed in Table 2 with their weights. All were perfused with formalin except the human brains which were fixed by immersion in formalin. Frozen sections were cut at 20–30 μm. Before mounting on gelatinized slides, a piece was snipped off each section with scissors under water, and this piece was further fixed in osmium tetroxide, dehydrated in acetone and embedded flat in a thin layer of Araldite. Hand sections were then cut perpendicular to the piece of section, and mounted in balsam for measurement of section thickness under oil immersion. This method of measuring section thickness by re-embedding and cutting perpendicularly was described by Marengo (1944) who used paraffin embedding, and can be used in the present context because as mentioned above, it can be shown that the procedure described does not change the dimensions of formalin fixed tissue.

After the sections had been mounted on gelatinized slides, the gelatin was fixed in formaldehyde vapour to prevent movement (shrinkage) of the sections, and the latter were then dehydrated, and fat extracted in pyridine. Various Nissl stains were compared, and it was found that the clearest picture of the nucleoli was given by cresyl violet at a pH of about 3·0. The nucleoli were counted under oil immersion in an appropriate part of the section where the pial surface was flat, and the diameters of some were measured. A proportion of neuronal nuclei in the pig and rabbit cortex had two nucleoli each, but of course only one was counted, since neuronal density was required. The nucleolar diameter was 1–2 μm according to the species studied, and the section thickness was 20–30 μm. The nucleoli counted were distributed in a tissue thickness equal to the sum of the section thickness and the nucleolar diameter (Abercrombie, 1946), so the small size of the latter made it unnecessary to measure nucleolar diameter accurately at different depths. By contrast, if cells or cell

nuclei had been counted, a size distribution in depth would have been needed to apply an accurate correction.

The density of neurones varies with depth in the cortex, but since a dendritic tree ascending to the top of the cortex may arise from a cell body at any depth (Sholl, 1956, p. 14), it is not possible to calculate the average number of synapses on a neurone as a function of depth. Instead, the average density of neurones was calculated for the whole of the cortex including layer *I*, as was done for the density of the synapses. The motor and visual areas could be recognized on cytoarchitectonic grounds in all the species studied, the absence in the former and presence in the latter of a distinct granular layer being particularly helpful. Parasagittal sections were cut to intersect the expected position of the motor and visual areas as described in the standard texts such as Kappers, Huber & Crosby (1936) and in other publications on the less familiar forms (Zuckerman & Fulton, 1941; Rose, 1929; Kanagasuntheram, Leong & Mahran, 1966).

The remaining difficulty is to distinguish neurones from glia, and the most valuable criterion was found to be the invariable possession by all neurones of a distinct nucleolus. In most glial cells in the species studied the nuclear chromatin is distributed in several small irregularly shaped bodies, and these cells are easily distinguished from neurones. In some species, however, a proportion of the glial cells does appear to have a distinct nucleolus and in mouse cortex some neurones have several particles of chromatin in their nuclei. It is then necessary to identify glial cells by their proximity to the pia, blood vessels or nerve fibres, to study the exact characteristics and sizes of these cells in the particular preparation, and apply the result to differentiating neurones from glia in the rest of the preparation. Cresyl violet often stains the neuronal nuclei blue, and the glial nuclear chromatin violet, but in a series of species there are considerable variations in detailed appearance, and some reformulation of the criterion for neurones or glia is needed after studying the easily identified cells. There is, nevertheless, a proportion of cortical cells whose assignment to either class is arbitrary, but this proportion is less than 10% in the areas and species studied.

RESULTS

Density of synapses

Synapses were counted and measured in blocks of tissue taken from the upper, middle and lower thirds of the cortex, but the results showed little variation in either size or number with depth, and were averaged (Fig. 1). Sections from several blocks at each level in each tissue were included in order to sample the tissue adequately. Table 1 shows the number of synapses counted and the estimate of average synaptic diameter. The latter, together with the section thickness (assumed to be 600 Å) represents the thickness of tissue in which the synapses were distributed, so this thickness multiplied by the area covered by the micrographs gives the volume which contained the synapses counted. In the last column of Table 1, 1 cc is divided by this volume and the result multiplied by the number of synapses counted to give an estimate of the number of synapses in 1 cc of tissue. It will be seen that the density of synapses does not vary widely within the tissues studied, and this is also the qualitative impression given by the micrographs (see Fig. 2). For comparison, 648

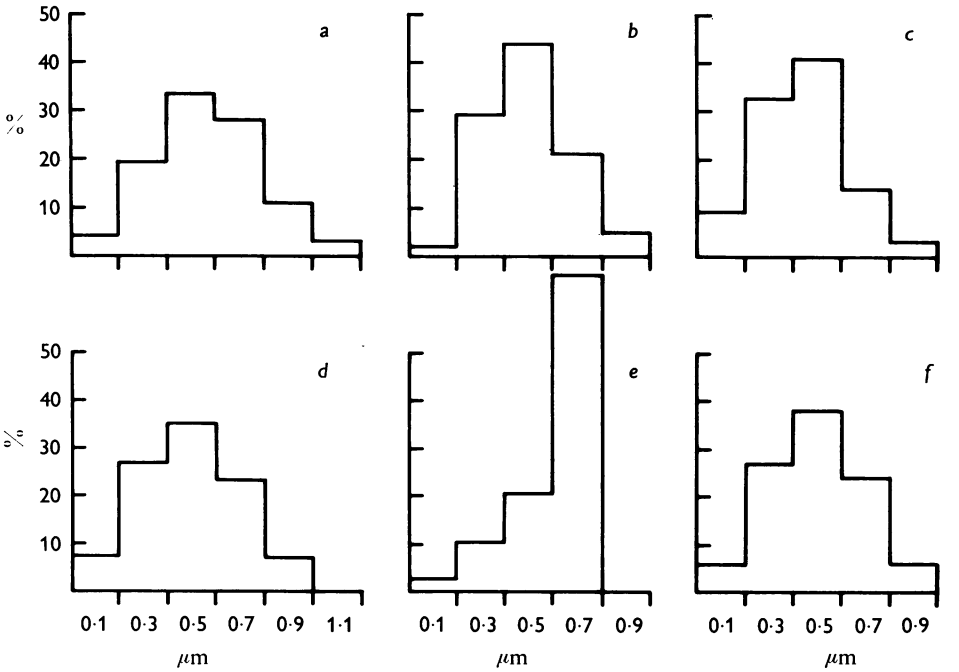
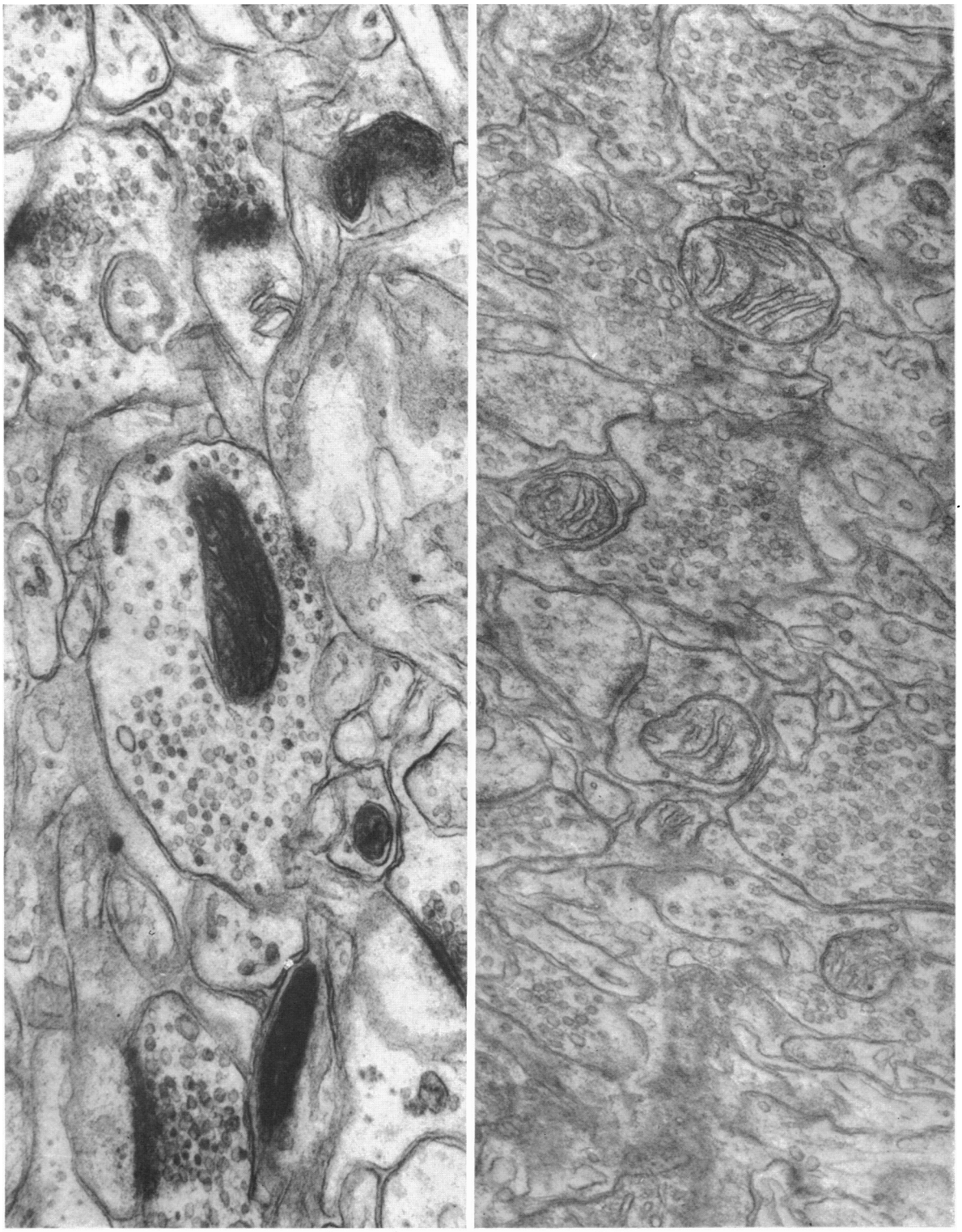


Fig. 1. The frequency distribution of synaptic profiles in cerebral cortex. (*a*) Monkey visual cortex. (*b*) Mouse visual cortex. (*c*) Monkey motor cortex. (*d*) Mouse motor cortex. (*e*) Expected distribution of profiles if all the synapses were of diameter 0·8 μm. (*f*) Expected distribution of profiles if 10 % of synapses were of diameter 1·0 μm, 33 % of 0·8μm, 40 % of 0·6 μm and 17 % of 0·4 μm.

Table 1. *The size and density of synapses in cerebral cortex*

Cortex	Profiles counted	Mean diameter of synapses (μm)	No. of synapses per cc
Mouse visual	365	0·64	$6{\cdot}6 \times 10^{11}$
Mouse motor	440	0·63	$8{\cdot}5 \times 10^{11}$
Monkey visual	713	0·72	$6{\cdot}2 \times 10^{11}$
Monkey motor	468	0·56	$9{\cdot}6 \times 10^{11}$

synaptosomes were counted in separated homogenates of guinea-pig cortex and estimated at $3–5 \times 10^{11}$ synaptosomes per g tissue by Clementi *et al.* (1966). Dr E. G. Gray (personal communication 1965) counted 969 synapses of average profile diameter 0·73 μm in an area of 3078 μm^2 of rat visual cortex, giving a synaptic density of $3{\cdot}2 \times 10^{11}$ per cc. In this case the cortex had been fixed by immersion in osmium tetroxide and stained with phosphotungstic acid, and the micrographs had been selected for other purposes. These results are dependent on the quality of the micrographs, for if the smaller synaptic profiles are missed this will not only decrease the total number of synapses counted, but also shift apparent average synaptic diameter towards a larger value, thus further reducing the apparent synaptic density.


(a)
1 μm
(b)

Considering the different methods employed, the different species and areas studied and the various approximations made to the synaptic geometry, the agreement between these results is surprisingly good.

Density of neurones

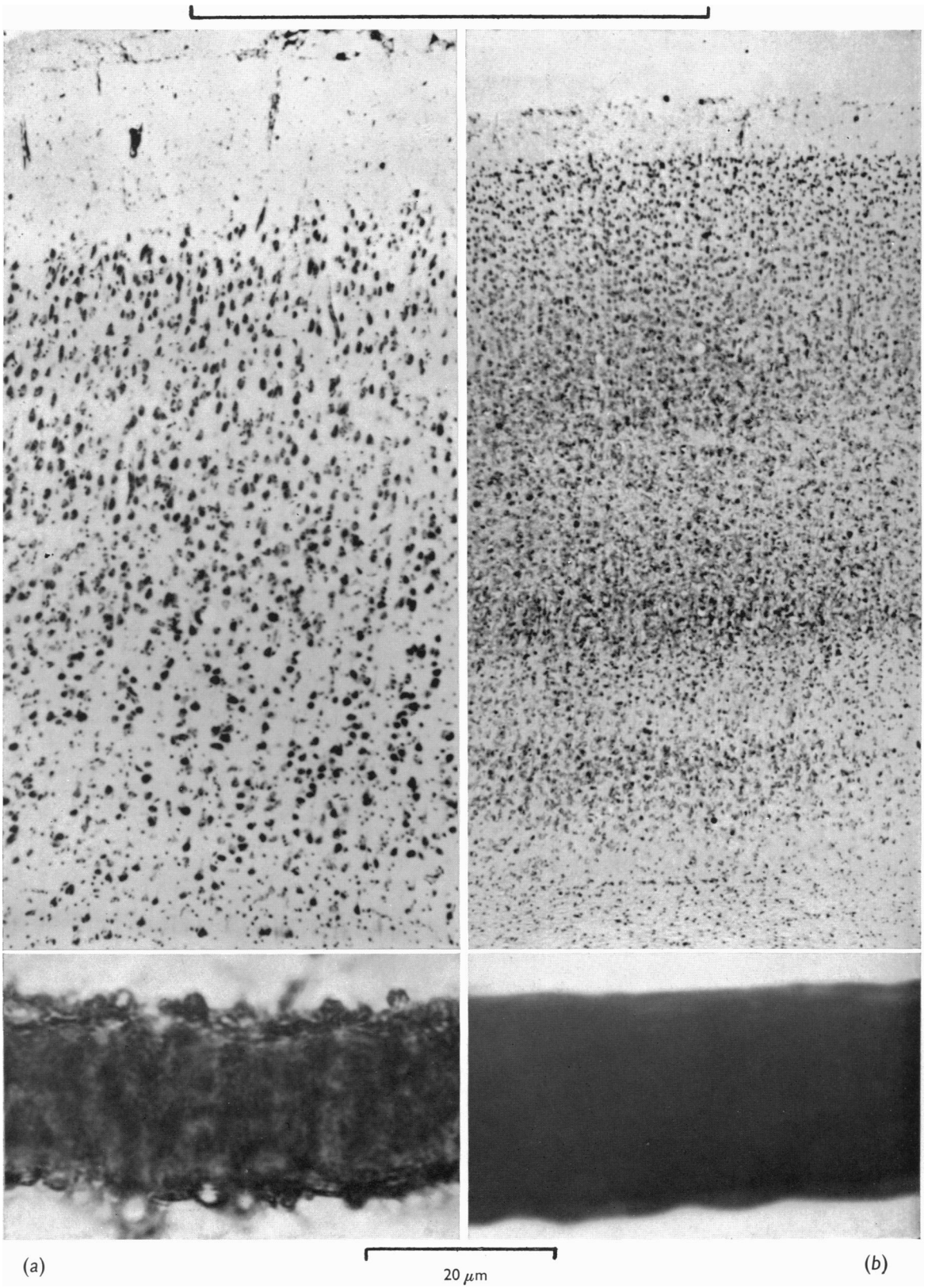
It was found that successive neuronal counts in adjacent sections of corresponding strips from pia to white matter yielded results that differed by as little as 10–15 %. Counts of other parts of the areas studied, or of other brains of the same species, showed greater variations. This study does not include a sufficient number of brains of each species to give a reliable measure of the variation that may exist within species. It was felt that an extensive survey of several species by one author was chiefly needed to determine the general pattern of neuronal density, for the several intensive studies of single species that already exist in the literature are difficult to compare because of the unknown shrinkages involved.

Table 2. *The density of neurones in cerebral cortex* (*no. per cc* $\times 10^{-6}$)

Species	Brain weight (g)	Visual cortex	Motor cortex
Mouse	0·45	87·3	46·2
	0·49	107·4	64·4
	0·52	92·4	56·4
Rat	1·8	48·8	—
(Wistar albino)	2·2	31·6	—
	2·6	46·8	—
	1·9	53·9	—
	2·0	47·2	25·1
Galago sen. sen.	4·9	95·7	33·5
(Bush-baby)	5·1	106·7	49·3
Rabbit	8·5	47·0	22·8
	7·5	41·3	27·6
Cat	29	34·0	—
	34	49·4	25·4
Pig	76	21·5	15·7
Monkey	86	104·2	—
(*Macaca mulatta*)	72	126·7	16·1
	75	110·3	20·7
Man		51·9	—
	1400	27·9	9·9

The results are set out in Table 2 for the eight species studied. Each result is the average of about 10 traverses from pia to white matter, and is based on a count of about 1000 neurones. There is clearly a considerable variation in the estimated neuronal density among the specimens representing each species, and this is particularly severe in the striate areas of the human brains. Sections from the latter were

Fig. 2. Electron micrographs used for counting synapses in: (*a*) monkey visual cortex, and (*b*) mouse motor cortex. The tissue was perfused with Pease formaldehyde, further fixed in osmium tetroxide in phosphate buffer, block stained in uranyl acetate in acetone, embedded in Araldite and stained on the grid in lead citrate.


1 mm
20 μm
(a)
(b)

taken at two levels, close to the occipital pole where the centre of macular vision is represented, and 4 cm anteriorly. The counts showed a slightly higher density of the anterior level in both brains but the brains differed consistently by a factor of about **2**. The first brain was that used by Sholl (1959) and was taken from a 50-year-old male, but the weight of this brain was unknown as was the age of the brain used in the present study. A considerable loss of neurones with age in the human brain has been described by Brody (1955). This may be responsible for the large difference between the two human brains, but is probably not a factor in the variation among the animal brains studied. None of the latter was taken from an aged animal, and in a study of senile rats at over 2 years of age I have found no spontaneous axonal degeneration stainable by the Nauta–Gygax technique in the cerebral cortex. When small lesions were made in the rat cerebral cortex, degeneration products were still stainable by the Nauta–Gygax method 8 months later. There is thus no evidence of spontaneous axonal degeneration in the rat cerebral cortex during the last third of the natural life span.

The counts of neurones in the motor cortex shown in Table 2 indicate that neuronal density decreases fairly steadily with increasing brain or body weight among the eight species studied. This is in agreement with the original observation of Nissl (1898) and with a comparison of eleven species by Tower & Elliot (1952) and Tower (1954). The latter studies were made in the motor cortex, but not specifically in the agranular area which was counted in the present work. The possible inclusion of a granular layer in Tower's counts, and the fact that no mention is made of a correction for volume shrinkage caused by dehydration and embedding no doubt account for Tower's estimates of density being higher than those of the same species included in the present study. The same considerations apply to the comparison in four species of neuronal densities in the mid-parietal field *j'* by Bok (1959). An estimate of neuronal density corrected for volume shrinkage has been published by Ramon-Moliner (1961) for the postcruciate gyrus of the cat. This estimate is 24×10^6 neurones per cc which is close to the value in Table 2, although the latter was obtained from the pre-cruciate gyrus. Hess (1961) has applied approximate corrections to the counts made by Rowland & Mettler (1949) in human frontal cortex, and deduced a density of 18×10^6 neurones per cc.

Comparison of the densities of neurones in the primary visual cortex in Table 2 shows a striking anomaly: the density tends to decrease with increasing brain weight in the non-primate species as expected, but among primates the Galago and rhesus brains show a higher density of neurones than even the mouse, and in the two human brains the density in the striate area is much higher than in the motor cortex. Two brains of approximately equal weight are the rhesus and pig brains, and these show closely similar values for neuronal density in the motor cortex, but a much higher density in the rhesus striate area. Such a striking increase in neuronal density in primate striate cortex should have been noticed previously, but the published estimates of density present a confusing picture. Thus Shariff (1953)

Fig. 3. Photomicrographs of Nissl stained preparations used of counting neurones in (*a*) pig visual cortex, (*b*) monkey visual cortex. Transverse sections were cut in Araldite to measure section thickness, as shown at the bottom of each figure, and the section thickness was found to be 20·4 μm (*a*) and 22·4 μm (*b*).

presented very high values for neuronal density in primate striate cortex, but these results were shown to be subject to a technical error by Haug (1956). The rather high value of 160×10^6 neurones per cc was reported by Chow, Blum & Blum (1950) for monkey striate cortex, but no mention was made of correction for volume shrinkage. A lower average value of 69×10^6 neurones per cc was found by Cowey (1964) and again no shrinkage correction was applied. Since these authors did not report corresponding results for non-primate species, the anomaly was not apparent. Nurnberger & Gordon (1957) used a homogenization procedure, and found a low neuronal density of about 14×10^6 neuronal nuclei per cc in human, monkey and rat striate cortex. The accuracy of the differentiation of neurones from glia with this method has been questioned by Brizzee, *et al.* (1964). Examination of the micrographs of visual cortex reproduced in Fig. 3 confirms that there is a qualitative difference between primate and non-primate visual cortex, indicating that the anomaly discussed above is not due to an artefact connected with the method of counting. When the visual cortex of the pig is compared with that of the monkey it is clear that the former has much larger neurones spread more widely apart, while the latter has a denser layer of granule cells.

Table 3. *The density of synapses and neurones per cc of cortex and the average number of synapses to each neurone*

Cortex	Synapses	Neurones	Synapses per neurone
Mouse visual	$6{\cdot}6 \times 10^{11}$	$92{\cdot}4 \times 10^6$	7000
Mouse motor	$8{\cdot}5 \times 10^{11}$	$64{\cdot}4 \times 10^6$	13000
Monkey visual	$6{\cdot}2 \times 10^{11}$	$110{\cdot}3 \times 10^6$	5600
Monkey motor	$9{\cdot}6 \times 10^{11}$	$16{\cdot}1 \times 10^6$	60000

The number of synapses on each neurone

The estimates of synaptic density in the mouse and monkey visual and motor cortical areas were divided by the corresponding neuronal densities measured in the same brains, to estimate the average number of synapses on each neurone (Table 3). Some of these results are larger than previous estimates made without electron microscopy. Thus Wyckoff & Young (1956) estimated that there are not less than 2000 end feet on each cat motor horn cell, and Armstrong & Young (1957) calculated that a large neurone in the cat's sensorimotor cortex should have more than 8000 synapses upon it. David (1957) arrived at a value of 3200 boutons on a single mesencephalic neurone in cat, and Hyden & Pigon (1960) suggested 10000 end feet on a neurone in Deiter's nucleus in the rabbit. Aitken & Bridger (1961) increased the Wyckoff & Young estimate to 16000 synapses on a large motor horn cell in the cat.

The striking differences between the individual values entered in Table 3 are mainly due to the large differences in neuronal density, the density of synapses being less widely variable. In passing from mouse to monkey, there are clearly opposite trends in the motor and visual areas. The motor area has far more synapses on each neurone in the monkey than in the mouse, and the visual area rather fewer. Intuitively, it seems reasonable that an improved quality of cortex should be associated

with increased neuronal interconnexion in motor and association cortex, as von Economo (1926) suggested. In the visual area, the larger number of smaller cells in the monkey have on average fewer synapses each, and it seems possible that this makes for a sharper representation of the visual field.

DISCUSSION

The measurements of neuronal density in the motor area confirm the work of other authors in showing that in a range of species the density tends to decrease with increasing brain or body weight (Tower & Elliot, 1952; Tower, 1954; Bok, 1959; Nissl, 1898). In the visual area also there is a fall in density with increasing brain size among the non-primate species studied, though the density in the visual area is greater than that in the motor area in each species, the ratio being 1·4–1·8. Among the primates, however, the neuronal density is much higher in the visual area, being 2·4–6·3 times that of the motor cortex of the same species. The primates possess colour vision and a high visual acuity. Both are better developed in near-macular vision than in peripheral vision (Weymouth, 1958; Duke-Elder, 1932, p. 906). The part of the striate cortex representing macular vision in macaca shows a slightly lower neuronal density than regions representing peripheral vision, but this difference is not statistically significant in the counts so far published (Chow, *et al.* 1950; Cowey, 1964). A slightly greater depth of cortex has been described in the macular part of the striate area by Solnitzky & Harman (1946). Thus neuronal density is high in parts of the striate cortex representing peripheral vision where visual acuity and colour vision are poorly developed. It is thus not easy to suggest a functional correlation of the high neuronal density in the primate visual area.

Such a variety of neuronal densities has been found by different authors using different methods in different species that it might be thought that no consensus exists. In fact, however, the few replications of counts on the same tissue by different authors that have been published clearly distinguish the areas and species, and rank them in the same order, in spite of variations in the numerical density, which must be attributed in part to uncorrected tissue shrinkage. Table 4 assembles the replicated results on a common basis of number of neurones in 1 cc of cortex. Shariff's results are systematically too high owing to a depth of focus error pointed out by Haug (1956), and only the estimates marked by an asterisk are free from errors due to shrinkage.

The present high estimates of synaptic density in cortical tissue do not indicate how much margin is left for further multiplication of synapses as a possible anatomical basis for learning. It has, however, been estimated that only about 15% of the surface area of the perikaryon is in contact with synapses in the hippocampus of the rat (Blackstad & Dahl, 1961). Estimates for dendrites are not yet available.

The measurements of neuronal and synaptic density yield an estimate of the average number of synapses on each neurone as in Table 3. This number sets an upper limit to the average number of neurones that can be interconnected directly with any one neurone in the areas concerned. It would be of great interest to know how much lower this average interconnexion is than the limit set by the average number of synapses on each neurone. At least two factors reduce the connectivity below this

limit: a proportion of the synapses are derived from thalamic axons and so do not take part in intracortical neuronal interconnexion, and a proportion of the synapses may constitute multiple junctions between the same two neurones.

The order of magnitude of the first factor was estimated by Sholl (1955) who found about 100000 fibres crossing 1 mm^2 of the grey-white boundary of cat visual cortex, and 75000 neurones above the same area possessing axons leaving the cortex. There are then 25000 afferent axons per mm^2, or about one to each three neurones with axons leaving the cortex, and thus one to each four cortical neurones altogether. If we make the further questionable assumption that each thalamic axon ends in about as many terminals as the axon of an average cortical neurone, then four-fifths of the synapses are of cortical origin. The only direct test of this question, however, is to denervate a piece of cortex by making a suitable thalamic lesion, and to compare the density of intact synapses in this area with that in the corresponding contralateral cortex. Material has been obtained from striate cortex of monkeys with lesions of the lateral geniculate nucleus to carry out this estimation.

Table 4. *Comparison of replicate estimates of neuronal density in cerebral cortex (nr. per cc* $\times 10^{-6}$)

Cat sensorimotor	Monkey visual	Human visual	Monkey motor	Human motor
*23·9*a*	265*e*	97·2*e*	62*e*	18·2*e*
*25·4*b*	*110*b*	*50*b*	*18*b*	*9·9*b*
31*c*	158*f*	77·7*d*	21*c*	10*c*
10·5*d*	68·6*g*	—	—	26·6*d*

a, Ramon-Moliner (1961); *b*, Cragg; *c*, Tower & Elliot (1952); *d*, Sholl (1959); *e*, Sharrif (1953); *f*, Chow, Blum & Blum (1950); *g*, Cowey (1964).
* Not subject to shrinkage error.

The second factor, multiplicity of connections between the same two neurones, cannot be measured by present techniques. If one neglects subcortical connexions, the axon of a cortical neurone must give rise to the same average number of synaptic boutons as a cortical neurone receives. No technique permits us to visualize these thousands of boutons in continuity with the parent axon and in relationship with the post-synaptic structures. However, the rapid Golgi and Ehrlich methods stain cortical axons, and studies of cortex with these methods have not given any indication that the branches of an axon are commonly directed predominantly towards the dendrites of one particular neurone. Moreover, it is relevant that Sholl (1953) calculated that in cat visual cortex, the territory defined by the dendritic arborization of one stellate neurone contained the cell bodies of about 4000 other neurones. Thus the dendritic processes of one neurone are intermixed with the dendrites of thousands of other neurones. If the axon of one neurone is then to make a large proportion of its synapses upon a small number of other neurones, an effective taxis or tropism of some kind operating on a microscopic scale is needed to cause the axon to seek out the appropriate dendrites among thousands of dendrites belonging to neighbouring neurones. No such microscopic taxis is known at present. The ordering of amphibian or fish optic nerve fibres regenerating into the tectum may take place at least partly within the optic tract before the fibres have reached the tectal neurones (Attardi & Sperry, 1963; Jacobson, 1961). The regenerating fibres when

they first reach the tectum may have widespread terminal arborizations which subsequently shrink to the appropriate locus (Gaze & Jacobson, 1963). A tropism on a much finer scale would be needed to confine a cortical axon to a particular dendritic tree.

It is conceivable that an axon makes synaptic contacts at random with the thousands of dendrites that it encounters, but that subsequently some of these synapses become inoperative. This possibility would further reduce the effective neuronal connectivity below the limit set by the average number of synapses on each neurone. It is not known whether the structural integrity of synapses is compatible with their being inoperative for prolonged periods, but there is a small amount of experimental evidence that can be interpreted to support this possibility. Thus Wall (1960) recorded monosynaptic responses in a single dorsal horn cell to tactile stimulation of a small patch of skin, surrounding areas of skin being unable to elicit a response. The response of the cell to touching the skin was unchanged when successive sensory rootlets were cut, until one particular 'microbundle' was reached, when the response totally disappeared. It was then found (Wall, personal communication, 1966) that elec.rical stimulation of this rootlet, and also of other neighbouring rootlets, elicited monosynaptic responses in the same dorsal horn cell. Accepting that the responses were monosynaptic and that there was no spread of stimulating current among the rootlets, it would seem to follow that the dorsal horn cell received synapses from axons in the neighbouring rootlets innervating surrounding areas of skin, and that these synapses were not operated by cutaneous stimulation, but became operative when the rootlets were stimulated electrically. This interpretation implies the structural persistence of synapses that are not functionally operative. If ultrastructural changes can be recognized in synapses that are artificially rendered inoperative, perhaps by local anaesthetic block of an afferent nerve, it may become possible to estimate whether inoperative synapses are present in sufficient numbers to reduce neuronal interaction substantially.

Let us suppose that on average half the synapses counted are of non-cortical origin, half the remainder are inoperative, and that if one neurone makes contact with another an average of twenty-five synapses is involved. Cortical neuronal connectivity would then be about one-hundredth of the limit set by the number of synapses on each neurone. With these arbitrary assumptions, the present data (Table 3) indicate an average of fifty-six neurones interconnected with each neurone in monkey visual cortex, and about 600 neurones connected to each neurone in monkey motor cortex. These levels of connectivity are much higher than those encountered in computers or in any other human artefact. There is at present no theoretical treatment of the properties of a neuronal assembly with so high a degree of interconnexion. The co-operative analogy proposed by Cragg & Temperley (1954, 1955) does not necessitate so great a multiplicity of interconnexions, and most treatments apply to low levels of neuronal interaction only. The factor of one hundred used to reduce the connectivity below the anatomical limit is only a guess. But if this factor was increased to one thousand, a typical visual cortex neurone in monkey would then be interconnected with five other neurones, and a motor neurone with sixty other neurones. Although the former figure is within the range encountered in simulation experiments (e.g. Anderson, Gillow & Rudjord, 1966) the

latter is not. It is thus difficult to avoid the conclusion that the high level of connectivity may set cerebral cortex apart from those non-biological analogies whose behaviour has been treated theoretically.

The big increase in neuronal interconnexion in motor cortex on passing from mouse to monkey is correlated with a reduction in neuronal density. The latter is lower still in man than in monkey, so it is possible that the connectivity is even higher in human cortex. The same argument, however, applies to the brain of the elephant or whale, which have a lower neuronal density than the human brain (Tower & Elliot, 1952; Tower, 1954) and a larger number of glial cells to each neurone (Hawkins & Olszewski, 1957). When it becomes possible to count synapses in human cortex, and in larger non-primate brains, it will become clear whether the level of neuronal interconnexion is a vital determinant of the complexity of behaviour that can be developed by the species concerned.

SUMMARY

1. The number of synapses in a volume of cortex has been estimated by electron microscopy for the mouse and monkey visual and motor areas. The synaptic density is $6-9 \times 10^{11}$ per cc, and thus does not vary widely between these tissues.

2. The number of neurones in a volume of cortex has been estimated by light microscopy, using methods free from shrinkage, for the mouse, rat, rabbit, cat, pig, bush-baby, monkey and human visual and motor areas. The neuronal density varies from 10^8 in monkey visual cortex to 10^7 per cc in human motor cortex.

3. Neuronal density falls with increasing brain weight in the motor cortex, and in the visual cortex of the non-primate species. In primate visual cortex neuronal density is much higher than in non-primate brains of comparable weight.

4. The average number of synapses of each neurone has been found for the mouse and monkey visual and motor areas. This varies from 5600 in monkey visual cortex to 60000 in monkey motor cortex.

5. In an attempt to deduce the corresponding levels of cortical connectivity, certain points requiring further work have been identified. Some implications of the seemingly high level of cortical connectivity are discussed.

REFERENCES

AITKEN, J. T. & BRIDGER, J. E. (1961). Neuron size and neuron population density in the lumbosacral region of the cat's spinal cord. *J. Anat.* **95**, 38–53.

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

ANDERSEN, P., GILLOW, M. & RUDJORD, T. (1966). Rhythmic activity in a simulated neuronal network. *J. Physiol., Lond.* **185**, 418–428.

ARMSTRONG, J. & YOUNG, J. Z. (1957). End-feet in the cerebral cortex. *J. Physiol., Lond.* **137**, 10–11 *P.*

ATTARDI, D. G. & SPERRY, R. W. (1963). Preferential selection of central pathways by regenerating optic fibres. *Expl Neurol.* **7**, 46–64.

BLACKSTAD, T. W. & DAHL, H. A. (1961). Quantitative evaluation of structures in contact with neuronal somata. *Acta morph. neerl. scand.* **4**, 329–343.

BRIZZEE, K. R., VOGT, J. & KHARETCHKO, X. (1964). Postnatal changes in glia/neuron index with a comparison of methods of cell enumeration in the white rat. In *Progress in Brain Research*, vol. 4; *Growth and Maturation of the Brain*, Ed. Purpura, D. P. and Schadé, J.P. New York: Academic Press.

BOK, S. T. (1959). *Histonomy of the cerebral cortex*. New York: Elsevier.
BRODY, H. (1955). Organisation of the cerebral cortex. III A study of aging in the human cerebral cortex. *J. comp. Neurol.* **102**, 511–556.
CHOW, K. L., BLUM, J. S. & BLUM, R. A. (1950). Cell ratios in the thalamo-cortical visual system of macaca mulatta. *J. comp. Neurol.* **92**, 227–239.
CLEMENTI, F., WHITTAKER, V. P. & SHERIDAN, M. N. (1966). The yield of synaptosomes from the cerebral cortex of guinea pigs estimated by a polystyrene bead "Tagging" procedure. *Z. Zellforsch. mikrosk. Anat.* **72**, 126–138.
COWEY, A. (1964). Projection of the retina on to striate and prestriate cortex in the squirrel monkey, *Saimiri sciureus*. *J. Neurophysiol.* **27**, 366–393.
CRAGG, B. G. & TEMPERLEY, H. N. V. (1954). The organisation of neurones: A co-operative analogy. *Electroenceph. clin. Neurophysiol.* **6**, 85–92.
CRAGG, B. G. & TEMPERLEY, H. N. V. (1955). Memory: the analogy with ferromagnetic hysteresis. *Brain* **78**, 304–316.
DAVID, G. B. (1957). On the structure of the synapse. In *Metabolism of the Nervous System*. Ed. D. Richter. London: Pergamon.
DUKE-ELDER, W. S. (1932). *Textbook of Ophthalmology*, vol. 1. London: Kimpton.
VON ECONOMO, C. (1926). Ein Koeffizient für die Organisationshöhe der Grosshirnrinde. *Klin. Wsch.* **5**, 593–595.
GAZE, R. M. & JACOBSON, M. (1963). A study of the retinotectal projection during regeneration of the optic nerve in the frog. *Proc. R. Soc.* B **157**, 420–448.
HAUG, H. (1956). Remarks on the determination and significance of the gray cell coefficient. *J. comp. Neurol.* **104**, 473–492.
HAWKINS, A. & OLSZEWSKI, J. (1957). Glia/nerve cell index for cortex of the whale. *Science* **126**, 76–77.
HESS, H. H. (1961). The rates of respiration of neurones and neuroglia in human cerebrum. In *Regional Neurochemistry*. Eds. S. S. Kety & J. Elkes. London: Pergamon Press.
HYDEN, H. & PIGON, A. (1960). A cytophysiological study of the functional relationship between oligodendroglial cells and nerve cells of Deiter's nucleus. *J. Neurochem.* **6**, 57–72.
JACOBSON, M. (1961). The recovery of electrical activity in the optic tectum of the frog during early regeneration of the optic nerve. *J. Physiol., Lond.* **157**, 27–29*P*.
KANAGASUNTHERAM, R., LEONG, C. G. & MAHRAN, Z. Y. (1966). Observations on some cortical areas of the Lesser Bush Baby (*Galago senegalensis senegalensis*). *J. Anat.* **100**, 317–334.
KAPPERS, A. C. U., HUBER, G. C. & CROSBY, E. C. (1936). *The Comparative Anatomy of the Nervous System of Vertebrates, including Man*, vol. 2. New York: MacMillan.
MARENGO, N. P. (1944). Paraffin section thickness—a direct method of measurement. *Stain Technol.* **19**, 1–10.
MILLONIG, G. (1961). Advantages of a phosphate buffer for OsO_4 solutions in fixation. *J. app. Phys.* **32**, 1637.
NISSL, F. (1898). Nervenzellen und graue Substanz. *Münch. med. Wschr.* **45**, 988–992, 1023–1029, 1060–1062.
NURNBERGER, J. I. & GORDON, M. W. (1957). The cell density of neural tissues: direct counting method and possible applications as a biological referent. *Prog. Neurobiol.* **2**, 100–138.
PEACHEY, L. D. (1958). Thin sections. 1. A study of section thickness and physical distortion produced during microtomy. *J. biophys. biochem. Cytol.* **4**, 233–242.
PEASE, D. C. (1962). Buffered formaldehyde as a killing agent and primary fixative for electron microscopy. *Anat. Rec.* **142**, 342.
RAMON-MOLINER, E. (1961). The histology of the post-cruciate gyrus in the cat. I. Quantitative studies. *J. comp. Neurol.* **117**, 43–62.
ROBINS, E., SMITH, D. E. & EYDT, K. M. (1956). The quantitative histochemistry of the cerebral cortex. 1. Architectonic distribution of ten chemical constituents in the motor and visual cortices. *J. Neurochem.* **1**, 54–67.
ROSE, M. (1929). Cytoarchitectonischer Atlas der Grosshirnrinde der Maus. *J. Psychol. Neurol. Lpz.* **40**, 1–51.
ROWLAND, L. P. & METTLER, F. A. (1949). Cell concentration and laminar thickness in the frontal cortex of psychotic patients; studies on cortex removed at operation. *J. comp. Neurol.* **90**, 255–280.
SHARRIF, G. A. (1953). Cell counts in the primate cerebral cortex. *J. comp. Neurol.* **98**, 318–400.
SHOLL, D. A. (1953). Dendritic organization in the neurons of the visual and motor cortices of the cat. *J. Anat.* **87**, 387–406.
SHOLL, D. A. (1955). The organisation of the visual cortex in the cat. *J. Anat.* **89**, 33–46.
SHOLL, D. A. (1956). *The Organisation of the Cerebral Cortex*. London: Methuen.
SHOLL, D. A. (1959). A comparative study of the neuronal packing density in the cerebral cortex. *J. Anat.* **93**, 143–158.

SOLNITZKY, O. & HARMAN, P. J. (1946). A comparative study of the central and peripheral sections of the visual cortex in primates, with observations in the lateral geniculate body. *J. comp. Neurol.* **85**, 313–420.

TOWER, D. B. (1954). Structural and functional organisation of mammalian cerebral cortex: the correlation of neurone density with brain size. *J. comp. Neurol.* **101**, 19–52.

TOWER, D. B. & ELLIOTT, K. A. C. (1952). Activity of the acetylcholine system in cerebral cortex of various unanaesthetized animals. *Am. J. Physiol.* **168**, 747–759.

WALL, P. D. (1960). Cord cells responding to touch, damage and temperature of skin. *J. Neurophysiol.* **23**, 197–210.

WESTRUM, L. E. (1965). A combination staining technique for electron microscopy. 1. Nervous tissue. *J. Microscopie* **4**, 275–278.

WEYMOUTH, F. W. (1958). Visual sensory units and the minimal angle of resolution. *Am. J. Ophthal.* **46**, 102–113.

WYCKOFF, R. W. G. & YOUNG, J. Z. (1956). The motor neuron surface. *Proc. R. Soc.* B, **144**, 440–450.

ZUCKERMAN, S. & FULTON, J. F. (1941). The motor cortex in *Galago* and *Perodicticus*. *J. Anat.* **75**, 447–456.